

**EVALUATION OF SULPHUR AND SALICYLIC ACID ON  
GROWTH, PHYSIOLOGY, YIELD AND MOLECULAR  
EXPRESSION OF INDIAN MUSTARD**

Thesis Submitted for the Award of the Degree of

**DOCTOR OF PHILOSOPHY**

in

**Agronomy**

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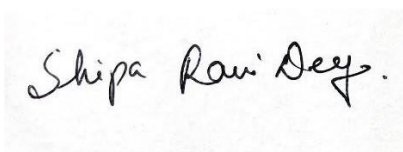
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**2024**

## **DECLARATION**

I, hereby declared that the presented work in the thesis entitled “**EVALUATION OF SULPHUR AND SALICYLIC ACID ON GROWTH, PHYSIOLOGY, YIELD AND MOLECULAR EXPRESSION OF INDIAN MUSTARD**” in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of Dr. Prasann Kumar, UID: 21784, working as Assistant Professor, in the School of Agriculture (Agronomy) of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.

A handwritten signature in black ink that reads "Shipa Rani Dey." The signature is written in a cursive style and is centered within a light gray rectangular box.

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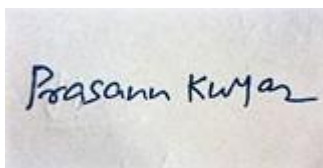
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## CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled “**EVALUATION OF SULPHUR AND SALICYLIC ACID ON GROWTH, PHYSIOLOGY, YIELD AND MOLECULAR EXPRESSION OF INDIAN MUSTARD**” submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the Department of Agronomy/ School of Agriculture, is a research work carried out by **Shipa Rani Dey, 11915166**, is bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.



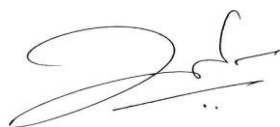
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## Abstract

The study entitled "**Evaluation of Sulphur and Salicylic Acid on Growth, Physiology, Yield and Molecular Expression of Indian Mustard**" was carried out during two consecutive Rabi seasons, specifically from 2021 to 2022 and 2022 to 2023, at Lovely Professional University in Punjab. This study aimed to assess the impact of Sulphur and Salicylic acid on the growth, physiology, yield, and molecular expression of the Indian mustard crop variety RH725 at various growth stages, precisely at 30, 60, 90, and 120 days after sowing (DAS). The mustard seeds utilised in this study were obtained from a reputable and certified seed producer named 'Good Grow' in Phagwara, Punjab. The research used an experimental plot with dimensions of 15 square metres, consisting of a width of 5 metres and a length of 3 metres. The seed-sowing process was efficiently executed within the research fields using a randomised block design (RBD). Treatments were applied at designated time intervals, specifically 15, 45, and 75 days after sowing (DAS). The concentrations of exogenous sulphur and salicylic acid utilised in the experimental treatments were established according to prior research findings. Data was gathered during the four pivotal growth stages specified, and the treatments encompassed a spectrum of concentrations that involved both separate and combined applications of Thiourea and Salicylic acid. Plant morphological traits that can be measured include plant height, leaf number, leaf area, leaf area index, node number, internodal length, number of primary branches, number of secondary units, and stem girth. The biochemical and physiological parameters under consideration are the Chlorophyll Index, plant turgid weight, plant dry weight, relative water content (RWC), membrane stability index (MSI), Chlorophyll a, chlorophyll b, chlorophyll a and b combination, and the chlorophyll a to b ratio, carotenoids, total phenol, flavonoids, and flavanols, total soluble protein, total free amino acids, total lipid, total soluble sugar, total starch, phenylalanine ammonia-lyase (PAL) activity, and total free proline, net assimilation rate (NAR), leaf area-based crop growth rate (CGR), and relative growth rate (RGR). The oil quality parameters of interest include acid value, iodine value, p-anisidine value, peroxide value, totox value, saponification value, refractive index, oil density, viscosity. The yield parameters of interest include economic yield, biological yield, stover yield, harvest index, test weight, oil content, oil cake. To check the soil health the parameters studied include soil pH, electrical conductivity (EC), and cation exchange capacity (CEC), nitrogen (N), phosphorus (P), potassium (K), and sulphur (S) and organic carbon.

A thorough analysis of the outcomes, encompassing the findings derived from diverse instrumental techniques, such as X-ray Diffraction (XRD), which was employed to verify the infiltration of sulphur and salicylic acid into the leaves after foliar application. Scanning

Electron Microscopy (SEM) is used to analyse the structural alterations in the leaves after the foliar administration of various treatments. Energy-dispersive X-ray Spectroscopy (EDX) is utilised to assess the changes in elemental composition within the leaves of mustard following the application of said treatments. Gas chromatography-mass spectrometry (GC-MS) is employed to analyse the oil extracted from seeds after harvest qualitatively. Fourier transform infrared spectroscopy (FTIR) examines alterations in the functional groups associated with the compounds generated. Additionally, Zeta potential, particle size analysis, and other pertinent methodologies are employed in this context. The research endeavour followed a rigorous trajectory, ultimately uncovering notable discrepancies and favourable results from the interventions involving Sulphur and Salicylic acid. The observations above were not solely based on personal anecdotes but underwent thorough examination through a rigorous analysis process facilitated by the SPSS software. The comprehensive analysis of the data revealed significant findings regarding the effects of these interventions on the growth, physiology, yield, and molecular expression of the Indian mustard.

As the results emerged, it became increasingly apparent that this study contributes substantially to our understanding of how interventions involving Sulphur and Salicylic acid influence the course of Indian mustard cultivation. The comprehensive body of evidence gathered during the two-year research period offers a nuanced comprehension of the complex interplay between these interventions and the diverse facets of the mustard crop. One of the primary strengths of this study resides in its rigorous methodology. The study was carried out meticulously, following established methods and protocols commonly accepted in the discipline. The utilization of this particular methodology effectively safeguarded the integrity of the data that was gathered, thereby establishing a dependable basis upon which to construct meaningful insights and draw valid conclusions. The study employed a Randomized Block Design and implemented treatments at predetermined intervals, effectively mitigating potential biases. As a result, the collected data is deemed credible and reflective of agricultural conditions in real-world settings. The observed variations and outcomes in this study should be considered. The authors emphasize the possibility of substantial enhancements in the cultivation methods of Indian mustard. Sulphur and Salicylic acid interventions significantly influence important parameters, including plant height, chlorophyll content, silique number and length, and oil quality, which hold considerable implications for the agricultural community. The research has significant potential to influence farming practices, improving crop productivity and quality. In an era characterized by dynamic shifts in climate patterns, population expansion, and resource limitations, the imperative for implementing sustainable and efficient agricultural

methodologies has become increasingly paramount. The results obtained from this study can influence decision-making processes, encompassing both the individual farm level and broader agricultural policy deliberations. Moreover, these research findings' significance is the broader objective of ensuring food security. In light of ongoing global environmental changes, such as climate change, limited resources, and shifting dietary preferences, there is an increasing urgency to develop innovative and efficient approaches to safeguard reliable food provision. This study establishes a foundation for making better-informed agricultural decisions, promoting the advancement of sustainable and resilient food production systems. The objective is not solely on augmenting crop production but on guaranteeing that.

**Keywords:** Sulphur and Salicylic acid nutrition, Physiological parameters, Yield attributes, Quality, Gene expression.

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*Place: LPU,*

*Phagwara*

*Shipa Rani Devi*

## TABLE OF CONTENT

<b>Particulars</b>	<b>Page No.</b>
<b>CHAPTER-1</b>	<b>22-43</b>
Introduction	22-42
Objectives	42-43
<b>CHAPTER-2</b>	<b>44-88</b>
Review of Literature	
<b>CHAPTER-3</b>	<b>89-134</b>
Material and Methods	
<b>CHAPTER-4</b>	<b>135-617</b>
Results and Discussions	
<b>CHAPTER-5</b>	<b>618-631</b>
Summary and conclusion	
<b>REFERENCES</b>	<b>632-655</b>

### List of Tables

<b>Table No.</b>	<b>Title</b>	<b>Page No.</b>
2.1	Main Information about data and its results	49-50
2.2	Annual Total Citation	51-52
2.3	Most Relevant Sources	54
2.4	Main Information about Data	56-57
2.5	Most Relevant Sources	59
3.1	Soil data of Mustard during Rabi 2021-22& 2022-23	91



3.2	Details of Treatment	92-93
3.3	Details of Layout	94
3.4	Cultivation Details	94-96
3.5	Weather Details of 2021-2022	97
3.6	Weather Details of 2022-2023	97-98
4.1	Plant height (cm) of Mustard during <i>Rabi</i> 2021-2023	141
4.2	Leaves number plant <sup>-1</sup> in Mustard during <i>Rabi</i> 2021-2023	148
4.3	Leaf area (cm <sup>2</sup> ) of Mustard during <i>Rabi</i> 2021-2023	154
4.4	Leaf area Index of Mustard during <i>Rabi</i> 2021-2023	163
4.5	Node number of Mustard during <i>Rabi</i> 2021-2023	169
4.6	Internodal length of Mustard During <i>Rabi</i> 2021-2023	175
4.7	Number of Primary Branches of Mustard During <i>Rabi</i> 2021-2023	182
4.8	Number of Secondary Branches of Mustard During <i>Rabi</i> 2021-2023	188
4.9	Stem Diameter (cm) of Mustard During <i>Rabi</i> 2021-2023	195
4.10	Chlorophyll Index of Mustard During <i>Rabi</i> 2021-2023	201
4.11	Plant fresh weight of Mustard During <i>Rabi</i> 2021-2023	207
4.12	Plant turgid Weight of Mustard During <i>Rabi</i> 2021-2023	212
4.13	Plant dry weight of Mustard During <i>Rabi</i> 2021-2023	218
4.14	RWC (%) of Mustard During <i>Rabi</i> 2021-2023	224
4.15	CGR of Mustard During <i>Rabi</i> 2021-2023	232
4.16	RGR of Mustard During <i>Rabi</i> 2021-2023	237
4.17	NAR of Mustard During <i>Rabi</i> 2021-2023	246
4.18	Siliqua Number of Mustard During <i>Rabi</i> 2021-23	252
4.19	Siliqua length (cm) of Mustard During <i>Rabi</i> 2021-2023	257

4.20	MSI (%) of Mustard During Rabi 2021-2023	263
4.21	Chlorophyll a of Mustard During Rabi 2021-2023	268
4.22	Chlorophyll b of Mustard During Rabi 2021-2023	273
4.23	Chlorophyll a+b of Mustard During Rabi 2021-2023	278
4.24	Chlorophyll ab ratio of Mustard During Rabi 2021-2023	283
4.25	Carotenoids of Mustard During Rabi 2021-2023	289
4.26	Total Phenol of Mustard During Rabi 2021-2023	295
4.27	Flavanols of Mustard During Rabi 2021-2023	301
4.28	Flavonoids of Mustard During Rabi 2021-2023	307
4.29	Total Soluble Protein of Mustard During Rabi 2021-2023	312
4.30	Total Free Amino Acids of Mustard During Rabi 2021-2023	318
4.31	Total Lipids of Mustard During Rabi 2021-2023	324
4.32	Total Soluble sugar of Mustard During Rabi 2021-2023	330
4.33	Total Starch of Mustard During Rabi 2021-2023	336
4.34	PAL of Mustard During Rabi 2021-2023	341
4.35	Total Free Proline of Mustard During Rabi 2021-2023	346
4.36	Economic Yield of Mustard During Rabi 2021-2023	352
4.37	Biological yield of Mustard During Rabi 2021-2023	352
4.38	Stover yield of Mustard During Rabi 2021-2023	352
4.39	Harvest Index of Mustard During Rabi 2021-2023	365
4.40	Test Weight of Mustard During Rabi 2021-2023	365
4.41	Oil content of Mustard During Rabi 2021-2023	374

4.42	Oil Cake of Mustard During Rabi 2021-2023	374
4.43	Acid value of Mustard During Rabi 2021-2023	383
4.44	Iodine Value of Mustard During Rabi 2021-2023	383
4.45	P-anisidine Value of Mustard During Rabi 2021-2023	392
4.46	Peroxide value of Mustard During Rabi 2021-2023	392
4.47	Totox Value of Mustard During Rabi 2021-2023	401
4.48	Saponification Value of Mustard During Rabi 2021-2023	401
4.49	Refractive Index of Mustard During Rabi 2021-2023	401
4.50	Oil Density of Mustard During Rabi 2021-2023	414
4.51	Oil Viscosity of Mustard During Rabi 2021-2023	414
4.52	Soil pH of Mustard During Rabi 2021-2023	424
4.53	Soil EC of Mustard During Rabi 2021-2023	424
4.54	Cation Exchange Capacity of Mustard During Rabi 2021-2023	424
4.55	Soil Available Nitrogen of Mustard During Rabi 2021-2023	437
4.56	Soil Available Phosphorus of Mustard during Rabi 2021-2023	437
4.57	Soil Potassium of Mustard During Rabi 2021-2023	446
4.58	Sulphur of Mustard During Rabi 2021-2023	446
4.59	Soil Organic Carbon of Mustard During Rabi 2021-2023	456
4.60	Economic Analysis	460
4.61	GC-MS analysis(qualitative) of oil of seed harvest from T0-Control	467-468
4.62	GC-MS analysis(qualitative) of oil of seed harvest from Treatment T1- (Thiourea recommended dose (1000ppm)	470-471
4.63	T2- GC-MS analysis(qualitative) of oil of seed harvest from Salicylic Acid (300ppm)	474-475
4.64	GC-MS analysis(qualitative) of oil of seeds harvest from Treatment- T3 Thiourea (1000ppm) + Salicylic Acid (300ppm)	480

4.65	GC-MS analysis(qualitative) of oil of seeds harvest from Treatment- T4- Thiourea (1500ppm) + Salicylic Acid (300ppm)	487
4.66	GC-MS analysis(qualitative) of oil of seeds harvest from treatment- T5- (Thiourea (1000ppm) + Salicylic Acid (450ppm)	494-495
4.67	GC-MS analysis(qualitative) of oil of seeds harvest from treatment- T6-Thiourea (500 ppm) + Salicylic Acid (300ppm)	500-501
4.68	GC-MS analysis(qualitative) of oil of seeds harvest from treatment- T7-Thiourea (1000ppm) + Salicylic Acid (150ppm)	507
4.69	GC-MS analysis(qualitative) of oil of seeds harvest from Treatment- T8- Thiourea (500ppm) + Salicylic Acid (600ppm)	513-514
4.70	GC-MS analysis(qualitative) of oil of seeds harvest from Treatment- T9-Thiourea (2000ppm) + Salicylic Acid (600ppm)	520
4.71	GC-MS analysis(qualitative) of oil of seeds harvest from Treatment- T10- Thiourea (2000ppm) + Salicylic Acid (600ppm)	526-527
4.72	GC-MS analysis(qualitative) of oil of seeds harvest from Treatment- T11- Thiourea (500ppm) + Salicylic Acid (150ppm)	532-533
4.73	CT mean of all the genes used for expression test	551-552
4.74	EDS- T0	568
4.75	EDS- T1	571
4.76	EDS-T2	574
4.77	EDS- T3	576-577
4.78	EDS- T10	579
4.79	EDS- T11	582
4.80	Zeta Potential	608
4.81	Particle size	616

### List of Figures

<b>Table No.</b>	<b>Title</b>	<b>Page No.</b>
2.1	Keywords analysis search option "Salicylic acid*" AND "Mustard*"	46
2.2	Keywords analysis search option "Mustard*" And "Sulphur*"	47
3.1	Photographs of Field work and Lab work	123-134

4.1	Plant Height (cm) of Mustard During Rabi 2021-2023 & 2022-23	142
4.2	Leaf Number of Mustard During Rabi 2021-2023 & 2022-23	149
4.3	Leaf Area (Cm <sup>2</sup> ) of Mustard During Rabi 2021-2023 and 2022-23	155
4.4	Leaf Area Index of Mustard During Rabi 2021-2023 & 2022-23	163
4.5	Node Number of Mustard During Rabi 2021-2023 & 2022-23	170
4.6	Internodal Length(cm) of Mustard During Rabi 2021-2023 & 2022-23	176
4.7	Number of primary branches of Mustard During Rabi 2021-2023 & 2022-23	183
4.8	Number of secondary branches of Mustard During Rabi 2021-2023 & 2022-23	189
4.9	Stem Girth(cm) of Mustard During Rabi 2021-2023 & 2022-23	196
4.10	Chlorophyll Index (Sped unit) of Mustard During Rabi 2021-2023 & 2022-23	<b>202</b>
4.11	Plant fresh weight of Mustard During Rabi 2021-2023	<b>208</b>
4.12	Plant turgid Weight of Mustard During Rabi 2021-2023	213
4.13	Plant dry weight of Mustard During Rabi 2021-2023	219
4.14	RWC (%) of Mustard During Rabi 2021-2023	225
4.15	CGR of Mustard During Rabi 2021-2023	233
4.16	RGR of Mustard During Rabi 2021-2023	238
4.17	NAR of Mustard During Rabi 2021-2023	247
4.18	Siliqua Number of Mustard During Rabi 2021-23	253
4.19	Siliqua length (cm) of Mustard During Rabi 2021-2023	257
4.20	MSI (%) of Mustard During Rabi 2021-2023	264
4.21	Chlorophyll a of Mustard During Rabi 2021-2023	269

4.22	Chlorophyll b of Mustard During Rabi 2021-2023	274
4.23	Chlorophyll a+b of Mustard During Rabi 2021-2023	279
4.24	Chlorophyll ab ratio of Mustard During Rabi 2021-2023	284
4.25	Carotenoids of Mustard During Rabi 2021-2023	290
4.26	Total Phenol of Mustard During Rabi 2021-2023	296
4.27	Flavanols of Mustard During Rabi 2021-2023	302
4.28	Flavonoids of Mustard During Rabi 2021-2023	308
4.29	Total Soluble Protein of Mustard During Rabi 2021-2023	313
4.30	Total Free Amino Acids of Mustard During Rabi 2021-2023	319
4.31	Total Lipids of Mustard During Rabi 2021-2023	325
4.32	Total Soluble sugar of Mustard During Rabi 2021-2023	331
4.33	Total Starch of Mustard During Rabi 2021-2023	337
4.34	PAL of Mustard During Rabi 2021-2023	342
4.35	Total Free Proline of Mustard During Rabi 2021-2023	347
4.36	Economic Yield of Mustard During Rabi 2021-2023	353
4.37	Biological yield of Mustard During Rabi 2021-2023	357
4.38	Stover yield of Mustard During Rabi 2021-2023	361
4.39	Harvest Index of Mustard During Rabi 2021-2023	366
4.40	Test Weight of Mustard During Rabi 2021-2023	370
4.41	Oil content of Mustard During Rabi 2021-2023	375
4.42	Oil Cake of Mustard During Rabi 2021-2023	379
4.43	Acid value of Mustard During Rabi 2021-2023	384

4.44	Iodine Value of Mustard During Rabi 2021-2023	388
4.45	P-anisidine Value of Mustard During Rabi 2021-2023	393
4.46	Peroxide value of Mustard During Rabi 2021-2023	397
4.47	Totox Value of Mustard During Rabi 2021-2023	402
4.48	Saponification Value of Mustard During Rabi 2021-2023	406
4.49	Refractive Index of Mustard During Rabi 2021-2023	410
4.50	Oil Density of Mustard During Rabi 2021-2023	415
4.51	Oil Viscosity of Mustard During Rabi 2021-2023	419
4.52	Soil pH of Mustard During Rabi 2021-2023	425
4.53	Soil EC of Mustard During Rabi 2021-2023	429
4.54	Cation Exchange Capacity of Mustard During Rabi 2021-2023	433
4.55	Soil Available Nitrogen of Mustard During Rabi 2021-2023	438
4.56	Soil Available Phosphorus of Mustard during Rabi 2021-2023	442
4.57	Soil Potassium of Mustard During Rabi 2021-2023	447
4.58	Sulphur of Mustard During Rabi 2021-2023	452
4.59	Soil Organic Carbon of Mustard During Rabi 2021-2023	457
4.60	Different genes and their expression	548-551
4.61	FTIR T0	556
4.62	FTIR T1	557
4.63	FTIR T2	558
4.64	FTIR T3	559

4.65	FTIR T4	560
4.66	FTIR T5	561
4.67	FTIR T6	562
4.68	FTIR T7	563
4.69	FTIR T8	564
4.70	FTIR T9	565
4.71	FTIR T10	566
4.72	FTIR T11	567
4.73	EDS result T0	568
4.74	EDS result T1	571
4.75	EDS result T2	573
4.76	EDS result T3	576
4.77	EDS result T10	578
4.78	EDS result T11	581
4.79	XRD result T0	587
4.80	XRD result T1	588
4.81	XRD result T2	589
4.82	XRD result T3	590
4.83	XRD result T4	591
4.84	XRD result T5	592
4.85	XRD result T6	593
4.86	XRD result T7	594



4.87	XRD result T8	595
4.88	XRD result T9	596
4.89	XRD result T10	597
4.90	XRD result T11	598

**LIST OF  
ABBREVIATIONS**

**SYMBOLS USED**

;	Semicolon
%	Per cent
:	Colon
<sup>0</sup> C	Degree Celsius
CAT	Catalase
Chl a	Chlorophyll a
Chl b	Chlorophyll b
cm <sup>2</sup>	Square Centimeter
cm	Centimetre
CO <sup>2</sup>	Carbon dioxide
RBD	Randomized Block Design
Conc.	Concentration
DAS	Days after sowing
DDW	Double distilled water
EC	Electrical Conductivity
EDTA	Ethylenediaminetetraacetic acid
et al.,	Co-worker
FW	Fresh Weight

DW	Dry Weight
g	Gram
g cm <sup>-3</sup>	Gram per cubic centimetre
HCl	Hydrochloric Acid
H <sub>2</sub> SO <sub>4</sub>	Sulphuric Acid
H <sub>2</sub> O <sub>2</sub>	Hydrogen Peroxide
q ha <sup>-1</sup>	Quintal per hectare
kg ha <sup>-1</sup>	Kilogram per hectare
lakh ha	Lakh hectare
lakh tons	Lakh tons
M	Molar
t ha <sup>-1</sup>	Tonne per hectare
mg	Milligram
mg kg <sup>-1</sup>	Milligram per kilogram
ml	Milliliter
mM	Millimolar
N	Normal
NaOH	Sodium Hydroxide
Nm	Nano Meter
NO	Nitrous Oxide
POD	Peroxidase

ppm	Parts Per Million
R	Replication
rpm	Rotation Per Minute
RWC	Relative Water Content
PAL	Phenylalanine ammonia lyase
T	Treatment
CGR	Crop Growth Rate
RGR	Relative Growth Rate
NAR	NET Assimilation Rate
LAI	Leaf Area Index
XRD	X-ray Diffraction
FTIR	Fourier transform infrared
EDS	Energy dispersivspectroscopy
GC-MS	Gas chromatography- Mass spectrometry
R	Replication
B	Boron
S	Sulphur
Cyt.	Cytokinin
UV	Ultra Violet
w/v	weight by volume

MSI	Membrane Stability Index
MII	Membrane Injury Index
v/v	volume by volume
OP	Osmotic Potential
PV	Peroxide value
H.I.	Harvesting index
E.Y.	Economical yield
B.Y.	Biological yield

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**INTRODUCTION**

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Oilseeds play a significant role in India's agricultural sector, occupying a prominent position after cereal grains regarding their impact on land area, production output, and economic value (Kumar et al., 2022). It is important to note that India's contribution to global vegetable oil production underscores its prominence. India ranks as the fifth-largest producer globally, falling behind the economies of the United States of America, China, Brazil, and Argentina. However, India's contribution to global vegetable oil production underscores the country's significance. As a result of this ranking, India is responsible for approximately 11.2% of the world's oil imports, contributes 6.32% to the global production of vegetable oil, and accounts for 9.3% of the worldwide consumption of edible oil (Iqrar et al., 2022). The rapid increase in the number of people living in the world is mainly responsible for the skyrocketing demand for seed oils of the highest possible quality. On the international stage, Glycine max, more commonly known as soybeans, is the crop that holds the preeminent position among oilseed crops. Oilseed rape or canola (*Brassica napus* L.) comes in a close second. The growing of mustard and rapeseed crops is practised in more than fifty countries across Asia, Africa, Europe, the Americas, and Australia. Canada, China, and India account for a combined total of 67.7% of the total cultivated area and 55.83% of the overall production output compared to the other nine countries that rank among the top ten producers of rapeseed and mustard. India, a prominent global producer of rapeseed and mustard, ranks third in production volume, trailing behind Canada and China. India's significance is further emphasised by its ranking as the third-largest country in cultivated land area, accounting for 18.58% of the total global land area dedicated to agriculture. Additionally, India's production output contributes 9.50% to the overall global aggregate, solidifying its position as a crucial global producer of these essential crops. Rapeseed and mustard hold significant prominence within India's agricultural sector, positioning the country as one of the leading international producers of these crops. These crops consist of a wide range of varieties, including both traditional indigenous species, such as yellow sarson (*B. campestris* L. var. yellow sarson), black mustard (*B.*

*nigra*), brown sarson (*B. campestris* L. var. brown sarson), toria (*Brassica campestris* L. var. toria), Indian mustard [*B. juncea* (L.) Czern. & Coss.], and taramira (*Eruca sativa/vesicaria* Mill.), which have been cultivated since the emergence of agriculture approximately 3500 BC. Furthermore, unconventional species such as Gobhi sarson (*B. napus* L.) and Ethiopian mustard, also called Karan rai (*B. carinata*, *A. braun*), add to the diverse mustard and rapeseed varieties. *B. juncea* (L.) Czern. & Coss., more commonly referred to as "Indian mustard," emerges as the leading and most widely cultivated variety among these diverse species. It occupies over 80% of the cultivated land dedicated to rapeseed and mustard, totalling approximately 6.02 million hectares (Nad et al., 2001). It is worth mentioning that Indian mustard has exhibited a higher growth rate than the cultivation of brown sarson and yellow sarson in regions of India that are widely recognised for their rapeseed and mustard production. This preference is due to the inherent resistance that Indian mustard possesses against significant diseases, major pests, and environmental stresses. As a result, Indian mustard is the preferred choice among cultivars. India, as a significant centre of crop diversity for Indian mustard, showcases a remarkable and unparalleled array of plant life. The scope of this diversity includes the wild counterparts of cultivated species and botanical varieties that are more distantly related. In addition, India is recognised as one of the primary centres of crop diversity for Indian mustard, highlighting this nation's substantial role in preserving the genetic heritage of this priceless agricultural resource. Oils derived from vegetables are common in human diets because of their high energy concentration and their role in transporting vitamins soluble in fat. The nutritional characteristics of an oil are the primary determinant of its quality. This is followed by considerations of the oil's industrial applicability, culinary characteristics, consumer preferences, physical characteristics, market acceptance, shelf life, palatability, and many other factors (Shah et al., 2022). The proportional composition of different fatty acids is referred to as the rich acid profile, and it is one of the most important factors that goes into determining an oil's nutritional and functional properties. The percentage of lipids found in allotetraploid *Brassica* species, including *Brassica juncea* and *Brassica napus*, can range anywhere from 35% to 45%, while the rate of proteins can

be anywhere from 20% to 25%. It is important to note that unsaturated fatty acids comprise most rapeseed-mustard oil's composition, with saturated fatty acids comprising less than 7% of the total design. This oil is considered one of the healthiest options due to its high polyunsaturated fatty acids (PUFAs) and presence of only 7% saturated fatty acids, which places it among the top choices for healthy oils (SAFAs). Excessive consumption of saturated fatty acids has been linked to atherosclerosis, the primary precursor to coronary heart disease. On the other hand, rapeseed-mustard oil presents itself as a relatively less risky alternative. Its higher content of monounsaturated fatty acids (MUFAs), along with a well-balanced ratio of monounsaturated-to-polyunsaturated fatty acids, leads to reduced levels of harmful cholesterol and low-density lipoproteins in the bloodstream while simultaneously improving the oil's overall stability. Moreover, the oil contains abundant linoleic and linolenic acids, crucial for human health (Singh & Meena, 2004). Regarding its nutritional value, rapeseed meal is among the best sources of plant-based proteins because it has an impressive protein content of approximately 40%. The amino acid content of mustard oil cake exceeds that of soybean meal, making it a preferable option for monogastric digestive systems. Nevertheless, glucosinolates with high sulphur content in the meal restrict its suitability as animal feed, specifically for pigs and poultry. This is primarily due to producing harmful and goitrogenic breakdown products and an undesirable strong taste. An increased level of glucosinolate content is responsible for causing changes in the thyroid's function, size, and structure and damage to the hepatic and renal systems. As a result, the breeding imperative in mustard production has centred on achieving "0" erucic acid content (less than 2% in seed oil), as well as "00" (the "0" classification coupled with less than 30 moles/g of glucosinolates in the defatted seed meal). This is because "0" erucic acid content is optimal for mustard production. As a result of the ever-increasing demand for edible oils across the globe, there is an urgent requirement to raise crop production levels in various agricultural settings. This necessity is made even more pressing by the persistent growth in the consumption of edible oils. Because of the unpredictability of production, which is caused by a wide variety of biotic and abiotic stressors, it is



necessary to have a multifaceted strategy combining agronomic and breeding approaches to strengthen crop resilience. A substantial increase in yield potential bolstered by a greater tolerance to biotic and abiotic adversities can be anticipated through genetic manipulation and substantial breeding efforts (Abbas et al., 2022; Adhikari et al., 2022). At the same time, taking a focused approach to preserving crop genetic resources and putting them to use is necessary. The cultivation of Indian mustard is more formally known by its scientific name, *Brassica juncea* (L.) Czernj. & Cosson is most prevalent in parts of the country, including Rajasthan, Uttar Pradesh, Haryana, Madhya Pradesh, and Gujarat. The adaptability of mustard species, specifically brown sarson (*B. rapa*), encompasses irrigated and rainfed agricultural systems. This species exhibits two distinct ecotypes, namely login and toria. On the other hand, yellow sarson, also known as *B. rapa* var. *trilocularis*, does particularly well during the rabi season in the states of Assam, Bihar, Orissa, and West Bengal. It also serves as a catch crop in Punjab, Haryana, Himachal Pradesh, Uttar Pradesh, and Madhya Pradesh. Taramira, also known as *Eruca sativa*, is a plant grown in the arid regions of North-West India, specifically in Rajasthan, Haryana, and Uttar Pradesh. Gobhi sarson (*Brassica napus* L. ssp. *oleferia* DC. Var *annua* L.) and Karan rai (*Brassica carinata*) occupy limited cultivation areas. Still, they are quickly becoming important new players in the oilseed industry. Gobhi Sarson, distinguished by its extended growth cycle, is most commonly cultivated in the Indian states of Himachal Pradesh, Haryana, and Punjab because these regions offer favourable yield potential, adaptability, and high-quality oil content. In contrast, Karan rai has remarkable pest and disease resistance, excellent environmental adaptability, and high yield potential. The country experienced a significant increase in production and productivity, commonly called the "yellow revolution." This period saw a remarkable rise from 2.68 million tons and 650 kg/ha in 1985-86 to 6.96 million tons and 1022 kg/ha in 1996-1997, respectively. However, there is a significant gap between the potential for production and the actual amount of output. There are 5.53 million hectares dedicated to cultivating rapeseed mustard in India, which helps contribute to the country's respective production and productivity figures of 6.41 million tonnes and 1157 kg/ha (Zhao et al., 2022; Zhao

et al., 2022; Zhao et al., 2022; Zhu et al., 2022; Zhu et al., 2023; Zulfiqar et al., 2022). The cultivation of mustard is most successful in climates classified as temperate; however, it can also manifest as a cold-season crop in certain tropical and subtropical regions. Indian mustard exhibits tolerance to annual precipitation ranging from 500 to 4200 mm, annual temperature fluctuations spanning 6 to 27°C, and soil pH levels within the range of 4.3 to 8.3. The rapeseed-mustard plant, which utilises the C<sub>3</sub> carbon assimilation pathway, reaches its maximum photosynthetic efficiency between 15 and 20 degrees Celsius. After reaching this temperature range, its efficiency begins to decrease. Rai is a crop that does best when grown in areas that receive much rainfall and has a pH range of between 5.5 and 6.8. It is only moderately resistant to soil acidity. It thrives in climates with warm days and cool nights, and it can somewhat tolerate dry conditions. Because of its low water requirement (240–400 mm), mustard grows best in well-drained sandy loam soil. Additionally, mustard's low water requirement works harmoniously with rainfed cropping systems, accounting for nearly 20% of its total cultivation area (Stavridou et al., 2012).

Salicylic acid (SA) and its derivatives are essential components of the phenolic acid group because they are endogenous hormones in plants. These compounds originate from the transformation of cinnamic acid and are predominantly synthesised within the cytoplasm of plant cells. They have a ring structure composed of intertwined hydroxyl and carboxyl groups. The presence of SA in *Salix* spp., where it can be found either as free phenolic acids or bound to amino compounds, most notably salicin, which makes up 9.5–11% of the plant's overall composition, was the impetus for the discovery of Salicylic acid. SA, also known as ortho-hydroxy benzoic acid, is denoted by the chemical formula C<sub>7</sub>H<sub>6</sub>O<sub>3</sub> and can be written out as such (Aghdam et al., 2023; Ahammed & Yu, 2023).

In plant growth and development, SA plays a central role, performing essential functions that help the plant become more resistant to various biotic and abiotic stresses. This is accomplished through a process known as System Acquired Resistance (SAR), which involves the activation of transformative internal signalling cascades that give

the plant the ability to withstand adverse conditions. Sodium chloride (NaCl) induced salt stress and stress from drought, cold, heat, heavy metals, ammonia, and other environmental factors are mitigated by the presence of SA. Salicylic acid (SA) significantly establishes alliances with amino acids such as proline and arginine, enhancing the plant's capacity to counteract environmental challenges while maintaining systemic acquired resistance. The ability of SA to stimulate the production of antioxidants is further evidence of this substance's profound impact. These antioxidants serve as the first line of defence against the ravages caused by reactive oxygen species (ROS), protecting the plant from oxidative damage caused by heat and drought stress. The effects of SA are not limited to merely physiological responses; instead, they extend into genetics, where they stimulate the genes that encode antioxidant enzymes, such as the manganese superoxide dismutase (Masud) gene. SA strengthens the plant's resistance and improves its response to several diseases. The plant's defence mechanisms against pathogens are activated when the internal concentrations of SA reach a high level. In addition to this, SA participates in a wide variety of physiological processes, including the stimulation of flowering and the uptake of ions, as well as the facilitation of the transport of nutrients, the enhancement of CO<sub>2</sub> assimilation, the regulation of stomatal opening and closing, photosynthesis, gas exchange, and the production of proteins. Furthermore, it plays a role in synthesising nucleic acids, amino acids, and dry matter, thereby facilitating the generation of a wide range of plant pigments, such as chlorophyll and carotene. In addition, it is worth noting that salicylic acid (SA) can counteract the synthesis of ethylene gas, in contrast to the actions of abscisic acid (ABA), which promotes leaf abscission. Salicylic acid (SA) stimulates metabolic rates, enhancing plant energy utilisation efficiency by activating alternative pathways and regulating nucleic and amino acid levels. The word "Salix," which refers to the willow tree in Latin, is where the term "salicylic acid" (SA) comes from in its nomenclature. Salicin is a glucoside of salicylic alcohol. In the following years, synthetic SA production for commercial use began in Germany in 1874. The bark of the willow tree contains salicin, which is also found in the bark of 36 other plant species. Salicin is an essential component of the bark of the willow tree and is also

prevalent in these other plant species. Humans make extensive use of a variety of drugs that are classified as anti-inflammatory agents, including aspirin, morphine, taxol, digitalis, and codeine. The synthetic derivatives of SA are used as the precursors for these medications. Acetylsalicylic acid was given the trade name Aspirin, quickly becoming the most popular selling drug globally. SA affects the pathways involved in taxol and isopentenyl pyrophosphate production in *Taxus chinensis* cultures, affecting biomass and high taxol production. The connection between salicylic acid and the growth and development of plants has been the subject of a significant number of studies.

A research investigation was conducted on *Brassica juncea* L., commonly known as Indian mustard, to examine the effects of two different concentrations of salicylic acid (35 and 70 mg) on various vegetative traits (such as plant height, branch number, and leaf area) as well as crop parameters (including seed weight, total seed yield, and seed yield). The study's results demonstrated significant enhancements in these traits and parameters following exposure to the salicylic acid treatments. Compared to a concentration of 35 mg/l and a spray made with only distilled water, the effects were most pronounced when plants were sprayed at 70 mg/L. The most common form of SA found in plants is a powdered crystalline form with a melting point of 157 to 159 degrees Celsius and a pH level of 2.4. SA may be found in a glycosylated form, a methylated form, or both forms simultaneously. When SA's hydroxyl group is conjugated with glucose, a significant conjugate is produced, known as SA glucosides. Salicylic acid (SA) is a highly significant phenolic compound crucial in enhancing various aspects of plant physiology, including plant growth, photosynthesis, flowering, and post-harvest longevity. Furthermore, it has been shown that salicylic acid (SA) effectively mitigates biotic and abiotic stresses. SA has emerged as a crucial phytohormone that plays a significant role in plant life, particularly in plant defence mechanisms. The amount of SA present in different plants, species, organs, and subcellular locations varies, and these differences are caused by fluctuations in both the developmental stages of the plant and the environmental stressors to which it is exposed. Research has shown that SA can be found in various plants, including barley,

rice, crabgrass, and soybeans, with concentrations averaging around one microgram per gram of fresh weight. Because of the exponential growth in the world's population, the demand for food production has skyrocketed to levels that have never been seen before. Conversely, plants must contend with a formidable obstacle in biotic and abiotic stresses, stunting their growth and reducing output. A promising strategy for increasing crop yield in both standard and stress-induced conditions, SA is an attractive option because it is cost-effective, biodegradable, and possesses potent growth-regulating properties. It presents an attractive option for satisfying the ever-increasing requirement for food all over the world. The germination process is a critical stage in plant development influenced by various phytohormones at various points. It is worth mentioning that gibberellins (GAs) have been observed to promote germination, whereas a heightened level of abscisic acid (ABA) demonstrates inhibitory effects. Salicylic acid (SA) is a significant factor in this context, demonstrating a substantial influence on the seed germination process, particularly when subjected to unfavourable environmental conditions (Ahmad et al., 2022; Shu, et al., 2022).

As was elucidated, the participation of SA in seed germination is inextricably linked to the glutathione pool found within the plant. SA's beneficial effect on seed germination is further supported by evidence gathered from experiments. It is imperative to acknowledge that SA's impact on seed germination depends on various factors, including the plant's genetic makeup, the nature of the stress imposed, and the conditions under which the experiment is conducted. The efficacy of SA in promoting seed germination has been observed across multiple plant species, resulting in increased growth rates and productivity. Barley and wheat are among the examples that have been studied. In addition, the supplementation of SA has been demonstrated to positively affect the germination process in marigold seeds under challenging conditions, such as water and heat stress. Similarly, SA supplementation has been found to enhance seedling growth and improve qualitative and quantitative characteristics in sweet peppers subjected to salt stress. Additionally, the effects of SA can be seen in crop plants. External application of SA at a concentration of  $130 \text{ mg L}^{-1}$  and seed treatment at a volume of  $2 \text{ ml kg}^{-1}$  has been shown to have the ability to improve rice seed vigour

and yield.

It has been demonstrated that the foliar application of salicylic acid (SA) on the leaves of two different African violet cultivars significantly improves the overall growth of the plants as well as the flowering of the plants. This treatment has resulted in significant improvements in various floral characteristics, such as flower shape, size, rosette diameter, flower bud count, and flower colour, compared to the controls, which were not subjected to any treatment (Arif et al., 2022; Azeem et al., 2023; Aziz et al., 2022).

Applying SA from an exogenous source effectively promotes the transition to the flowering stage in conditions where UV-C light has been shown to induce stress. In their study on cut roses, increasing the amount of SA in the solution also increased the amount of sucrose, extending the vase life of the flowers. Applying SA to the outside of five important cut flower varieties increased the amount of water contained in the petals while extending the vase life of the flowers (Bae et al., 2023; Bagautdinova et al., 2022; Banerjee & Roychoudhury, 2022).

It has been demonstrated that SA can increase flowering in Anthurium flowers and delay flower senescence, particularly when the flowers are subjected to chilling stress. Salicylic acid treatment can promote flowering, reduce stress, and lengthen plants' time after harvesting. Additionally, it has been discovered that the presence or absence of the *siz1* gene affects the amount of SA contained in plants. This has the effect of delaying flowering in plants with the *siz1* gene, while *siz1* mutants have an early onset of flowering.

It was discovered that applying SA and glycine betaine together to hybrid sunflower plants increased the flowering stage, head diameter, achene quantity in the head, and oil content. This was the case regardless of whether the hybrid sunflower plants were grown organically. The application of salicylic acid (SA) to cut gladiolus flowers has been found to extend their vase life and delay the senescence process. Additionally, SA promotes flowering in *Pharbitis nil*, mainly when grown in nutrient and mineral-deficient environments. Applying pre-harvest salicylic acid (SA) treatment

in tomato plants has been associated with a rise in crop yield and flowering. Salicylic acid treatment positively impacts Chrysanthemum flowering, petal water content, and vase life extension through the delay of senescence. In nutrient-deficient environments, the administration of SA to *Pharbitis nil*, also known as Japanese morning glory, has been linked to increased flowering, which can be attributed to an increased expression of the PnFT2 gene.

Regarding post-harvest preservation, plants are vulnerable to attacks from various pathogens, decreasing yields and making plants economically unviable. In the past, post-harvest pathogens have typically been fought using artificial chemicals such as fungicides and insecticides. Nevertheless, these chemicals' environmental and health implications have motivated researchers to investigate environmentally sustainable alternatives, such as SA. The exogenous administration of salicylic acid (SA) or its derivative, acetylsalicylic acid, can augment the transcription of genes associated with pathogenesis and confer resistance against various pathogens. The phenolic compound known as SA is essential in preventing post-harvest damage in horticultural crops. Chitosan--salicylic acid has been shown to prevent post-harvest decay in cucumbers potentially. This method works by gradually releasing SA and lowering malondialdehyde (MDA) levels, a damaging compound known to cause membrane instability. It has also been discovered that SA can increase proline and antioxidant content, thereby protecting against chilling conditions and the rapid production of reactive oxygen species (ROS). In Rock and Hami melons, it has been demonstrated that synthetic SA analogues such as acibenzolar-S-methyl can resist various diseases. Exogenous application of SA has been shown to reduce the rate of fungal decay in Selva strawberries; the effects of this treatment are concentration-dependent, however. When applied in non-toxic concentrations to susceptible fruits and vegetables, scientists have discovered that SA induces resistance to post-harvest decay and resistance to pathogens. It has been discovered that SA, when applied at a concentration of 0.5 mM, can prevent

post-harvest decay in sweet cherries without causing surface damage, thereby providing resistance against *Alternaria* rot and blue mould. In healthy and infected plant tissues, it has been demonstrated that methyl salicylate, also known as MeSA, can induce disease resistance and regulate the expression of genes related to pathogen defence. The application of methyl salicylate (MeSA) to Hayward kiwifruit plants resulted in a notable decrease in the rate of post-harvest decay when compared to untreated control plants (Brilli et al., 2022; Butt & Gul, 2023; Çam et al., 2022; Campos et al., 2023). The application of MeSA vapour during the ripening process of tomatoes resulted in an augmentation of red pigmentation and a regulation of ACS gene expression. The post-harvest fruit storage at a colder temperature increases the fruit's resistance to pathogens. However, it also causes detrimental physiological changes and reduces the fruit's quality because of the effects of cold stress. When applied to post-harvest fruit, SA and other stress regulators have been shown to boost antioxidant activity and increase the amount of phenols, proline, and polyphenol oxidase activity in the fruit. These treatments have also prevented the pericarp from hardening, improving the quality of the fruit and its monetary value. Previous studies have indicated that the application of salicylic acid (SA) in pomegranate fruit has the potential to mitigate the loss of ascorbic acid, suppress the activity of phenylalanine ammonia-lyase (PAL), alleviate chilling injury, minimise electrolyte leakage, and prolong the post-harvest longevity of the fruit. Strawberry plants treated with SA at a concentration of 2 millimoles per litre were more resistant to disease and experienced less decay in their fruit. The exogenous application of SA on freshly cut water chestnut fruit acted as a potent anti-browning agent, enhancing colouration and overall fruit quality. The authors of the study above showed this. This treatment also decreased the activity of enzymes that were responsible for the browning of the fruit's flesh, such as POD, polyphenol oxidase (PPO), and PAL. The combination of pre-harvest SA application and post-harvest treatment resulted in increased fruit weight and quality in peach (*Prunus persica*). Salicylic acid (SA) plays a crucial role in regulating plant growth and significantly impacts the complex process of photosynthesis. The regulatory function of this entity encompasses a wide range of environmental conditions, including both optimal and stressful circumstances.



Under typical circumstances, mustard plants experience advantageous effects from the presence of salicylic acid (SA), resulting in improvements in photosynthesis, rubisco activity, and nitrate reductase function. On the other hand, when plants are under stress, SA slows down photosynthesis, primarily by increasing the amount of proline in their bodies. Barley's growth, yield, and photosynthetic rate improve when given the SA treatment, and it also sees an increase in its stomatal conductance during this time. The most important anti-nutritional compounds are called glucosinolates, and they can be found in rapeseed mustard. These naturally occurring plant components, distinguished by the amount of nitrogen and sulphur they contain, have emerged as entities with a growing significance due to their many facets. Sugars significantly impact every aspect of the plant life cycle because they can act as both energy and carbon sources and because of the regulatory power they possess. According to the authoritative sources consulted for this article, their complex interplay extends to interactions with various signalling molecules, including phytohormones (Chen et al., 2022; Chen et al., 2023). As a result, plant growth and development are intricately guided. Various factors, including cellular physiological activity, plant organs, environmental conditions, circadian rhythms, and developmental stages, play significant roles in the intricate orchestration of sugar levels within plant cells and their transport, utilisation, and storage. Sugar-induced signals are first received and interpreted within the apoplast and during transmembrane transit or intracellular transport, most notably in the cytosol. During this complex process, glucose and sucrose membrane transporters, invertases, and hexokinases (HXK) often work together as reliable glucose sensors. Additionally, it involves subtle shifts in the ratio of AMP to ATP, which adds another layer of complexity to the process. Sugars have been shown to play a crucial part in plant defence mechanisms, which are activated in response to a wide variety of biotic and abiotic stressors (Karaman, 2023; Karamat et al., 2022; Katoch et al., 2022; Kaviani et al., 2023; Kaya et al., 2023; Khalid et al., 2023; Khan et al., 2022, 2023; Khan et al., 2022). A significant body of research has demonstrated this. These sugars, known as primary substrates fuelling respiration, supply the necessary energy for the plant's cellular defence against pathogens. Moreover, they are the primary carbon structure for

synthesising defence compounds, including secondary metabolites such as flavonoids, stilbenes, and lignins. Moreover, saccharides, such as sucrose, glucose, fructose, and trehalose, play a crucial role as metabolic signalling molecules in the cellular environment of host plants. The presence of these entities elicits the activation of numerous genes, particularly those associated with immune defence mechanisms. The realm of flavonoids, specifically flavanols, reveals a remarkable collection of naturally occurring compounds whose structural makeup is characterised by a ketone group. Products commonly used in cooking, such as onions, kale, lettuce, tomatoes, apples, grapes, and berries, all contain significant amounts of flavanols in their natural form. Notably, beverages such as tea and red wine also contribute to the flavanol intake resulting from dietary consumption. This consumption has been linked to various health benefits, including their anti-oxidant potential and ability to reduce the risk of developing cardiovascular disease. The more general category of phenolic compounds includes flavonoids as a subcategory because flavonoids are considered a significant subgroup within the category of secondary metabolites (Chen et al., 2022; Chen et al., 2022; Chen et al., 2023; Chen, Zhang, et al., 2022; Cheng et al., 2022). They are found in prokaryotic organisms and various plant species across the entire plant kingdom. Unexpectedly, the total number of flavonoids that have been characterised now exceeds 6,500 unique compounds. These multifunctional molecules protect plant health, defending the plant against various biotic and abiotic stresses it may be exposed to. They demonstrate a diverse array of biological functions and play a pivotal role in directing the complex dynamic between plants and the environment in which they are situated. Flavonoids have an exceptional capacity to absorb the damaging effects of UV radiation and mitigate those effects, which protects plant cells from damage caused by radiation (Ji et al., 2022; Jia et al., 2022; Jin et al., 2022; Jofre et al., 2023; Kandhol et al., 2023; Kapoor et al., 2022). Flavonoids are endowed with bioactivity and influence vital physiological processes even though they are not essential for plant survival. Most notably, they control the movement of auxin, an essential plant hormone that controls various aspects of plant growth and development. Flavonoids are responsible for the captivating colours that can be seen in flowers and their physiological functions. In

addition, they perform the function of a natural defence mechanism, thereby enhancing plants' resistance to the attack of microorganisms and protecting them from the ravages of insect predators. Flavonoids are a structural class that can be traced back to their parent compound, flavones. Flavones are found everywhere in the cellular sap of higher plants that are still developing their tissues. Most flavonoids are found in nature as glycosides, one of the characteristics that gives these compounds their ability to dissolve in water. Within their chemical structure, sugars and hydroxyl groups confer this solubility. On the other hand, lipophilicity can be conferred upon flavonoids through the introduction of methyl groups and isopentyl units (Ciarkowska et al., 2023; Costa-Gutierrez et al., 2022; da Cunha et al., 2023; del Pilar Cordovilla et al., 2023; Deolu-Ajayi et al., 2022).

Oils are analysed to determine their physicochemical properties to establish their quality, purity, and identification. Properties that are characterised by the nature of the oil itself are known as characteristic properties. These are used to characterise oil and are not dependent on the location of the oil or source. At a temperature of 20 degrees Celsius, the specific gravity of an oil or fat is determined and calculated as a ratio of the mass in air of a given volume of the oil or fat to that of the same volume at 20 degrees Celsius. The degree to which a beam of light is bent as it travels from one transparent medium to another is measured by a property known as the refractive index. With the help of a refractometer, one can determine an oil's refractive index in degrees, typically using 20 degrees Celsius as the reference temperature. Because the obtained value is specific to a given oil, it can be applied to determine whether or not the oil has been adulterated and, if so, to what degree. The free fatty acid value is frequently employed as an indicator of the quality and suitability for consumption of various types of oils. The iodine value is utilised to quantify the extent of unsaturation or the presence of double bonds within the fatty acids found in an oil. Consequently, it does not indicate the specific composition of fatty acids in any given oil. The iodine value, also known as iodine number, is a valuable tool for assessing oil adulteration and as a means of process control in the oil industry. The saponification value can be considered a rough indicator of the molecular weight of the fat or oil. In general, a higher molecular weight

corresponds to a lower saponification value. In addition, it specifies the amount of alkali required to convert a predetermined quantity of oil or fat into soap. It is used to determine whether or not fats and oils have been tampered with. The saponification value of fats and oils is subtracted from the value of their free fatty acids to arrive at the esterification value of those fats and oils. The level of rancidity can be estimated using the peroxide value, which measures the amount of peroxides already present in the oil (Dhiman et al., 2022; Sabagh et al., 2022; Elnahal et al., 2022; Faisal et al., 2023; Felipez et al., 2022; Feng et al., 2023; Ferreira et al., 2023; Fidler et al., 2022). The standard peroxide value for edible oils that have not gone rancid should be significantly lower than ten meq/kg. Oil can also vary in p-anisidine value, solubility, freezing point, colour, odour, and boiling point. Other properties that oil can change include these. The removal of air, the addition of antioxidants and chelating agents, and the process of hydrogenation are all methods that can be used to prevent the rancidity of oil. Plants have developed multiple defence signalling pathways to survive hostile environmental conditions and attacks from pathogens. Secondary metabolites, such as phenylpropanoids, are regulated in response to cues from their surrounding environment. The development of the phenylpropanoid pathway in plants throughout evolution was an essential adaptation that allowed plants to defend themselves against biotic and abiotic stresses. Phenylpropanoids are synthesised by converting cinnamic acid derived from the amino acid phenylalanine. The enzymatic reaction facilitated by phenylalanine ammonia-lyase (PAL) involves the non-oxidative deamination of phenylalanine, producing trans-cinnamate. The initial stage in the phenylpropanoid pathway is a pivotal regulatory checkpoint that governs the transition from primary to secondary metabolism. The enzyme phenylalanine ammonia-lyase (PAL) is known to be responsive to both biotic and abiotic stresses, including pathogens, ultraviolet (UV) irradiation, and low temperatures. The phenylalanine ammonia-lyase (PAL) enzyme plays a crucial role in plant defence mechanisms. It is responsible for salicylic acid (SA) biosynthesis, a critical signalling molecule in establishing systemic resistance in plants. The expression of the PAL gene exhibits responsiveness to a diverse range of environmental stressors, such as pathogenic infection, physical injury, scarcity of

nutrients, exposure to ultraviolet radiation, and extreme temperatures (Fierli et al., 2022; Ganz et al., 2022; Garcya-Laynes et al., 2022; Geng et al., 2022; González-Pérez et al., 2022; Gul et al., 2023; Guo et al., 2023).

To determine the compositional quality of mustard oil, researchers investigate a variety of its physical and chemical properties, such as its density, viscosity, boiling point, saponification value (SV), iodine value (IV), and peroxide value (PV). Mustard oil's most important chemical constituent is allyl isothiocyanate, which makes up approximately 92% of the oil. Due to the high levels of allyl isothiocyanate that it contains, mustard oil is considered the most hazardous kind of oil. Mustard seeds are comprised of various chemical constituents, such as phytoalexins (specifically sinalenin, sinalbins A and B), sterols and sterol esters (mainly sitosterol and campesterol), and flavonoids (for instance, apigenin and chalcone). The flavour of mustard seeds is derived from glucosinolates and thiocyanate glycosides. The crude mucilage extracted from mustard seeds contains between 80% and 94% carbohydrates, 1.7% to 15% ash, and 2.2% to 4.4% protein. Secondary metabolites that contain sulphur but are not toxic are called glucosinolates. The flavour of white mustard seeds comes from a compound called sinalbin, while the flavour of yellow and brown mustard seeds comes from a compound called sinigrin. The pungency of a substance is generated through the hydrolysis of glucosinolates by the enzyme myrosinase, also known as thioglucoside glucohydrolase. This process results in the formation of isothiocyanates, commonly referred to as mustard oils, which contribute to the flavour profile. Glucosinolates in mustard oil contribute to its medicinal properties, including antibacterial, antifungal, and anticarcinogenic effects. The protein content of mustard oil is thirty per cent and contains calcium, phytins, phenolics, and other natural antioxidants (Gupta et al., 2022; Hajiboland et al., 2022; Hartmann et al., 2022; Hernandez-Leon & Valenzuela-Soto, 2022; Hernández et al., 2023; Hernández et al., 2022; Hilal et al., 2023; Hong et al., 2022; Huang et al., 2022; Hudeček et al., 2023). Certain aspects of mustard oil's physical makeup are discussed here. As examples of physical characteristics, mustard oil is evaluated based on its specific gravity, refractive index, acidic value, and iodine content. A significant amount of sulphur is found in

mustard oil, which may contribute to the oil's increased stability. Argemone oil, which is highly poisonous, is sometimes added to mustard oil during refining. The adulteration should be checked, and the results should come back negative. The relative density of Mustard oil (KachhiGhani) ranges from 0.907 to 0.910 throughout the analysis. Researchers determined that the refractive index of unmixed mustard oil ranged from 1.4662 to 1.4662. The temperature significantly impacted the SV value obtained for mustard oil, which ranged from 168 to 177 mg KOH/g. The iodine values of Mustard oils ranged from 96 to 112, as observed. The exceptional oxidative storage stability of the product may be attributed to its low iodine values. The acid value of unblended mustard oil reached a maximum of 1.5%. Three different fatty acids can be found in mustard oil: oleic acid, linoleic acid, and erucic acid. The monounsaturated and polyunsaturated fats and omega-3, omega-6, and omega-9 fatty acids are all found in high concentrations in mustard oil. The oxidative and chemical changes that occur in oils during storage are characterised by an increase in the total unsaturation of oils and a decrease in the amount of free fatty acids in the oils (Huh, 2022; Hui et al., 2022; Huntenburg et al., 2022; Hussein et al., 2023; Iftikhar et al., 2023; Islam et al., 2023; Javadipour et al., 2022).

### **Objectives of the proposed work**

1. To assess the effect of Sulphur and Salicylic acid on Growth, Physiology, and yield,
2. To evaluate the biochemical behaviour of crops under different treatments,
3. Analysis of oil profile (GC-MS) and Gene expression in the grains and leaves.

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**REVIEW OF LITERATURE**

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The literature review is presented in section A. Bibliometric Analysis of Database of Scopus; [B] Systematic Review.

**Section A. Bibliometric Analysis:** Search strategy and document evaluation A Comprehensive search of global literature was conducted in the Scopus database. Scopus was chosen because it is regarded as the most complete and extensively used database archiving literature in reviews and bibliometric analyses. The search keywords were "Salicylic acid\*" AND "Mustard," and the Second Search option was "Mustard" And "Sulphur\*" covering long years. No language restriction was applied because most articles were written in English. The different search yield from 2022 to 23 after the post-COVID period has been represented in the figures (Figure 1 & 2) and table below. It is intended to export information about the authors, such as their names, affiliations, countries, the document's title, the abstract, the publication date, and the journal name. As part of the bibliographic analysis, we used the VOS viewer (Version 1.6.17) bibliographic metric tool to determine the co-authorship (country, organization), co-occurrence of keywords (most significant, all), and total number of links for each article (Figure 1 & 2). The results of the studies have been visualised and mapped out so that potential gaps can be identified and knowledge limits can be highlighted about the regions where the studies have been carried out. The extraction and analysis of document metadata are essential to bibliometric analysis, a quantitative methodology used to evaluate the scholarly influence and patterns within academic literature. Document metadata encompasses organised and structured data about various documents, particularly research papers. This includes pertinent information such as the author's name, publication date, the journal or conference in which the document was presented, and associated citations. The metadata collection presents a valuable information source for scholars engaged in bibliometric research. In bibliometric analysis, extracting metadata entails systematically gathering, refining, and structuring relevant information from an extensive collection of scholarly articles. This procedure

enables researchers to generate extensive bibliographic databases, which form the basis for subsequent analysis. Tools and software are frequently employed to automate data extraction, improving efficiency and accuracy. After collecting metadata, the subsequent phase involves the commencement of the analytical process. Researchers can utilise this information to assess the productivity and influence of individual authors, research institutions, or journals. Citation networks can be established to discern influential papers and their interconnections, providing insights into research patterns and collaborative efforts. Furthermore, the utilisation of metadata analysis facilitates the evaluation of scholarly outputs across temporal dimensions, thereby facilitating the identification of nascent domains of inquiry, monitoring the progression of particular disciplines, and appraising the influence of pivotal scholarly works. Scholars can assess academic publications' influence using diverse bibliometric measures, such as the h-index, impact factor, and citation counts.



Figure 2.1: Keywords analysis search option "Salicylic acid\*" AND "Mustard\*"

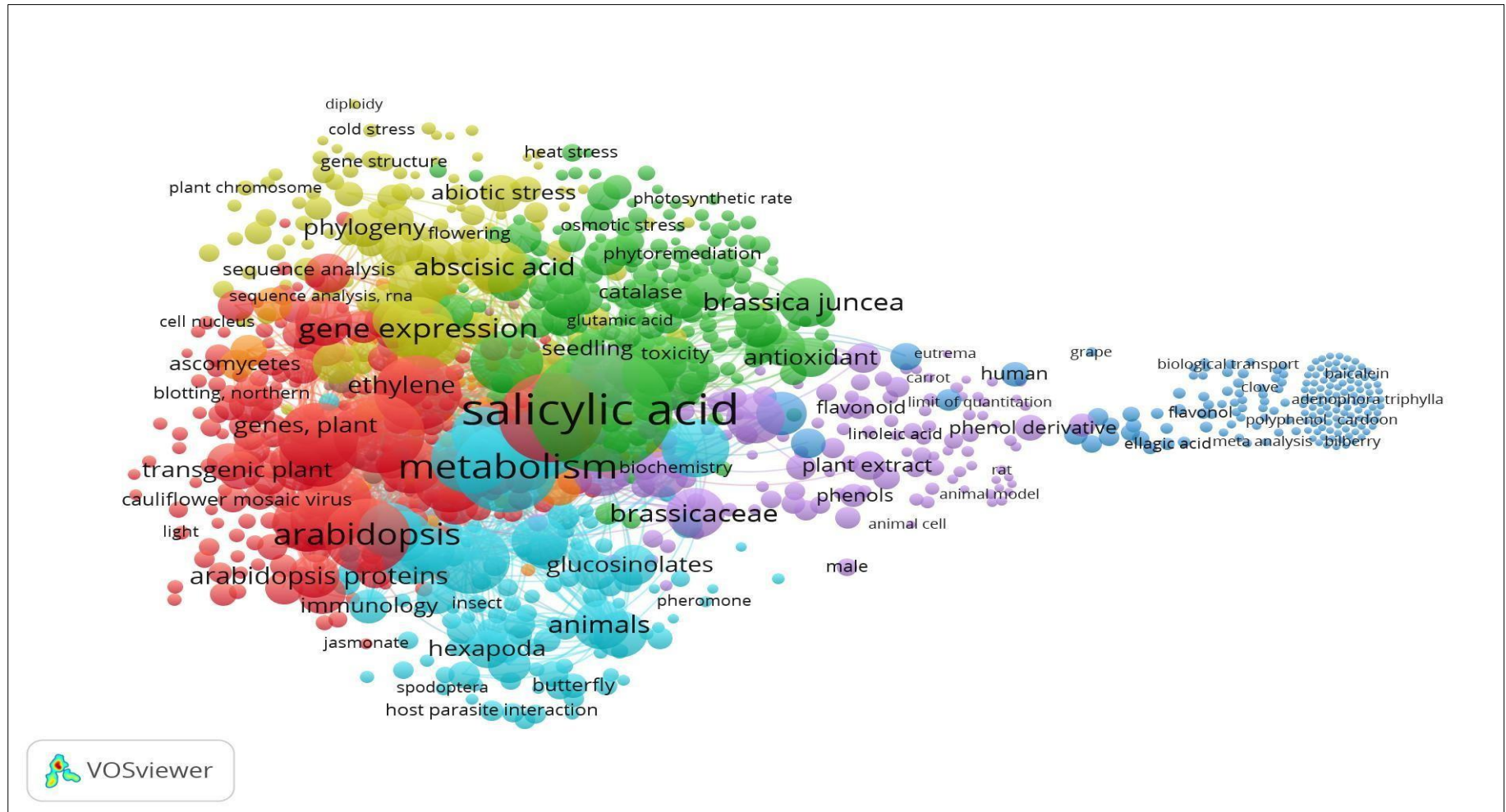
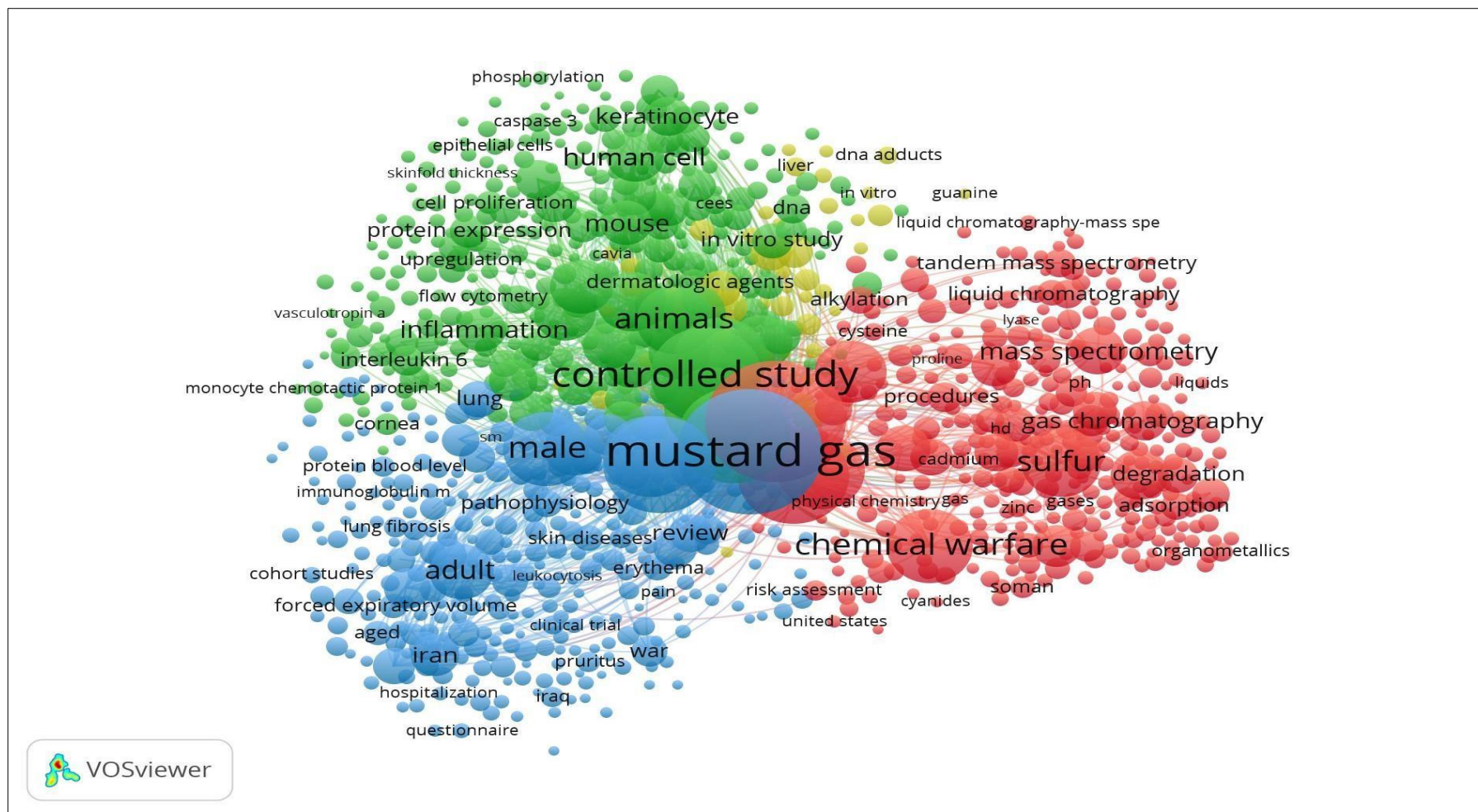


Figure 2.2. Keywords analysis search option “Mustard\*” And “Sulphur\*”



## **Bibliometric analysis based on keywords Salicylic acid and Mustard**

**Primary Information Extracted:** The data presented in this study provides a comprehensive bibliometric analysis, encompassing a wide range of document metadata and publication patterns from 1972 to 2023. The following is an analysis of the data: The primary details regarding the data are as follows: Temporal Range (Years): The dataset covers the period from 1972 to 2023, constituting 51 years. References (scholarly journals, books, etc.): The data collection process involved utilising a comprehensive set of 281 sources, encompassing various publication types such as journals, books, and other relevant scholarly materials. The dataset comprises a total of 710 documents. The dataset exhibits an annual growth rate of publications of approximately 7.65%. This observation suggests a steady upward trend in the quantity of publications over time. Average Age of Documents: The dataset exhibits an average document age of 8.52 years, indicating that the documents under consideration in this analysis are comparatively recent. The average number of citations per document in the dataset is 38.42, which indicates the research's impact and influence. The dataset encompasses a comprehensive collection of 36,167 references, highlighting the substantial interconnections among scholarly materials within these documents. The document's contents are as follows: The "Keywords Plus" feature encompasses 4,376 distinct terms that index and categorise various documents. Author's Keywords (DE): The dataset encompasses 1,780 distinct keywords generated by the authors. This inclusion of author-generated keywords offers supplementary understanding and perspective regarding the content of the documents. The individuals responsible for the creation of a written work: The dataset contains a total of 2,744 unique authors who have made contributions to the documents. The individuals responsible for creating single-authored documents are referred to as authors. A limited number of authors (precisely 13) have been responsible for producing documents as sole authors, thereby highlighting the prevalence of research conducted through collaboration. The phenomenon of authors collaborating on a project has been observed and studied in various academic contexts. The dataset contains 13 documents that a single individual has authored. The average number of co-authors per document is

approximately 5.74, indicating the prevalent collaborative aspect of academic research—the percentage of international co-authorships. The proportion of international co-authorships in this academic field is estimated to be around 27.46%, indicating a significant level of global collaboration. The various types of documents that exist. The dataset comprises diverse documents, predominantly classified as "articles" (n=664). Additional types of documents that can be found in the dataset include "book chapter" (4 occurrences), "conference paper" (9 occurrences), "conference review" (1 occurrence), "erratum" (1 occurrence), "letter" (1 occurrence), "note" (3 occurrences), "retracted" (1 occurrence), and "review" (26 occurrences). The presence of various document types within the dataset suggests the inclusion of research from multiple disciplines, highlighting its multidisciplinary nature. The provided information offers a comprehensive analysis of the dataset, encompassing its temporal expansion, the variety of document genres, the degree of author collaboration, and the significance of the documents based on their citations and references. Researchers' utilisation of this data enables them to acquire valuable insights about the academic landscape and prevailing trends within the field (Table 1).

**Table 2.1: Main Information about data and its results**

<b>Description</b>	<b>Results</b>
<b>MAIN INFORMATION ABOUT DATA</b>	
Timespan	1972:2023
Sources (Journals, Books, etc)	281
Documents	710
Annual Growth Rate %	7.65
Document Average Age	8.52
Average citations per doc	38.42
References	36167
<b>DOCUMENT CONTENTS</b>	
Keywords Plus (ID)	4376
Author's Keywords (DE)	1780
<b>AUTHORS</b>	
Authors	2744
Authors of single-authored docs	13
<b>AUTHORS COLLABORATION</b>	
Single-authored docs	13
Co-Authors per Doc	5.74

International co-authorships %	27.46
DOCUMENT TYPES	
article	664
book chapter	4
conference paper	9
conference review	1
erratum	1
letter	1
note	3
retracted	1
review	26

**Annual Total Citation:** The data provided presents an analysis of citation patterns over some time, specifically examining the average total number of citations per article (referred to as Mean TC per Art), the number of articles (N) published within a particular year, the average total number of citations per year (referred to as Mean TC per Year), and the duration of citable years for each respective year. The following is a comprehensive examination of each data point: In 1972, The average total citations per article in the given year was 0, suggesting that the articles published during this period did not receive any citations on average. N: One article was published in 1972. The total citation count per year for the given article is 0.00, indicating that only one article received no citations. The number of citable years, considering a standard 2-year citation window, since 1972 is 52. The year 1975 marks an important milestone in history. The average number of citations per article in the field of art in 1975 was 34. The dataset includes one article that was published in 1975. The average number of citations per year for articles published in 1975 is 0.69. The number of years that can be referenced or cited is 49, denoting the period from 1975 to the present year of 2023. The year 1992 marks an important milestone in history. The average number of citations per article in the field of art in 1992 was 59. The dataset includes one article that was published in 1992. The average total citations per year for articles published in 1992 was 1.84. The number of years that can be cited is 32, indicating the time elapsed since 1992 as of 2023. The year 1994 marks an important milestone in history. The average number of citations per article in the field of art in 1994 was 108.25. The number of articles published in 1994 was 4.00. The average number of citations per year for articles published in 1994 is 3.61.

Citable Years: 30 (as of 2023, there are 30 years since 1994). The year 1995 marks an important milestone in history. The average total citations per article in the field of art for 1995 was 35. Two articles were published in 1995, with a N value of 2.00. The average number of citations per year for articles published in 1995 is 1.21. The number of years since 1995 that can be cited is 29 as of 2023. The trend above persists throughout the subsequent years, exhibiting fluctuations in citation impact and the number of articles published annually. The dataset offers significant insights into the temporal accumulation of citations for research articles and the distribution of citations across various publication years. These findings can be instrumental in evaluating the influence and impact of academic publications (Table 2).

**Table 2.2: Annual Total Citation**

Year	Mean TC per Art	N	Mean TC per Year	Citable Years
1972	0	1.00	0.00	52
1975	34	1.00	0.69	49
1977	0	1.00	0.00	47
1992	59	1.00	1.84	32
1994	108.25	4.00	3.61	30
1995	35	2.00	1.21	29
1996	150.67	6.00	5.38	28
1997	102	5.00	3.78	27
1998	207.33	3.00	7.97	26
1999	88.4	5.00	3.54	25
2000	103.4	5.00	4.31	24
2001	125.4	5.00	5.45	23
2002	89.57	14.00	4.07	22
2003	80.07	14.00	3.81	21
2004	93.53	15.00	4.68	20
2005	45.4	10.00	2.39	19
2006	36.41	17.00	2.02	18
2007	71.28	18.00	4.19	17
2008	94.22	23.00	5.89	16
2009	43.9	20.00	2.93	15
2010	52.61	18.00	3.76	14
2011	68.6	15.00	5.28	13

2012	65	27.00	5.42	12
2013	32.33	21.00	2.94	11
2014	46.88	33.00	4.69	10
2015	37.46	37.00	4.16	9
2016	28.39	31.00	3.55	8
2017	28.41	32.00	4.06	7
2018	30.52	46.00	5.09	6
2019	24.9	59.00	4.98	5
2020	18.18	60.00	4.54	4
2021	7.92	61.00	2.64	3
2022	4.7	57.00	2.35	2
2023	0.72	43.00	0.72	1

**Most Relevant Sources:** The provided data presents information regarding the origins of the articles and the corresponding quantity of publications in each source. Below is a comprehensive elucidation of every data point: The dataset includes a significant contribution from *Frontiers in Plant Science*, which has published 27 articles. This platform is highly esteemed for its contributions to the field of plant science, encompassing a diverse array of topics within this discipline. *Plant physiology* is a notable scholarly resource, encompassing a collection of 20 published articles. The aforementioned scholarly publication is renowned for its significant contributions to plant physiology, encompassing investigations into various aspects of plant function, such as growth, development, and biochemical processes. *The International Journal of Molecular Sciences* has recently published 19 articles. The source in question is an interdisciplinary resource expected to encompass various topics related to molecular research within plant sciences. The following collection comprises 15 articles on the topic of molecular plant-microbe interactions. This source comprises 15 articles that predominantly examine the interplay between plants and microorganisms. This particular area of investigation holds significant importance within plant science. The journal *Planta* has published a total of 14 articles. The subject matter of this field encompasses a broad spectrum of topics about the biology and physiology of plants. *PLOS ONE*, a multidisciplinary open-access journal, has published 14 articles. The

platform is renowned for its comprehensive approach to scientific research, encompassing various disciplines, such as plant science while emphasising inclusivity. The Plant Journal, comprising a collection of 13 articles, is a scholarly publication that likely caters to the specific field of plant biology, encompassing a range of topics such as molecular and cellular research. This source's focus pertains to plant physiology and biochemistry, encompassing the physiological and biochemical aspects of plant science. A total of 13 articles have been published within this source. The field of plant science, encompassing 13 articles, comprehensively explores diverse subjects such as genetics and plant breeding. The journal Molecular Plant Pathology primarily focuses on investigating these mechanisms underlying plant diseases. This journal has published a total of 12 articles in this field. The source under consideration encompasses a collection of twelve articles that collectively address various subjects about plants. These topics span various aspects of plant biology, such as physiology, genetics, and ecology. The journal Phytochemistry comprises a collection of 11 scholarly articles that primarily focus on investigating plant compounds and their corresponding chemical properties. BMC Plant Biology, an open-access publication, encompasses a collection of ten articles that delve into diverse facets of plant research. The Journal of Experimental Botany, comprising a collection of ten articles, primarily focuses on disseminating experimental research within the field of plant science. Scientific Reports, a journal encompassing multiple disciplines and following an open-access model, has recently published ten scholarly articles. This encompasses a broad range of scientific research subjects. The journal Acta Physiologiae Plantarum comprises nine articles primarily focusing on plant physiology. Physiologia Plantarum, comprising nine articles, is a scholarly publication primarily focusing on plant physiology. Food Chemistry encompasses eight articles that primarily examine plant-derived food's chemical composition and nutritional properties. Each source within this dataset represents a distinct platform dedicated to the dissemination of research in the domain of plant science. The quantity of articles published in each source indicates its importance within the academic community. Scholars frequently utilise such data to evaluate the calibre and influence of research disseminated in these publications and identify prospective avenues for their scholarly contributions (Table 2.3).



**Table 2.3: Most Relevant Sources**

Sources	Articles
FRONTIERS IN PLANT SCIENCE	27
PLANT PHYSIOLOGY	20
INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES	19
MOLECULAR PLANT-MICROBE INTERACTIONS	15
PLANTA	14
PLOS ONE	14
PLANT JOURNAL	13
PLANT PHYSIOLOGY AND BIOCHEMISTRY	13
PLANT SCIENCE	13
MOLECULAR PLANT PATHOLOGY	12
PLANTS	12
PHYTOCHEMISTRY	11
BMC PLANT BIOLOGY	10
JOURNAL OF EXPERIMENTAL BOTANY	10
SCIENTIFIC REPORTS	10
ACTA PHYSIOLOGIAE PLANTARUM	9
PHYSIOLOGIA PLANTARUM	9
FOOD CHEMISTRY	8

**Bibliometric analysis based on keywords Sulphur and Mustard**

**Primary Information Extracted:** The data provided presents a comprehensive overview of various facets of a bibliometric analysis, encompassing the duration of time considered, the sources utilised, the number of documents, the rate of growth, the age of the documents, the citations and references, the contents of the documents, the authors involved, the collaboration among authors, and the types of documents. The following is a comprehensive elucidation of each data point: The primary details regarding the data are as follows: Timespan (1972:2023): The data spans over 51 years, covering research and publications from 1972 to 2023. The user has provided a list of sources, including journals, books, and other materials, totalling 281. A comprehensive compilation of 281 sources has been assembled, encompassing a variety of scholarly materials such as journals, books, and other

forms of publications. The sources above function as the channels through which the documents incorporated in the analysis are disseminated. The collection of documents consists of 710 items. The dataset consists of 710 documents. These documents represent the primary focus of the analysis and likely contain various research articles. The dataset exhibits an annual growth rate of approximately 7.65%, signifying a steady upward trend in the quantity of publications throughout the years.

**Average Age of Documents (8.52):** The dataset reveals an average document age of 8.52 years, indicating that the documents under consideration in this analysis are comparatively recent. The average number of citations per document in the dataset is 38.42, indicating the significant impact and influence of the research. The dataset comprises a substantial number of references, precisely 36,167, highlighting the extensive scholarly interconnections within the documents.

**The document's contents are as follows:** The dataset contains 4,376 distinct "Keywords Plus" terms. These terms serve the purpose of indexing and categorising documents, thereby enhancing the contextual and informational aspects of the dataset. The author's designated keywords for this study are DE - 1780. The dataset comprises 1,780 distinct keywords generated by the authors, providing valuable insights into the precise content and emphasis of the documents. The individuals responsible for creating written works are commonly referred to as authors. The dataset contains a total of 2,744 unique authors who have made contributions to the documents. These authors represent a broad spectrum of researchers in the field. The list comprises a total of thirteen authors who have written single-authored documents. A limited number of authors (13) have produced documents as sole authors, indicating the prevalence of collaborative research within the dataset.

**Collaboration among authors:** The dataset contains 13 documents that a single individual authored. This suggests that most of the documents in the dataset were produced through a collaborative effort, highlighting the collaborative nature inherent in academic research. The average number of co-authors per document is 5.74, indicating the collaborative nature of academic research in this field. The percentage of international co-authorships is 27.46%. The proportion of international co-authorships in this academic field is approximately 27.46%, indicating a significant level of global collaboration. The various types of

documents that exist. The dataset comprises diverse document types, with the largest category being "articles" (664 instances). Additional document types that can be found within the dataset include "book chapter" (4 occurrences), "conference paper" (9 occurrences), "conference review" (1 occurrence), "erratum" (1 occurrence), "letter" (1 occurrence), "note" (3 occurrences), "retracted" (1 occurrence), and "review" (26 occurrences). The presence of a wide variety of document types emphasises the interdisciplinary nature of the research included in the dataset. The comprehensive data presented offers significant contributions to our understanding of the dataset's attributes, encompassing its expansion over time, the variety of document genres, the level of author collaboration, and the significance of the documents based on their citations and references. Researchers can utilise this data to comprehend better the scholarly environment and patterns within the specific discipline being investigated (Table 2.4).

**Table 2.4: Main Information about Data**

<b>Description</b>	<b>Results</b>
<b>MAIN INFORMATION ABOUT DATA</b>	
Timespan	1972:2023
Sources (Journals, Books, etc)	281
Documents	710
Annual Growth Rate %	7.65
Document Average Age	8.52
Average citations per doc	38.42
References	36167
<b>DOCUMENT CONTENTS</b>	
Keywords Plus (ID)	4376
Author's Keywords (DE)	1780
<b>AUTHORS</b>	
Authors	2744
Authors of single-authored docs	13
<b>AUTHORS COLLABORATION</b>	

Single-authored docs	13
Co-Authors per Doc	5.74
International co-authorships %	27.46
DOCUMENT TYPES	
article	664
book chapter	4
conference paper	9
conference review	1
erratum	1
letter	1
note	3
retracted	1
review	26

**Most Relevant Sources:** The data comprises diverse sources, including journals, books, and other relevant publications, and the corresponding count of articles published within each source. The following is a comprehensive account of each data point: Frontiers in Plant Science emerges as a prominent contributor within the dataset, having published 27 articles. This source is expected to encompass a diverse array of research topics within the plant science discipline, serving as a forum for researchers to disseminate their discoveries. Plant physiology is a notable scholarly resource, encompassing 20 published articles. The journal above has gained recognition for its significant contributions to plant physiology, encompassing various aspects such as plant growth, development, and biochemical mechanisms. The International Journal of Molecular Sciences has recently published 19 scholarly articles. This source is most likely an interdisciplinary publication that comprehensively addresses multiple facets of molecular research within the field of plant sciences. The following collection comprises 15 articles that delve into the intricate dynamics of molecular interactions between plants and microbes. This source consists of 15 articles and is presumably centred on examining the interplay between plants and microorganisms, a pivotal field of investigation within plant

science. The journal *Planta* has published a total of 14 articles. The subject matter of this field encompasses a broad spectrum of topics about the biology and physiology of plants. *PLOS ONE*, a multidisciplinary open-access journal, has published 14 articles. The platform is renowned for its comprehensive and inclusive approach to scientific research, encompassing various disciplines, including plant science. The *Plant Journal*, comprising a collection of 13 articles, is a scholarly publication that is likely dedicated to exploring diverse facets within the field of plant biology, encompassing investigations into molecular and cellular phenomena. The focus of this source pertains to the field of plant physiology and biochemistry, encompassing the examination of physiological and biochemical aspects within the realm of plant science. A total of 13 articles have been published within this source. *Plant Science* encompasses many topics, as evidenced by the 13 articles in this collection. These articles comprehensively explore various aspects of the discipline, such as genetics and plant breeding. The journal *Molecular Plant Pathology* primarily investigates the molecular mechanisms underlying plant diseases. To date, it has published 12 articles in this field. The provided source comprises a collection of articles encompassing a diverse array of plant subjects. These articles encompass various aspects containing plant physiology, genetics, and ecology, offering a comprehensive coverage of plant-related topics. The journal *Phytochemistry* comprises a collection of 11 scholarly articles that primarily focus on investigating plant compounds and their corresponding chemical properties. *BMC Plant Biology*, an open-access publication, encompasses a collection of ten articles that delve into diverse facets of plant research. The *Journal of Experimental Botany*, comprising a collection of ten articles, primarily focuses on disseminating experimental research within the field of plant science. *Plants*, a journal encompassing various disciplines and following an open-access model, has recently published ten scholarly articles. This encompasses a broad range of scientific research subjects. The journal *Acta Physiologiae Plantarum*, comprising nine articles, appears to primarily focus on plant physiology. *Plant Physiology*, comprising nine articles, is a scholarly publication likely dedicated to plant physiology. *Food Chemistry* encompasses eight articles examining plant-derived food's chemical composition and properties. The sources above encompass various platforms through which scholarly investigations

in pcontainnce are communicated. The quantification of articles published in various sources offers valuable insights into their prominence within the academic community. Researchers can utilise this information to evaluate the calibre and influence of research disseminated through these channels (Table 2.5).

**Table 2.5: Most Relevant Sources**

Sources	Articles
FRONTIERS IN PLANT SCIENCE	27
PLANT PHYSIOLOGY	20
INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES	19
MOLECULAR PLANT-MICROBE INTERACTIONS	15
PLANTA	14
PLOS ONE	14
PLANT JOURNAL	13
PLANT PHYSIOLOGY AND BIOCHEMISTRY	13
PLANT SCIENCE	13
MOLECULAR PLANT PATHOLOGY	12
PLANTS	12
PHYTOCHEMISTRY	11
BMC PLANT BIOLOGY	10
JOURNAL OF EXPERIMENTAL BOTANY	10
SCIENTIFIC REPORTS	10
ACTA PHYSIOLOGIAE PLANTARUM	9
PHYSIOLOGIA PLANTARUM	9
FOOD CHEMISTRY	8

## **B. Systematic Review.**

### **1. A marine-based supramolecular ionic salt that enhances the water solubility, transdermal delivery, and bioactivity of salicylic acid**

Wang et al., 2023- Salicylic acid is a notable bionic acid extensively employed in the cosmetic and pharmaceutical industries for its remarkable attributes in treating

various skin conditions. It boasts excellent anti-acne, anti-inflammatory, antibacterial, oil-regulation, stratum corneum conditioning, and anti-photoaging properties. However, H[Sal] is associated with relatively irritative solid effects, rendering it unsuitable for individuals with sensitive skin. Furthermore, its limited water solubility poses formulation challenges, leading to suboptimal transdermal efficiency and reduced bioavailability. In this research endeavour, we introduce matrinium salicylate ([Mat][Sal]), an innovative supramolecular ionic salt. [Mat][Sal] exhibits significantly reduced cytotoxicity and is less irritating to the skin when compared to H[Sal]. It demonstrates enhanced water solubility, improved transdermal delivery capabilities, and heightened bioactivity. The comprehensive evaluation of [Mat][Sal] extends to its anticancer, antioxidant, anti-inflammatory, and antibacterial properties, which either surpass or are on par with those exhibited by H[Sal]. The molecular structure of [Mat][Sal] undergoes thorough scrutiny, including meticulous single-crystal structure refinement, to fully understand its intrinsic properties. Furthermore, through accurate in-vitro skin penetration experiments, we elucidate that the formation of a salt with marine substantially enhances the transdermal efficiency of H[Sal] while preserving the skin's natural barrier. The mechanisms governing the transdermal behaviour of both H[Sal] and [Mat][Sal] are meticulously examined, employing a blend of experimental techniques and sophisticated molecular dynamics simulations. These investigations unveil that, in comparison to H[Sal], [Mat][Sal] exhibits heightened reactivity with the skin. Notably, [Sal]<sup>-</sup> ions exhibit more rapid diffusion within the skin, driven by the elevated water solubility and the ion-pair effect shown by [Mat][Sal]. Furthermore, [Mat][Sal] demonstrates impressive efficacy in alleviating acne-related concerns in a clinical-efficacy trial, underscoring its substantial potential for applications in biomedicine and cosmetics.

## **2. An integrated transcriptomic and metabolomic analysis for changes in rose plants induced by rose powdery mildew and exogenous salicylic acid**

Yang et al., 2022- We investigated the transcriptomic and metabolomic alterations in *Rosa chinensis* following infection with *Podosphaera pannosa* and subsequent treatment with exogenous salicylic acid (SA), each analyzed separately. The response of the rose to mildew infection exhibited apparent similarities to its response to SA

treatment. Through comprehensive omics analysis, it was evident that following induction by both *P. pannosa* and SA, *R. chinensis* consistently exhibited reactions involving MAPK cascades, activation of the plant-pathogen interaction pathway, and the expression of resistance (R) genes. Furthermore, compared to the control group, significant enrichments were observed in triterpenoid biosynthesis, glutathione metabolism, and linoleic acid metabolism. Notably, triterpenoids with the most substantial fold change values, such as dehydrousolic acid lactone and maslinic acid, were significantly up-regulated. This suggests that these pathways and metabolites were pivotal in conferring resistance to *P. pannosa*. Additionally, salicylic acid beta-D-glucoside, methyl salicylate, and methyl jasmonate increased significantly in response to *P. pannosa* infection and exogenous SA treatment.

### **3. Antiamoebic properties of salicylic acid-based deep eutectic solvents for the development of contact lens disinfecting solutions against *Acanthamoeba***

Siddiqui et al., 2022- *Acanthamoeba castellanii*, a protist pathogen, can potentially cause severe conditions such as sight-threatening keratitis and the life-threatening central nervous system infection known as granulomatous amoebic encephalitis. This study aimed to investigate the impact of five deep eutectic solvents (DES) based on malonic acid and salicylic acid on *A. castellanii*. These solvents were named as follows: salicylic acid-trioctylphosphine (DES 1), salicylic acid-trihexylamine (DES 2), salicylic acid-trioctylamine (DES 3), malonic acid-trioctylphosphine (DES 4), and malonic acid-trihexylamine (DES 5). Experiments included amoebicidal, encystment, excystment, cytopathogenicity, and cytotoxicity assays. DES 2 and 3 exhibited significant amoebicidal effects at micromolar dosages ( $P < 0.05$ ). They also inhibited encystment and excystment processes, reduced cell-mediated cytopathogenicity caused by *A. castellanii*, and demonstrated minimal cytotoxicity to human cells. Conversely, the individual chemical components of these solvents, namely salicylic acid, trihexylamine, and trioctylamine, had limited effects when tested separately. These findings are highly promising and, to our knowledge, represent the first report on the impact of deep eutectic solvents on amoebae. This research can potentially be applied in developing new formulations for innovative contact lens disinfectants targeted against *Acanthamoeba castellanii*.



#### **4. Application of salicylic acid to cv. Muscat Hamburg grapes for quality improvement: Effects on typical volatile aroma compounds and anthocyanin composition of grapes and wines**

Yue et al., 2023, Aromas and anthocyanins are essential factors affecting grapes and wine quality. Monoterpenes are regarded as typical volatile aroma compounds in Muscat Hamburg grapes. This study determined the effects of exogenous salicylic acid (SA) on anthocyanins and monoterpene components of Muscat Hamburg (*Vitis vinifera*) berries and wines. Berries were treated with aqueous solutions of SA (0.0, 50, 100 and 200 mg/L) at E-L 34 (berry colour change stage) during the 2018 and 2019 seasons. 50 mg/L salicylic acid (SA50) significantly increased the sum of anthocyanin contents in ripe berries, while 100 mg/L and 200 mg/L salicylic acid had the opposite effect. Additionally, SA50 significantly promoted the free or bound monoterpenes accumulation in mature grapes. The concentrations of geraniol, neral, geranic acid, nerol, nerol oxide, and phellandrene were higher in the wine produced from SA50 treatment grapes than in the wine made from control groups. The SA50 treatments increased the transcription of some essential genes related to monoterpene biosynthesis, namely, VviPNLinNer1, VviPNLinNer2, VviPNLNGI2, VviPNLNGI4, VviTer, and VviGT14, which accelerated the synthesis and accumulation of monoterpene components in grapes. These findings can provide fundamental knowledge for future grape aromas and anthocyanin improvements.

#### **5. Assessing a double silicon decorated fullerene for the delivery of interacting flurbiprofen and salicylic acid drugs: A DFT approach**

Çatal et al., 2023- Drug interactions have become increasingly critical as the number of available drugs rises steadily. Furthermore, the effective administration of multiple medications has emerged as another vital topic for discussion, emphasising the growing importance of targeted and selective drug delivery methods. One promising avenue in this context is the utilisation of impurity-doped C60 fullerenes, incorporating various dopant atoms like silicon or boron, as potential drug delivery vehicles. Our study delved into the interaction between salicylic acid and flurbiprofen and their controlled delivery using double silicon-decorated C60 fullerenes, employing density functional theory. We also scrutinised stability and reactivity by examining crucial

structural parameters, interaction energies, and frontier molecular orbitals. Additionally, we monitored these interactions by investigating critical diagnostic vibrational bands. Using the quantum theory of atoms in molecules, we identified the strength of the interactions between particles at the interaction sites.

## **6. Cerium oxide- salicylic acid nanocomposite foliar use impacts physiological responses and essential oil composition of spearmint (*Mentha spicata* L.) under salt stress**

Shiri et al., 2023- Utilising certain compounds, such as salicylic acid and cerium oxide, presents a valuable strategy for enhancing abiotic stress tolerance in plants. To assess the effects of salicylic acid nanoparticles (SA-NPs), cerium oxide nanoparticles (CeO<sub>2</sub>-NPs), and the CeO<sub>2</sub>-SA nanocomposite on the physiological and biochemical attributes, as well as the essential oil yield of spearmint plants (*Mentha spicata* L.) subjected to salinity stress, we conducted a factorial experiment under controlled greenhouse conditions. This experiment followed a completely randomised design comprising two factors and three replications. The first factor involved three levels of salinity stress (0, 50, and 100 mM), while the second factor included the following treatments: T2 (100 µM SA-NPs), T3 (50 mg L<sup>-1</sup> CeO<sub>2</sub>-NPs), T4 (25 mg L<sup>-1</sup> CeO<sub>2</sub>-SA nanocomposite and 50 µM CeO<sub>2</sub>- SA nanocomposite), and T5 (50 mg L<sup>-1</sup> and 100 µM CeO<sub>2</sub>- SA nanocomposite). We evaluated various parameters, including the activity of antioxidant enzymes such as superoxide dismutase, guaiacol peroxidase, and ascorbate peroxidase, as well as total soluble protein, malondialdehyde, hydrogen peroxide, proline, total ascorbate, reduced and entire AsA, DHA, AsA/DHA, elemental composition (Na, K, Na/K, Zn, Cu, Mn, Fe), chlorophyll fluorescence (Fm, Fv, Fm/Fv, F0), essential oil content, essential oil constituents, and photosynthetic pigment levels. Our findings revealed that as salinity stress increased, the levels of carbohydrates, protein, total antioxidants, carotenoids, chlorophyll a, chlorophyll b, and total chlorophyll declined compared to the control group. Conversely, applying the cerium oxide-salicylic acid nanocomposite significantly improved protein, carbohydrate, phenolics, flavonoids, essential oil yield, and overall antioxidant capacity. Proline, hydrogen peroxide, superoxide dismutase, guaiacol peroxidase, and ascorbate peroxidase substantially increased with escalating salinity levels compared to the

control. However, using the cerium oxide-salicylic acid nanocomposite mitigated these adverse effects. The dominant constituents of the essential oil included pulegone, L-enthone, 1,8-cineole,  $\alpha$ -terpinene, trans-caryophyllene, isopulegol,  $\beta$ -pinene, sabinene, and  $\beta$ -myrcene. In conclusion, the CeO<sub>2</sub>-SA nanocomposite demonstrated a positive ameliorative effect on the detrimental impacts of salinity stress.

## **7. Chlorophyll-sensitized and salicylic acid functionalised TiO<sub>2</sub> nanoparticles as a stable and efficient catalyst for the photocatalytic degradation of ciprofloxacin with visible light**

Krishnan and Shriwastav-2023 developing a highly efficient and durable visible light photocatalyst holds immense promise for addressing environmental challenges. While incorporating natural chlorophyll pigments through dye sensitisation of TiO<sub>2</sub> nanoparticles offers the potential for enhancing visible light activity, concerns about their long-term stability persist. In our study, we explored a novel approach: functionalising TiO<sub>2</sub> with salicylic acid, followed by sensitisation with chlorophylls, to bolster the catalyst's stability for the photocatalytic degradation of Ciprofloxacin (CPX) under visible light. Our investigations yielded remarkable results, revealing a substantial degradation efficiency and catalyst durability enhancement over five reuse cycles. Furthermore, we identified the optimal conditions for CPX degradation, achieving an impressive ~75% removal rate using a catalyst dosage of 0.75 g L<sup>-1</sup> of 0.1 chl/0.1 SA-TiO<sub>2</sub>, an initial pH of 6, and an initial CPX concentration of 10 ppm during a 2-hour exposure to visible light. The degradation process adhered to pseudo-second-order kinetics. In a real-world wastewater matrix, other scavenging species like chlorides, sulphates, and alkalinity reduced the efficiency of ciprofloxacin degradation. Nevertheless, our study revealed a significant reduction in the toxicity of degradation by-products compared to the parent CPX compound. Additionally, we proposed a plausible degradation pathway and mechanism for CPX, shedding light on its intricate degradation process. This research contributes to advancing environmentally friendly photocatalysis and offers a potential solution to the growing concern of pharmaceutical contaminants in water bodies.

## **8. Comprehensive quantification of flavonoids and salicylic acid representative of *Salix* spp. Using micro liquid Chromatography-Triple Quadrupole Mass**

## **Spectrometry: the importance of drying procedures and extraction solvent when performing classical solid-liquid extraction**

(Curtasu and Nørskov, 2023)- Willow trees (*Salix* spp.) have garnered growing interest due to their remarkable ability to rapidly develop and yield abundant biomass while demanding minimal agricultural inputs. Moreover, these trees harbour a wealth of potentially bioactive compounds. In this study, our primary objective was to devise a high-yield extraction procedure coupled with a robust, sensitive, and rapid microliquid Chromatography-Triple Quadrupole Mass Spectrometry (LC-MS/MS) method for the comprehensive quantification of flavonoids and salicylic acid within the bark of *Salix* spp. Our investigation encompassed an assessment of the impact of various drying techniques, including freeze-drying and oven-drying, and the influence of five distinct extraction solvents on the yields of individual flavonoids and salicylic acid during classical solid-liquid extraction. Free drying emerged as the superior method for preserving monomeric and polymeric flavan-3-ols, although its effect on other flavonoids was less pronounced. Interestingly, salicylic acid remained unaffected by the drying procedures. Among the array of solvents tested, the combination of methanol acidified with 1% hydrochloric acid stood out as the most effective in maximising individual flavonoid yield. Importantly, our LC-MS/MS method exhibited outstanding performance, boasting a recovery rate exceeding 80%, excellent precision, and overall robustness. These findings represent a significant step in the field, facilitating the precise quantification of valuable bioactive compounds in *Salix* spp. bark

## **9. Degradation of salicylic acid using electrochemically assisted UV/chlorine process: Effect of operating conditions, reaction kinetics, and mechanisms**

Liu et al., 2022- Salicylic acid (SA), an emerging pollutant, typically exhibits minimal decomposition during conventional wastewater treatment. We propose an innovative electrochemically assisted UV/chlorine reaction system to address this persistent pollutant. In this system, oxidants are continuously generated in real-time from chloride ions, eliminating the need for additional dosing agents. The electrogenerated free chlorine, in the form of hypochlorite ions and hypochlorous acid, demonstrates an enhanced capacity to oxidise SA. This approach offers a technically and chemically simple method for efficiently treating SA-contaminated wastewater.

The uniqueness of our work lies in integrating electrogenerated free chlorine with UV irradiation to enhance SA degradation. We also provide insights into the kinetics and mechanisms of SA degradation. Optimal conditions for electrochemical SA degradation were determined, including a current density of 5 mA cm<sup>-2</sup>, chloride ion concentration of 0.05 M, and pH 4, resulting in an impressive degradation efficiency (DE) of 96%. By incorporating UVC irradiation during electrochemical treatment, the DE of SA can be further elevated to > 99% due to the generation of free radicals. These radicals reduce the activation energy required for SA degradation, leading to exceptional DE. Furthermore, our study revealed that the degradation of SA in the electrochemically assisted UV/chlorine system follows a pseudo-first-order reaction and maintains high stability through repeated treatments. Using liquid chromatography-tandem mass spectrometry, we proposed potential SA degradation pathways, highlighting the predominant role of hydroxyl radicals in SA degradation rather than reactive chlorine species.

#### **10. Development of benzothiazole-derived rhodamine fluorescent probes for sensitive, rapid, and reversible detection and imaging of salicylic acid in food samples and plants**

Ma et al., 2023- Salicylic acid (SA) is a pivotal molecule, with roles encompassing plant immunity and a broad impact on ecological environments and human health. Nevertheless, the development of rapid, sensitive, and user-friendly tools for the selective detection of SA has posed a formidable challenge. In this study, our objective was to engineer a series of rhodamine fluorescent probes containing six- and five-membered benzothiazole rings. We employed a novel synthesis method and evaluated the fluorescence performance of these probes. Among the probes examined, Probe 1 exhibited outstanding sensing capabilities for SA, boasting rapid response times (< 1 minute), exceptional selectivity, sensitivity, photostability, a low detection limit (1 nM), and reversibility. Serving as a representative fluorescence probe, Probe 1 demonstrated its potential for visualising SA within plant tissues, opening avenues for research in plant biology. Additionally, it effectively detected elevated SA levels in various foods, such as grapes, bananas, and cucumbers, providing a valuable warning for individuals sensitive to SA. This proof-of-concept study underscores the prospective utility of

Probe 1 as a molecular tool for monitoring SA in plants and food. Furthermore, its application extends to sewage detection, the food industry, and cosmetics residue detection, where it can deliver cost-effective, reusable, and swift response performance.

### **11. Discovery of carbamate-based salicylic acid derivatives as a novel cholinesterase inhibitor**

Wang et al., 2023- In the realm of Alzheimer's disease (AD) treatment, cholinesterase inhibition stands as a well-established therapeutic strategy. Leveraging active substructure integration, we designed and synthesised a series of Cholinesterase (ChE) inhibitors featuring a carbamate group rooted in salicylic acid. Among this cohort, compounds 3l (with IC<sub>50</sub> values of 1.06  $\mu$ M for eqBChE and 2.08  $\mu$ M for eeAChE) and 3t (with IC<sub>50</sub> values of 0.82  $\mu$ M for eqBChE and 2.38  $\mu$ M for eeAChE) demonstrated superior dual inhibitory activity against both AChE and BChE compared to their counterparts. Computational modelling revealed that compounds 3l and 3t establish binding interactions with cholinesterase through hydrogen bonds and  $\pi$ - $\pi$  stacking interactions, further corroborating their potency. Notably, these compounds exhibited robust neuroprotective qualities and demonstrated anti-apoptotic effects in mouse hippocampal HT22 cells. Equally significant, comprehensive assessments of cell cytotoxicity and acute toxicity (up to 1000 mg/kg) in mice affirmed that compounds 3l and 3t maintain a favourable safety profile. Through a thorough evaluation at both the enzyme and cellular levels, compounds 3l and 3t have emerged as promising candidates. They represent privileged scaffolds with the potential to pave the way for the development of dual AChE/BChE inhibitors

### **12. Enhancing Antioxidant Capacity and Hormone Levels in Winter Jujube**

Yang et al., 2022- This study delved into the impact of exogenous salicylic acid (SA) treatment, specifically at a concentration of 3 mmol L<sup>-1</sup>, on the antioxidant capacity and hormone levels in winter jujube over a 20-day shelf life at 4°C. The findings revealed that the three mmol L<sup>-1</sup> SA treatment effectively preserved the fruit's firmness, colour, titratable acidity, and total soluble solids, reducing the respiratory intensity and the TSS/TA value by 13.08%. Additionally, compared to the control group, the SA-treated group exhibited higher levels of sucrose (14.03%) and malic acid

(29.13%). Furthermore, SA mitigated the accumulation of  $\text{H}_2\text{O}_2$  (27.73%) and  $\text{O}_2^-$  (45.44%) by enhancing the activity of antioxidant enzymes (such as superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase) and increasing the content of antioxidant substances (including ascorbic acid, total phenols, total flavonoids, and glutathione) within the fruit. The SA treatment also led to elevated levels of endogenous abscisic acid (18.49%) and SA (20.47%), along with a decrease in jasmonic acid (42.68%). However, it had a minor impact on indole acetic acid levels.

### **13. Salicylic Acid's Role in Melatonin-Induced Chilling and Oxidative Stress Tolerance in Kiwifruit**

Guo et al., 2023- This study explored the potential of melatonin (MT) in mitigating chilling injury (CI) in 'Xuxiang' kiwifruit during 90 days of storage at 1°C while also investigating the involvement of endogenous salicylic acid (SA) in MT-induced tolerance to chilling and oxidative stress. MT pretreatment effectively protected the kiwifruit against CI, as evidenced by reduced CI index, less firmness loss, and controlled accumulation of soluble solids, resulting in improved pulp appearance compared to untreated fruit. MT pretreatment stimulated the generation of endogenous SA by activating phenylalanine ammonia-lyase (PAL) gene expression and the activity of PAL and benzoic acid-2-hydroxylase. This, in turn, up-regulated the expression of SA-responsive pathogenesis-related gene 1, prompting a defence response in kiwifruit against chilling stress. Additionally, MT neutralised reactive oxygen species by increasing endogenous MT, ascorbic acid, glutathione contents, and the activity of superoxide dismutase, catalase, and ascorbate peroxidase, thus minimising membrane damage. However, the positive effects of MT were partially counteracted when combined with paclobutrazol (PAC), an SA biosynthesis inhibitor, highlighting the mediating role of endogenous SA in the chilling and oxidative stress tolerance induced by MT.

### **14. Solubility Investigation of Paracetamol, Salicylic Acid, and 5-Aminosalicylic Acid in PEGDME 250 + Water Mixtures**

Barzegar-Jalali et al., 2023- This study investigates the solubility of three poorly water-soluble drugs, namely 5-aminosalicylic acid, salicylic acid, and paracetamol, in

polyethene glycol dimethyl ether 250 (PEGDME 250) and water mixtures at temperatures ranging from 293.15 K to 313.15 K under atmospheric pressure (85 kPa). The solubility of these drugs in PEGDME 250 + water solutions follows a descending order: 5-aminosalicylic acid < paracetamol < salicylic acid. It was observed that the solubility of these drugs increased with higher temperatures and greater PEGDME 250 mass fractions. The study employed various data analysis models, including Jouyban-Acree-based models and the modified Wilson-van't Hoff, NRTL, Wilson, and UNIQUAC models. The results provide insights into the solubilisation capacity of PEGDME 250 and the thermodynamic properties associated with the dissolution and mixing of these drugs in PEGDME 250 + water mixtures.

### **15. Mitigating Salinity Stress in Maize with Sodium Hydrosulfide and Salicylic Acid**

Shoukat et al., 2023- Hydrogen sulfide (H<sub>2</sub>S) and salicylic acid (SA) have emerged promising agents for enhancing plant growth and development. This study explores the effects of individual and combined NaHS (H<sub>2</sub>S donor) and SA treatments in alleviating salt stress in maize plants. In vitro screening identified 0.1 mM SA and 0.5 mM NaHS as the most effective concentrations for increasing biomass and germination of maize plants under salt-stress conditions. Furthermore, both individual and combined treatments of H<sub>2</sub>S and SA improved various morphological, biochemical, and physiological attributes compared to untreated plants. Notably, the combined treatment demonstrated the highest salinity resistance. It resulted in increased levels of chlorophyll a (165%), chlorophyll b (138%), and carotenoid (54.4%), as well as elevated activities of antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and polyphenol oxidase (PPO) by 441%, 416.7%, 501.4%, and 510%, respectively, while reducing Na<sup>+</sup> accumulation and enhancing K<sup>+</sup> accumulation. The combined treatment also reduced proline and MDA content by 300% and 108%, respectively, offering protection against salt stress. In silico analysis of the NaHS-SA pathway aligned with experimental results, affirming the role of endogenous SA in mediating chilling and oxidative stress tolerance induced by MT pretreatment and highlighting its potential use in enhancing genomic stability and stress resilience.

### **16. Alleviating Cadmium Toxicity in Chia with Salicylic Acid**



Rharbi et al., 2023- Cadmium (Cd) accumulation poses a significant global threat, impacting plants' physiological and metabolic aspects. This study conducted a potted experiment to assess the effectiveness of salicylic acid (SA) foliar spray in mitigating Cd toxicity in chia (*Salvia hispanica*) seedlings. Chia, known for its exceptional nutritional value and therapeutic potential, was subjected to Cd-induced stress, resulting in reduced growth, mainly shoot and root length, and a diminished number of leaves (25.5%, 21.5%, and 22.6%, respectively). However, SA treatment counteracted the adverse effects of Cd stress, significantly enhancing stem and root growth, particularly shoot length, which increased by 32.3% compared to the Cd treatment alone. Under the combined Cd + SA treatment, there was a marked increase in the accumulation of proline (85.3%) and anthocyanin (83.1%), along with a substantial enhancement in peroxidase activity (34.9%), compared to the control. These findings illustrate SA's role in alleviating Cd-induced oxidative damage and enhancing Cd tolerance in chia, emphasising its potential for improving chia seedling growth.

#### **17. Mitigating Drought Stress in Isabgol with Foliar Salicylic Acid Application**

Roumani et al., 2022- This study aimed to investigate the impact of drought stress on the physiological and biochemical characteristics of isabgol and determine whether foliar application of salicylic acid (SA) can mitigate the stress effects. The study involved a factorial field experiment in north-eastern Iran over two growing seasons. Drought stress was applied at three levels, and SA was sprayed at three concentrations. The results revealed that the highest seed yield was achieved with a foliar spray of 0.8 mM SA under moderate stress conditions. SA application at this concentration improved various parameters, including membrane stability index, catalase activity, proline content, soluble sugars content, total phenol content in leaves, and seed yield. The application of SA at 0.8 mM also enhanced relative water content and membrane stability index by 14% and 12%, respectively, under moderate and severe stress conditions. Moreover, it increased the seed yield, membrane stability, catalase activity, and proline concentration compared to regular irrigation, highlighting its positive effects.

#### **18. Mitigating Water Deficiency Stress in Potato's with Foliar Salicylic Acid Application**

Acevedo et al., 2023- Water deficiency (WD) significantly constrains crop growth and productivity worldwide. Salicylic acid (SA) has been suggested to mitigate the impact of physical stress in potato plants. This study evaluated potato plants' physiological and biochemical responses to SA foliar application under WD conditions. Moderate WD negatively affected metabolic parameters in potato plants, but SA application mitigated oxidative damage by reducing reactive oxygen species (ROS). This protection enhanced cellular membrane structure and reduced malondialdehyde accumulation. SA application also increased the antioxidative capacity of the plants, indicating its potential for alleviating water deficiency stress.

### **19. Enhancing Biochemical Compounds in Hybrid Chillies with Foliar Salicylic Acid and Ascorbic Acid**

Zahid et al., 2023- Chillies (*Capsicum annuum*) are valued for their high antioxidant and nutritional properties. This study examined the effects of foliar application of salicylic acid (SA) and ascorbic acid (AA) on hybrid chillies. Various treatments were applied, and results indicated improvements in yield, fruit dimensions, and biochemical attributes. Notably, the SA and AA combination treatment showed the most significant enhancements in various parameters, including plant height, shoot weight, root weight, pericarp thickness, and the content of carbohydrates, proteins, fibre, ash, proline, SOD, POD, and CAT. These treatments have the potential to enhance chilli growth and biochemical properties.

### **20. Efficient Removal of Salicylic Acid from Pharmaceutical Wastewater Using Composite Nanofiber Membrane**

Wang et al., 2023- The study presents a cost-effective and efficient adsorbent for removing salicylic acid (SA) from pharmaceutical wastewater. A composite nanofiber membrane modified with UiO-66(Hf)-NH<sub>2</sub> nanocrystals demonstrated exceptional flexibility and reusability, with a removal efficiency of 96.85% even after ten cycles. The adsorption process was described by pseudo-second-order kinetics and Langmuir isotherm models. Dynamic adsorption experiments showed complete SA removal at a 50 mg/L concentration. X-ray photoelectron spectroscopy (XPS) revealed

various interactions responsible for adsorption. The membrane holds promise for pharmaceutical wastewater treatment.

## **21. *Pseudomonas putida* and Salicylic Acid Interaction in Canola Growth Under Stress**

Tanveer et al., 2023- This research investigates the combined effects of *Pseudomonas putida* and salicylic acid (SA) on canola growth under stressed and non-stressed conditions. Results indicate that the combined application of *Pseudomonas putida* and SA significantly improved canola germination and morphological, physiological, and biochemical parameters. This combined treatment enhanced seedling vigour, membrane stability, proline content, flavonoids, phenol, and antioxidant enzyme activities. These findings suggest that the synergistic effect of *Pseudomonas putida* and SA induces drought tolerance in canola and enhances growth.

## **22. Jasmonic Acid and Salicylic Acid's Defensive Response in Wine Grapes Against *Drosophila Suzukii***

Hussain et al., 2023- This study explores the defensive response in wine grapes against *Drosophila suzukii* by applying jasmonic acid (JA) and salicylic acid (SA). Using JA and SA significantly impacted phenolic content in grapes and reduced injury from *D. suzukii*. *D. suzukii* females laid fewer eggs on treated plants, and behavioural studies indicated a preference for specific sugar solutions. Catechin exhibited high mortality in *D. suzukii*. These findings have implications for managing *D. suzukii* in wine grapes.

## **23. Molecular Regulation of the Salicylic Acid Hormone Pathway in Response to Changing Environments**

Rossi et al., 2023- discusses the regulation of the salicylic acid (SA) hormone pathway in plants under changing environmental conditions. SA is an essential plant hormone in immunity, growth, and development. The study highlights the sensitivity of the SA pathway to ecological factors and the plant microbiome. It delves into molecular mechanisms that govern SA pathway vulnerability or resilience, including thermosensitive responses, transcription factors, protein interactions, and biomolecular

condensates. This information provides insights into how external environments influence the SA pathway in plants.

#### **24. Optimizing the Elicitation Strategy of Salicylic Acid for Enhanced Flavonoid and Phenolic Production in Fed-Batch Cultured *Oplopanax elatus* Adventitious Roots**

Yu et al., 2023- *Oplopanax elatus* is a valuable medicinal plant, but its plant resources need improvement. Culturing adventitious roots (ARs) of *O. elatus* in a fed-batch system effectively produces plant materials. Salicylic acid (SA) has been shown to enhance metabolite synthesis in specific plant cell and organ culture systems. To investigate the elicitation effect of SA on fed-batch cultured *O. elatus* ARs, this study examined various factors, including SA concentration, elicitation timing, and duration. The results demonstrated that when ARs were treated with 100  $\mu\text{M}$  SA for 4 days starting on day 35, flavonoid and phenolic contents and antioxidant enzyme activity increased significantly. Under these elicitation conditions, total flavonoid and phenolic contents reached 387 mg/g DW and 128 mg/g DW, respectively, substantially higher than those in the SA-untreated control. Moreover, SA treatment significantly increased DPPH and ABTS<sup>+</sup> scavenging and Fe<sup>2+</sup> chelating rates, indicating elevated antioxidant activity. This study highlights the potential of SA as an elicitor to enhance flavonoid and phenolic production in fed-batch *O. elatus* AR culture.

#### **25. Polyfluorinated Salicylic Acid Analogs Do Not Interfere with Siderophore Biosynthesis**

Hegde et al., 2023- Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is a leading infectious disease worldwide. Small molecule siderophores known as mycobactins, derived from salicylic acid, play a critical role in Mtb's iron acquisition when iron is limited in the host environment. This study investigates polyfluorinated salicylic acid analogues, previously known for their potent antimycobacterial activity, to understand their mechanism of action. It was hypothesized that these fluorinated derivatives might inhibit mycobactin biosynthesis by interfering with late steps in mycobactin assembly through bioactivation and conversion to downstream metabolites. Enzymatic studies showed that some fluorinated salicylic acid derivatives were readily

activated by the enzyme MbtA, responsible for incorporating salicylic acid into the mycobactin biosynthetic pathway. However, these compounds did not inhibit mycobactin biosynthesis, as confirmed by LS-MS/MS using an authentic synthetic mycobactin standard. Further mechanistic analysis of the most active derivative (Sal-4), using an MbtA-overexpressing Mtb strain and complementation studies with iron and salicylic acid, revealed that Sal-4 could not be antagonized by MbtA overexpression or supplementation with iron or salicylic acid. These results suggest that the observed antimycobacterial activity of polyfluorinated salicylic acid derivatives is independent of mycobactin biosynthesis.

## **25. PpMYB44 Enhances Salicylic Acid Biosynthesis in *Pichia guilliermondii*-Induced Peach Fruit Resistance Against *Rhizopus stolonifera***

Li et al., 2023- Antagonistic yeast induces postharvest disease resistance and prolongs the shelf life of fruits. Salicylic acid (SA), a signalling molecule, activates induced disease resistance in various harvested fruits. However, limited information is available regarding the transcriptional regulatory mechanism of SA biosynthesis in peaches responding to *Rhizopus stolonifer*, a common postharvest pathogen. This study reveals that applying *Pichia guilliermondii* induces SA accumulation, increases the activity of SA biosynthetic enzymes such as PAL and ICS, and upregulates the expression of genes related to SA synthesis and defence in peach fruit. Additionally, an R2R3 MYB transcription factor, PpMYB44, is induced in *P. guilliermondii*-treated peach fruit in response to fungal disease. PpMYB44 is identified as a critical regulator of SA synthesis by binding to the promoters of PpPAL1 and PpSARD1. Furthermore, transient overexpression of PpMYB44 in peach fruit reduces disease incidence and severity, upregulates the expression of SA synthesis-related genes (PpPAL1 and PpSARD1), and enhances SA accumulation. Conversely, silencing PpMYB44 in peach fruit increases disease incidence and severity, downregulates the transcript levels of PpPAL1 and PpSARD1, and reduces SA accumulation. This study sheds light on the role of PpMYB44 in regulating yeast-induced disease resistance by increasing SA content in peaches.

## **26. Electrochemical Removal of Salicylic Acid Using Stainless Steel and Platinum Anodes**

Koktas et al., 2022- Salicylic acid is a crucial pharmaceutical compound widely used in plant hormones and personal care products. Peroxycatalysis (PC) is an electrochemical advanced oxidation process that removes organic pollutants. Carbon-based cathode materials like graphite and carbon fibre are typically used for in situ H<sub>2</sub>O<sub>2</sub> production. In contrast, stainless steel (SS316-L) is used as the anode for low iron production in PC studies as an efficient system modification. This study investigates the efficiency of electrochemical processes for removing salicylic acid from wastewater using stainless steel as the anode. The oxidation effect of stainless steel is believed to contribute to the partial removal of salicylic acid. The study employs stainless steel anode and graphite or carbon fibre cathodes in PC treatments to remove salicylic acid from aqueous solutions. Some model trials are conducted to examine in-situ Fe<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub> production. The findings indicate a total organic carbon (TOC) removal of 30.5% and a salicylic acid removal of 69.5% under optimised conditions. This study proposes electrochemical PC system modification as an accessible, cost-effective, and efficient treatment option for pharmaceutical industry waste containing salicylic acid.

## **27. Salicylic Acid Alleviates Salt Toxicity in Kenaf by Activating the Antioxidant System and Regulating Crucial Pathways and Genes**

Hu et al., 2023- High salinity is a major abiotic stressor that limits crop production. Salicylic acid (SA) has been shown to mitigate the adverse effects of environmental stress on plants, yet the molecular mechanisms of SA-mediated salinity tolerance in kenaf remain unclear. To elucidate the alleviation effects of SA on salt stress in kenaf, this study examined morphological changes, physiological parameters, and transcriptomic regulation in response to salt stress with or without prior SA treatment. The results demonstrate that exogenous SA significantly mitigated the detrimental effects of salt stress on kenaf, affecting agronomic traits, antioxidant enzyme systems, and various essential pathways. Several differentially expressed genes (DEGs) involved in these pathways and transcription factors (TFs), such as NAC, MYB, bHLH, and ERF, were significantly influenced by SA application. Virus-induced gene Silencing (VIGS)

of a differentially expressed TF, HcNAC29, reduced salt tolerance in kenaf. Integrating physiological and transcriptomic analyses revealed the importance of pathways such as phenylpropanoid and flavonoid techniques, plant hormone signalling, and specific TFs, such as NAC, in SA-mediated salinity tolerance in kenaf. This study provides insights into the multifaceted regulatory roles of SA in plant responses to abiotic stress.

## **28. Salicylic Acid Directly Binds to Ribosomal Protein S3 and Suppresses CDK4 Expression in Colorectal Cancer Cells**

Imai et al., 2022- Colorectal cancer poses a significant health challenge worldwide, necessitating the development of effective preventive strategies. Aspirin has garnered attention as a potential cancer prophylactic agent, with reported inhibitory effects on various cancers. However, the precise molecular mechanisms underlying aspirin's protective effects remain unclear. This study aimed to identify target proteins of aspirin using salicylic acid, its primary metabolite, through a chemical biology approach. Salicylic acid-presenting FG beads were employed to purify salicylic acid-binding proteins from human colorectal cancer HT-29 cells. The results identified ribosomal protein S3 (RPS3) as a potential target protein of salicylic acid. Depleting RPS3 through siRNA reduced CDK4 expression and induced G1 phase arrest in colorectal cancer cells. These effects were consistent with those caused by sodium salicylate treatment, suggesting that salicylic acid negatively regulates RPS3 function. This study highlights RPS3 as a potential novel target for salicylic acid in aspirin's protective effects against colorectal cancer, supporting its role as a target for cancer prevention.

## **29. Enhancing Antioxidant Activity in Spinach Leaves Through Salicylic Acid Elicitation: Increasing Phenolic Content and Enzyme Levels**

Singh, 2023- In agriculture, elicitation is employed to protect crops and boost the production of important nutraceutical or pharmaceutical metabolites using chemical stimuli known as elicitors. In this experiment, we harnessed the elicitor potential of Salicylic acid (SA) to induce metabolic changes in spinach plants. Various concentrations of Salicylic acid (ranging from 1 mg/ml to 0.01 mg/ml) were applied to spinach leaves through foliar spray, and the resulting biochemical changes were

observed. A significant increase in the levels of total phenolic and total flavonoid content was observed, along with heightened enzyme activity involving peroxidase, catalase, and superoxide dismutase, all in a dose-dependent manner. This elicitation led to increased antioxidant activity, closely correlated with the rise in phenolic compounds. However, high concentrations of SA negatively impacted growth parameters, such as total biomass and chlorophyll content. Metabolite profiling, conducted through Electrospray Ionization Mass Spectrometry (ESI-MS) in both positive and negative ion modes, revealed an increase in ion intensity peaks associated with phenolic acids, including gentisic acid, hydroxycinnamic acid, coumaric acid, ferulic acid, and caffeic acid, as well as significant flavonoids found in spinach, such as kaempferol, quercetin, isorhamnetin, and patulin glycosides. The concentrations of ferulic and caffeic acids were quantified by High-Performance Liquid Chromatography (HPLC) and shown to increase following elicitor treatment.

### **30. Interactions of Salicylic Acid with Other Plant Growth Regulators and Signalling Molecules under Stressful Plant Environments**

Kaya et al., 2023- Salicylic acid (SA) is a pivotal plant growth regulator (PGR) known to modulate various physiological and metabolic processes, playing a crucial role in plant growth, development, and defence against environmental stressors. SA is recognised as a critical component of plant defence mechanisms against environmental stimuli. Although it is well-established that SA plays a significant role in enhancing plant tolerance to diverse stresses, it remains to be seen whether low or high concentrations are more effective in optimising plant growth under stressful conditions. Additionally, the extent and manner in which SA interacts with other potential growth regulators and signalling molecules within the plant must be fully understood. This critical review delves into how SA mediates crosstalk with other important PGRs and molecular components of signalling pathways, particularly in plants exposed to various environmental cues. Furthermore, it elucidates the function of exogenously applied SA in regulating plant growth, development, and the reinforcement of oxidative defence systems under abiotic stress conditions.

### **31. Mitigating Drought Stress in Flowering Kale (cv. 'Red Pigeon F1') Through Seed Treatments with Salicylic Acid and Succinic Acid**



KILIÇ, 2023- Drought stress, exacerbated by climate change, substantially threatens bedding plants, including flowering kale (*Brassica oleracea*, Acephala group). This study aimed to evaluate the impact of seed treatments with salicylic acid (SA) and succinic acid (SAC) on the physiological and biochemical traits of flowering kale plants exposed to drought stress. Seeds of the 'Red Pigeon F1' cultivar were used for the study. The seeds were treated with three different concentrations of SAC (0.5 mM, 1.0 mM, and 2.0 mM) and SA (0.5 mM, 1.0 mM, and 2.0 mM). During the early seedling stage, untreated seedlings (negative control) and seedlings treated with SAC and SA were subjected to severe drought stress conditions, while the positive control received adequate watering. The experiment concluded when 50% of the seedlings in the negative control group displayed drought stress symptoms. Various parameters were measured, including relative chlorophyll content, stomatal conductance, leaf temperature, leaf relative water content, leaf area, membrane permeability, lipid peroxidation, proline content, and antioxidant enzyme activity. Results showed that both SAC and SA treatments effectively mitigated the adverse effects of drought stress on flowering kale seedlings. Seedlings treated with these compounds displayed improved growth, reduced oxidative stress, and enhanced drought tolerance. Additionally, SAC treatment resulted in green fuel ( $H_2$  gas) production during the treatment process. This study demonstrates the potential of SAC and SA seed treatments to enhance stress tolerance in flowering kale, offering valuable insights for better crop productivity under drought conditions.

### **32. Enhancing Salinity Tolerance in Sugarcane (*Saccharum officinarum* L.) Through Sett Priming with Salicylic Acid**

Apon et al., 2023- Sugarcane (*Saccharum officinarum* L.) is a globally significant crop for sugar production but is susceptible to soil salinity due to its glycophytic nature. High salinity levels can lead to water stress and metabolic changes, resulting in crop failure during early developmental stages. This study investigated the potential of salicylic acid (SA) as a sett priming material to alleviate the adverse effects of salt stress on sugarcane during germination and early growth. Various concentrations of SA (ranging from 0 to 2 mM) were applied to sugarcane setts, and their performance was evaluated under different salinity levels ( $0.5 \text{ dS m}^{-1}$ ,  $4 \text{ dS m}^{-1}$ , and  $8 \text{ dS m}^{-1}$ )

within a controlled environment. Results indicated that SA priming significantly improved germination, seedling growth, and several physiological traits under salt stress conditions. It increased root length, root-to-stem length ratio, antioxidant enzyme activity, ascorbic acid and phenol content while reducing sodium ion accumulation and the sodium-to-potassium ratio. Even under high salinity levels (8 dS m<sup>-1</sup>), SA-primed setts exhibited improved germination and seedling growth compared to non-primed setts. This study provides valuable insights into the management of salinity stress in sugarcane. It highlights the potential of SA priming as an effective strategy to enhance crop tolerance to abiotic stress.

### **33. Enhancing Salicylic Acid Solubility and Intermolecular Interactions in Aqueous Solutions of Choline Chloride-Based Deep Eutectic Solvents**

Nourizadeh et al., 2022- This study explores the solubility enhancement and molecular interactions of salicylic acid (SA) in aqueous solutions of deep eutectic solvents (DESs) based on choline chloride (ChCl). DESs are considered environmentally friendly solvents and have gained attention for their potential to enhance the solubility of poorly soluble drugs. In this research, the solubility of SA was examined in water and aqueous solutions of ChCl-based DESs at various temperatures. Computational studies were also conducted to gain insights into the molecular interactions between DESs and SA. The results revealed that SA solubility was significantly increased in DESs. These DESs also influenced the pH of the solution, further enhancing SA solubility. The improved solubility of SA in DESs was shown to be consistent between experimental and computational results. This research contributes to the development of solvents and methods for improving the solubility of drugs like SA.

### **34. Role of SsRSS1 in Salicylic Acid Tolerance and Virulence in Sugarcane Smut Fungus**

Hao-yang et al., 2023- Sugarcane smut, caused by the fungus *Sporisorium scitamineum*, severely threatens sugarcane production worldwide. However, the mechanisms underlying the pathogenicity of this fungus remain poorly understood. In this study, researchers investigated the role of the SsRSS1 gene, which encodes a

regulator sensitive to salicylic acid (SA), in the fungus's tolerance to SA stress and virulence. The results showed that disrupting the SsRSS1 gene did not affect the fungus's growth or sexual mating ability but reduced the tolerance of basidiospores (fungal spores) to SA stress. This reduction in SA tolerance was associated with the inhibition of a gene called SsSRG1, which is involved in the SA response of the fungus. Moreover, the deletion of SsRSS1 attenuated the virulence of the fungus, leading to a decrease in whip formation, a characteristic symptom of sugarcane smut. This study sheds light on the role of SsRSS1 in the fitness of the fungus and its ability to counteract SA stress.

### **35. Remediating Salicylic Acid from Wastewater Using Surfactant-Aided Electrocoagulation/Flotation with Punched Electrodes**

Ahmad et al., 2023- This study evaluates the performance of a batch electrocoagulation/floatation (ECF) system using punched aluminium electrodes for the removal of salicylic acid (SA) from wastewater, with the aid of the surfactant cetyltrimethyl ammonium bromide (CTAB). To achieve efficient SA removal, the researchers optimised various operational parameters, including electrolysis time, electrode gap, electrolyte concentration, pH, current density, and CTAB dosage. Under optimised conditions, the ECF process performed a remarkable 92.1% removal of SA from wastewater with an initial SA concentration of 50 mg/L. The study also investigated the kinetics of SA degradation, electrode corrosion, and sludge generation. After ECF treatment, the treated water exhibited reduced phytotoxicity and the age of green fuel, hydrogen gas ( $H^2$ ). Notably, adding CTAB to the electrolyte reduced sludge generation by approximately half. The study also calculated the energy consumption and treatment cost, making it a promising method for removing SA from contaminated water.

### **36. Sustained Release of Salicylic Acid from Ethyl Cellulose Microspheres Fabricated Using the Quasi-Emulsion Solvent Diffusion Method**

Pokharel et al., 2023- This research explores the use of ethyl cellulose (EC) microspheres for the sustained release of salicylic acid (SA). The quasi-emulsion solvent diffusion (QESD) method was employed to fabricate these microspheres,

offering an efficient and cost-effective approach. Poly (vinyl alcohol) in water served as the external phase, while EC and SA were dissolved in dichloromethane as the internal phase. SA is known for its anti-inflammatory properties and inhibitory effects on bacterial biofilm formation. The study successfully produced microspheres with various sizes ranging from 5 to 40  $\mu\text{m}$ . Extensive characterisation confirmed the suitability of the materials used. Cytotoxicity tests demonstrated the biocompatibility of the microspheres. In vitro drug release studies revealed sustained release profiles, with most of the drug being released after 48 hours, reaching a maximum of 63% of the total drug content. Adding polyethylene glycol (PEG) eliminated burst release, resulting in sustained release patterns over 48 hours, reaching saturation by day 5. Behavioural studies evaluating the microspheres revealed different kinetic models, including Higuchi and Korsmeyer-Peppas models, with varying diffusion mechanisms. The study suggests potential improvements to enhance drug release from the microspheres, such as increasing their porosity. It highlights the need for further investigations into longer-term drug release and using near-infrared light (NIR) to monitor drug release changes.

### **37. Diversity in Salicylic Acid Biosynthesis and Defense Signaling in Plants: Knowledge Gaps and Future Opportunities**

Ullah et al., 2023- Salicylic acid (SA) is a pivotal phytohormone known for its role in regulating plant immunity against pathogens. Plants can synthesise SA through two main pathways: the isochorismate synthase (ICS) pathway and the phenylalanine ammonia-lyase (PAL) pathway. *Arabidopsis thaliana* is a model plant with pathogen-induced SA accumulation through the ICS pathway. In contrast, some species, including *Populus* (poplar), rely on the less understood PAL pathway for constitutive and pathogen-stimulated SA production. SA-mediated defence mechanisms also display diversity in SA accumulation, redox regulation, and interactions with other hormones like jasmonic acid. This review highlights the contrasting characteristics between *Arabidopsis* and poplar, explores the factors contributing to SA diversity in plant defences, and identifies future research directions.

### **38. Preharvest and Postharvest Application of Salicylic Acid and Its Derivatives for Fruit and Vegetable Storage**

Chen et al., 2023- Salicylic acid (SA) and its derivatives, including acetylsalicylate (ASA) and methyl salicylate (MeSA), have gained widespread use for preserving fruits and vegetables. This comprehensive review summarises recent research on preharvest and postharvest treatment methods with SA and its derivatives, focusing on their effectiveness in safeguarding postharvest physiological processes in fruits. Preharvest spraying is recommended as the optimal method due to its efficient absorption of salicylates and reduced postharvest handling time. Applications of SA and its derivatives have been shown to enhance storage quality by reducing ethylene production, respiration rate, fruit softening, and colour changes while maintaining sugar, organic acid, and aroma content. They also inhibit chilling injury, improve pathogen resistance, and activate the antioxidant system. The review compares treatment methods and physiological analyses, offering insights into the practical applications of SA and its derivatives in fruit and vegetable storage.

### **39. Bulb Productivity and Quality of Monsoon Onion (*Allium Cepa* L.) as Affected by Transient Waterlogging at Different Growth Stages and Its Alleviation with Plant Growth Regulators**

Wakchaure et al., 2023- The productivity and quality of monsoon onions (*Allium cepa* L.) can significantly affect transient waterlogging during the rainy season. This study investigated the consequences of water stagnation at various growth stages. It explored potential solutions using plant growth regulators (PGRs) such as potassium nitrate (PN), thiourea (TU), salicylic acid (SA), and sodium benzoate (SB) during the period from 2018 to 2020. The results showed that bulb yield losses ranged from 7% to 60%. Bulb initiation was the most sensitive stage, with a yield loss of 20%, followed by vegetative growth (15%) and bulb development (7%). Yield losses were additive when waterlogging occurred at multiple growth stages, reaching up to 60%. However, applying PGRs significantly improved marketable bulb yields by 10.4% to 23.3%, enhancing water productivity (4.0 to 4.4–4.9 kg m<sup>-3</sup>). PGRs were crucial in mitigating waterlogging stress by maintaining higher leaf water content, preserving plant vigour, lowering canopy temperatures, and regulating stomatal openings. Potassium nitrate and

thiourea were particularly effective during the sensitive bulb initiation stage. Although waterlogging did impact bulb quality traits, applying PGRs helped recover these traits, highlighting their potential to alleviate the adverse effects of transient waterlogging on monsoon onion cultivation.

#### **40. Cerium Oxide- Salicylic Acid Nanocomposite Foliar Use Impacts Physiological Responses and Essential Oil Composition Of Spearmint (*Mentha Spicata* L.) Under Salt Stress**

Shiri et al., 2023- Applying certain compounds like salicylic acid and cerium oxide has shown promise in enhancing plant tolerance to abiotic stress, including salt stress. To assess the impact of salicylic acid nanoparticles (SA-NPs), cerium oxide nanoparticles (CeO<sub>2</sub>-NPs), and a cerium oxide-salicylic acid nanocomposite (CeO<sub>2</sub>-SA) on spearmint (*Mentha spicata* L.) under salt stress, a greenhouse experiment was conducted with a factorial design consisting of three levels of salinity stress (0, 50, and 100 mM) and various treatments including SA-NPs, CeO<sub>2</sub>-NPs, and CeO<sub>2</sub>- SA nanocomposite. The results demonstrated that increasing salinity stress decreased levels of carbohydrates, proteins, total antioxidants, carotenoids, and chlorophyll content in spearmint. However, applying the cerium oxide-salicylic acid nanocomposite positively influenced protein, carbohydrate, phenolic compounds, flavonoids, essential oil percentage, and total antioxidant capacity. Additionally, it helped alleviate oxidative stress by reducing proline and hydrogen peroxide levels and enhancing the activity of antioxidant enzymes such as superoxide dismutase and guaiacol peroxidase. The nanocomposite also positively influenced the content of essential oil compounds. Overall, the CeO<sub>2</sub>-SA nanocomposite demonstrated its potential to mitigate the detrimental effects of salt stress on spearmint plants and improve their overall quality.

#### **43. Diversity of Glucosinolates Among Common Brassicaceae Vegetables in China**

Zhu et al., 2023- Brassicaceae vegetables are a staple in Chinese and global diets, providing essential nutrients and phytochemicals that offer significant health benefits. This review focuses on the diversity of commonly consumed Brassicaceae vegetables and their glucosinolate (GSL) composition in their edible parts. It also discusses the factors influencing GSL content and their roles and functions in plant

health. By highlighting the variations in GSL content among different Brassicaceae vegetables, this review aims to guide consumers in selecting vegetables with high GSL content at optimal stages of maturity and suitable preparation methods. Furthermore, it provides valuable insights for crop molecular breeding and developing GSL-based products.

#### **44. Glucosinolates Extracts from Residues of Conventional and Organic Cultivated Broccoli Leaves (*Brassica oleracea* Var. *Italica*) As Potential Industrially-Scalable Efficient Biopesticides Against Fungi, Oomycetes and Plant Parasitic Nematodes**

Eugui et al., 2023- This study aimed to develop an efficient and scalable protocol for obtaining bioactive extracts from conventional and organically cultivated broccoli (*Brassica oleracea* var. *italica*) leaves, suitable for application as biopesticides against fungi, oomycetes, and plant parasitic nematodes. The study examined various extraction factors' influence on glucosinolate (GSL) content and assessed the phytotoxicity and cytotoxicity of the extracts. The results indicated that lyophilisation did not impact GSL content, and storage at different temperatures (-20°C and -80°C) had no significant effect on GSL content. The extracts showed phytotoxicity at concentrations above 10%, while cytotoxicity was low. In vitro tests against various plant pathogenic fungi and oomycetes revealed that the sections, particularly when combined with myrosinase enzyme, inhibited the growth of several pathogens. However, they had no significant effect on plant-parasitic nematodes. The main constituents of the essential oil extracted from the broccoli leaves were identified. In conclusion, the study demonstrated the potential of using broccoli leaf extracts as biopesticides against plant pathogenic fungi and oomycetes, offering an eco-friendly approach to crop protection.

#### **45. The Phytochemical Composition of Sprouts and Microgreens from Brassica Vegetables Affects the Sensory Profile and Consumer Acceptability**

Cano-Lamadrid et al., 2023- In recent decades, pre and postharvest strategies have been developed to enhance sprouts and microgreens' yield, quality, and bioactive compound levels. The quantity of phytochemicals in these plants varies, especially

during the early growth stages, and often has a notable impact on their sensory characteristics and overall consumer appeal. This study aimed to compare the content of critical compounds, such as organosulfur compounds and total phenolic content, with the sensory profiles of sprouts and microgreens in five Brassica species (kale, radish, rocket, broccoli, and mustard) at different growth stages. The research also involved conducting a penalty analysis based on consumer preference data to optimise harvest timing, thereby avoiding undesirable sensory attributes. An online survey was administered to populations in the Mediterranean basin, including Italy and Spain, to determine product intentions and consumption preferences. Overall, it was observed that Brassica sprouts generally exhibited higher levels of organosulfur compounds compared to microgreens of the same species. This study further confirms the association between certain organosulfur compounds and their hydrolysed derivatives with the spicy or pungent sensory characteristics in Brassica sprouts and microgreens. The findings presented in this study offer valuable insights for small-scale vegetable farmers, providing them with a tool to make informed decisions regarding the optimal harvest time based on phytochemical content and composition.

#### **46. Integrative Transcriptome and Metabolome Revealed the Molecular Mechanism of *Bacillus Megaterium* BT22-Mediated Growth Promotion in *Arabidopsis thaliana***

Liu et al., 2023- Plant growth-promoting rhizobacteria (PGPR) play a pivotal role in enhancing plant growth and protecting plants against pathogens, contributing to the sustainability of agricultural practices. Numerous studies have underscored their advantageous characteristics in promoting plant growth and development and fortifying plant resilience to stressors through various mechanisms. However, there remains a challenge in comprehending the molecular mechanisms governing plant responses to PGPR. In this study, we analysed the transcriptome and metabolome of *Arabidopsis thaliana* (*Arabidopsis*) to elucidate its reactions following inoculation with an isolated PGPR strain, BT22, derived from *Bacillus megaterium*. The application of BT22 resulted in increased fresh shoot weight, dry shoot weight, and leaf count in *Arabidopsis*, indicative of a positive influence on growth. The multi-omics analysis unveiled 878 differentially expressed genes (296 up-regulated and 582 down-regulated)



and 139 differentially expressed metabolites (66 up-regulated and 73 down-regulated) in response to BT22 inoculation. Gene Ontology (GO) enrichment analysis revealed that the up-regulated genes were predominantly associated with pathways related to growth regulation and auxin response. In contrast, the down-regulated genes were mainly linked to responses to wounding, jasmonic acid, and ethylene pathways. BT22 inoculation exerted a regulatory effect on plant hormone signal transduction in *Arabidopsis*, involving auxin and cytokinin response genes such as AUX/IAA, SAUR, and A-ARR, which are associated with cell enlargement and cell division. Additionally, the levels of nine flavonoids and seven phenylpropanoid metabolites increased, contributing to the induction of systemic resistance in plants. These findings suggest that BT22 promotes *Arabidopsis* growth by modulating plant hormone balance and initiating a reprogramming of the metabolome.

#### **47. Microbial Community Succession in Soil is Mainly Driven by Carbon and Nitrogen Contents Rather Than Phosphorus and Sulphur Contents**

Tang et al., 2023- Applying organic manure is a common agricultural practice to enhance soil nutrient cycling and improve various physicochemical properties. However, there is still a need for a comprehensive understanding of the biotic and abiotic mechanisms governing the cycling of carbon (C), nitrogen (N), phosphorus (P), and sulphur (S) following manure application. This study collected soil samples from long-term experimental plots that had received farmyard manure or mineral fertilisers since 1964. To investigate C, N, P, and S dynamics and their relationships with microbial community composition and functions in response to different fertilisation regimes, we employed isotope labelling with  $^{15}\text{N}$ ,  $^{33}\text{P}$ , and  $^{35}\text{S}$ , metagenomics, and high-throughput sequencing. Our findings revealed distinct niche differentiation between bacteria and fungi in soils treated with mineral fertilisers versus organic manure. Network analysis indicated that prolonged manure application reduced the complexity and stability of the soil microbial network. Furthermore, based on redundancy analysis, variation partitioning analysis highlighted that changes in soil carbon and nitrogen content were predominant in driving variations in the microbial community. Dissolved organic carbon emerged as the most influential factor shaping microbial community structure, explaining 43.5% of the variation in bacterial

communities and 37.9% in fungal communities. Conversely, soil phosphorus and sulfur content contributed 29.9% of the variation in bacterial communities and 20.3% in fungal communities. Long-term manure application was associated with an increased abundance of functional genes related to C, N, P, and S cycling, ultimately resulting in elevated C and N cycling rates due to ample microbial growth substrates. Partial least squares path modelling revealed that soil physicochemical properties, particularly on dissolved organic carbon, directly impacted C and S cycling. In contrast, alterations in microbial community composition indirectly influenced the N and P cycles. These findings provide novel insights into the direct and indirect effects of organic manure and inorganic fertilisers on soil nutrient cycling processes, all mediated by the soil microbial community."

#### **48. Nitric Oxide Co-Ordination with Nitrogen Reverses Cadmium-Inhibited Photosynthetic Activity by Interacting with Ethylene Synthesis, Strengthening the Antioxidant System, and Nitrogen and Sulphur Assimilation in Mustard (*Brassica Juncea* L.)**

Mir et al., 2023- Nitric oxide (NO) is a gaseous signalling molecule that plays significant roles in plant development and adaptation to abiotic stress. This study sheds light on how the combination of NO and nitrogen (N) effectively mitigated the inhibitory effects of cadmium (Cd) on photosynthetic activity in mustard plants (*Brassica juncea* L.) cv. Giriraj. This was achieved by influencing ethylene synthesis and modifying the mechanisms of antioxidant enzymes and N and sulphur (S) assimilation. In the experiment, mustard plants grown in soil containing 200 mg Cd kg<sup>-1</sup> exhibited reduced photosynthetic activity due to Cd-induced stress. However, when treated with 100 µM sodium nitroprusside (SNP), an NO donor, and 200 mg N kg<sup>-1</sup> soil in the form of ammonium nitrate, these Cd-exposed plants experienced a noticeable reduction in oxidative stress, including levels of superoxide radicals, hydrogen peroxide, and thiobarbituric acid reactive species. This reduction in oxidative stress was accompanied by a decrease in cell death, brought about by enhanced synthesis of metal chelators such as non-protein thiols and phytochelatins and restricted Cd translocation within the plant. The decline in cellular reactive oxygen species (ROS) levels was attributed to the combined action of NO and N, which facilitated increased

assimilation of sulphur (S) and nitrogen (N), as well as the activation of antioxidants. Additionally, the higher expression of *psbA* and *psbB* genes involved in the D1 protein of Photosystem II (PSII) was observed under the influence of NO and N, leading to improved photosynthetic performance, even under elevated Cd levels. Furthermore, when NO and N were applied alongside aminoethoxyvinylglycine (AVG), an inhibitor of ethylene biosynthesis, the growth and photosynthetic attributes of the plants were compromised, resulting in heightened oxidative stress. This underscores the role of ethylene in the NO and N-mediated reversal of Cd-induced photosynthetic inhibition. Principal component analysis revealed a positive correlation between the SNP + N + Cd treatment and various factors, including NO production, antioxidant enzymes, glutathione, and metal chelators. These factors contributed to enhanced N and S assimilation, improved growth, and increased photosynthetic capacity under Cd-induced stress conditions. This study demonstrates that the alleviation of Cd toxicity and the protection of photosynthetic activity through NO were significantly enhanced in the presence of available N, acting in coordination with ethylene synthesis. These findings hold promise for managing soils contaminated with potentially toxic metals such as Cd.

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**MATERIALS AND METHODS**

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The project work entitled “**Evaluation of Sulphur and Salicylic Acid on Growth, Physiology, Yield and Molecular Expression of Indian Mustard**” was conducted during the *Rabi* season of the year 2021-2023 and 2022-23 at Lovely Professional University field, Jalandhar, Punjab. The materials and methods used in this investigation are described below in this chapter. A brief description of all materials and methods, like the location of the experiments, properties of soil, climatic conditions, treatments, and all agronomical operations, are given in this chapter. Statistical analysis by SPSS data collected from the experimental field is described in a tabular and graphical form.

**3.1 Description of the Experimental Site****3.1.1 Location of the Experiments**

This experiment was conducted at Lovely Professional University, Phagwara, Punjab's agricultural research farm, during the 2021-2023 and 2022-23 *Rabi* seasons. The research field is graphically located at latitude 31°22'31.8" North, longitude 75°23'03.02" East, and 252m above sea level.

**3.2 Experimental Design and Treatments Details**

**3.2.1 Experimental Design:** The experiment was laid with the Randomized Block Design. The total area required for the experiment was approx. 650 m<sup>2</sup>. The experiment was conducted with 12 treatments and 3 replications; thus, the total number of plots was 36. All the treatments were arranged with randomization (unbiased) in the plots. Each subplot size was 5m × 3 m = 15 sq. m. There were separated irrigation channels of 1m width to provide irrigation to each plot separately.

**3.2.2 Weather and Climate:** The research trial site reported different hot and cold climate conditions. The average temperature goes up to approx. 50°C. Weather parameters like average minimum and maximum temperature, rainfall, and evaporation rate were taken from the website during the season from sowing to harvesting.

**3.2.3 Properties of Soil:** The different standard procedures determine soil properties. Determination of soil pH, soil EC, and macronutrients were distinguished by other following procedures.

**Soil pH:** A pH meter was used to estimate the pH of the soil. Soil samples were taken from the field at different layers and collected in polythene bags. Take 12.5g of soil in 150 ml of the beaker. There should be no clods in the soil. Add 50 ml of distilled water to the same cup. Stir the soil solution for half an hour. Switch on the pH meter and calibrate it with the buffer pH solutions. Dip the rod of the pH meter in the soil solution and note the readings (Sawarkar, 2012).

**Soil EC:** Soil Electrical conductivity was estimated using the instrument EC meter. 10 g of air-dry soil was taken into a bottle, and 50 ml of distilled water was added to it, followed by shaking on a mechanical shaker for 1 hour to properly dissolve soluble salts. EC meter calibration was carried out with 0.01 M KCl solution before feeding the sample to the electrode. After that, an electric conductivity cell was inserted into the soil solution without disturbing the soil sample and readings were noted down (Sawarkar, 2012).

**Available Nitrogen Content:** Nitrogen contents of both plant and soil samples were estimated. The procedure was completed in three parts, i.e. digestion, distillation, and titration. In digestion -1 mg of soil sample was transferred to the digestion tube, 10 ml of concentrated sulphuric acid and 5 g of catalyst mixture was added, and digestion was initiated. The initial temperature was 100°C, and effective digestion started when the temperature reached 360°C. At the end of the digestion process, the colour changed to light green, and finally, a colourless solution was obtained. Further distillation was carried out after cooling the digestion material. One side hose should be kept in 20 ml of 4 % boric acid solution, and 40 ml NaOH was added to the distillation unit. Heat can pass with the stream, and ammonia gas is absorbed in boric acid. The colour of the sample was changed from pinkish to green colour. A blank sample was run simultaneously without the soil. In the third and final step, titration was carried out using 0.02N sulphuric acid. At the endpoint, the colour was changed from greenish to pinkish again. The volume of the sulphuric acid used for titration was noted down (Upadhyay & Sahu, 2012).

**Available Phosphorus:** Olsen's method is the most suitable to estimate the available Phosphorus in the soil sample. Take 1g of soil sample in the 150 ml conical flask and add a pinch of charcoal in the same flask. After this, add 0.5 N NaOHCO<sub>3</sub> into the same flask and shake it with the help of an electric shaker for half an hour. After that, filter

the content through Whatman's number 1 filter paper. From the filter content, Pipette out 5 ml content into 25 ml volumetric flask. Run blank solution side by side. Then, add 0.5 ml of 5N of H<sub>2</sub>SO<sub>4</sub> and shake it well for CO<sub>2</sub> evaluations to disappear. After that, add 4 ml of Ascorbic acid and distilled water to the mark on the volumetric flask. Then, mix the content and measure the intensity of the blue colour in a colourimeter at 760 µm wavelength and note down the readings (Thakur, 2012).

**Available Potassium:** A Flame photometer was used to determine the exchangeable potassium in the experimental soil. Take 5g of soil sample and add to 50 ml conical flask. Then, add 25ml of ammonium acetate solution in the same conical flask and shake well for five minutes. Filter the solution through Whatman's number 1 filter paper. Pipette out 5ml filter content and transfer it to a 25 ml volumetric flask. Make a standard potassium (k) solution with different ppm (0, 2, 4, 6, 8, 10 ppm). Feed the prepared solution in a flame photometer and note the reading (Baghel, 2012).

**Table 3.1: Soil data of Mustard during Rabi 2021-2023& 2022-23**

Sr No.	Particulars	Initial reading	Initial reading
	1 <sup>st</sup> trail	1 <sup>st</sup> trail	2 <sup>nd</sup> trail
<b>Chemical Properties</b>			
1	pH	7.2	6.9
2	E.C (dS/m)	0.14	0.16
<b>Nutrient Availability</b>			
1	Available N	185.6 kg/ha	201.3 kg/ha
2	Available P	10.97 kg/ha	13.01 kg/ha
3	Available K	62.68 kg/ha	66.14 kg/ha

### 3.3 Experimental Site and Details of the Experiment

**3.3.1 Experimental Site:** The field experiment was conducted at the Agronomy Research Farm, Department of Agronomy, Lovely Professional University fields, during the *Rabi* season of 2021-2022 and 2022-2023. Experimental plots are fertile with uniform topography and even textural makeup, and it is attached to the main irrigation channel, which connects to the tube well for fast, appropriate, and timely irrigation. It also has a good drainage facility for removing additional water during experimental time.

**3.3.2 Details of the Experiment:** The field experiment was conducted during Rabi 2021-2022 and 2022-2023 by Randomized Block Design (RBD) with three replications and twelve treatments. Experimental details are given below: Title: Evaluation of Sulphur and Salicylic Acid on Growth, Physiology, Yield and Molecular Expression of Indian Mustard, Experimental Design: Randomized block design (RBD), Replications- 3, Treatment- 12

#### 3.3.3: TREATMENT DETAILS

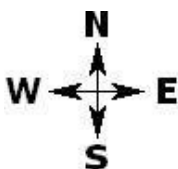
**Table 3.2: Details of Treatments**

Treatments (T0)	Control (RDF)
Treatment (T1)	Thiourea (TU) Recommended dose (1000 ppm)
Treatment (T2)	Salicylic Acid (SA) Recommended dose (300 ppm)
Treatment (T3)	Thiourea (TU) (1000 ppm) + Salicylic acid (SA) (300 ppm)
Treatment (T4)	Thiourea (TU) (1500 ppm) + Salicylic acid (SA) (300 ppm)
Treatment (T5)	Thiourea (TU) (1000 ppm) + Salicylic acid (SA) (450 ppm)
Treatment (T6)	Thiourea (TU) (500 ppm) + Salicylic acid (SA) (300 ppm)
Treatment (T7)	Thiourea (TU) (1000 ppm) + Salicylic acid (SA) (150 ppm)

Treatment (T8)	Thiourea (TU) (500 ppm) + Salicylic acid (SA) (600 ppm)
Treatment (T9)	Thiourea (TU) (2000 ppm) + Salicylic acid (SA) (150 ppm)
Treatment (T10)	Thiourea (TU) (2000 ppm) + Salicylic acid (SA) (600 ppm)
Treatment (T11)	Thiourea (TU) (500 ppm) + Salicylic acid (SA) (150 ppm)

where “T” stands for Treatment, “TU” is Thiourea, “ppm” is parts per million, “SA” is salicylic acid

### 3.3.4: LAYOUT: -



R1		R2		R3
T0	<b>Irrigation channel</b>	T11	<b>Irrigation channel</b>	T8
T1		T4		T10
T2		T9		T7
T3		T5		T11
T4		T10		T6
T5		T2		T0
T6		T8		T9
T7		T3		T2
T8		T7		T5
T9		T0		T3
T10		T6		T1
T11		T1		T4



### 3.3.5: Details of Layout:

**Table 3.3: Details of Layout**

Sl. No	Particulars	Remark
1	Design	Randomized Block Design
2	No. of treatments	12
3	No. of replications	3
4	Total no. of plots	12x3= 36
5	Field size	600m <sup>2</sup>
6	Net plot size	5x3m <sup>2</sup>
7	Sowing method	Line sowing
8	Replication border	1.0m
9	Plot border	0.5m
10	Row-to-row spacing	30cm
11	Plant-to-plant spacing	10cm
12	No. of rows per plot	10
13	No. of plants per row	30
14	Variety	RH725
15	Treatment application time	15, 45 and 75DAS
16	Method of application	Foliar spray
17	Date of first sowing	10/10/2021
18	Date of first harvest	27/03/2022
19	Date of second sowing	15/10/2022
20	Date of second harvest	07/04/2023

### 3.3.5: Cultivation Details:

**Table-3.4.: Cultivation Details**

<b>S. No</b>	<b>Operations</b>	<b>Date</b>
1	Preparatory tillage	
	(a) Ploughing with tractor-drawn disc plough	01-10-2021
	(b) Followed by disc harrow and rotavator	01-10-2021
	(c) Planking	05-10-2021
2	Layout	05-10-2021
3	Fertilizer Application	10-10-2021
4	Sowing	10-10-2021
	Thinning	26-11-2021
	(a) 1 <sup>st</sup> Top dressing	08-12-2021
	(b) 2 <sup>nd</sup> Top dressing	20-01-2022
5	Intercultural operations	10-01-2022
6	Irrigation	
	(a) First	26-11-2021
	(b) Second	22-01-2022
7	Treatment Spray (First Trail): (a) 15DAS (b) 45DAS (c) 75DAS	25-11-2021 27-12-2021 25-01-2022

8	Treatment Spray (Second trail): (a) 15DAS (b) 45DAS (c) 75DAS	30-11-2022 31-12-2022 29-01-2023
9	Observation Taken (First Trail): (a) 30DAS (b) 60DAS (c) 90DAS	11-12-2021 12-01-2022 11-02-2022
10	Observation Taken (second Trail): (a) 30DAS (b) 60DAS	17-11-2022 15-12-2022
	(c) 90DAS	15-01-2023
11	Harvesting (1 <sup>st</sup> trail) Harvesting (2 <sup>nd</sup> trail)	27-03-2022 07-04-2023

### 3.1 Weather Details of 2021-2022 and 2022-23

**Table 3.5. Weather Details of 2021-2022**

Nov-21			Dec-21			Jan-22			Feb-22			Mar-22		
Temperature														
Wee k	Min .	Ma x.	Wee k	Min .	Ma x.	Wee k	Mi n.	Ma x.	Wee k	Min .	Ma x.	Wee k	Min .	Ma x.
1st	11.4 3	29.4 3	1st	10.1 4	24.0 0	1st	8.7 1	16.5 7	1st	6.43	20.8 6	1st	11.1 4	27.5 7
2nd	10.1 4	27.1 4	2nd	9.00	18.4 3	2nd	7.4 3	13.5 7	2nd	9.29	21.0 0	2nd	13.2 9	27.8 6
3rd	9.57	21.8 6	3rd	2.71	15.5 7	3rd	5.1 4	15.7 1	3rd	10.1 4	21.5 7	3rd	14.0 0	28.5 7
4th	9.29	21.7 1	4th	3.86	15.4 3	4th	4.4 3	18.2 9	4th	12.1 4	27.4 3	4th	14.0 0	28.5 7

**Table 3.6. Weather Details of 2022-2023**

Nov-22			Dec-22			Jan-23			Feb-23			Mar-23		
Temperature														
Wee k	Min .	Max .	Wee k	Mi n.	Max .	Wee k	Mi n.	Max .	Wee k	Min .	Max .	Wee k	Min .	Max .
1st	16.8 6	29.2 9	1st	7.7 1	24.1 4	1st	6.5 7	11.5 7	1st	7.57	23.0 0	1st	13.2 9	27.5 7
2nd	13.2 9	26.2 9	2nd	8.7 1	24.2 9	2nd	6.8 6	16.0 0	2nd	7.86	22.2 9	2nd	15.0 0	30.0 0
3rd	9.71	25.1 4	3rd	6.5 7	20.5 7	3rd	4.2 9	18.1 4	3rd	11.7 1	26.4 3	3rd	15.2 9	25.8 6
4th	7.86	25.4 3	4th	5.5 7	13.0 0	4th	4.8 6	19.0 0	4th	11.2 9	27.1 4	4th	14.1 4	26.2 9

**3.5: Cultivation details:** The cultivation practices which are followed during the experiment are detailed below.

**3.5.1: Selection and preparation of field:** The experiment will take place on a plot explicitly chosen for its level topography and consistent fertility. The land was prepared by performing two rounds of deep ploughing, then two rounds of cross harrowing using a tractor-drawn disc harrow, and finally performing a levelling operation before being subdivided into plots according to the layout and requirements.

**3.5.2: Layout preparation:** Labourers prepare the experimental layout by the requirements and the plan with a disc plough, which is used to divide the plots uniformly.

**3.5.3: Planting material:** Indian Mustard variety “RH-725” was selected for conducting this research. It was obtained from an authorized certified seed producer, ‘Good Grow’, Phagwara, Punjab. Healthy and undamaged seeds are used for sowing.

**3.5.4: Sowing:** Seeds were sown in 4-5 cm deep lines with a 30x10 cm planting distance.

**3.5.5: Description of variety: RH-725:** Plants produced are tall, erect, and compact with green foliage. It matures within 130-150 days and yields around 6.74q/acre. Seeds are round, bold, brown-coloured, and uniform. This variety is resistant to various soil and seed-borne diseases. Oil content is 39-40%

**3.5.6: Application of fertilizers:** Uniform quantities of nitrogen and phosphorus are applied in plots other than the control plots through urea (46%N) and Single Super Phosphate (16% P<sub>2</sub>O<sub>5</sub>), (12% S). The rate of fertilizer used 80 kg N, 40 kg P and 40 kg K per hectare. Half dose of nitrogen, even dose of phosphorus, and potassium doses were applied as basal doses at the time of sowing of seeds in lines. A remaining half dose of nitrogen was applied at 30 DAS (25%) and the remaining 25% after irrigation.

**3.5.7: Treatment application:** Treatments were applied as a foliar spray by knap-sack sprayer at 30-day intervals. Treatments were applied at 15DAS, 45DAS, 75DAS of the sowing, and the observations were taken at 30DAS, 60DAS, 90DAS, and 120DAS of the crop. At different intervals, sulphur, boron, and cytokinin were applied as a foliar spray to the crop canopy. The cytokinin used as described below:

### **3.6 : CULTURAL OPERATIONS AFTER SOWING:**

**3.6.1 : Weeding:** Manually, two to three weeding were done during the crop period. First-hand weeding is done at 30 DAS, and second is at 70 DAS. The third weeding is done at 120 DAS to clean the bunds.

**3.6.2 : Irrigation:** Mustard crop only requires a little irrigation. Two irrigations were provided during the whole lifespan of the crop. The initial irrigation was given after the first weeding. The flood irrigation method is used to irrigate each plot. 2<sup>nd</sup> irrigation was given to each plot at the flowering stage so that effective pod formation could occur.

**3.6.3 : Plant protection measures:** Immaculate care for plant protection was taken during the crop's lifetime. During the crop season, some severe insect pests and diseases were observed. Proper care at different intervals was done to the mustard crop when insect pest was seen in the experimental field.

**3.6.4 : Harvesting:** The crop is harvested when pods mature and its foliage is completely dry. Harvesting is done after 140 DAS.

**3.6.5 : Sampling technique:** For data sampling, three plants were selected randomly in every plot and then tagged for easy identification for taking the intervallic observations. The treatments were applied at 15, 45, 75 DAS. The first observation was noted at 30 DAS, and afterwards, observations were taken at 30 days, i.e., 60, 90, and 120 DAS. The average value of recorded data was calculated and the final observation was statistically analysed using SPSS software.

### **3.7: OBSERVATIONS RECORDED:**

#### **A. Pre-harvest studies:**

#### **3.7.1 : MORPHOLOGICAL PARAMETERS:**

**3.7.1.2 : Plant height (cm):** Initially, three plants were selected randomly in every plot and then tagged for taking the observations. The main shoot length from the ground level up to the growing point tip was measured with the help of a meter scale at 30, 60, 90, and 120DAS and at the time of harvest.

**3.7.1.3 : Number of leaves per plant:** Initially, three plants were selected randomly in every plot and then tagged for taking the observations. Then, the number of leaves per plant was counted at 30, 60, 90, and 120DAS and at harvest time.

**3.7.1.4 : Stem girth (cm):** The stem girth was recorded from the base of the plant to the tip of the stem at 30, 60, 90, and 120 DAS intervals using the instrument Vernier

calliper. The mean stem girth of the tagged plant of each replicate was calculated and expressed in the cm.

**3.7.1.5 : Leaf area (cm<sup>2</sup>):** The area covered by a particular plant's leaf in its canopy was calculated in the laboratory after collection of the plant's leaf at 30, 60, 90, and 120 DAS with the help of a leaf area meter.

**3.7.1.6 : Fresh weight of plant (g):** The fresh weight of the plant was taken after the removal of three plants with the help of sickle in each plot at 30, 60, 90, 120DAS and at the time of harvest and after that immediately weighed in grams.

**3.7.1.7 : Dry weight of plant (g):** Dry weight is taken after fresh weight by air drying in the sun for 3-4 days before it is placed in a hot air oven at 50<sup>0</sup>C for 36 hours for moisture removal. The average of three plants was taken for average dry matter accumulation at different stages of the crop. After drying in a Hot air oven, then weighed, and observations were taken.

### **3.7.2 PHYSIOLOGICAL PARAMETERS:**

**3.7.2.3: Leaf Area Index:** Williams (1946) proposed the term Leaf Area Index (LAI). It is the ratio of the crop leaf to the ground area throughout an interval of time. The value of LAI should be optimum at the maximum ground cover area at which the crop canopy receives maximum solar radiation. Leaf area was measured by leaf area meter.

$$\text{LAI} = \frac{\text{Total Leaf Area of a plant}}{\text{Ground area occupied by the plant}}$$

**3.7.2.3 : Net Assimilation Rate:** Williams (1946) used the term NAR term. NAR is defined as dry matter increment per unit leaf area or per unit leaf dry weight per unit of time. The NAR measures the average photosynthetic efficiency of leaves in a crop community.

$$\text{NAR} = \frac{(W_2 - W_1)}{(t_2 - t_1)} \times \frac{(\log_e L_2 - \log_e L_1)}{(L_2 - L_1)}$$

Where  $W_1$  and  $W_2$  are the dry weight of the whole plant at time  $t_1$  and  $t_2$ , respectively,  $L_1$  and  $L_2$  are leaf weights of leaf area at  $t_1$  and  $t_2$ , NAR is expressed as the grams of dry weight increase per unit dry weight or area per unit time ( $\text{g g}^{-1}\text{day}^{-1}$ ).

**3.7.2.4 : Relative Growth Rate:** The term was coined by Williams (1946). Relative Growth Rate (RGR) expresses the total plant dry weight increase in a time interval about the initial weight or Dry matter increment per unit biomass per unit time or grams of dry weight increase per gram of dry weight and expressed as unit dry weight/unit dry weight/unit time ( $\text{g g}^{-1}\text{day}^{-1}$ ).

$$\text{RGR} = (\log_e W_2 - \log_e W_1) / (t_2 - t_1)$$

Where,  $W_1$  and  $W_2$  are whole plant dry weight at  $t_1$  and  $t_2$  respectively  $t_1$  and  $t_2$  are time interval in days

**3.7.2.5 : Crop Growth Rate:** The method was suggested by Watson (1956). The CGR explains the dry matter accumulated per unit land area per unit time ( $\text{g m}^{-2} \text{day}^{-1}$ ).

$$\text{CGR} = ((W_2 - W_1) / (t_2 - t_1))$$

Where  $W_2$  &  $W_1$  are plant dry weight at time  $T_2$  &  $T_1$ .

### **3.7.3 : BIOCHEMICAL PARAMETERS:**

**3.7.3.1 : Chlorophyll Content ( $\text{mg g}^{-1}$  Fresh Weight):** Arnon DI's method estimated chlorophyll content in the Mustard crop leaf (1949). Chlorophyll was extracted in 80% acetone, and the absorbance was measured at 645nm and 663nm. The amount of chlorophyll is calculated using the absorbance coefficient.

**Reagent:** Acetone (80%, pre-chilled).

**Instrument used:** Visible Spectrophotometer.



**Procedure:** Chlorophyll was extracted from a 100mg leaf sample using 20 ml of 80% acetone. The supernatant was transferred to a volumetric flask after centrifugation at 5000 rpm for 10 minutes. The extraction was repeated until the residue became colourless. The volume in the flask was made up to 100ml with 80% acetone. The absorbance of the extract was read in a spectrophotometer at 645nm and 663nm against 80% acetone blank. The amount of chlorophyll content was calculated by using the formula given.

Chlorophyll 'a' (mg/g Fresh Weight) =  $12.7(A_{663}) - 2.69(A_{645}) \times V1000 \times W$ ,  
Chlorophyll 'b' (mg/g Fresh Weight) =  $22.9(A_{645}) - 4.68(A_{663}) \times V1000 \times W$ , Total  
chlorophyll (mg/g Fresh Weight) =  $20.2(A_{645}) + 8.02(A_{663}) \times V1000 \times W$

Where V= Final volume of the extract, W= Fresh weight of the leaves, A= absorbance at the specific wavelength. The value is expressed as the mg/g fresh weight (FW)

**3.7.3.2 : MSI and MII:** The MSI was calculated using the formula described by Premachandran et al. (1990). Membrane damage can be evaluated indirectly by measuring solute leakage (electrolyte leakage) from cells and the MSI. The stimulation effect of stress on Electro Leyte leakage might be attributed to the injury of the plasma membrane.

**Reagent:** Double Distilled Water.

**Procedure:** Leaves were taken from the youngest fully-grown leaf. The membrane stability index (MSI) and Membrane Injury Index were estimated by placing 200 mg of leaves in 10 ml double-distilled water in two sets. One set was heated at 40°C for 30 minutes in a water bath and the electrical conductivity (C1) was measured. The second set was boiled at 100 °C in a boiling water bath for 10 min and the conductivity (C2) was measured; both conductivities were measured using a conductivity meter (ME977-C, Max Electronics, India). The MSI and MII were calculated using the formula described below:

$$MII = \frac{EC1}{EC2} \times 100$$

$$MSI = 100 - MII$$

**3.7.3.3 : Total Soluble Sugar:** The total soluble sugar content in the plant sample was estimated following the method proposed by Sadasuvam and Manickam (1992). The Anthrone reaction is the basis of a rapid and convenient method for determining total

soluble sugar in the plant sample. Carbohydrates are dehydrated by conc.  $\text{H}_2\text{SO}_4$  to form furfural. Furfural condenses with Anthrone to form a blue-green-coloured complex measured calorimetrically at 630 nm.

**Reagents:**

- Ethanol (80%)
- Anthrone reagent: Dissolve 200 mg anthrone in 100 ml of ice-cold 95% sulphuric acid. Prepare fresh before use.
- Standard glucose: Stock-dissolve 100mg of glucose in 100ml water, working standard-10 ml of the stock diluted to 100ml with distilled water.

**Procedure:** 100 mg of leaf sample was homogenised with 10 ml of ethanol till all the leaf tissues were fully digested. Then, the extract of the sample was centrifuged at 5000 rpm for 15 min. The volume of the extract was 100 ml, adding distilled water. One ml of the extract was taken in a test tube and 6 ml of the anthrone reagent were added to each test tube. The tube was then placed in a boiling water bath for 10 min, after which they were allowed to cool in running water. A blank was prepared similarly but without a leaf sample. After some time, a blue colour developed in the test tubes, and the intensity of the blue colour was measured at 620 nm by a spectrophotometer. The standard curve calculated the amount of sugar in the leaf sample.

**Preparation of The Standard Curve for Estimation of Total Soluble Sugar:** 10 mg of the glucose was dissolved in 100 ml of distilled water, or a working standard was prepared by diluting 10 ml of standard glucose stock with 100 ml of distilled water. Different concentrations of the sugar solution were prepared from this stock solution by taking 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the stock solution in a separate test tube. The final volume of these test tubes was made to 3 ml by adding distilled water, and after that, 6 ml of the Anthrone reagent was added to each test tube. They were boiled in a water bath, as described above. The solution was cooled and the intensity of the blue colour was read at 620 nm. The standard curve was prepared by plotting the absorbance value on the y-axis against the sugar concentration in the solution on the x-axis.

**3.7.3.4 : Total Soluble Protein:** The total soluble protein content in the plant sample was estimated following the method proposed by Bradford (1976) method. The assay is based on the observation that the absorbance maximum for an acidic Coomassie Brilliant Blue G-250 solution shifts from 465 nm to 595 nm when binding to protein

occurs. Both hydrophobic and ionic interaction stabilises the anionic form of the dye, causing a visible colour change. The assay is helpful since the extinction coefficient of a dye-albumin complex solution is constant over a 10-fold concentration range.

**Reagents:**

- Sodium phosphate buffer (pH 7.4)
- **Solution A:** To prepare the Sodium phosphate buffer, 13.9 g of 0.1 M sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ ) was dissolved in distilled water, and the volume was up to 1000 ml.
- **Solution B:** To prepare the Sodium phosphate buffer, 26.82 g of 0.1 M disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ) was dissolved in distilled water, and the volume was up to 1000 ml.
- Solution A and solution B were mixed in a ratio of 19:81, and the final pH (7.4) was adjusted with the help of a pH meter.
- **Dye concentration:** Dissolve 100mg of Coomassie brilliant blue G 250 in 50 ml of 95% ethanol. Add 100 ml of concentrated orthophosphoric acid. Add distilled water to a final volume of 200 ml. Store in an amber bottle in the refrigerator, the solution is stable for at least six months. Mixed concentrated dye solution with distilled water at the ratio of 1:4. Filter with Whatman No. 1 paper if any precipitate occurs.

**Procedure:** 100 mg of plant sample was taken and transferred into a mortar. The 10 ml of cold extraction was added. The mortar was put into the ice bucket and cursed with a pestle's help till a fine slurry was made. The homogenates were centrifuged at 15,000 rpm for 15 minutes. The supernatant was collected and used as crude protein extract. Took 5ml diluted dye, 0.2 ml of leaf crude protein extract, and 0.8 ml of distilled water; mix well and allow the colour to develop for at least five minutes but not longer than 30 minutes. The red dye turns blue when it binds to proteins, Read the absorbance at 595 nm in the spectrophotometer.

**Preparation of the standard curve for estimating total soluble protein:** The standard curve was prepared using 0.1-1.0 ml BSA (Bovine Serum Albumin). The standard curve was prepared by plotting the absorbance value on the y-axis against the sugar concentration in the solution on the x-axis. The amount of total soluble protein expressed in mg/g of sample.

**3.7.3.5 : Estimation of total phenol:** The amount of total phenol was measured according to the protocol given by Mahadevan and Sridhar (1982). Phenol reacts with an oxidizing agent phosphomolybdate in Folin-Ciocalteu reagent under alkaline conditions and results in the formation of a blue-coloured complex, the molybdenum blue, which is measured at 650nm with the help of a spectrophotometer.

**Reagents:**

- 80% Ethanol
- Folin-Ciocalteu reagent (FCR)
- 20% Na<sub>2</sub>CO<sub>3</sub>
- Stock Standard (100mg catechol in 100ml of water). Working standard is prepared by dilution of Stock Standard 10 times.

**Procedure:** Five hundred mg leaf samples were crushed in 3 ml 80% ethanol and centrifuged at 10,000 rpm for 20 min. Residue and supernatant were separated. The supernatant was saved. The residue was rewashed with 2 ml of 80% ethanol, and the supernatant was saved. Finally, the supernatants were mixed, and the final volume was 5 ml with 80% ethanol. 1 ml of the above supernatant (extract) was taken, and to it, 1 ml of Folin Ciocalteu reagent and 2 ml of sodium carbonate were added. The mixture was heated for 1 minute, and absorbance was recorded at 650 nm. A calibration curve of the known dilution of pyrocatechol was made following the same procedure as that of the sample, and the number of phenols in the sample was expressed as mg g<sup>-1</sup> fresh weight (FW).

**Preparation of the standard curve for estimation of total phenol:** Catechol (100 mg) was taken and dissolved in 100 ml distilled water, and then the stock solution was diluted to 1:10. The 0.2, 0.4, 0.6, 0.8-, and 1.0-ml aliquots were taken into different test tubes, and the volume was raised to 1ml by adding distilled water. The pink colour was developed the same way as for the sample, and absorbance was determined with the help of a spectrophotometer.

**3.7.3.9 : Estimation of l-phenylalanine ammonia lyase (pal) activity:** The activity of the L-Phenylalanine Ammonia Lyase (PAL) enzyme was measured according to the protocol given by Subba Rao *et al.* (1970). The enzyme may be assayed by measuring the appearance of trans-cinnamic acid from phenylalanine spectrophotometrically at 650 nm.

**Reagents:**

- 0.2M Sodium Borate Buffer, pH 8.7
- 0.01M L-Phenylalanine (pH 8.7): Prepared in sodium borate buffer
- 0.05M Tris-HCl buffer, pH 8.8
- 1N HCl
- Peroxide-free ether
- 0.05N NaOH: prepared fresh from 1N NaOH
- Standard Cinnamic Acid: Prepared in borate buffer
- Mercaptoethanol (0.8ml/litre)

**Procedure:** 3.0 grams of fresh leaf sample was ground in 2.6 ml sodium borate buffer containing 2-mercaptoethanol (0.8ml/litre). This was centrifuged at 7000g for 10 min at 2-4°C. The supernatant was saved. The pH of the supernatant was adjusted to 5.5 with the help of acetic acid (1M). Incubate 1 ml of 0.05M Tris-HCl buffer, 0.5 ml of 0.01M L-phenylalanine, and 0.4 ml of water at 30°C for 5 min. The reaction was initiated by adding 0.1 ml of the enzyme, which was incubated for 60 min at 30°C. A blank sample was prepared without phenylalanine. The reaction was stopped by the addition of 0.5 ml of 1N HCl. The mixture was extracted with the help of 3.5 ml of ether twice. The ether phase was removed, and the residue pooled and dried under air. The residue was dissolved in 3 ml of 0.05N NaOH. This was kept at room temperature overnight. The mixture was centrifuged at 2000g for 15 min, and the supernatant was saved. 1 ml of the above supernatant (extract) was taken, and 1 ml of Folin Ciocalteu reagent was added. The mixture was heated for 1 min, and absorbance was recorded at 650 nm. A calibration curve of known dilution of cinnamic acid was made following the same procedure as that for the sample. The amount of PAL in the sample was expressed as  $\mu$ moles of cinnamic acid produced /min/mg protein.

**Preparation of the standard curve for estimation of pal activity:** Cinnamic acid (100 mg) was taken and dissolved in 100 ml borate buffer, and then the stock solution was diluted to 1:10. The 0.2, 0.4, 0.6, 0.8, and 1.0 ml of working standard solution were taken into different test tubes, and the volume was raised to 1ml by adding borate buffer. Absorbance was determined with the help of a spectrophotometer at 650 nm.

**3.7.3.10 : Estimation of free proline:** Free proline content in the leaves was determined by the following method of Bates *et al.* (1973). Proteins are precipitated as a protein-

sulphosalicylic acid complex during tissue extraction with sulphosalicylic acid. The extracted proline reacts with ninhydrin under acidic conditions to form a red colour.

**Reagents:**

- Acidic ninhydrin reagent. Dissolved 1.25 g of ninhydrin in a mixture of warm 30 ml of glacial acetic acid and 20 ml of 6M phosphoric acid (pH 1.0) with agitation until it is dissolved. Stored at 4°C and used within 24 hours.
- 3% Aqueous sulphosalicylic acid
- Glacial Acetic Acid
- Toluene
- Standard proline solution.

**Procedure:** A leaf sample of 100 mg was homogenised in 10 ml of sulphosalicylic acid (3%) using mortar and pestle. It was centrifuged at 6000 rpm for 10 min, and the supernatant was collected. The 2.0 ml of the extract was taken in the test tube with 2 ml each of glacial acetic acid and ninhydrin reagent. The reaction mixture was boiled in a water bath at 100°C for 30 min till brick red colour developed. After cooling the reaction mixture, 5 ml of toluene was added and transferred to a separating funnel. The absorbance was read at 520 nm using a spectrophotometer against toluene as blank.

**Preparation of the standard curve for estimating proline:** Proline (10 mg) was dissolved in 3% aqueous sulphosalicylic acid and then diluted to 100 ml. The 0.2, 0.4, 0.6, 0.8-, and 1.0-ml aliquots were taken into different test tubes, and the volume was raised to 2 ml by adding 3% aqueous sulphosalicylic acid solution. Colour was developed the same way as for the sample, and absorbance was determined with the help of a spectrophotometer.

**3.7.3.11 : Estimation of total flavanol content:** The flavanol content was estimated by the protocol given by Akkol *et al.*, 2008. The basic principle of the Aluminium chloride colourimetry method is that Aluminium chloride forms acid-stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of Flavonols and flavonoids.

**Reagents:** Methanol 80%, Sodium Acetate, Aluminium Chloride.

**Procedure:** To estimate Flavonols, a 0.05g plant sample was extracted by boiling 80% methanol for 3 hours. One ml of methanol extract, 3 ml of sodium acetate and 1 ml of aluminium chloride solution were mixed, and the absorbance was recorded at 445nm after 2.5hrs.

**3.10.12 Total flavonoid content:** Formation of acid-stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavanols in addition to aluminium chloride. Aluminium chloride also forms acid labile complexes with the ortho-dihydroxyl groups in flavonoids' A- or B-ring. For building the calibration curve, quercetin is used as a standard material. Various concentrations of standard quercetin solution were used for a standard calibration curve.

**Reagents:** quercetin, methanol, aluminium chloride, potassium acetate.

**Procedure:** The aluminium chloride colourimetric method was used to determine the total flavonoid content of the sample. Quercetin was used to make the standard calibration curve for total flavonoid determination. Stock quercetin solution was prepared by dissolving 5.0 mg quercetin in 1.0 mL methanol, and then the standard solutions of quercetin were prepared by serial dilutions using methanol (5–200  $\mu\text{g/mL}$ ). An amount of 0.6 mL diluted standard quercetin solutions or extracts were separately mixed with 0.6 mL of 2% aluminium chloride. After mixing, the solution was incubated for 60 min at room temperature. The absorbance of the reaction mixtures was measured against a blank at 420 nm wavelength with a Varian UV-Vis spectrophotometer (Cary 50 Bio UV-Vis Spectrophotometer, Varian). The concentration of total flavonoid content in the test samples was calculated from the calibration plot and expressed as mg quercetin equivalent (QE)/g of dried plant material. All the determinations were carried out in triplicate.

**3.7.3.13 : Relative Water Content (RWC):** Relative water content (RWC) is the most appropriate measure of plant water status regarding the physiological consequence of cellular water deficit. Water potential as an estimate of the energy status of plant water helps deal with water transport in the soil-plant-atmosphere continuum.

**Reagents:** Distilled water.

**Procedure:** Fresh samples of leaves were collected from each cultivar to determine the relative water content (RWC). 500mg of plant leaves were taken in a 500ml beaker containing 500ml of distilled water. Dip the samples properly in water. Leave it for 30 minutes. Place the samples in the boiling paper, dry them and measure the weight. The measured weight is turgid. Then, dry it and measure the dry weight.

**RWC (%) = Fresh Weight-Dry weight/Turgid Weight-Dry weight $\times$ 100**

**3.7.3.14 : Chl. Index (SPAD Unit):** The chlorophyll index was estimated by using a SPAD meter.

**3.7.4.15. Estimation of total free amino acid:** The plant sample's total free amino acid content was estimated following the method proposed by Moore and Stein (1948). Ninhydrin (triketohydrindene hydrate), a powerful oxidizing agent, reacts with  $\alpha$ -amino acids between pH 4 and 8 and decarboxylates to give an intensity bluish-purple-coloured compound.

Reagents

1. 80 % Ethanol
2. 0.2M Citrate buffer, pH 5.0
3. Ninhydrin reagent: Dissolved 0.8g of stannous chloride in 500 ml of 0.2M citrate buffer, pH 5.0 and added this solution to 20 g ninhydrin in 500 ml of methyl cellosolve (2-methoxyethanol).
4. Diluent solvent: Mixed equal volume of water and n-propanol
5. Stock standard leucine solution: Dissolved 50 mg of leucine in 50 ml water.
6. Working standard leucine solution: Diluted 10 ml of stock leucine solution to 100 ml with water.

**Procedure:** A leaf sample of 500 mg was homogenised in a pestle and mortar with a small quantity of acid-washed sand. 5-10 ml of 80% ethanol was added. It was centrifuged at 5000 rpm. Repeated the extraction twice, and all the supernatants were extracted. The 0.1 ml of extract was taken in the test tube, and 1 ml of ninhydrin reagent was added and mixed thoroughly. The final volume was made up of 2 ml of distilled water. The reaction mixture was boiled in a water bath for 20 min. The 5 ml of water and n-propanol were added while still in the bath. After 15 min of cooling, read the absorbance of the purple colour against the blank at 570 nm. The calculation of total free amino acids was derived from the standard curve prepared from leucine.

**3.7.4.16. Estimation of Total Lipid:** The total lipid content in the leaves was determined by the following method by Jayaraman (1981). The tissue is extracted in a 3:1 ether and ethanol mixture followed by centrifugation and separated by adding KCl solution, which helps in layer separation, and salt prevents emulsification. The lipid layer is then dried and weighed, and the amount of the lipid is calculated.



## Reagents

1. Ethyl Ether: Ethanol (3:1, v/v)
2. 0.05M KCl solution

**Procedure:** 1.0 g of fresh sample was homogenised with 10 ml of the solvent mixture, i.e., ethyl and ethanol. This was centrifuged at 2000g for 10 min, and then the supernatant was transferred into a separatory funnel. The 2 ml of 0.05 M KCl solution was added to the extract and shaken well. Two layers were separated. One layer was of lipid, and the other was of water. Both layers were decanted carefully. The lipid layer was then dried and weighed. Based on weight, the amount of lipids was calculated.

## B. Post-harvest studies:

### 3.7.4 : OIL QUALITY PARAMETERS:

**3.7.4.1 : Peroxide value (POV):** The Peroxide values were predicted using the ISO 3960-2007 standard. 5g of the oil sample was dissolved in glacial acetic acid: chloroform (3:2, v/v, 30 ml) mixture, followed by 1ml of saturated potassium iodide solution. Further on, the desired amount of distilled water was added and then titrated gradually against sodium thiosulphate solution (0.01ml), where the starch solution (1%<sub>s</sub>) was used as an indicator (Sharma et al., 2006).

$$\text{Formula: } \text{POV (meq per1000g)} = \frac{(V_S - V_b) \times F \times N \times 1000}{W} = \frac{(V_S - V_b) \times F \times 1 \times 1000}{W \times 100}$$
$$= \frac{(V_S - V_b) \times F \times 10}{W}$$

Where,  $V_s$  = Titration volume of sample (ml),  $V_b$  = Titration volume of blank (ml),  $F$  = Factor of 0.01N  $\text{Na}_2\text{S}_2\text{O}_3$  solution,  $W$  = Weight of oil,  $N$  = Normality of  $\text{Na}_2\text{S}_2\text{O}_3$  solution

**3.7.4.2 : p-Anisidine value (p-AV):** The ISO 6885:2006 standards were followed to predict Anisidine values. In acidic conditions, the resultant sample has undergone a reaction with p-anisidine, and the values have been collected (WHO, 1983).

### Procedure:

1. Take 0.3g oil sample in 10ml flask.
2. Dissolve it in iso-octane in 10ml flask.
3. Measure O.D. of 2.5ml sample at 350nm against blank (O.D. of iso-octane).
4. 0.5ml of p-anisidine reagent added to cuvette.
5. Place in incubator for 10 min.
6. Measure its O.D. at 350nm.

**Formula:** 
$$\frac{10\text{ml} \times (1.2 \times (\text{AS}_2 - \text{AB}_2) - (\text{AS}_1 - \text{AB}_1))}{W \text{ sample}}$$

**3.7.4.3 : Totox value (TV):** The Totox value of oil is measured using the formula, (de Abreu et al., 2010),

**Formula:**  $Tv = (2 * Pv) + pAv$ , where Pv = Peroxide value, p-Av = para Anisidine value.

**3.7.4.4 : Density:** The density of the various oil mixtures has been calculated using a Relative Density (R. D) bottle with a capacity of 10 mL (Zahir *et al.*, 2017).

**3.7.4.5 : Viscosity:** Using Cannon-Fenske (Fisher Scientific, Pitts-burgh, PA) glass capillary kinematic viscometers in a steady-temperature bath. A programmable water bath (Model F25-HE, Julabo USA Inc. Allentown, PA) was utilized to confirm the exact and concurrent results. The trials have been made with ASTM D445 for viscosity determination. The difference in viscosity as a function of temperature was calculated using this formula (Noureddini et al., 1992),  $V = c \times t$ , where c = Viscometer Constant ( $\text{mm}^2/\text{s}^2$ ), t = Time,  $\mu = v \times p$ , where, v = Viscosity in  $\text{mm}^2/\text{s}^2$ , p = Density of the oil.

**3.7.4.6 : Saponification value:** The known amount of oil sample is mixed with 10 mL 1 N KOH. In addition, 10 mL of de-ionized water is added and the resultant combination is heated below the reserved condenser for 30–40 min and chilled. Titrated against 0.5 M of HCl, using an indicator to get the pale pink colour. Same conditions were followed for the blank (Firestone, 2007).

**Calculation:** Saponification Value =  $\frac{56.1 (B-S)N}{W}$ , Where B = Volume in ml of standard hydrochloric acid required for the blank, S = Volume in ml of standard hydrochloric acid required for the sample, N = Normality of the standard hydrochloric acid and, W = Weight in gm of the oil/fat taken for the test.

**3.7.4.7 : Iodine value:** About 5g of the sample were treated with an excess of Iodobromine (IBr) in glacial acetic acid. The reaction between Iodobromine and Potassium iodide gives the amount of iodine from the given sample. The determination of iodine was calculated using the formula, (Crowe and White, 2001).

**Reagents:**

- Iodine monochloride Reagent
- Potassium Iodide
- 0.1N Sod. thiosulphate

**Procedure:** 1. Take 10ml of oil sample., 2. Add 20ml Iodine monochloride reagent in flask and mix it., 3. Incubate for ½ an hr. in the dark. 4. Make a blank by adding 10ml chloroform to the flask. 5. Add 10ml of potassium iodide solution. 6. Rinse the sides of the flask using 10ml distilled water.

7. Titrate against sod. Thiosulphate solution until pale straw colour. 8. Add 1 ml starch indicator (purple colour observed). 9. Titrate until the solution turns colourless. 10. Follow the same for blank and observe colour.

**Calculation:** Vol. of Sod. Thiosulphate used= (Blank-Test) ml.

$$\text{Formula: IV} = \frac{(B - S) \times N \times 126.9}{w}$$

126.9 is the molecular weight of iodine

B = quantity of sodium thiosulphate used for blank, S = quantity of thiosulphate for sample,

N = normality of thiosulphate solution, w = weight of the oil sample

**3.7.4.8 : Acid value:** The acid value of the cooking oil is measured using the titration method by AOCS [Cd 38-63] (Alimentarius, 1999).

**Reagents:**

1. **Phenolphthalein Indicator:** Add 1g phenolphthalein in 100ml ethanol
2. **Sodium hydroxide titrant:** Add 4g of sod. Hydroxide in 1000ml distilled water.
3. **Ethanol ether sol.:** Prepare mixture of ethanol and diethyl ether (1:1 v/v). Neutralize with sod. Hydroxide titrant and add 1ml phenolphthalein indicator until pink colour observed.

**Procedure:**

1. Take a known amount of oil sample.
2. Place it in 250ml conical flask.
3. Add 50ml ethanol ether solution and shake well.
4. Titrate against sodium hydroxide titrant until solution turns pink for 30s.

$$\text{Formula: } \frac{\text{Titre value} \times 0.1 \times 56.1}{10}$$

**3.7.4.9 : Refractive index:** The refractive index was measured by a digital refractometer or a hand operator refractometer. The values were recorded and should be write on a notebook to keep it safe.

**Procedure:**

1. Take a drop of oil sample with the help of dropper.
2. Place it over stage of refractometer.
3. Reading will show on the digital box.

**3.7.4.10 : Oil content:** Oil content from the mustard seeds can be extracted using an oil expeller machine. It should be spotless before use. The seeds of mustard are inserted on the top (bowl) attached to the machine. The oil is extracted automatically from the tube attached to the bottom of the machine. The oil cake and oil come out separately from different openings. The oilcake comes out from the front of the tube inserted in the chamber of the oil expeller. The oil is collected in a beaker and poured into a measuring cylinder to measure the content of the oil. Similarly, the oil cake is collected in a bowl and then weighed using a weighing balance to check the weight of the extracted oilcake.

**3.7.5 : Oil cake parameters: -**

**3.7.5.1 : Oil cake wt. /100g seed:** The weight of the oil cake can be estimated by measuring the oil cake obtained from 100g mustard seeds during the oil extraction process. Weigh the oil cake using a weighing balance.

**3.7.5.3 : Nitrogen content:** Nitrogen content in mustard oil cake was determined by a modified method by Jackson (1967). For the determination of nitrogen content, the wet digestion method was used. Oven-dried samples (0.5g) from each treatment were subjected to wet digestion using H<sub>2</sub>SO<sub>4</sub>, potassium sulphate, copper sulphate, and selenium powder. Digested material was taken in a 100 ml volumetric flask, and the volume was made to 100 ml with distilled water. Five ml of distilled sample was taken in a distillation tube, 10 ml NaOH was poured into the tube, and then water was added. The flask containing 5 ml boric acid was kept under the condenser until the appearance of purple colour. Then, the distilled sample was titrated against conc. H<sub>2</sub>SO<sub>4</sub> until the appearance of pink colour. The final volume used was taken for final calculation.

$$\text{Nitrogen content (\%)} = \frac{R (\text{sample-blank}) \times \text{Normality of acid} \times \text{Atomic weight of N} \times 100}{\text{Weight of Sample (g)} \times 1000}$$

**3.7.5.4 : Protein:** For determination of protein content the following formula was used:

$$\text{Protein content (\%)} = \text{micro-Kjeldahl nitrogen content (\%)} \times 6.25$$

**3.7.6 : Yield Parameters: -**

**3.7.6.1 : No. of primary branches:** No. of primary branches were counted at 90DAS of the crop. Branches were counted manually and data was noted in a notebook.

**3.7.6.2 : No of secondary branches:** No. of secondary branches were counted at 120DAS of the crop. All the branches were counted manually and data was noted in a notebook.

**3.7.6.3 : No. of siliquae plant<sup>-1</sup>:** No. of siliquae plant<sup>-1</sup> were counted manually at 120DAS and harvest, and the data was recorded in a notebook.

**3.7.6.4 : Length of siliquae:** Siliqua length can be measured using a 15cm ruler scale, and the data was recorded in a notebook for further use.

**3.7.6.5 : No. of seeds siliqua<sup>-1</sup>:** No. of seeds siliqua<sup>-1</sup> were counted manually by opening the Siliqua (Pod) into 2 halves. Seeds were counted, and the data was recorded in a notebook for further use.

**3.7.6.6 : Seed Yield (/m<sup>2</sup>):** Plants from 1m<sup>2</sup> area were harvested and grains were separated after threshing. From seed yield per m<sup>2</sup> area, yield per plot can be determined. Later, plot yield was converted into quintal per hectare (q/ha).

**3.7.6.7 : Stover yield (/m<sup>2</sup>):** Plants after threshing were weighed, and stover yield per m<sup>2</sup> was calculated. Plants were harvested from the ground level; yield was calculated per plot.

**3.7.6.8 : Harvest index (%):** The harvest index was calculated as the ratio of economic yield to biological yield based on the following formulae.

It was given by Fisher in 1962.

$$\text{Harvest Index \%} = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

**3.8. Methodology for XRD detection:** X-ray diffraction (XRD) is a powerful technique used to analyse the crystallographic structure of materials, including the leaves of a mustard plant. You must follow a specific methodology to perform XRD detection on mustard plant leaves. Here is a detailed step-by-step procedure:

**Materials and Equipment:**

- Mustard plant leaves (fresh or freeze-dried)
- Mortar and pestle or a grinder
- Liquid nitrogen (if using fresh leaves)
- X-ray diffractometer
- Sample holder (e.g., glass slides or aluminium sample holders)
- X-ray source (typically Cu K $\alpha$  radiation at  $\lambda = 1.5406 \text{ \AA}$ )
- Detector (scintillation or semiconductor detector)
- Computer with data analysis software
- Safety equipment (lab coat, gloves, safety glasses)

**Procedure: Sample Preparation:** The dry leaves were cut into small pieces and frozen with liquid nitrogen. The leaves were ground into a fine powder using a mortar, pestle, or grinder. The sample was ensured to be as homogeneous as possible, and it was stored in a sealed container to prevent moisture absorption or contamination.

**Mounting the Sample:** A small amount (usually a few milligrams) of the powdered leaf sample was placed on a glass slide or an aluminium sample holder. The sample surface was carefully made flat and even, and slight pressure was applied to create a uniform layer.

**X-ray Diffractometer Setup:** The X-ray diffractometer was turned on and allowed to warm up according to the manufacturer's instructions. The X-ray source was set to emit Cu K $\alpha$  radiation at the appropriate wavelength ( $\lambda = 1.5406 \text{ \AA}$ ), and the X-ray beam was aligned to focus on the sample.

**Data Collection:** The sample holder was placed in the diffractometer's sample stage. The sample stage was rotated to different angles ( $2\theta$ ) while X-ray diffraction data were collected. Typically, a scan was conducted from  $2\theta = 5^\circ$  to  $2\theta = 90^\circ$ , although the range

may have varied depending on the specific research goals. The diffracted X-ray intensities were recorded as a function of the diffraction angle.

**3.9. Methodology for FTIR Analysis:** Fourier-transform infrared spectroscopy (FTIR) is a widely used technique for analysing the molecular composition of samples, including dry powder samples. Here is a detailed methodology for performing FTIR analysis on dry powder samples:

**Materials and Equipment:**

- FTIR spectrometer
- Dry powder sample
- Sample holder or cell (typically made of potassium bromide, KBr, or other suitable material)
- Pestle and mortar (for sample preparation)
- Clean, lint-free tissue or lens paper
- Nitrile gloves (to prevent contamination)
- Computer with FTIR software for data analysis

**Procedure:** Sample Preparation: Ensured that the dry powder sample was representative and homogeneous. When necessary, ground the sample into a fine powder using a pestle and mortar. Ensured that the piece was thoroughly mixed to avoid any concentration variations. For susceptible models, it was essential to prevent contamination. Cleaned all equipment and wore nitrile gloves during handling. Preparation of KBr Pellet (if using): If the sample is compatible with KBr pellets (standard in FTIR analysis), mixed a small amount of the powdered sample (usually a few milligrams) with KBr powder (typically 100 mg) in a mortar and pestle. Ground the mixture gently to ensure even distribution. Transferred the mixture into a pellet press and applied sufficient pressure to form a transparent pellet. Alternatively, a hydraulic press could be used. FTIR Spectrometer Setup: Turned on the FTIR spectrometer and allowed it to warm up for the recommended duration specified by the manufacturer (usually 15-30 minutes). Background correction was performed by measuring an empty sample holder (KBr cell) to account for any atmospheric interference. Sample Measurement: Place the prepared sample (the KBr pellet or the

dry powder) in the sample holder or cell. Carefully positioned the sample holder in the FTIR spectrometer's sample compartment. **Data Acquisition:** Started the FTIR software and set the desired scanning parameters, such as the wavenumber range and resolution. Standard wavenumber ranges were between  $4000\text{ cm}^{-1}$  and  $400\text{ cm}^{-1}$ . Initiated the scan to collect the FTIR spectrum. Typically, the instrument recorded absorbance or transmittance as a function of wavenumber.

**Data Analysis:** Analyzed the obtained spectrum using FTIR software. Identified the sample's peaks and bands corresponding to functional groups and molecular vibrations. If necessary, compare the sample spectrum to reference spectra or databases for compound identification. FTIR analysis provided valuable information about the chemical composition of the dry powder sample, allowing for the identification of functional groups, chemical bonds, and structural features. It was essential to carefully follow the instrument manufacturer's guidelines and calibration procedures for accurate and reliable results.

**3.10.SEM-EDX Analysis Methods:** Scanning Electron Microscopy with energy-dispersive X-ray Spectroscopy (SEM-EDX) is a powerful technique for imaging and elemental analysis of samples, including mustard plant leaves. Below is a detailed analysis method for SEM-EDX on mustard plant leaves:

**Materials and Equipment:**

- Mustard plant leaves (fresh or prepared, as needed)
- SEM-EDX instrument
- Liquid nitrogen (if using fresh leaves)
- Conductive adhesive or carbon tape
- Gold or carbon coating unit (optional)
- Vacuum desiccator (optional)
- Computer with SEM-EDX software for data analysis
- Safety equipment (lab coat, gloves, safety glasses)

**Sample Preparation:** **Sample Collection:** Collected representative mustard plant leaves from the sample source. We minimized the time between collection and analysis when using fresh leaves to prevent sample degradation. **Sample Preservation:** Fresh



leaves were collected, frozen with liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until analysis.

**Sample Preparation (for SEM):** Removed a small portion and froze it with liquid nitrogen when using fresh leaves. This frozen section was fractured to expose the inner structures. When using dry leaves, a small piece of the leaf was carefully mounted on conductive adhesive or carbon tape, ensuring the sample was securely attached.

**Sample Coating:** Using a sputter coater to enhance surface conductivity and prevent charging effects during SEM imaging, Coating the sample with a thin layer of gold or carbon. This step was beneficial for non-conductive samples.

**SEM Imaging: Instrument Setup:** Turned on the SEM instrument and allowed it to reach the appropriate vacuum conditions and operating temperature. Aligned the electron beam, adjusted the working distance, and set the desired electron beam energy (typically 5-30 kV) based on the sample's characteristics.

**Sample Loading:** Carefully loaded the prepared sample (with or without coating) onto the SEM stage.

**SEM Imaging:** Captured high-resolution SEM images of the mustard plant leaf surfaces and any specific regions of interest and adjusted the magnification and focus settings as needed.

**EDX Elemental Analysis: EDX Setup:** Activated the EDX detector on the SEM instrument and properly calibrated it.

**EDX Spectrum Acquisition:** Selected the regions of interest on the SEM images where elemental analysis was desired and initiated the EDX analysis to collect energy-dispersive X-ray spectra.

**Data Analysis:** Used the SEM-EDX software to analyze the collected spectra, identifying and quantifying the elements in the mustard plant leaves. If necessary, compare the elemental composition of different regions or samples and generate basic distribution maps. SEM-EDX analysis provided valuable information about mustard plant leaves' surface morphology and elemental composition, essential for various research applications, including plant physiology, environmental studies, and material characterization.

**3.11. Measurement of Zeta Potential of Mustard Leaves:** Measuring mustard leaves' zeta potential involves assessing their surface charge, which can provide valuable information about their colloidal stability and interactions with other particles or substances. Here is a detailed methodology for measuring the zeta potential of mustard leaves:

**Materials and Equipment:**

- Mustard plant leaves (fresh or freeze-dried)
- Deionized or distilled water
- Zeta potential analyzer (e.g., Zeta Sizer)
- Zeta potential cells or cuvettes
- pH meter and pH buffer solutions
- Ultrasonic bath or probe sonicator
- Centrifuge
- Conductivity meter (optional)
- Safety equipment (lab coat, gloves, safety glasses)

**Procedure: Sample Preparation:** We thoroughly washed and rinsed fresh leaves with deionized or distilled water to remove contaminants. The leaves were then dried using a clean, lint-free cloth or paper towel, ensuring they were scorched before proceeding. When using freeze-dried leaves, we provided they were correctly stored and moisture-free. **Leaf Extraction:** We cut the mustard leaves into small pieces to increase the surface area for analysis. Depending on our experiment, we removed the leaf cuticles to expose the cell surfaces more effectively. This was done by gently scraping the surface with a spatula or a similar tool. **Suspension Preparation:** We prepared a stock suspension of the mustard leaf fragments in deionized or distilled water. The concentration could vary depending on our research goals, but we typically used a 0.1-1.0% w/v concentration. **pH Adjustment:** We measured the pH of the suspension using a pH meter and adjusted the pH to the desired value using pH buffer solutions (e.g., phosphate buffers) or acid/base solutions. We noted that the zeta potential is pH-dependent, so measurements were taken at multiple pH values to understand the surface charge behaviour of the leaves. **Ultrasonication:** To ensure uniform dispersion of the leaf fragments in the suspension, we subjected the mixture to ultrasonication using an ultrasonic bath or probe sonicator. This step helped break down agglomerates and ensured a stable, homogeneous suspension. **Centrifugation:** If necessary, we centrifuged the suspension at a low speed to remove large particles or debris. We collected the supernatant for zeta potential analysis.

**3.12. Zeta Potential Measurement:** We transferred the prepared suspension into the zeta potential cells or cuvettes. The cells or cuvettes were placed in the zeta potential

analyzer, and we followed the instrument's operating instructions to measure the zeta potential. Typically, the zeta potential was determined by measuring the electrophoretic mobility of particles in an applied electric field.

**Data Analysis:** We recorded the zeta potential values obtained at different pH values or conditions. We analyzed the data to conclude the surface charge behaviour of the mustard leaves under various conditions. Measuring the zeta potential of mustard leaves helped us understand their surface charge characteristics, which were essential for applications such as studying interactions with nanoparticles, agglomeration behaviour, and stability in various environments.

**3.13. Measurement of Particle Size:** Particle size analysis of mustard leaves using a particle size analyzer typically involved laser diffraction or dynamic light scattering techniques. Here's a methodology for conducting particle size analysis in mustard leaves using a particle size analyzer:

**Sample Preparation:** a. Collected fresh mustard leaves from the desired source or location. b. Washed the leaves thoroughly to remove any dirt or contaminants. c. Patted dry the leaves using paper towels to remove excess moisture. d. Cut the leaves into small, uniform pieces to facilitate the analysis.

**Sample Homogenization:** a. Placed the prepared mustard leaf samples in a suitable container. b. Homogenized the samples to create a uniform paste or slurry. Ensured that the sample was mixed correctly to avoid aggregation of particles. Preparation of Dispersant: a. Depending on the particle size analyzer, we needed to prepare a dispersing medium, typically water or an appropriate solvent, if the sample did not disperse nicely in the instrument.

**Calibration of the Particle Size Analyzer:** a. Ensured that the particle size analyzer was calibrated correctly according to the manufacturer's instructions. This step was critical for accurate results. **Sample Analysis:** a. Introduced a small amount of the homogenized mustard leaf sample into the sample chamber of the particle size analyzer. b. If necessary, add the dispersant to create a suitable suspension. c. Started the analysis according to the instrument's operating instructions. d. Allowed the instrument to

perform the analysis involving laser diffraction or dynamic light scattering to determine the particle size distribution.

### **3.15. Methods for gene expression analysis**

#### **3.15.1. RNA isolation cDNA synthesis and Quantitative Real-Time PCR (qRT-PCR) analysis**

Total RNA was isolated from *Brassica* sp. Leaf samples (Treated and untreated) using Trizol RNA isolation System (Invitrogen)) following manufacturer's protocol followed by DNase treatment to remove contaminations of DNA molecules. On column DNase-I (Qiagen) treatment was given to remove contaminating DNA in the RNA preparations. The quality of purified RNA samples was analyzed both on 1.2% denaturing Agarose gel. A total of 1 µg of total RNA was used in the cDNA preparation reaction using the FIREScript RT cDNA Synthesis Kit (Solis BioDyne) following the manufacturer's protocol. Total cDNA was diluted up to 20 ng/µl, and a total 80 ng was used in a 10 µl reaction mixture using Power SYBR® Green PCR Master Mix (Life Technologies), and the reaction was performed on StepOnePlus™ Real-time PCR system (Life Technologies). The relative expression levels of target genes were examined using primers designed to give an amplicon of 150-250 bp and anneals at 60 °C temperature. The RNA concentration in different samples was normalized using *G3PDH*, which has been established as most stable gene in various tissues and stress conditions.

#### **cDNA synthesis reaction mixture composition**

Template RNA- 1.0 µg in 10 µl	10.0 µl
Oligo dT/Random primers (100µM)-	1.0 µl
dNTP Mix (20 mM each)-	0.5 µl
10X RT reaction buffer	2.0 µl
FIREScript RT	1.0 µl
RiboGrip RNase Inhibitor	0.5 µl
Nuclease free Water-	5.0 µl
<b>Total Reaction Volume-</b>	<b>20.0 µl</b>

### Thermal Program used to synthesize cDNA

Priming	10 min at 25°C
Reverse Transcription	30 min at 42°C
RT Inactivation	5 min at 85°C
Storage	hold at 4°C

### qPCR Reaction Setup

2X SYBR green buffer-	10.0 µl
cDNA dil. to 20 ng/µl -	5.0 µl
Primer 10 µM Fwd -	1.0µl
Primer 10 µM Rev -	1.0µl
Nuclease free water -	3.0µl
<b>Total Volume -</b>	<b>20.0 µl</b>

For each reaction, three technical replicates were set in a 96 well format. Simultaneously, a no template control (NTC) for each set of primer was kept to put a check for contaminants or non-specific amplicons or primer dimmers, if any.

The following thermal cycling program was used:

1. 10 min at 95 °C (enzyme activation),
2. 10 sec at 95 °C (cyclic denaturation),
3. 30 sec at 60 °C (annealing/extension) for 40-45 cycles, which includes data acquisition.

A dissociation curve (Melting Curve) analysis was performed from 55°C-95°C in increments of 0.3 °C, each lasting for 5 sec, to ensure the presence of a specific product. The RNA concentration was normalized using housekeeping gene *G3PDH* transcript abundance in all the four samples.

We analyzed a total of 6 genes (Table 1) along with transcript abundance of *G3PDH* gene as an endogenous control to analyze the differential expression the selected genes in Brassica leaf tissues.

### 3.1. Photographs of Field work and Lab work

#### Field preparation



#### Irrigation





**Spraying**



**Measuring morphological parameters**





**Photos of different interval**









**Harvesting**

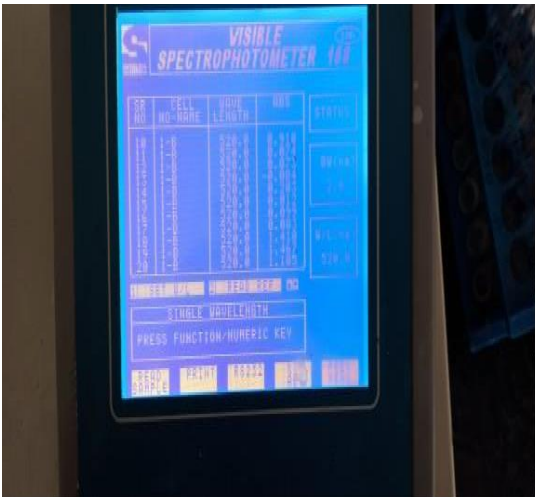


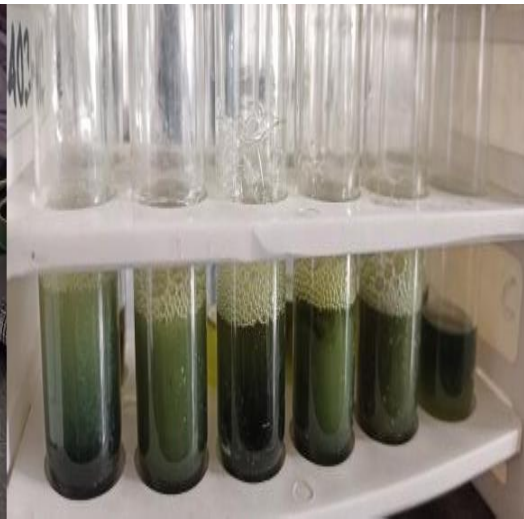


**Biochemical Parameters**









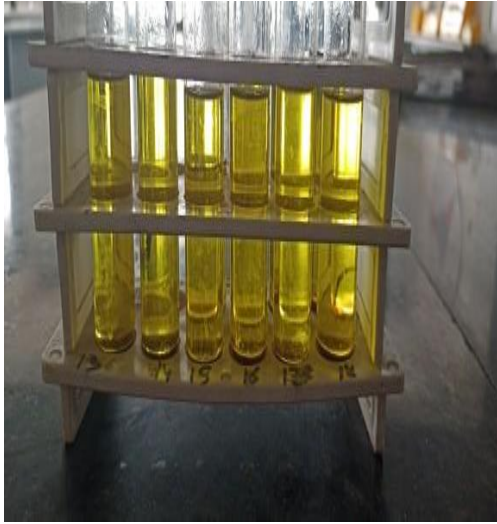


**Quality parameters**











## RESULTS AND DISCUSSION

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The present research work entitled “**Evaluation of Sulphur and Salicylic Acid on Growth, Physiology, Yield and Molecular Expression of Indian Mustard**” was carried out during two Rabi seasons of 2021-2023 and 2022-2023 respectively, in the Department of Agronomy as the field experiment in the Lovely Professional University, Punjab. The results obtained are presented and discussed in this chapter.

### **4A. Thiourea (sulphur) and salicylic acid-mediated effects on morphological parameters of Indian mustard grown under the open filed condition**

**Plant Height (cm):** The effect of Sulphur and Salicylic acid and their combination on plant height was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, 90 and 120 days after sowing (DAS) (Table 4.1, Fig.4.1). In 2021-2022, there was a significant difference in plant height compared to T0 (Control) at 30, 60, 90 and 120 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the plant height was observed at 30,60,90 and 120 DAS. Therefore, at 30 DAS the percentage increase as compared to T0 was found highest in T11 followed by T9, T8, T3, T10, T4, T6, T7, T2, T5, T1, and the percentage values were 43.45%, 41.15%, 40.23%, 36.41%, 35.10%, 34.30%, 33.11%, 31.29%, 31.18%, 23.94%, and 17.50% respectively. At 60DAS the percentage increase as compare to T0 was found highest in T9 followed by T8, T11, T3, T1, T6, T2, T4, T7, T10, T5 and the percentage values were 22.88%, 22.32%, 21.68%, 18.30%, 16.83%, 14.50%, 13.96%, 11.87%, 10.49%, 8.34%, 6.70% respectively. At 90DAS the percentage increase as compare to T0 was found highest in T11 followed by T8, T2, T9, T3, T1, T4, T10, T7, T6, T5 and the percentage values were 24.18%,19.64%, 17.92%, 17.37%,14.92%,13.85%, 13.03%, 12.44%, 11.56%, 11.48%, 8.25% respectively. At 120DAS the percentage increase as compare to T0 was found highest in T11 Followed by T8, T9, T3, T2, T10, T6, T1, T4, T5, T7 and the percentage values were 29.15%, 24.47%, 22.62%, 21.09%, 19.53%, 18.34%, 17.86%, 15.02%, 12.10%, 8.55%, 7.23% respectively. 2022-2023, plant height significantly differed from T0 (Control) at 30, 60, 90 and 120 DAS. The percentage

increase was calculated by comparing all the treatments with T0. Therefore, At 30 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T8, T9, T4, T10, T3, T2, T7, T5, T6, T1 and the percentage values were 41.78%, 40.01%, 39.48%, 37.73%, 37.45%, 36.94%, 33.12%, 30.73%, 25.03%, 22.29%, 21.9% respectively. At 60 DAS the percentage increase as compare to T0 was found highest in T9 followed by T11, T8, T2, T7, T1, T3, T10, T4, T6, T5 and the percentage values were 20.97%, 20.62%, 17.16%, 16.56%, 14.91%, 13.37%, 10.64%, 10.37%, 10.32%, 7.29%, 3.89% respectively. At 90 DAS the percentage increase as compare to T0 was found highest in T11 followed by T9, T8, T2, T7, T3, T6, T10, T1, T4, T5 and the percentage values were 28.08%, 20.89%, 19.58%, 17.43%, 17.33%, 16.99%, 15.67%, 14.55%, 13.01%, 11.38%, 8.57% respectively. At 120 DAS the percentage increase as compare to T0 was found highest in T11 followed by T9, T8, T3, T2, T10, T6, T1, T4, T7, T5 and the percentage values were 26.49%, 20.26%, 18.82%, 18.40%, 15.41%, 14.05%, 13.52%, 9.97%, 9.09%, 8.39%, 6.37% respectively. Sulphur is a vital macronutrient crucial in numerous physiological processes within plants. Manganese is an essential component of amino acids, vitamins, and coenzymes, and it plays a crucial role in plant metabolism. The influence of sulphur on plant height primarily occurs through its participation in synthesising cysteine and methionine, two amino acids containing sulphur. Cysteine and methionine are indispensable constituents of proteins. The proteins under consideration play a crucial role in various cellular processes such as cell division, elongation, and structural development, thereby directly influencing plant height (Kochanek et al., 2023; Kosakivska et al., 2022; Kour et al., 2023; Kudoyarova, 2022; Kumar & Ohri, 2023; Kurepa & Smalle, 2022; Kurniawan & Chuang, 2022; Kuromori et al., 2022; Lajayer et al., 2022; Li et al., 2022). The presence of sulphur directly influences the rate at which protein synthesis occurs, consequently affecting the growth process. Sulphur-containing amino acids are precursors for enzymes that participate in diverse metabolic pathways. Enzymes play a critical role in facilitating nutrient absorption, photosynthetic processes, and the synthesis of hormones, thereby significantly impacting plant growth and development. Sulphur significantly impacts the regulation of hormones in plants, thereby influencing the modulation of growth processes. Sulphur plays a crucial role in the biosynthesis of indole-3-acetic acid (IAA), a prominent plant hormone called auxin. Auxins are a class

of vital hormones that play a crucial role in stimulating the elongation and expansion of cells, thereby contributing to the overall increase in plant height. Sulphur impacts the equilibrium of cytokinin, which are hormones that play a crucial role in cell division. Maintaining appropriate levels of cytokinin is imperative for achieving optimal plant growth, and the homeostasis of cytokinin can be indirectly affected by the presence of sulphur. Sulphur exhibits interactions with gibberellin signalling pathways as well. Gibberellins are a class of hormones that play a crucial role in regulating plant development, particularly in stem elongation (Li et al., 2022; Li et al., 2022; Li, Ren, et al., 2023; Li, Zhang, et al., 2022; Li, Zheng, et al., 2023; Li et al., 2022). The availability of sulphur can influence both the production and sensitivity of gibberellins. Salicylic acid (SA) is a plant hormone primarily recognised for its involvement in the defence mechanisms against biotic stressors, particularly pathogens. Nevertheless, recent studies have revealed the diverse functions that it plays in the growth and development of plants. Sociocultural factors can influence the regulation of gene expression associated with growth and development. This encompasses genes implicated in cellular expansion, elongation, and division. The Sonic hedgehog (SHH) signalling pathway initiates the activation of distinct transcription factors, which subsequently govern the modulation of growth-associated genes. Succinic acid (SA) affects cell expansion by modulation of proton pump activity within the cell wall. The acidification of the cell wall creates a favourable environment for cell elongation, thus playing a role in the promotion of plant height. Although the main function of SA is commonly linked to defence mechanisms, its impact on stress management can indirectly affect plant growth. When plants experience decreased stress levels due to SA-mediated defence mechanisms, they may allocate more resources towards adaptation. The redistribution of resources, encompassing energy and nutrients, can augment vegetative growth and promote an elevation in plant stature. The concurrent utilisation of sulphur and salicylic acid elicits synergistic impacts on the growth of Indian mustard plants. The observed synergy can be ascribed to the complementary functions of S and SA in plant physiology. Sulphur facilitates the absorption of vital nutrients, such as nitrogen, phosphorus, and potassium. These nutrients are essential for the optimal growth and development of plants (Li, Han, et al., 2022; Li, He, et al., 2023; Li et al., 2022; Li, Han, et al., 2023; Li, Huang, et al., 2022; Li, Luo, et al., 2022). When the application of SA is

combined with S, it can potentially augment nutrient uptake efficiency. The presence of SA significantly impacts the morphology of roots, increasing the surface area of roots and enhancing the process of nutrient absorption. Enhanced nutrient availability facilitates the overall growth of plants, leading to an increase in plant height. The integration of S and SA has the potential to induce complex hormonal intercommunication, thereby promoting and enhancing the growth of plants. The compound S has been observed to enhance the process of auxin biosynthesis, whereas SA has been found to promote both auxin transport and sensitivity in various plant tissues. The concurrent execution of these processes enhances cellular elongation and promotes the growth of stems, thereby facilitating the development of taller plants. The interactions between S-mediated gibberellin signalling and SA-induced gene expression lead to the synergistic activation of growth pathways dependent on gibberellin. The combined effect of this synergistic action results in the expedited growth of stems and an overall increase in plant height. The inclusion of salicylic acid (SA) in the growth medium has positively impacted plants' ability to tolerate stress. In contrast, sulphur plays a crucial role in facilitating the presence of essential components required for stress-related compounds, such as glutathione, which exhibits potent antioxidant properties. The synergistic interaction between stress tolerance and growth promotion facilitates the establishment of a favourable milieu that supports vigorous and towering plant growth. The plant height of Indian mustard (*Brassica juncea* L.) is significantly influenced by sulphur and salicylic acid, which operate through complex molecular, physiological, and biochemical mechanisms. As a crucial macronutrient, Sulphur influences various physiological processes such as protein synthesis, enzyme activity, and hormonal regulation, promoting vegetative growth. Salicylic acid, renowned for its involvement in stress responses, is crucial in regulating growth-related gene expression and cellular expansion. The combined application of sulphur and salicylic acid demonstrates synergistic effects, enhancing nutrient uptake, hormonal crosstalk, and stress tolerance. The interaction between these factors enhances cellular elongation and the growth of stems, leading to increased height in Indian mustard plants. The comprehension of these mechanisms offers significant knowledge for agricultural practices, presenting potential approaches to enhance crop productivity and quality while safeguarding the well-being of plants in cultivating Indian mustard (Li,

Tong, et al., 2023; Liu et al., 2023; Liu, Wang, et al., 2022; Liu, Li, et al., 2022; Liu,Cui, et al., 2022; Liu, Zong, et al., 2022; Liu, Liu, et al., 2022; Liu, Xiao, et al., 2022; Liu, Meng, et al., 2022; Lombardino et al., 2022).

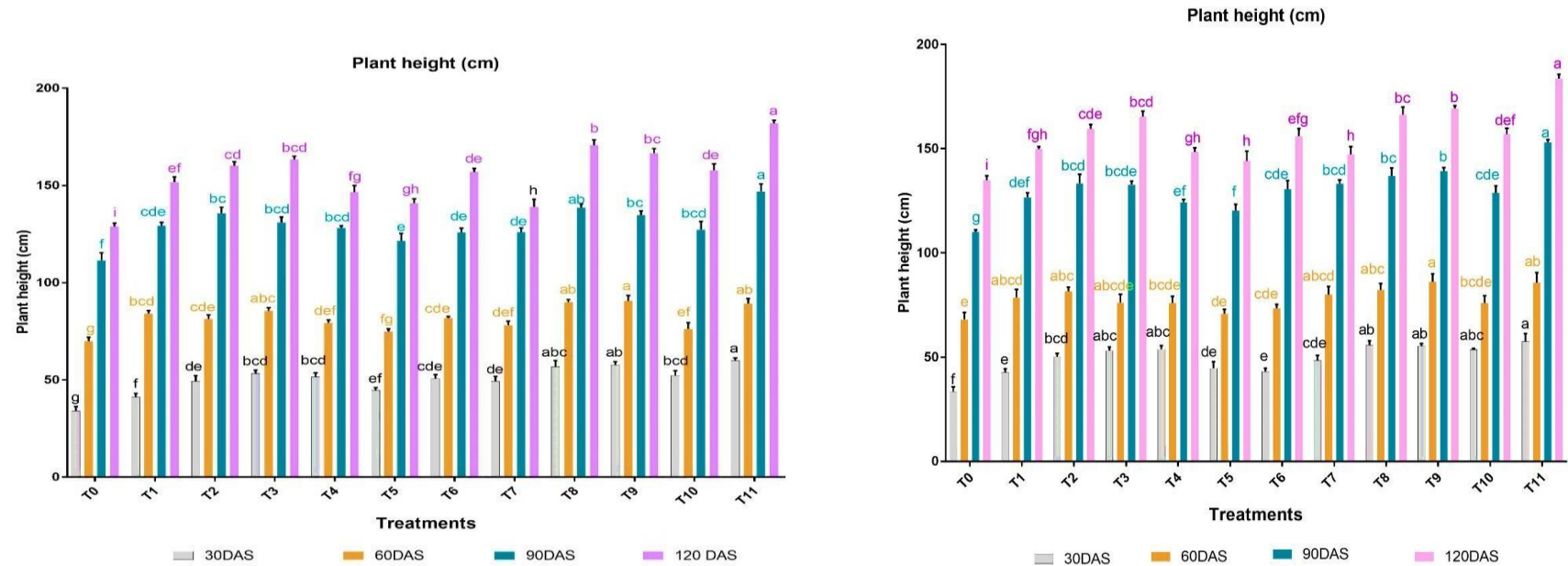
**Table 4.1. Impact of Different Treatments on Plant Height (cm) of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS		120 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	33.94 <sup>g</sup> ±2.33	33.50 <sup>f</sup> ±2.30	69.88 <sup>g</sup> ±2.08	68.06 <sup>e</sup> ±3.29	111.40 <sup>f</sup> ±3.93	110.10 <sup>g</sup> ±0.99	128.91 <sup>i</sup> ±1.77	134.93 <sup>i</sup> ±2.11
<b>T1 (Thiourea-1000 ppm)</b>	41.14 <sup>f</sup> ±1.84	42.73 <sup>e</sup> ±1.60	84.02 <sup>bcd</sup> ±1.64	78.57 <sup>abcd</sup> ±3.89	129.31 <sup>cde</sup> ±1.67	126.56 <sup>def</sup> ±2.24	151.71 <sup>ef</sup> ±2.67	149.89 <sup>efg</sup> ±1.08
<b>T2 (Salicylic Acid-300 ppm)</b>	49.32 <sup>de</sup> ±2.84	50.10 <sup>bcd</sup> ±1.68	81.22 <sup>cde</sup> ±2.19	81.57 <sup>abc</sup> ±2.01	135.73 <sup>bc</sup> ±3.02	133.35 <sup>bcd</sup> ±4.35	160.20 <sup>cd</sup> ±1.94	159.53 <sup>cde</sup> ±2.08
<b>T3 (Thiourea-1000 ppm + Salicylic Acid-300 ppm)</b>	53.38 <sup>bcd</sup> ±1.64	53.13 <sup>abc</sup> ±1.72	85.54 <sup>abc</sup> ±1.56	76.17 <sup>abcde</sup> ±3.91	130.94 <sup>bcd</sup> ±2.78	132.65 <sup>bcd</sup> ±1.90	163.37 <sup>bcd</sup> ±1.63	165.37 <sup>bcd</sup> ±2.62
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	51.66 <sup>bcd</sup> ±1.93	53.80 <sup>abc</sup> ±1.69	79.29 <sup>def</sup> ±1.51	75.90 <sup>bcd</sup> ±3.25	128.10 <sup>cde</sup> ±1.20	124.25 <sup>ef</sup> ±1.46	146.66 <sup>fg</sup> ±3.32	148.43 <sup>gh</sup> ±1.96
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	44.63 <sup>ef</sup> ±1.38	44.69 <sup>de</sup> ±3.11	74.90 <sup>fg</sup> ±1.41	70.82 <sup>de</sup> ±2.09	121.42 <sup>e</sup> ±3.85	120.42 <sup>f</sup> ±2.88	140.98 <sup>gh</sup> ±2.20	144.11 <sup>h</sup> ±4.71
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	50.74 <sup>cde</sup> ±1.98	43.11 <sup>e</sup> ±1.50	81.73 <sup>cde</sup> ±0.85	73.42 <sup>cde</sup> ±1.86	125.85 <sup>de</sup> ±2.18	130.56 <sup>cde</sup> ±4.24	156.96 <sup>de</sup> ±1.83	156.03 <sup>efg</sup> ±3.65
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	49.40 <sup>de</sup> ±2.40	48.36 <sup>cde</sup> ±2.51	78.07 <sup>def</sup> ±2.15	79.99 <sup>abcd</sup> ±3.94	125.97 <sup>de</sup> ±2.22	133.18 <sup>bcd</sup> ±1.87	138.97 <sup>h</sup> ±3.97	147.30 <sup>h</sup> ±3.75
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	56.79 <sup>abc</sup> ±3.12	55.85 <sup>ab</sup> ±1.97	89.96 <sup>ab</sup> ±1.42	82.17 <sup>abc</sup> ±3.13	138.63 <sup>ab</sup> ±1.86	136.92 <sup>bc</sup> ±3.82	170.68 <sup>b</sup> ±2.73	166.21 <sup>bc</sup> ±3.74
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	57.68 <sup>ab</sup> ±1.76	55.36 <sup>ab</sup> ±1.12	90.62 <sup>a</sup> ±2.77	86.13 <sup>a</sup> ±3.87	134.82 <sup>bc</sup> ±2.12	139.18 <sup>b</sup> ±1.76	166.60 <sup>bc</sup> ±2.42	169.23 <sup>b</sup> ±1.36
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	52.30 <sup>bcd</sup> ±2.40	53.56 <sup>abc</sup> ±.62	76.24 <sup>ef</sup> ±3.26	75.94 <sup>bcd</sup> ±3.53	127.23 <sup>cde</sup> ±4.30	128.85 <sup>cde</sup> ±3.25	157.87 <sup>de</sup> ±3.24	156.99 <sup>def</sup> ±2.73
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	60.03 <sup>a</sup> ±1.26	57.55 <sup>a</sup> ±3.72	89.23 <sup>ab</sup> ±2.63	85.75 <sup>ab</sup> ±4.77	146.93 <sup>a</sup> ±3.77	153.09 <sup>a</sup> ±1.24	181.96 <sup>a</sup> ±1.61	183.56 <sup>a</sup> ±2.15
<b>CD</b>	3.786	3.472	3.387	4.962	5.174	4.665	4.441	5.033
<b>CV</b>	4.436	4.131	2.432	3.739	2.341	2.093	1.677	1.884

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.



**Figure 4.1. Plant Height (cm) of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars shows a level of significance, treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Leaf Number:** The effect of Sulphur and Salicylic acid and their combination on leaf number was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, 90 and 120 days after sowing (DAS) (Table 4.2 Figure 4.2). In 2021-2022, there was a significant difference in leaf number compared to T0 (Control) at 30, 60, 90 and 120 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the leaf number was observed at 30, 60, 90 and 120 DAS. Therefore, At 30 DAS the percentage increase as compared to T0 was found highest in T11 and T5 followed by T6, T3, T1, T7, T4, T8, T10, T9, T2, and the percentage values were 33.34%, 33.34%, 30.09%, 28.00%, 24.20%, 23.42%, 20.87%, 20.00%, 19.11%, 17.24%, 10.01% respectively. At 60 DAS the percentage increase as compared to T0 was found highest in T8 followed by T5, T11, T7, T3, T6, T9, T10, T4, T2, T1, and the percentage values were 47.92%, 47.45%, 46.16%, 41.07%, 40.88%, 39.87%, 36.60%, 36.14%, 32.17%, 24.24%, 16.26% respectively. At 90 DAS the percentage increase as compared to T0 was found highest in T5 followed by T8, T11, T6, T7, T3, T10, T4, T9, but the percentage also decrease in T1 and T2 compare to the T0 and the percentage values were 24.23%, 21.68%, 20.72%, 19.34%, 16.66%, 15.36%, 10.94%, 10.46%, 3.55% respectively. But the percentage also decreased in T1 and T2 compared to T0, and the percentage values were -5.18% and -5.18%. At 120 DAS the percentage increase as compared to T0 was found highest in T5 followed by T8, T6, T4, T3, T7, T11, T10, T9, T2, T1, and the percentage values were 31.09%, 27.11%, 22.62%, 20.40%, 20.17%, 17.56%, 17.31%, 11.50%, 4.81%, 4.16%, 2.80% respectively. In 2022-2023, there was a significant difference in leaf number compared to T0 (Control) at 30, 60, 90 and 120 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T5, followed by T3, T11, T6, T1, T4, T7, T8, T9, T10, T2 and the percentage values were 33.63%, 30.01%, 26.69%, 24.53%, 23.01%, 19.81%, 17.23%, 17.23%, 14.47%, 4.96%, 2.54% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T5, T8, T6, T3, T7, T10, T9, T4, T1, T2 and the percentage values were 43.41%, 43.07%, 38.83%, 35.71%, 33.68%, 30.77%, 28.67%, 24.39%, 23.17%, 18.18%, 10.00% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T5 followed by T8, T11, T3, T6, T7,

T10, T4, T9, T1, T2 and the percentage values were 28.67%, 27.84%, 25.42%, 24.88%, 24.52%, 13.37%, 12.84%, 10.11%, 6.61%, 5.47%, 3.12% respectively. At 120 DAS, the percentage increase as compared to T0 was found highest in T8 followed by T5, T6, T11, T3, T7, T9, T10, T4, T2, T1 and the percentage values were 28.86%, 26.59%, 24.79%, 22.03%, 19.53%, 17.11%, 10.97%, 9.50%, 7.68%, 5.73%, 1.42% respectively. Sulphur (S) and salicylic acid (SA) have been recognised as significant determinants in the modulation of leaf numbers in plants. Nonetheless, the complexities of the underlying mechanisms of the phenomenon above continue to be the focus of ongoing research. This discussion aims to investigate the molecular and physiological mechanisms responsible for the ability of S and SA to regulate leaf numbers in Indian mustard (*Brassica juncea* L.)(Ma et al., 2022; Mabasa, 2023; Maheshwari et al., 2022; Maia et al., 2022; Makete et al., 2022; Manepalli et al., 2022; Mangena, 2022; Manjunatha et al., 2022; Masmoudi et al., 2023; Megala et al., 2022; Mittal et al., 2022). The number of leaves, a crucial factor in plant structure and overall development, significantly influences important physiological processes, including photosynthesis, nutrient uptake, and agricultural productivity. Although the involvement of S and SA in the control of leaf number has been observed in various plant species, their specific mechanisms of action in the context of Indian mustard remain incompletely understood. This extended dialogue explores the intricate mechanisms that govern the influence of S and SA on leaf number in Indian mustard. Doing so aims to provide valuable insights into potential strategies for enhancing crop productivity. Sulphur, an essential macronutrient, plays a crucial role in the growth and development of plants. Various fundamental mechanisms influence the impact of this phenomenon on the number of leaves. To begin with, sulphur is a fundamental component of amino acids, particularly cysteine and methionine. The amino acids in question serve as the fundamental constituents for protein synthesis. Proteins play a crucial role in the initiation and development of leaves. Additionally, amino acids containing sulphur are essential for synthesising enzymes involved in numerous metabolic pathways. These enzymes directly impact leaf number as they are crucial in hormone biosynthesis, nutrient uptake, and cell division (Mohammadi et al., 2023; Molinari et al., 2023; Moustakas et al., 2022; Mugwanya et al., 2023; Mukarram et al., 2022; Mukhtar et al., 2023; Myers Jr. et al., 2023; Nadeem, 2022; Nam et al., 2023; Napieraj et al., 2023; Nasir &

Toth, 2022; Negi & Kumar, 2023; Nimsi et al., 2023; Niu & Fu, 2022). Moreover, the regulation of plant hormonal activity by sulphur can indirectly influence the development of leaf numbers. It is worth mentioning that sulphur plays a significant role in the biosynthesis of indole-3-acetic acid (IAA), a well-known plant hormone called auxin. Auxins play a crucial role in the regulation of leaf initiation and development. Sufficient levels of sulphur are essential in facilitating the synthesis of an adequate amount of indole-3-acetic acid (IAA), which in turn promotes the development of new leaves. Furthermore, sulphur availability can impact the equilibrium of cytokinins, hormones that play a significant role in promoting cell division. Ensuring appropriate cytokinin levels is crucial for facilitating the transition of leaf primordia into fully developed and functional leaves. Salicylic acid (SA), traditionally known for its role in plant defence mechanisms, also regulates leaf development. This is accomplished through a variety of mechanisms. First and foremost, the expression of genes associated with leaf initiation and development is regulated by SA. Using its activity, SA initiates the activation of distinct transcription factors, thereby coordinating the regulation of genes accountable for the process of leaf growth. Additionally, shoot apical meristem (SAM) promotes cellular proliferation and growth in leaf primordia. Cell elongation and leaf expansion can be facilitated by modulating the activity of proton pumps in the cell wall, thereby creating a favourable environment (Ortiz-García et al., 2022, 2023; Ozturk & Unal, 2023; Paalli et al., 2022; Pal et al., 2023; Parada et al., 2022; Parrey et al., 2023; Patel et al., 2022; Peng et al., 2022; Pérez-Llorca et al., 2023; Phokas et al., 2023; Phour & Sindhu, 2022; Poór et al., 2022; Quesada, 2022; Rai & Kaushik, 2023; Raja Gopalan et al., 2022). Although SA is primarily linked to defence-related functions, its ability to improve plant stress tolerance can indirectly influence the number of leaves. When plants detect reduced stress levels due to SA-mediated defence mechanisms, they allocate more resources, such as energy and nutrients, towards various growth processes, including the initiation and development of leaves. The simultaneous application of sulphur and salicylic acid has the potential to produce synergistic effects on leaf number. Sulphur plays a crucial role in enhancing the absorption of vital nutrients, such as nitrogen and phosphorus, which are necessary for the growth and development of leaves. The concurrent implementation of SA has improved nutrient uptake efficiency, thereby creating a favourable environment for leaf initiation and expansion by increasing nutrient availability. Additionally, the synergistic impact of sulphur and salicylic acid (SA) initiates complex hormonal communication, exerting

additional influence on leaf count. Sulphur has been found to enhance the process of auxin biosynthesis, whereas salicylic acid (SA) has been observed to enhance both auxin transport and sensitivity. The collaborative process described enhances the process of leaf initiation at axillary buds and promotes the growth of emerging leaves. Furthermore, the impact of sulphur on the levels of cytokinins, combined with the role of salicylic acid (SA) in enhancing the responsiveness to cytokinins, results in achieving an optimal balance of cytokinins. This balance is crucial for developing leaf primordia into fully mature and functional leaves (Ramakrishnan & Zhou, 2022; Rani et al., 2023; Rizvi et al., 2022; Roussos, 2023; Ruidas et al., 2022; Rybczyński et al., 2022; Sachan & Krishna, 2022; Salih, Wu, et al., 2022; Salih, Zhou, et al., 2022; Salwan et al., 2023; Samad et al., 2023; Sampedro- Guerrero et al., 2022; Samtani et al., 2022; Shah et al., 2022, 2023; Shang et al., 2022; Sharma & Verma, 2023; Sheikhalipour et al., 2023; Shekhawat et al., 2023). Moreover, including salicylic acid (SA) in the growth environment enhances the ability of plants to withstand and adapt to stressful conditions. In the present context, sulphur plays a crucial role in facilitating the availability of essential components necessary for producing stress-alleviating substances, such as glutathione, which possesses strong antioxidant properties. The combination of stress resilience and growth promotion creates a favourable environment for increasing leaf numbers. In summary, the intricate mechanisms that regulate leaf number in Indian mustard (*Brassica juncea* L.) are influenced by sulphur and salicylic acid (Sun et al., 2022; Sun et al., 2023; Sun et al., 2022; Taira & Shiono, 2022; Takeuchi et al., 2022; Talaat, 2023). As a vital macronutrient, Sulphur substantially influences the initiation and development of leaves by facilitating the synthesis of amino acids and enhancing enzyme activity. Salicylic acid, a well-known compound recognised for its defensive properties, exerts its influence on the expression of growth-related genes and the expansion of cells, thereby regulating the development of leaf numbers. The simultaneous utilisation of sulphur and salicylic acid results in synergistic outcomes that enhance the absorption of nutrients, initiate intercellular communication through hormones and enhance the ability to withstand stress, ultimately leading to an increase in leaf growth. An in-depth

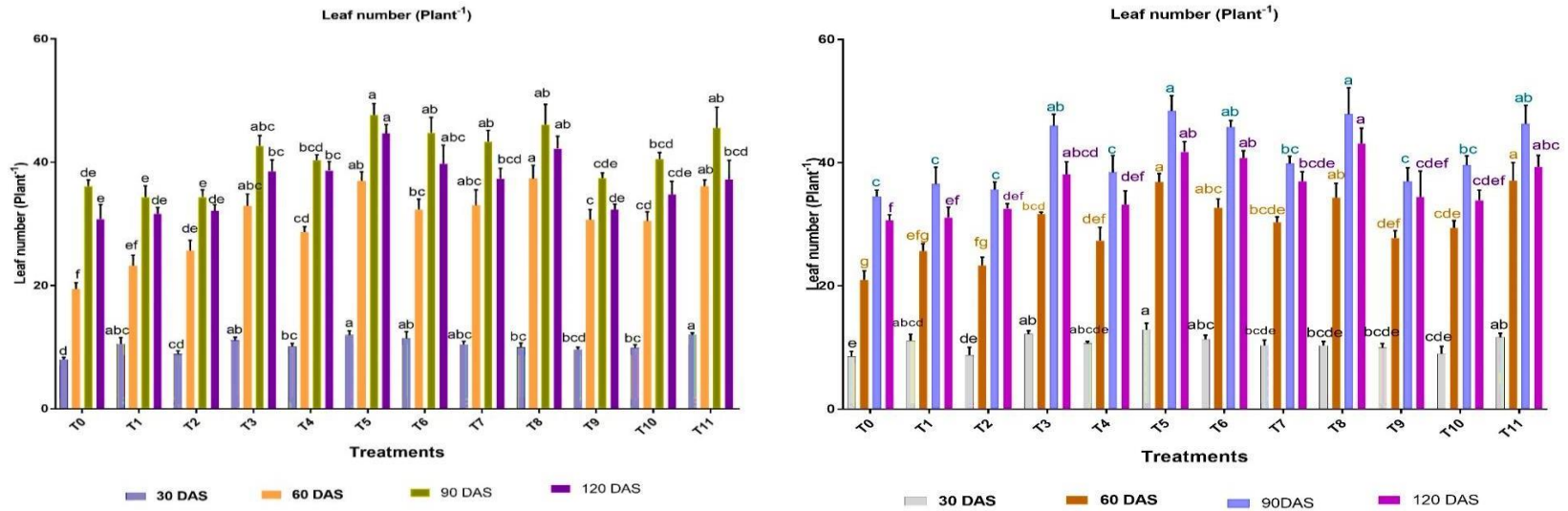
understanding of these mechanisms offers significant implications for agricultural practises, guiding maximising leaf number and crop yield in mustard cultivation. Additional investigation into the intricate molecular mechanisms involved in the interactions between sulphur and salicylic acid can reveal further opportunities for enhancing crop productivity (Shi et al., 2023; Shi et al., 2022; Si et al., 2023; Singh et al., 2022; Singh & Roychoudhury, 2023; Singh & Nandi, 2022; Singhal et al., 2023; Sivanesan et al., 2022; Song et al., 2023; Sousa et al., 2022; Spinoso-Castillo & Bello-Bello, 2022; Suliman et al., 2022).

**Table 4.2. Impact of Different Treatments on Leaf Number of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS		120 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	7.99 <sup>d</sup> ±0.33	8.55 <sup>e</sup> ±0.84	19.44 <sup>f</sup> ±1.01	20.99 <sup>g</sup> ±1.45	36.11 <sup>de</sup> ±1.01	34.55 <sup>c</sup> ±1.01	30.77 <sup>e</sup> ±2.36	30.66 <sup>f</sup> ±0.87
<b>T1 (Thiourea-1000 ppm)</b>	10.55 <sup>abc</sup> ±1.01	11.10 <sup>abcd</sup> ±1.07	23.21 <sup>ef</sup> ±1.70	25.66 <sup>efg</sup> ±1.20	34.33 <sup>e</sup> ±1.85	36.55 <sup>c</sup> ±2.71	31.66 <sup>de</sup> ±1.00	31.10 <sup>ef</sup> ±1.67
<b>T2 (Salicylic acid-300 ppm)</b>	8.88 <sup>cd</sup> ±0.50	8.77 <sup>de</sup> ±1.26	25.66 <sup>de</sup> ±1.66	23.33 <sup>fg</sup> ±1.33	34.33 <sup>c</sup> ±1.19	35.66 <sup>c</sup> ±1.20	32.11 <sup>de</sup> ±1.01	32.53 <sup>def</sup> ±0.83
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	11.10 <sup>ab</sup> ±0.50	12.21 <sup>ab</sup> ±0.50	32.88±1.95	31.66 <sup>bcd</sup> ±0.33	42.66 <sup>abc</sup> ±1.66	45.99 <sup>ab</sup> ±1.85	38.55 <sup>bc</sup> ±1.83	38.10 <sup>abcd</sup> ±2.03
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	10.10 <sup>bc</sup> ±0.50	10.66 <sup>abcde</sup> ±0.33	28.66 <sup>cd</sup> ±0.87	27.33 <sup>def</sup> ±2.18	40.33 <sup>bcd</sup> ±0.88	38.44 <sup>c</sup> ±2.71	38.66 <sup>bc</sup> ±1.45	33.21 <sup>def</sup> ±2.22
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	11.99 <sup>a</sup> ±0.66	12.88 <sup>a</sup> ±1.07	36.99 <sup>ab</sup> ±1.45	36.88 <sup>a</sup> ±1.34	47.66 <sup>a</sup> ±1.85	48.44 <sup>a</sup> ±2.45	44.66 <sup>a</sup> ±1.45	41.77 <sup>ab</sup> ±1.64
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	11.44 <sup>ab</sup> ±1.07	11.33 <sup>abc</sup> ±0.67	32.33 <sup>abc</sup> ±1.67	32.66 <sup>abc</sup> ±1.45	44.77 <sup>ab</sup> ±2.50	45.77 <sup>ab</sup> ±1.07	39.77 <sup>abc</sup> ±3.01	40.77 <sup>ab</sup> ±1.17
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	10.44 <sup>abc</sup> ±0.50	10.33 <sup>bcd</sup> ±0.88	32.99 <sup>abc</sup> ±2.51	30.33 <sup>bcd</sup> ±0.88	43.33 <sup>ab</sup> ±1.85	39.88 <sup>bc</sup> ±1.17	37.33 <sup>bcd</sup> ±1.67	36.99 <sup>bcd</sup> ±1.52
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	9.99 <sup>bc</sup> ±0.66	10.33 <sup>bcd</sup> ±0.67	37.33 <sup>a</sup> ±2.183	34.33 <sup>ab</sup> ±2.33	46.10 <sup>ab</sup> ±3.28	47.88 <sup>a</sup> ±4.29	42.22 <sup>ab</sup> ±2.01	43.10 <sup>a</sup> ±2.50
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	9.66 <sup>bcd</sup> ±0.33	9.99 <sup>bcd</sup> ±0.66	30.66 <sup>c</sup> ±1.66	27.77 <sup>def</sup> ±1.17	37.44 <sup>cde</sup> ±0.84	36.99 <sup>c</sup> ±2.18	32.33 <sup>de</sup> ±0.88	34.44 <sup>cdef</sup> ±4.19
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	9.88 <sup>bc</sup> ±0.50	8.99 <sup>cde</sup> ±1.20	30.44 <sup>cd</sup> ±1.50	29.44 <sup>cde</sup> ±1.16	40.55 <sup>bcd</sup> ±1.01	39.64 <sup>bc</sup> ±1.47	34.77 <sup>cde</sup> ±2.14	33.88 <sup>cdef</sup> ±1.67
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	11.99 <sup>a</sup> ±0.33	11.66 <sup>ab</sup> ±0.66	36.11 <sup>ab</sup> ±1.01	37.10 <sup>a</sup> ±2.91	45.55 <sup>ab</sup> ±3.37	46.33 <sup>ab</sup> ±3.00	37.21 <sup>bcd</sup> ±3.09	39.33 <sup>abc</sup> ±1.85
<b>CD</b>	1.098	1.499	2.038	2.882	3.306	4.036	3.360	3.598
<b>CV</b>	6.232	8.322	3.913	5.677	4.720	5.728	5.376	5.812

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.2: Leaf Number of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show an extra level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).



**Leaf Area (cm<sup>2</sup>):** The effect of Sulphur and Salicylic acid and their combination on leaf area was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.3, Figure 4.3). In 2021-2022, there was a significant difference in leaf area compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the leaf area was observed at 30, 60, and 90 DAS. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T1, followed by T3, T5, T9, T11, T4, T6, and T7, and the percentage values were 36.66%, 36.45%, 34.36%, 30.47%, 21.26%, 14.33%, 12.33%, 9.77% respectively. But the percentage also decrease in T2, T10, T8 as compare to T0 and the percentage values were -1.65%, -12.07%, -19.10% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T1 followed by T3, T9, T4, T5, T11, T6, T7, T2, T8, T10 and the percentage values were 43.32%, 40.34%, 36.37%, 29.30%, 28.19%, 27.49%, 25.41%, 19.21%, 17.77%, 16.86%, 10.73% respectively. At 90DAS, the percentage increase as compared to T0 was found highest in T3 followed by T9, T6, T4, T1, T8, T11, T10, T7, T2, T5 and the percentage values were 35.31%, 32.77%, 31.71%, 29.75%, 25.74%, 23.55%, 20.43%, 15.64%, 15.60%, 12.19%, 11.92% respectively. In 2022-2023, there was a significant difference in leaf area compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found highest in T1 followed by T3, T9, T4, T5, T2, T6, T7, T11, T8, T10 and the percentage values were 40.91%, 38.77%, 38.26%, 36.04%, 29.55%, 27.88%, 26.62%, 21.75%, 18.40%, 8.48%, 5.44% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T1 followed by T9, T11, T10, T6, T3, T4, T2, T5, T8, T7 and the percentage values were 44.86%, 40.27%, 36.91%, 31.73%, 30.04%, 29.37%, 27.20%, 26.20%, 21.72%, 21.40%, 17.28% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T4 followed by T9, T3, T1, T8, T6, T11, T10, T2, T5, T7 and the percentage values were 29.49%, 25.90%, 25.21%, 23.82%, 22.58%, 21.68%, 15.79%, 12.84%, 12.44%, 11.14%, 8.20% respectively. The leaf area is a crucial factor in plant development and productivity as it directly impacts important processes, including photosynthesis, nutrient absorption, and, ultimately, the yield of crops. Hence, it is

imperative to comprehensively understand the influence of nutrients and phytohormones on leaf area modulation in mustard (*Brassica juncea* L.) to enhance agricultural practices. Within the realm of plant growth, there exist numerous factors that exert influence. Two such elements, Sulphur (S) and salicylic acid (SA), have emerged as multifaceted entities whose impact on leaf area necessitates thorough investigation. Sulphur, as a crucial macronutrient for the growth and development of plants, exerts its influence on leaf area through various significant mechanisms. The pivotal aspect of its impact lies in its involvement in the process of amino acid synthesis, specifically in the formation of cysteine and methionine. These amino acids play a crucial role as the fundamental constituents for protein synthesis. Proteins are crucial in various cellular processes, including synthesising enzymes, structural proteins, and regulatory proteins that coordinate leaf expansion and growth. In addition, it is important to acknowledge Sulphur's substantial role in chlorophyll production. This element is an essential requirement for synthesising this crucial photosynthetic pigment. The maintenance of optimal levels of sulphur is crucial for the continuous synthesis of chlorophyll, which plays a fundamental role in enhancing the efficiency of photosynthesis. This, in turn, results in an increase in the overall leaf area. Sulfur-containing amino acids play an additional function as cofactors for enzymes involved in diverse metabolic pathways. The enzymes Sulphur activate play a crucial role in various processes related to leaf development, such as cell division and elongation. Therefore, Sulphur plays a direct role in regulating leaf area by participating in essential cellular processes. Nevertheless, the influence of Sulphur on leaf area extends beyond its direct interactions, as it also affects the synthesis and metabolism of plant hormones, including auxins and cytokinins. Auxins play a crucial role in the initiation and growth of leaves (Tan et al., 2022; Tariq et al., 2023; Tariq et al., 2022; Todorova et al., 2022; Topcu et al., 2022; Torres & Figueroa, 2023; Tyagi et al., 2022; Ullah et al., 2023; Ullah et al., 2022; Verma et al., 2023; Waadt et al., 2022; Wan & Xin, 2022; Wang et al., 2022; Wang et al., 2023; Wang et al., 2023). The influence of sulphur on auxin levels can significantly impact leaf expansion, thereby introducing an additional level of complexity to its regulatory effect on leaf area. Salicylic acid, traditionally recognised for its role in plant defence mechanisms, has also been identified as a regulator of leaf area. The mechanisms by which it operates encompass the regulation of gene

expression linked to the processes of growth and development, particularly the genes that control the expansion of leaf area. The mechanism by which salicylic acid exerts its effects involves activating specific transcription factors that intricately modulate the expression of genes involved in cell division and expansion. Moreover, previous studies have demonstrated that salicylic acid can stimulate cellular division and enlargement, specifically in leaf structures. The impact of this phenomenon is enhanced by its capacity to affect the activity of proton pumps in the cell wall, leading to favourable conditions for cell elongation and subsequently resulting in an increase in leaf area. The main function of salicylic acid is to provide defence against various environmental stressors. However, it is worth noting that its stress-response properties can indirectly impact leaf area. Activating salicylic acid-mediated defences can decrease stress levels, triggering the redistribution of resources such as energy and nutrients towards growth-related activities (Wang et al., 2022; Wang & Komatsu, 2022; Wang et al., 2022; Wang et al., 2022; Wang et al., 2022; Wante et al., 2022; Waters & Nelson, 2023; Wu et al., 2022; Wurms et al., 2023; Xiao et al., 2022; Xie et al., 2023). This reallocation encompasses a significant allocation towards the expansion of leaf area. The interaction between Sulphur and salicylic acid enhances their respective impacts on leaf area. The capacity of sulphur to enhance the assimilation of crucial nutrients, such as nitrogen and phosphorus, plays a pivotal role in facilitating leaf growth. Salicylic acid plays a role in improving nutrient uptake efficiency, thereby facilitating the provision of enriched nutrients that effectively support leaf area development. Moreover, the combined action of Sulphur and salicylic acid leads to complex hormonal interactions. Sulphur has been found to have a stimulatory effect on the synthesis of auxin, a plant hormone. In contrast, salicylic acid has been observed to facilitate the transport and enhance auxin sensitivity. The mutually beneficial interaction between different cellular processes enhances cell division and expansion, increasing leaf area overall. Furthermore, the inclusion of salicylic acid in the growth medium results in a significant improvement in plants' ability to withstand various forms of stress. Sulphur enhances this effect by facilitating the presence of crucial foundational components required for the formation of stress-related substances, including glutathione—an antioxidant with significant implications for the alleviation of stress. The simultaneous improvement in stress tolerance and stimulation of growth results in an environment that inherently

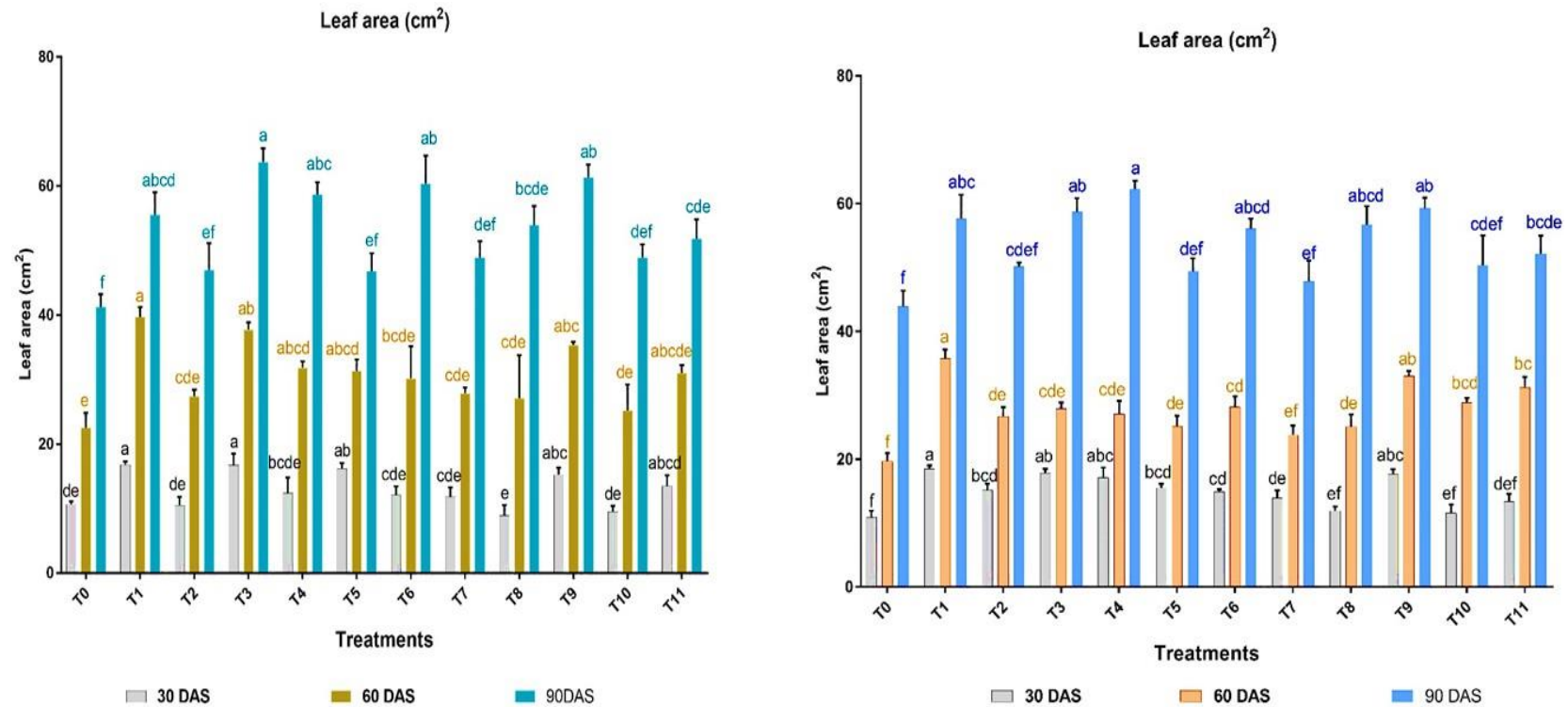
supports an increase in leaf area. In summary, the interplay of sulphur and salicylic acid governs complex mechanisms that impact leaf area in mustard (*Brassica juncea* L.). Sulphur, functioning as a vital nutrient, exerts a direct influence on the process of leaf expansion by participating in the synthesis of amino acids, the production of chlorophyll, the activation of enzymes, and the regulation of hormones. Salicylic acid, commonly recognised for its involvement in defence mechanisms, also significantly influences the expression of growth-related genes and the expansion of cells, thereby impacting leaf area. When Sulphur and salicylic acid combine, they demonstrate synergistic effects that result in improved nutrient uptake, enhanced hormonal crosstalk, increased stress tolerance, and expanded leaf area. The comprehensive comprehension of the mechanisms involved provides valuable insights for agricultural practices, presenting potential strategies to maximise leaf area and improve crop yield in mustard cultivation. Moreover, conducting additional investigations on the specific molecular mechanisms that underlie the interactions between Sulphur and salicylic acid could reveal further prospects for enhancing crop performance, thus initiating a novel phase of heightened agricultural efficiency (Yang et al., 2023; Yang & Lee, 2023; Yang et al., 2023; Yang et al., 2022; Yao et al., 2022; Yin et al., 2023; Yousaf et al., 2022; Yu et al., 2022; Yu et al., 2023).

**Table 4.3. Impact of Different Treatments on Leaf Area (cm<sup>2</sup>) of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	10.64 <sup>de</sup> ±0.46	10.93 <sup>f</sup> ±0.99	22.48 <sup>e</sup> ±2.34	19.71 <sup>f</sup> ±1.23	41.22 <sup>f</sup> ±2.01	43.91 <sup>f</sup> ±2.44
<b>T1 (Thiourea-1000 ppm)</b>	16.80 <sup>a</sup> ±0.48	18.50 <sup>a</sup> ±0.55	39.67 <sup>a</sup> ±1.55	35.76 <sup>a</sup> ±1.40	55.52 <sup>abcd</sup> ±3.49	57.64 <sup>abc</sup> ±3.74
<b>T2 (Salicylic acid-300 ppm)</b>	10.46 <sup>de</sup> ±1.34	15.16 <sup>bcd</sup> ±1.01	27.34 <sup>cde</sup> ±1.10	26.71 <sup>de</sup> ±1.44	46.95 <sup>ef</sup> ±4.20	50.15 <sup>cdef</sup> ±.61
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	16.74 <sup>a</sup> ±1.78	17.85 <sup>ab</sup> ±0.625	37.69 <sup>ab</sup> ±1.18	27.91 <sup>cde</sup> ±0.96	63.73 <sup>a</sup> ±2.12	58.72 <sup>ab</sup> ±2.11
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	12.42 <sup>bcd</sup> ±2.38	17.09 <sup>abc</sup> ±1.60	31.80 <sup>abcd</sup> ±1.01	27.08 <sup>cde</sup> ±2.04	58.68 <sup>abc</sup> ±1.88	62.28 <sup>a</sup> ±1.30
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	16.21 <sup>ab</sup> ±.83	15.52 <sup>bcd</sup> ±0.65	31.31 <sup>abcd</sup> ±1.80	25.19 <sup>de</sup> ±1.59	46.81 <sup>ef</sup> ±2.75	49.42 <sup>def</sup> ±2.00
<b>T6 (Thiourea-500 ppm) + (Salicylic Acid-300ppm)</b>	12.13 <sup>cde</sup> ±1.32	14.90 <sup>cd</sup> ±0.38	30.15 <sup>bcd</sup> ±5.00	28.18 <sup>cd</sup> ±1.65	60.37 <sup>ab</sup> ±4.32	56.07 <sup>abcd</sup> ±1.58
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	11.79 <sup>cde</sup> ±1.47	13.97 <sup>de</sup> ±1.14	27.83 <sup>cde</sup> ±0.93	23.83 <sup>ef</sup> ±1.42	48.85 <sup>def</sup> ±2.59	47.84 <sup>ef</sup> ±3.23
<b>T8 (Thiourea-500 ppm) + (Salicylic Acid-600ppm)</b>	8.93 <sup>e</sup> ±1.61	11.94 <sup>ef</sup> ±0.64	27.05 <sup>cde</sup> ±6.72	25.08 <sup>de</sup> ±1.91	53.93 <sup>bcd</sup> ±2.95	56.72 <sup>abcd</sup> ±2.86
<b>T9 (Thiourea-2000 ppm) + (Salicylic Acid-150ppm)</b>	15.30 <sup>abc</sup> ±1.03	17.71 <sup>abc</sup> ±0.71	35.34 <sup>abc</sup> ±0.50	33.01 <sup>ab</sup> ±0.79	61.33 <sup>ab</sup> ±1.99	59.26 <sup>ab</sup> ±1.65
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	9.49 <sup>de</sup> ±.91	11.56 <sup>ef</sup> ±1.34	25.19 <sup>de</sup> ±4.03	28.88 <sup>bcd</sup> ±0.71	48.87 <sup>def</sup> ±2.08	50.38 <sup>cdef</sup> ±4.64
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	13.51 <sup>abcd</sup> ±1.62	13.40 <sup>def</sup> ±1.18	31.01 <sup>abcde</sup> ±1.24	31.25 <sup>bc</sup> ±1.61	51.81 <sup>cde</sup> ±3.03	52.15 <sup>bcd</sup> ±2.83
<b>CD</b>	2.347	1.461	5.232	2.556	5.107	4.323
<b>CV</b>	10.702	5.761	10.04	5.411	5.635	4.722

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance

**Figure 4.3. Leaf Area (cm<sup>2</sup>) of Mustard During Rabi 2021-2023 and 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid (300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Leaf Area Index:** The effect of Sulphur and Salicylic acid and their combination on leaf area index was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.4, Figure 4.4). In 2021-2022, there was a significant difference in leaf area index compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the leaf area index was observed at 30, 60, and 90 DAS. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T1, followed by T3, T5, T9, T11, T4, T6, and T7, and the percentage values were 36.66%, 36.45%, 34.36%, 30.47%, 21.26%, 14.33%, 12.33%, 9.77% respectively. But in T2, T10, T8 percentage decrease as compared to T0 and the percentage values were -1.65%, -12.09%, and -19.10%. At 60 DAS, the percentage increase as compared to T0 was found highest in T1 followed by T3, T9, T4, T5, T11, T6, T7, T2, T8, T10 and the percentage values were 43.32%, 40.34%, 36.37%, 29.30%, 28.19%, 27.49%, 25.41%, 19.21%, 17.77%, 16.86%, 10.73% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T9, T6, T4, T1, T8, T11, T10, T7, T2, T5 and the percentage values were 35.31%, 32.77%, 31.71%, 29.75%, 25.74%, 23.56%, 20.43%, 15.64%, 15.60%, 12.19%, 11.92% respectively. In 2022-2023, there was a significant difference in leaf area index compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found highest in T1 followed by T3, T9, T4, T5, T2, T6, T7, T11, T8, T10 and the percentage values were 40.91%, 38.77%, 38.26%, 36.05%, 29.55%, 27.88%, 26.62%, 21.75%, 18.40%, 8.48%, 5.44% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T1 followed by T9, T11, T10, T6, T3, T4, T2, T5, T8, T7 and the percentage values were 44.86%, 40.27%, 36.91%, 31.73%, 30.04%, 29.37%, 27.20%, 26.20%, 21.72%, 21.40%, 17.28% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T4 followed by T9, T3, T1, T8, T6, T11, T10, T2, T5, T7 and the percentage values were 29.49%, 25.90%, 25.21%, 23.82%, 22.58%, 21.68%, 15.79%, 12.84%, 12.44%, 11.14%, 8.20% respectively. Brassica juncea, a botanical species commonly recognised as mustard, assumes significant prominence as a widely cultivated oilseed crop on a global scale. The plant in question is held in high

regard due to its notable contributions to the culinary field, as well as its substantial impact on agro-industries and the production of oil. The examination of various agronomic methodologies and the implementation of treatments by farmers and researchers have been conducted to enhance the productivity and quality of mustard crops. Considerable attention has been directed towards the utilisation of salicylic acid and sulphur in a specific domain. This essay investigates the impact of salicylic acid and sulphur on the leaf area index (LAI) in mustard plants, offering an analysis of the scientific findings and agricultural implications related to these interventions. The leaf area index (LAI) holds significant importance in the field of plant science as it is closely linked to a plant's photosynthetic capacity and overall growth. The leaf area index (LAI) is a metric used to measure the ratio of a plant's total leaf area to the area of ground it occupies. This metric functions as a reliable indicator of the plant's comprehensive vegetative vigour and overall health (Xing et al., 2022; Xu et al., 2023; Xu et al., 2022, 2023; Xu, Cao, et al., 2022; Xu, Zeng, et al., 2022; Yagci & Agar, 2022; Yan et al., 2022). The investigation of the Leaf Area Index (LAI) of mustard plants holds significant importance in evaluating both crop productivity and quality, rendering it a subject of substantial academic interest. Salicylic acid (SA) is a naturally occurring phytohormone that has gained significant recognition for its pivotal role in plant defence mechanisms and stress responses. However, recent research has provided insights into the potential benefits of this practise in augmenting plant growth and bolstering crop productivity. When administered externally, salicylic acid (SA) can induce a range of physiological and biochemical responses in plants, including increased photosynthesis, enhanced nutrient uptake, and improved tolerance to abiotic stressors. The aforementioned effects can have a substantial impact on the Leaf Area Index (LAI) of mustard plants. In recent years, empirical research has yielded evidence suggesting that the application of salicylic acid can significantly enhance the Leaf Area Index (LAI) of mustard plants. Salicylic acid (SA) functions as a signalling molecule that triggers the activation of genes associated with photosynthesis and the synthesis of chlorophyll. Consequently, there is an increase in leaf area as the plant allocates additional resources towards the growth and development of leaves. Furthermore, the application of salicylic acid (SA) has been shown to enhance the plant's capacity to endure adverse environmental conditions, including drought and pathogen-induced



assaults. Consequently, this phenomenon results in an elevation of the leaf area index (LAI) due to its ability to alleviate leaf damage and senescence. Sulphur (S) is a vital element in the growth and development of plants, as it plays a crucial role in various physiological processes. Sulphur is of significant importance as an essential component of amino acids, vitamins, and coenzymes that are involved in diverse metabolic pathways, such as photosynthesis. A deficiency of sulphur in mustard plants has the potential to reduce the Leaf Area Index (LAI) as it hampers the synthesis of chlorophyll and proteins that are crucial for leaf development and overall growth. Prior research has indicated that the application of sulphur to mustard plants has a notable and beneficial impact on the leaf area index (LAI). Sulphur plays a notable role in facilitating the synthesis of crucial biomolecules, including cysteine and methionine, which are essential for the production of chlorophyll and the activation of enzymes involved in the process of photosynthesis. As a result, mustard plants that have undergone sulphur treatment exhibit increased levels of chlorophyll and improved photosynthetic efficiency, resulting in an enlargement of leaf area and overall plant growth. It is important to highlight that the simultaneous application of salicylic acid and sulphur has demonstrated synergistic effects on the leaf area index (LAI) of mustard plants (Samad et al., 2023; Sampedro-Guerrero et al., 2022; Samtani et al., 2022; Shah et al., 2022, 2023; Shang et al., 2022; Sharma & Verma, 2023; Sheikhalipour et al., 2023; Shekhawat et al., 2023). When used together, these substances have a synergistic effect that enhances the positive impact on plant growth and resilience to stress. Systemic acquired resistance (SAR) is a phenomenon that augments the inherent defence mechanisms of plants, thereby enhancing their capacity to efficiently absorb and utilise sulphur. Sulphur, in a reciprocal manner, assumes a supportive function in the modulation of signalling pathways facilitated by salicylic acid (SA), thereby augmenting the plant's ability to withstand both biotic and abiotic stresses. Furthermore, the impact of salicylic acid and sulphur on the leaf area index (LAI) of mustard plants goes beyond simple vegetative growth. The application of these substances to mustard plants has resulted in the augmentation of both flower and seed production, thereby leading to a notable improvement in agricultural productivity. The potential economic implications for mustard farmers can be significant due to the augmentation of leaf area index (LAI), as a greater LAI is correlated with a more profitable crop yield. The

environmental benefits of utilising salicylic acid and sulphur in mustard cultivation warrant careful consideration. Through the augmentation of the Leaf Area Index (LAI) and subsequent enhancement of photosynthetic activity, these interventions possess the capacity to facilitate the process of carbon sequestration and mitigate the emission of greenhouse gases. Furthermore, the utilisation of salicylic acid (SA) and sulphur has the potential to enhance stress tolerance in plants, which could lead to a reduced dependence on chemical pesticides and fertilisers. This, in turn, may encourage the implementation of sustainable and ecologically sound agricultural practises. The application of salicylic acid and sulphur has a substantial impact on the leaf area index in mustard plants, leading to an augmentation of vegetative growth, enhancement of photosynthetic efficiency, and an increase in crop productivity. The implementation of these treatments presents a potentially beneficial strategy for enhancing mustard cultivation, thereby providing notable economic and environmental benefits. The progression of research offers farmers and agricultural scientists' valuable resources to improve mustard production, thereby making a substantial contribution to the global food security. The Leaf Area Index (LAI) holds significant importance in the field of crop science and agriculture due to its fundamental role in understanding and managing crop growth, productivity, and overall health. The Leaf Area Index (LAI) is a quantitative measure employed to assess the leaf area of plants in relation to the area of ground they cover. The recognition of its significance as an indicator of crop performance is widely acknowledged. This study aims to examine the importance of the Leaf Area Index (LAI) in agricultural crops and its relevance within current agricultural methodologies. The relationship between the photosynthetic efficiency of a crop and its Leaf Area Index (LAI) is highly correlated, as the LAI plays a crucial role in determining the crop's ability to capture sunlight and convert it into energy through the process of photosynthesis. Leaves serve as the primary sites for the process of photosynthesis, whereby plants convert light energy into chemical energy. An elevated Leaf Area Index (LAI) signifies a larger proportion of leaf surface area that is available for the absorption of light. As a result, this phenomenon leads to increased levels of photosynthetic activity, promoting enhanced growth and heightened agricultural productivity. The Leaf Area Index (LAI) is a reliable methodology that enables precise prediction of crop yields and efficient monitoring of crops. The utilisation of Leaf Area

Index (LAI) measurements at different growth stages allows farmers and researchers to make informed decisions regarding crop management practises, such as irrigation, fertilisation, and pest management, by estimating the potential harvest. The continuous monitoring of the Leaf Area Index (LAI) throughout the complete growth cycle of plants is advantageous for the timely identification of stress factors, facilitating prompt interventions. There exists a significant correlation between the Leaf Area Index (LAI) and the water requirements of a crop (Shi et al., 2022; Si et al., 2023; Singh et al., 2022; Singh & Roychoudhury, 2023; N. Singh & Nandi, 2022). Crops with a higher Leaf Area Index (LAI) typically demonstrate an increased rate of water transpiration, primarily as a result of an enlarged leaf surface area. The dissemination of this information holds significant importance in the context of optimising irrigation scheduling. Farmers have the ability to improve water management, reduce inefficiencies, and address the challenges of water stress or excessive irrigation through the acquisition of knowledge regarding a crop's Leaf Area Index (LAI). These practises are of utmost importance as they have a detrimental effect on the quality and productivity of the crop. The Leaf Area Index (LAI) is commonly employed as a dependable metric for evaluating the nutritional needs of a crop. Plants exhibiting a higher Leaf Area Index (LAI) require a greater availability of nutrients to support their growth and maintenance. Therefore, the utilisation of Leaf Area Index (LAI) measurements aids in tailoring fertiliser applications to effectively meet the nutritional requirements. The implementation of this targeted strategy serves to alleviate the inefficiency associated with the usage of fertilisers and the potential adverse outcomes resulting from nutrient runoff, thus effectively mitigating the negative environmental impacts. The evaluation of crop health. The examination of alterations in the Leaf Area Index (LAI) can yield valuable insights regarding the general well-being and levels of stress experienced by crops. A reduction in Leaf Area Index (LAI) may suggest the existence of multiple factors, including the presence of diseases, nutrient deficiencies, or water stress. Farmers have the ability to implement remedial measures by promptly identifying these issues, which has the potential to mitigate significant reductions in crop productivity. The incorporation of Leaf Area Index (LAI) data can yield significant insights for crop selection and enable the adjustment of agricultural practises to effectively address the consequences of evolving climatic conditions (Zhao & Hu, 2023; Zhao et al., 2022;

Zhu et al., 2022; Zhu et al., 2023; Zulfiqar et al., 2022). The selection of crops based on their Leaf Area Index (LAI) requirements has the potential to improve resilience to various environmental factors, including temperature, precipitation, and radiation levels. The maintenance of an optimal Leaf Area Index (LAI) is associated with notable ecological advantages, thereby contributing to the overarching objective of environmental sustainability. The presence of ample vegetation cover has been observed to be an effective strategy in mitigating soil erosion, improving soil quality, and promoting the sequestration of carbon dioxide. As a result, it plays a significant role in addressing climate change. Furthermore, crops exhibiting a higher Leaf Area Index (LAI) demonstrate increased resistance to pests and diseases, thereby reducing the need for chemical interventions that could potentially have adverse effects on the environment. Precision agriculture combines leaf area index (LAI) data with modern technologies such as remote sensing and GPS to produce detailed crop maps and enable location-specific management approaches. This practise enhances resource efficiency, diminishes input costs, and optimises crop productivity. Within the realm of research and development, scientists employ the Leaf Area Index (LAI) as a pivotal parameter in the context of crop modelling and simulations. The application of this technology enables the creation of predictive models that can anticipate different aspects of crop cultivation, such as growth patterns, yield estimations, and responses to variations in environmental conditions (Singhal et al., 2023; Sivanesan et al., 2022; Song et al., 2023; Sousa et al., 2022; Spinoso-Castillo & Bello-Bello, 2022). The information provided possesses substantial importance in the advancement of agricultural science and technology. The Leaf Area Index (LAI) plays a pivotal role in crop management and the formulation of agricultural strategies. It has a significant influence on the process of photosynthesis, the regulation of water and nutrient levels, the evaluation of crop vitality, and the capacity to adapt to shifting climatic conditions. The incorporation of technology and sustainable practises in the field of agriculture has resulted in continuous progress and development. The Leaf Area Index (LAI) holds significant importance in the present context as it plays a crucial role in the efficient management of crop production while simultaneously mitigating the environmental impact. The accurate evaluation and efficient application of the Leaf Area Index (LAI) are paramount in modern agriculture, as they are essential for meeting the needs of a

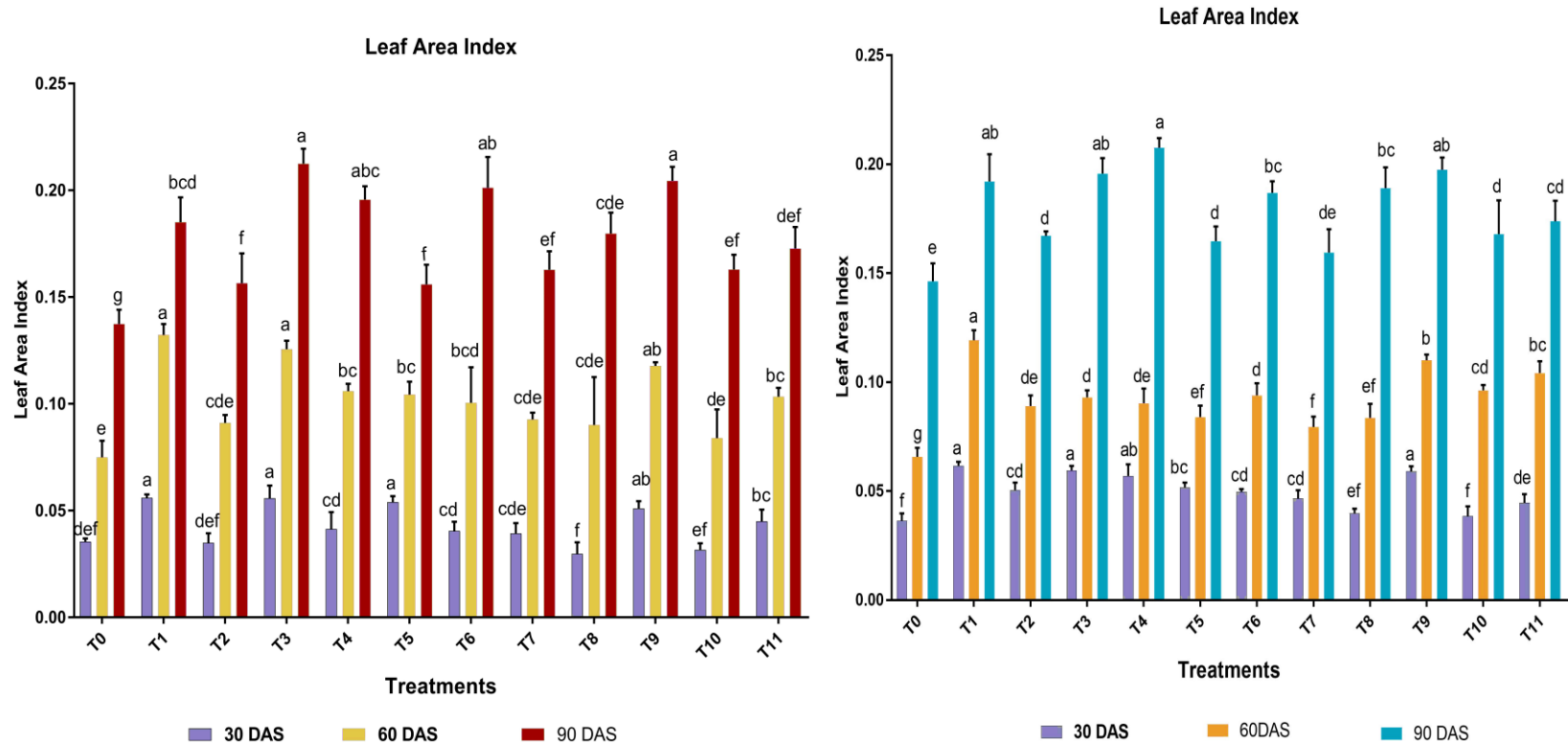
growing global population while safeguarding our limited natural resources (Yuan et al., 2022; Zahid et al., 2023; Zang et al., 2022; Zhang et al., 2023; Zhang et al., 2022).

**Table 4.4. Impact of Different Treatments on Leaf Area Index of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	0.035 <sup>def</sup> ±0.001	0.036 <sup>f</sup> ±0.003	0.075 <sup>e</sup> ±0.007	0.065 <sup>s</sup> ±0.004	0.137 <sup>s</sup> ±0.006	0.146 <sup>e</sup> ±0.008
<b>T1 (Thiourea-1000 ppm)</b>	0.056 <sup>a</sup> ±0.001	0.061 <sup>a</sup> ±0.001	0.132 <sup>a</sup> ±0.005	0.119 <sup>a</sup> ±0.004	0.185 <sup>bcd</sup> ±0.011	0.192 <sup>ab</sup> ±0.012
<b>T2 (Salicylic Acid-300 ppm)</b>	0.034 <sup>def</sup> ±0.004	0.050 <sup>cd</sup> ±0.003	0.091 <sup>cde</sup> ±0.003	0.089 <sup>de</sup> ±0.004	0.156 <sup>f</sup> ±0.014	0.167 <sup>d</sup> ±0.002
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	0.055 <sup>a</sup> ±0.005	0.059 <sup>a</sup> ±0.002	0.125 <sup>a</sup> ±0.003	0.093 <sup>d</sup> ±0.003	0.212 <sup>a</sup> ±0.007	0.195 <sup>ab</sup> ±0.007
<b>T4 (Thiourea-1500 ppm) + (Salicylic Acid-300 ppm)</b>	0.041 <sup>cd</sup> ±0.007	0.057 <sup>ab</sup> ±0.005	0.106 <sup>bc</sup> ±0.003	0.090 <sup>de</sup> ±0.006	0.195 <sup>abc</sup> ±0.006	0.207 <sup>a</sup> ±0.004
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	0.054 <sup>a</sup> ±0.002	0.051 <sup>bc</sup> ±0.002	0.104 <sup>bc</sup> ±0.006	0.084 <sup>ef</sup> ±0.005	0.156 <sup>f</sup> ±0.009	0.164 <sup>d</sup> ±0.006
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	0.040 <sup>cd</sup> ±0.004	0.049 <sup>cd</sup> ±0.001	0.100 <sup>bcd</sup> ±0.016	0.094 <sup>d</sup> ±0.005	0.201 <sup>ab</sup> ±0.014	0.186 <sup>bc</sup> ±0.005
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	0.039 <sup>cde</sup> ±0.004	0.046 <sup>cd</sup> ±0.003	0.092 <sup>cde</sup> ±0.003	0.079 <sup>f</sup> ±0.004	0.162 <sup>ef</sup> ±0.008	0.159 <sup>de</sup> ±0.010
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	0.029 <sup>f</sup> ±0.005	0.039 <sup>ef</sup> ±0.002	0.090 <sup>cde</sup> ±0.022	0.083 <sup>ef</sup> ±0.006	0.179 <sup>cde</sup> ±0.009	0.189 <sup>bc</sup> ±0.009
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	0.051 <sup>ab</sup> ±0.003	0.059 <sup>a</sup> ±0.002	0.117 <sup>ab</sup> ±0.001	0.110 <sup>b</sup> ±0.002	0.204 <sup>a</sup> ±0.006	0.197 <sup>ab</sup> ±0.005
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	0.031 <sup>ef</sup> ±0.003	0.038 <sup>f</sup> ±0.004	0.084 <sup>de</sup> ±0.013	0.096 <sup>cd</sup> ±0.002	0.162 <sup>ef</sup> ±0.006	0.167 <sup>d</sup> ±0.015
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	0.045 <sup>bc</sup> ±0.005	0.044 <sup>de</sup> ±0.003	0.103 <sup>bc</sup> ±0.004	0.104 <sup>bc</sup> ±0.005	0.172 <sup>def</sup> ±0.010	0.173 <sup>cd</sup> ±0.001
<b>CD</b>	0.008	0.005	0.017	0.009	0.017	0.014
<b>CV</b>	10.702	5.762	10.040	5.411	5.636	4.722

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance

**Figure 4.4. Leaf Area Index of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm);

**T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).**



**Node number:** The effect of Sulphur and Salicylic acid and their combination on Node number was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.5, Figure 4.5). In 2021-2022, there was a significant difference in node number compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the node number was observed at 30, 60, and 90 DAS. Therefore, at 30 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T3, T9, T6, T8, T2, T4, T10, T1, T7, T5 and the percentage values were 53.84%, 50%, 50%, 45.45%, 45.45%, 40%, 40%, 40%, 33.33%, 33.33%, 25% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T6, T2, T9, T1, T3, T8, T4, T7, T10, T5 and the percentage values were 38.46%, 28.57%, 24.52%, 23.07%, 18.36%, 18.36%, 18.36%, 16.66%, 13.04%, 11.11%, 9.09% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T8, T9, T6, T2, T3, T1, T4, T7, T10, T5 and the percentage values were 33.85%, 26.31%, 24.32%, 23.63%, 22.22%, 20.75%, 20%, 16%, 14.28%, 12.5%, 9.67% respectively. In 2022-2023, there was a significant difference in node number compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found highest in T8 and T11 followed by T5, T7, T1, T6, T9, T10, T2, T3 and the percentage values were 46.15%, 46.15%, 41.66%, 36.36%, 29.99%, 29.99%, 29.99%, 29.99%, 22.22%, 22.22% respectively. But in T4, there was no impact of treatment, and the value is also the same as T0. At 60 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T6, T9, T3, T2, T8, T4, T1, T10, T5, T7 and the percentage values were 31.03%, 21.56%, 21.56%, 20%, 16.66%, 16.66%, 14.89%, 13.04%, 9.09%, 4.76%, 2.43% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T8, T6, T3, T9, T1, T4, T2, T5, T10, T7 and the percentage values were 37.12%, 30.83%, 27.82%, 24.54%, 24.54%, 23.85%, 23.14%, 20.95%, 15.30%, 13.54%, 11.70% respectively. The parameter referred to as "node number" is important in determining plants' architectural structure and growth patterns. This parameter signifies the quantity of distinct attachment points for leaves, stems, or branches along

the primary stem. A comprehensive understanding of the intricate relationship between factors affecting the total number of nodes in Indian mustard (*Brassica juncea* L.) is crucial for improving crop productivity. Sulphur (S) and salicylic acid (SA) are widely recognised as influential factors due to their significant contributions in shaping plant growth and development. This study examines the observable effects of sulphur and salicylic acid on the nodulation process in Indian mustard. The objective is to uncover the intricate mechanisms involved and explore the potential implications for agricultural practices. Sulphur, a crucial macronutrient for plant life, can multifacetedly influence the number of nodes within a network. This influence is demonstrated through various manifestations, including the following: Sulphur is a crucial constituent in synthesising amino acids, specifically cysteine and methionine, and assumes a particularly significant role in this biochemical pathway. The amino acids, which play a crucial role as the basic constituents of proteins, also impact the initiation of nodes and subsequent developmental processes (Kochanek et al., 2023; Kosakivska et al., 2022; Kour et al., 2023; Kudoyarova, 2022; Kumar & Ohri, 2023; Kurepa & Smalle, 2022). The sulphur availability directly affects protein synthesis, which regulates the intricate node formation and differentiation processes. Amino acids possessing a high concentration of sulphur have been identified as indispensable cofactors for enzymes involved in a diverse range of metabolic pathways. Enzymes activated by sulphur play a crucial role in coordinating hormonal processes, facilitating nutrient assimilation, and regulating cell division. These processes are closely interconnected with the determination of node count. Furthermore, sulphur substantially influences the regulation of node number by participating in the synthesis and metabolism of crucial plant hormones, such as auxins and cytokinin. The harmonious balance of hormonal players emerges as a crucial determining factor in initiating nodes and the subsequent development of branches and leaves. Salicylic acid, a compound with notable historical importance in the modulation of plant defence mechanisms, demonstrates a broader array of functions, including regulating node number. Several notable aspects characterise this phenomenon: Salicylic Acid (SA) can effectively modulate the expression of genes that are pivotal in growth and development processes, particularly those that substantially influence node number determination. The activation of specific transcription factors is induced by salicylic acid (SA). Subsequently, these transcription

factors exert their regulatory influence over genes' expression in initiating node formation and branching development. The scientific community has provided evidence indicating that SA can stimulate cell division and enlargement in various plant tissues (Zhao & Hu, 2023; Zhao et al., 2022; Zhu et al., 2022; Zhu et al., 2023; Zulfiqar et al., 2022). This particular ability manifests as a noticeable enhancement of lateral branch and leaf development, leading to a rise in the number of nodes within the specified context. Although the primary purpose of SA is defence-oriented, its distinctive stress-responsive properties have been found to play a significant role in modulating node numbers. When plants are subjected to lower stress levels due to the protective shield provided by SA-mediated defences, they can allocate a greater proportion of their resources towards growth processes, such as the formation of nodes and branches. The concurrent application of sulphur and salicylic acid on Indian mustard plants results in a synergistic interaction, leading to a potent amalgamation of effects that significantly influence the overall node count in the plant. Sulphur plays a significant role in facilitating the developmental progression of nodes by serving as a potent facilitator of nutrient absorption, including essential elements such as nitrogen and phosphorus (Kurniawan & Chuang, 2022; Kuromori et al., 2022; Lajayer et al., 2022; Li et al., 2022; Li, Ren, et al., 2023). Furthermore, applying salicylic acid enhances nutrient absorption, leading to an augmented nutrient reservoir that facilitates the development of nodes and the proliferation of branches. The coalescence of sulphur and salicylic acid engenders an environment distinguished by the intricate interplay of hormones. The observed augmentation in the number of nodes can be attributed to a collaborative endeavour involving sulphur, which exhibits a propensity to enhance the synthesis of auxin, and salicylic acid, which promotes the transport and sensitivity of auxin. The inclusion of salicylic acid within the growth medium has been found to have a substantial impact on the ability of plants to withstand and adapt to stressful conditions, ultimately facilitating their overall growth. The sulphur partnership ensures the availability of essential constituents required for synthesising stress-responsive compounds, such as glutathione. Glutathione, an antioxidant with notable implications for stress mitigation, is made more accessible. Considering both stress resistance and vigorous growth, the collaborative effort creates an environment that is particularly favourable for significant augmentation in the number of nodes. The total number of

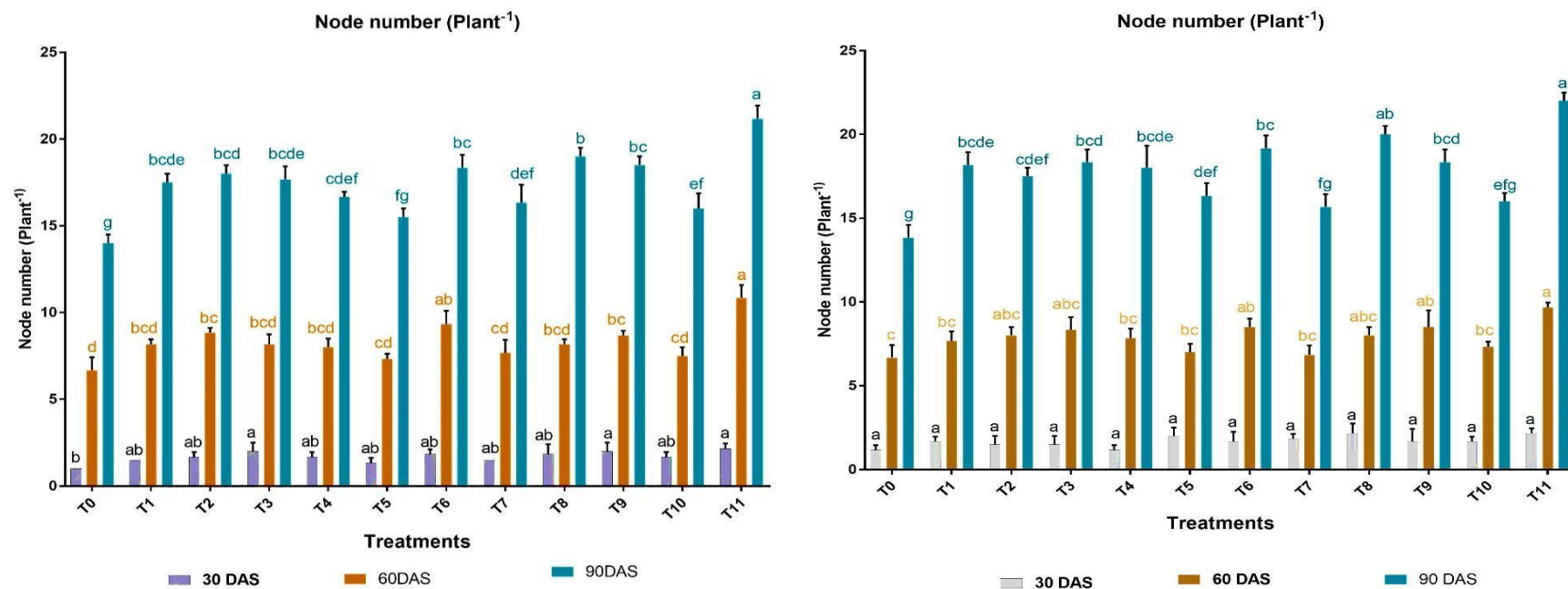
nodes in Indian mustard (*Brassica juncea* L.) is significantly influenced by complex mechanisms governed by sulphur and salicylic acid. Sulphur is highly regarded for its indispensable role as a non-replaceable nutrient (Yuan et al., 2022; Zahid et al., 2023; Zang et al., 2022; Zhang et al., 2023; Zhang et al., 2022).

**Table 4.5. Impact of Different Treatments on Node Number of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	1.00 <sup>b</sup> ±0.00	1.16 <sup>a</sup> ±0.28	6.66 <sup>d</sup> ±0.76	6.66 <sup>c</sup> ±0.76	14.00 <sup>e</sup> ±0.50	13.83 <sup>e</sup> ±0.76
<b>T1 (Thiourea-1000 ppm)</b>	1.50 <sup>ab</sup> ±0.00	1.66 <sup>a</sup> ±0.28	8.16 <sup>bcd</sup> ±0.28	7.66 <sup>bc</sup> ±0.57	17.50 <sup>bcd</sup> ±0.50	18.16 <sup>bcd</sup> ±0.76
<b>T2 (Salicylic acid-300 ppm)</b>	1.66 <sup>ab</sup> ±0.28	1.50 <sup>a</sup> ±0.50	8.83 <sup>bc</sup> ±0.28	8.00 <sup>abc</sup> ±0.50	18.00 <sup>bcd</sup> ±0.50	17.50 <sup>cdef</sup> ±0.50
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	2.00 <sup>a</sup> ±0.50	1.50 <sup>a</sup> ±0.50	8.16 <sup>bcd</sup> ±0.57	8.33 <sup>abc</sup> ±0.76	17.66 <sup>bcd</sup> ±0.76	18.33 <sup>bcd</sup> ±0.76
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	1.66 <sup>ab</sup> ±0.28	1.16 <sup>a</sup> ±0.28	8.00 <sup>bcd</sup> ±0.50	7.83 <sup>bc</sup> ±0.57	16.66 <sup>cdef</sup> ±0.28	18.00 <sup>bcd</sup> ±1.32
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	1.33 <sup>ab</sup> ±0.28	2.00 <sup>a</sup> ±0.50	7.33 <sup>cd</sup> ±0.28	7.00 <sup>bc</sup> ±0.50	15.50 <sup>fg</sup> ±0.50	16.33 <sup>def</sup> ±0.76
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	1.83 <sup>ab</sup> ±0.28	1.66 <sup>a</sup> ±0.57	9.33 <sup>ab</sup> ±0.76	8.50 <sup>ab</sup> ±0.50	18.33 <sup>bc</sup> ±0.76	19.16 <sup>bc</sup> ±0.76
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	1.50 <sup>ab</sup> ±0.00	1.83 <sup>a</sup> ±0.28	7.66 <sup>cd</sup> ±0.76	6.83 <sup>bc</sup> ±0.57	16.33 <sup>def</sup> ±1.04	15.66 <sup>g</sup> ±0.76
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	1.83 <sup>ab</sup> ±0.57	2.16 <sup>a</sup> ±0.57	8.16 <sup>bcd</sup> ±0.28	8.00 <sup>abc</sup> ±0.50	19.00 <sup>b</sup> ±0.50	20.00 <sup>ab</sup> ±0.50
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	2.00 <sup>a</sup> ±0.50	1.66 <sup>a</sup> ±0.76	8.66 <sup>bc</sup> ±0.28	8.50 <sup>ab</sup> ±1.00	18.50 <sup>bc</sup> ±0.50	18.33 <sup>bcd</sup> ±0.76
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	1.66 <sup>ab</sup> ±0.28	1.66 <sup>a</sup> ±0.28	7.50 <sup>cd</sup> ±0.50	7.33 <sup>bc</sup> ±0.28	16.00 <sup>ef</sup> ±0.86	16.00 <sup>efg</sup> ±0.50
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	2.16 <sup>a</sup> ±0.28	2.16 <sup>a</sup> ±0.28	10.83 <sup>a</sup> ±0.76	9.66 <sup>a</sup> ±0.28	21.16 <sup>a</sup> ±0.76	22.00 <sup>a</sup> ±0.50
<b>CD</b>	0.573	N/A	0.922	0.857	1.084	1.301
<b>CV</b>	20.003	28.09	6.538	6.400	3.658	4.294

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.5. Node Number of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm);

**T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).**

**Internodal length-** The effect of Sulphur and Salicylic acid and their combination on Internodal length was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 60, 90 and 120 days after sowing (DAS) (Table 4.6, Figure 4.6). In 2021-2022, there was a significant difference in internodal length compared to T0 (Control) at 60, 90 and 120 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the internodal length was observed at 60, 90 and 120 DAS. Therefore, at 60 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T8, T2, T3, T9, T7, T10, T1, T6, T5 and the percentage values were 34.91%, 29.71%, 26.97%, 24.06%, 22.69%, 18.81%, 17.43%, 14.72%, 7.85%, 3.44% respectively. However, the percentage is decreased in T4 compared to T0, and the percentage value is -9.38%. At 90 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T2, T8, T3, T1, T10, T9, T4, T6, T5, T7 and the percentage values were 38.83%, 32.81%, 31.72%, 31.54%, 26.83%, 18.30%, 18.05%, 13.52%, 10.76%, 6.82% respectively. At 120DAS, the percentage increase as compared to T0 was found highest in T11 followed by T2, T1, T8, T3, T9, T10, T6, T4, T5, T7 and the percentage values were 38.78%, 37.06%, 34.56%, 33.46%, 31.42%, 21.51%, 21.31%, 17.75%, 14.67%, 11.94%, 6.20% respectively. In 2022-2023, there was a significant difference in internodal length compared to T0 (Control) at 60, 90, and 120 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 60 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T8, T2, T9, T3, T6, T10, T7, T4, T1, T5 and the percentage values were 36.23%, 33.39%, 27.53%, 25.61%, 23.42%, 22.54%, 22.21%, 19.17%, 17.11%, 11.81%, 3.49% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T2, T8, T1, T3, T9, T5, T10, T6, T4, T7 and the percentage values were 38.34%, 35.78%, 32.17%, 31.58%, 29.68%, 22.55%, 19.37%, 16.93%, 15.96%, 13.40%, 10.40% respectively. At 120 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T2, T1, T3, T8, T9, T10, T6, T4, T5, T7 and the percentage values were 37.73%, 37.38%, 34.08%, 30.42%, 27.86%, 22.85%, 22.11%, 19.75%, 17.42%, 16.15%, 15.84% respectively. The length of internodes plays a crucial role in determining the structural arrangement of plants, thereby influencing their growth patterns and ultimately affecting the productivity of crops. Comprehending the



factors that impact internodal length is imperative for optimising agricultural practices. The roles of sulphur (S) and salicylic acid (SA) in plant growth and development have been recognised. However, there is a lack of research on their specific effects on the internodal length of mustard (*Brassica juncea* L.). This study comprehensively analyses the molecular, physiological, and metabolic mechanisms through which sulphur (S) and salicylic acid (SA) modulate the internodal length in mustard plants. This study aims to offer valuable insights that can be utilised to enhance crop management strategies and augment mustard crop yield by elucidating the underlying mechanisms involved. The measurement of internodal length, which refers to the spacing between consecutive nodes along the primary stem, plays a significant role in influencing the overall development and the plant's vertical stature and branching pattern. The manipulation of internodal space length can influence plant density, light interception, and nutrient distribution, thereby impacting crop yield. Sulphur (S) and salicylic acid (SA) can influence plant growth and development. Further investigation is needed to determine the specific effects of these factors on the internodal length of mustard plants. This comprehensive investigation examines the intricate mechanisms by which S and SA impact the internodal length of the mustard plant to elucidate the underlying processes involved. We aim to establish a scientific basis for enhancing plant architecture and increasing mustard crop yield. This is achieved through the elucidation of the underlying mechanisms involved. Sulphur is an essential macronutrient for plants, and its impact on internode elongation can be attributed to various mechanisms. Amino acids such as cysteine and methionine are reliant on the presence of sulphur, an indispensable element. Including these specific amino acids is essential for the intricate process of protein synthesis, a fundamental mechanism that plays a crucial role in cellular proliferation and development (Li, Zhang, et al., 2022; Li, Zheng, et al., 2023; Li, Han, et al., 2022; Li, He, et al., 2023; Li et al., 2022; Li, Han, et al., 2023; Li, Huang, et al., 2022). The processes of cell expansion and internodal elongation are reliant on adequate protein synthesis. This biological mechanism necessitates the presence of an ample supply of sulphur within the cellular environment. A subset of amino acids with sulphur atoms function as cofactors for enzymes involved in various metabolic pathways. The enzymatic processes involved in cell wall modification, which directly impact internodal length, are significantly influenced by the activation of enzymes

through the presence of sulphur. The internodal length of plants is indirectly influenced by sulphur through regulating hormonal equilibrium. The examined process significantly impacts the synthesis and translocation of hormones, specifically auxins and gibberellins, which are pivotal in the regulation of cellular elongation and internode length. Salicylic acid is a plant hormone predominantly linked to defensive reactions, although it also influences the elongation of internodal spaces. The regulation of gene expression related to growth and development can be influenced by salicylic acid (SA). These genes encompass those that influence the elongation of internodes. Salicylic acid (SA) can regulate the activity of genes that are involved in the process of cell expansion and internodal growth. This regulation occurs through the influence of SA on specific transcription factors (Poór et al., 2022; Quesada, 2022; Rai & Kaushik, 2023; Raja Gopalan et al., 2022; Ramakrishnan & Zhou, 2022; Rani et al., 2023; Rizvi et al., 2022; Roussos, 2023; Ruidas et al., 2022). The growth and division of plant cells are stimulated by salicylic acid (SA) in various plant tissues. Previous studies have demonstrated that salicylic acid (SA) in internodes play a role in stimulating cellular elongation, thereby leading to an augmentation in the length of internodes. The involvement of the sympathetic-adrenal (SA) axis in the physiological stress response may exert an indirect influence on the length of internodes. When plants experience reduced stress levels due to SA-mediated defences, they can allocate a larger proportion of their energy and nutrient resources towards growth-related processes. One of the processes involved in this phenomenon is known as internodal elongation. The potential synergistic impact of the interaction between sulphur and salicylic acid on the elongation of the internodal space is worth investigating. The facilitation of nutrient uptake, specifically nitrogen and phosphorus, crucial for the elongation of internodes, is supported by sulphur, which additionally promotes internodal growth. Research has demonstrated that applying salicylic acid can enhance the efficacy of nutrient absorption, resulting in a greater availability of nutrients for internodal growth. Combining sulphur and salicylic acid can lead to intricate interactions among hormonal processes. The promotion of cell elongation and internodal growth can be facilitated by the influence of sulphur on the synthesis of auxin, as well as the impact of salicylic acid (SA) on auxin transport and sensitivity. The presence of salicylic acid (SA) in the growth environment has the potential to enhance a plant's stress tolerance. Sulphur

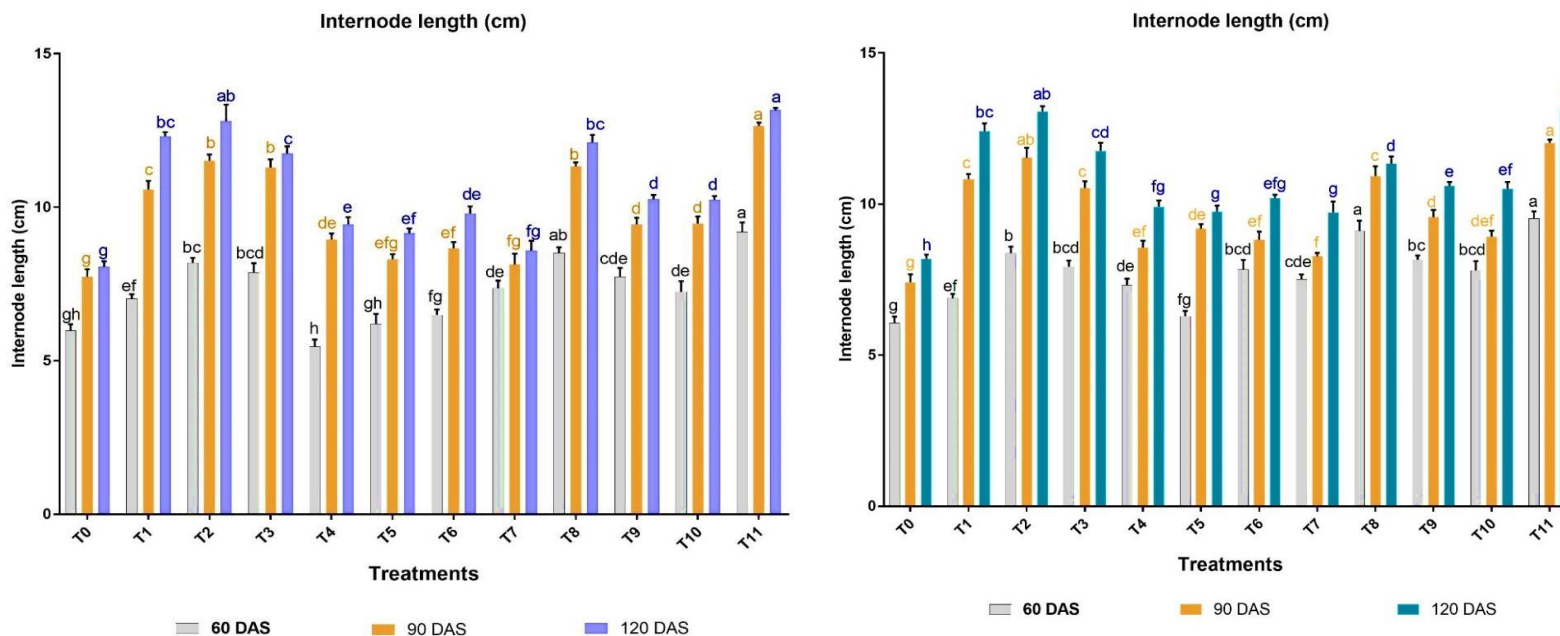
plays a crucial role in facilitating the availability of essential components for stress-related compounds, such as glutathione, which serves as an antioxidant within the human body (Peng et al., 2022; Pérez-Llorca et al., 2023; Phokas et al., 2023; Phour & Sindhu, 2022). The concurrent presence of stress resistance and growth stimulation fosters an environment conducive to augmenting internodal length. The internodal length of the mustard plant (*Brassica juncea* L.) is regulated by intricate mechanisms influenced by sulphur and salicylic acid. Sulphur directly impacts the internodal elongation of plants using amino acid synthesis, enzyme activation, and hormone regulation. Salicylic acid, a compound recognised primarily for its role in defence mechanisms, influences the length between nodes by modulating the expression of genes associated with growth, promoting cellular expansion, and augmenting cellular resilience to stressors. The combined application of sulphur and salicylic acid to mustard plants exhibits a synergistic effect. The observed effects encompass enhanced absorption of nutrients, intercellular communication through hormones, improved ability to withstand stress, and, ultimately, the elongation of internodes. A comprehensive comprehension of these mechanisms in mustard cultivation establishes a robust scientific basis for enhancing plant architecture and maximising crop yield. The potential discovery of novel approaches to enhance mustard crop productivity may arise from additional research on the precise molecular mechanisms underlying the interactions between sulphur and salicylic acid (Zhao et al., 2022; Zhu et al., 2022; Phour & Sindhu, 2022; Poór et al., 2022; Quesada, 2022; Rai & Kaushik, 2023; Raja Gopalan et al., 2022; Ramakrishnan & Zhou, 2022).

**Table 4.6. Impact of Different Treatments on Internodal Length of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	60 DAS		90 DAS		120 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	5.98 <sup>gh</sup> ±0.20	6.07 <sup>g</sup> ±0.20	7.73 <sup>g</sup> ±0.24	7.40 <sup>g</sup> ±0.27	8.06 <sup>g</sup> ±0.18	8.18 <sup>h</sup> ±0.14
<b>T1 (Thiourea-1000 ppm)</b>	7.01 <sup>ef</sup> ±0.15	6.88 <sup>ef</sup> ±0.14	10.57 <sup>c</sup> ±0.29	10.82 <sup>c</sup> ±0.17	12.31 <sup>bc</sup> ±0.12	12.41 <sup>bc</sup> ±0.26
<b>T2 (Salicylic acid-300 ppm)</b>	8.19 <sup>bc</sup> ±0.15	8.37 <sup>b</sup> ±0.21	11.51 <sup>b</sup> ±0.20	11.53 <sup>ab</sup> ±0.33	12.80 <sup>ab</sup> ±0.53	13.06 <sup>ab</sup> ±0.17
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	7.88 <sup>bcd</sup> ±0.30	7.92 <sup>bcd</sup> ±0.20	11.29 <sup>b</sup> ±0.27	10.53 <sup>c</sup> ±0.22	11.75 <sup>c</sup> ±0.22	11.75 <sup>cd</sup> ±0.27
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	5.47 <sup>h</sup> ±0.22	7.32 <sup>de</sup> ±0.20	8.94 <sup>de</sup> ±0.20	8.55 <sup>ef</sup> ±0.23	9.44 <sup>e</sup> ±0.23	9.90 <sup>fg</sup> ±0.21
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	6.19 <sup>gh</sup> ±0.33	6.29 <sup>fg</sup> ±0.17	8.30 <sup>efg</sup> ±0.17	9.18 <sup>de</sup> ±0.15	9.15 <sup>ef</sup> ±0.15	9.75 <sup>g</sup> ±0.19
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	6.49 <sup>fg</sup> ±0.17	7.83 <sup>bcd</sup> ±0.31	8.66 <sup>ef</sup> ±0.20	8.81 <sup>ef</sup> ±0.27	9.80 <sup>de</sup> ±0.22	10.19 <sup>efg</sup> ±0.11
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	7.37 <sup>de</sup> ±0.23	7.51 <sup>cde</sup> ±0.15	8.13 <sup>fg</sup> ±0.35	8.26 <sup>f</sup> ±0.11	8.59 <sup>fg</sup> ±0.31	9.72 <sup>g</sup> ±0.36
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	8.51 <sup>ab</sup> ±0.18	9.11 <sup>a</sup> ±0.33	11.32 <sup>b</sup> ±0.13	10.92 <sup>bc</sup> ±0.33	12.11 <sup>bc</sup> ±0.24	11.34 <sup>d</sup> ±0.23
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	7.74 <sup>cde</sup> ±0.28	8.16 <sup>bc</sup> ±0.14	9.43 <sup>d</sup> ±0.22	9.56 <sup>d</sup> ±0.24	10.27 <sup>d</sup> ±0.13	10.60 <sup>e</sup> ±0.13
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	7.24 <sup>de</sup> ±0.35	7.80 <sup>bcd</sup> ±0.30	9.46 <sup>d</sup> ±0.23	8.91 <sup>def</sup> ±0.20	10.24 <sup>d</sup> ±0.12	10.50 <sup>ef</sup> ±0.23
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	9.19 <sup>a</sup> ±0.31	9.52 <sup>a</sup> ±0.23	12.64 <sup>a</sup> ±0.11	12.01 <sup>a</sup> ±0.12	13.16 <sup>a</sup> ±0.07	13.13 <sup>a</sup> ±0.26
<b>CD</b>	0.412	0.367	0.396	0.415	0.37	0.378
<b>CV</b>	3.3	2.786	2.36	2.505	2.042	2.038

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.6. Internodal Length(cm) of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm);

**T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).**

**Number of Primary Branches:** The effect of Sulphur and Salicylic acid and their combination on the number of primary branches was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 60, 90 and 120 days after sowing (DAS) (Table 4.7, Figure 4.7). In 2021-2022, there was a significant difference in the number of primary branches compared to T0 (Control) at 60, 90 and 120 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the number of primary branches was observed at 60, 90 and 120 DAS. Therefore, at 60 DAS, the percentage increase as compared to T0 was found to be highest in T9, followed by T8, T11, T6, T1, T10, T3, T7, T4, T2, and the percentage values were 45.45%, 36.84%, 33.33%, 33.33%, 25%, 25%, 20%, 20%, 14.28%, 7.69% respectively. But in T5, there was no impact of treatment, and the value is also the same as T0. At 90 DAS, the percentage increase as compared to T0 was found highest in T8 followed by T11, T10, T6, T9, T4, T7, T5, T2, T3, T1 and the percentage values were 51.66%, 49.12%, 46.29%, 43.13%, 42%, 40.81%, 40.81%, 39.58%, 34.09%, 32.55%, 30.95% respectively. At 120 DAS, the percentage increase as compared to T0 was found highest in T8 followed by T9, T11, T2, T10, T6, T1, T7, T4, T3, T5 and the percentage values were 47.05%, 45.45%, 44.61%, 44.61%, 40%, 38.98%, 36.84%, 35.71%, 33.33%, 32.07%, 32.07% respectively. In 2022-2023, there was a significant difference in the number of primary branches compared to T0 (Control) at 60, 90 and 120 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 60 DAS, the percentage increase as compared to T0 was found to be highest in T11 and T9, followed by T6, T8, T3, T7, T1, and T10, and the percentage values were 31.57%, 31.57%, 27.77%, 27.77%, 23.52%, 18.74%, 13.33%, 13.33% respectively. But there was no impact on treatment in T2, T4, and T5, and the values were the same as in T0. At 90 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T8, T9, T6, T10, T4, T5, T7, T3, T2, T1 and the percentage values were 48.33%, 47.45%, 44.64%, 39.21%, 39.21%, 36.73%, 34.04%, 32.60%, 31.11%, 27.90%, 20.51% respectively. At 120 DAS, the percentage increase as compared to T0 was found highest in T8 followed by T9, T11, T2, T6, T10, T3, T4, T5, T1, T7 and the percentage values were 48.61%, 44.77%, 43.93%, 38.33%, 38.33%, 37.5%, 36.20%, 35.08%, 35.08%, 30.18%, 28.84% respectively. The architectural configuration of the

mustard plant (*Brassica juncea* L.) is significantly influenced by primary branches, which play crucial roles in determining overall growth, development, and yield. The primary branches of the mustard plant are fundamental constituents and play a crucial role in the overall structure and functioning of the plant. Investigating the importance of primary components and comprehending their reactions to external stimuli, such as sulphur (S) and salicylic acid (SA), is fundamental to enhancing mustard cultivation and augmenting crop productivity. These factors can exert a direct influence on the mustard plant. The primary branches directly impact the plant's reproductive capacity as they are significant sites for initiating flower and subsequent pod development (Zhao et al., 2022; Zhu et al., 2022). The degree to which plants can undergo flowering and seed production directly correlates with their numerical abundance. Moreover, these principal divisions significantly facilitate a substantial portion of the plant's capacity to undergo photosynthesis. This phenomenon is facilitated by the extensive leaf surfaces on these branches, which exhibit high efficiency in capturing solar radiation. The heightened photosynthetic activity ultimately leads to augmented energy production and improved nutrient assimilation, contributing to sustained growth. The principal ramifications of the plant are also implicated in the process of nutrient allocation within the plant. The branches of a plant serve as conduits, facilitating the transportation of water, minerals, and vital nutrients from the root system to the reproductive organs and foliage. Ensuring a sufficient availability of nutrients is imperative for promoting optimal vegetative growth and facilitating successful seed formation. Furthermore, the principal divisions of the plant are essential for upholding the plant's structural stability. They assume the crucial role of redistributing the weight of the plant, thus preventing lodging, which is a state characterised by the undesired bending or collapsing of the plant. The importance of this structural support is greatly enhanced during the critical stages of flowering and seed maturation. The growth and formation of primary branches in mustard plants are significantly influenced by sulphur, a crucial macronutrient for mustard plants (Rani et al., 2023; Rizvi et al., 2022; Roussos, 2023; Ruidas et al., 2022; Rybczyński et al., 2022; Sachan & Krishna, 2022; Salih, Wu, et al., 2022; Salih, Zhou, et al., 2022; Salwan et al., 2023LAI). The synthesis of amino acids represents a pivotal process within the intricate network of mechanisms regulated by sulphur. Sulphur is found to be a vital constituent of amino acids such as cysteine and methionine, which



play a crucial role as fundamental constituents in protein synthesis. These proteins significantly influence the primary branching process, as they are involved in its initiation and growth. The involvement of sulphur is not restricted solely to the synthesis of amino acids; instead, it encompasses the stimulation of enzymes employed in diverse metabolic pathways. Enzymes that activate in the presence of sulphur significantly impact hormonal regulation, cellular division processes, and nutrient absorption (Mukhtar et al., 2023; Myers Jr. et al., 2023; Nadeem, 2022; Nam et al., 2023; Napieraj et al., 2023). The development of primary branches is inherently interconnected with all of these processes. The impact of sulphur extends beyond its primary effects, encompassing plant hormones such as auxins and cytokinins. The macronutrient in question plays a crucial role in regulating the synthesis and metabolism of hormones, which impacts the delicate balance required to initiate primary branch growth and development. Similarly, salicylic acid, renowned for orchestrating plants' defensive responses, significantly impacts the main branches. Salicylic acid exerts precise regulatory control over gene expression, thereby influencing the genes associated with growth and development, including those responsible for initiating primary branching and extending pre-existing primary branching. The influence of this phenomenon extends to the activation of specific transcription factors, which are crucial in regulating the patterns of gene expression that delineate the progression of fundamental components. Furthermore, salicylic acid can induce cellular division and promote cellular expansion in diverse plant tissues. This characteristic results in a notable facilitation of branching and elongation in the context of primary branches, which can be perceived as a favourable attribute. The stress-responsive properties of salicylic acid have notable impacts on primary constituents, albeit through an indirect mechanism. When plants experience reduced stress levels due to the protective shield provided by SA-mediated defences, a subsequent redistribution of resources occurs (Rani et al., 2023; Rizvi et al., 2022; Roussos, 2023; Ruidas et al., 2022; Rybczyński et al., 2022; Sachan & Krishna, 2022; Salih, Wu, et al., 2022; Salih, Zhou, et al., 2022; Salwan et al., 2023). Reallocating resources entails an augmentation of investments in providing energy and nutrients, which carries substantial implications in primary branch development. The combination of sulphur and salicylic acid induces synergistic effects, resulting in noticeable alterations in the primary branches of mustard

plants. In this particular context, using a combination of sulphur and salicylic acid facilitates the augmentation of nutrient absorption. Sulphur is known to significantly facilitate the uptake of essential nutrients such as nitrogen and phosphorus, which are crucial for developing primary branches. The inclusion of salicylic acid enhances this process, leading to enhanced efficacy in the absorption of nutrients. Simultaneously, the mutual influence between sulphur and salicylic acid engenders an intricate intercellular communication network characterised by the interplay of various signalling molecules. The inclusion of Sulphur promotes the facilitation of auxin synthesis, while simultaneously, salicylic acid enhances both auxin transport and sensitivity (Parada et al., 2022; Parrey et al., 2023; Patel et al., 2022; Peng et al., 2022; Pérez-Llorca et al., 2023; Phokas et al., 2023; Phour & Sindhu, 2022; Poór et al., 2022; Quesada, 2022; Rai & Kaushik, 2023; Raja Gopalan et al., 2022; Ramakrishnan & Zhou, 2022). The effective incorporation of these elements ultimately facilitates the stimulation of cellular proliferation and expansion, thereby yielding a notable augmentation of primary branch development. Furthermore, the inclusion of salicylic acid in the growth medium leads to an augmentation in the plant's resilience to adverse conditions. Furthermore, sulphur plays a crucial role in facilitating the availability of essential elemental components required for synthesising stress-responsive compounds, including antioxidants (Samad et al., 2023; Sampedro-Guerrero et al., 2022; Samtani et al., 2022; Shah et al., 2022, 2023; Shang et al., 2022; Sharma & Verma, 2023; Shekhalipour et al., 2023; Shekhawat et al., 2023). A notable illustration of such a compound is glutathione. The confluence of heightened stress resilience and robust proliferation within this alliance engenders a milieu that is especially conducive to the maturation of principal appendages. When considering the development and efficiency of a mustard plant, primary branches hold a significant role in the framework. Both sulphur and salicylic acid possess intricate mechanisms that enable them to elucidate their effects on the growth of primary branches. The influence of sulphur on primary processes is primarily manifested through the production of amino acids, the activation of enzymes, and the regulation of hormones. Historically recognised for its involvement in defence mechanisms, salicylic acid exhibits a broader impact by modulating growth-related gene expression and cellular expansion, thereby influencing primary branch development (Nasir & Toth, 2022; Negi & Kumar, 2023; Nimsi et al., 2023; Niu & Fu,

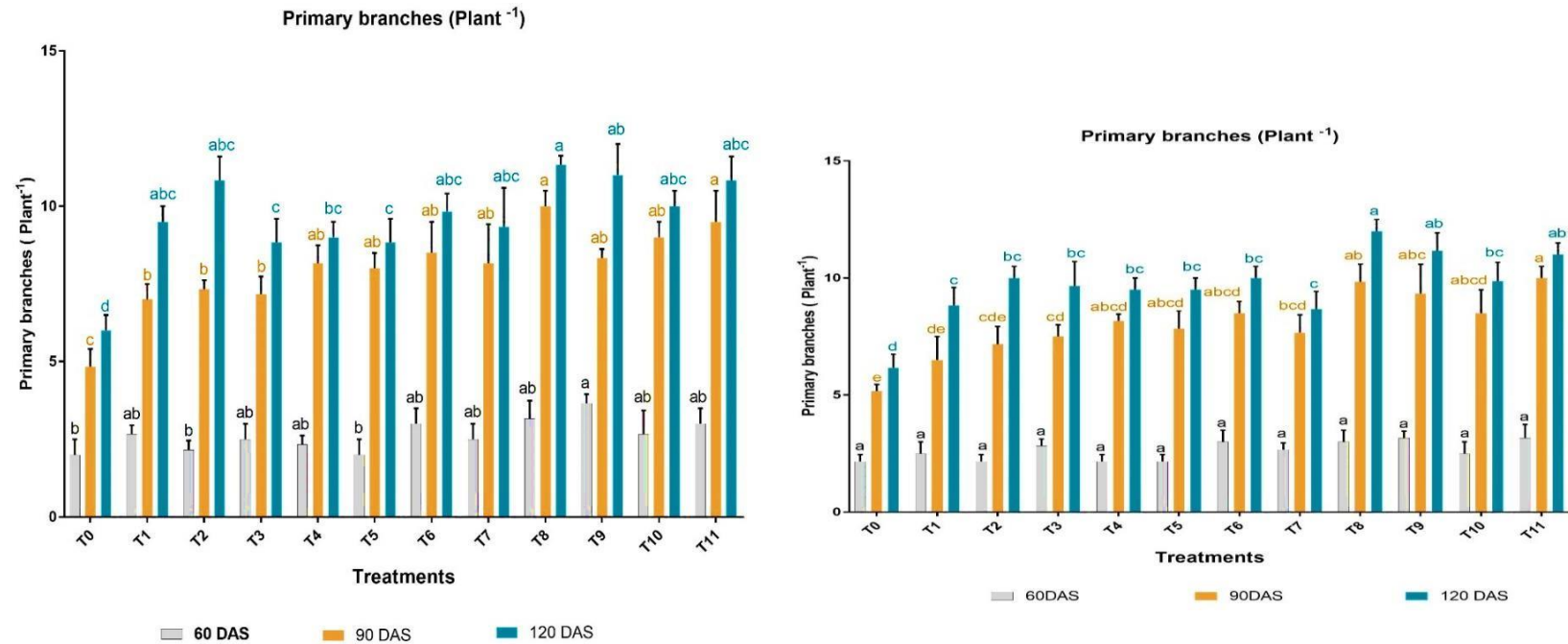
2022; Ortiz-García et al., 2022, 2023; Ozturk & Unal, 2023; Paalli et al., 2022; Pal et al., 2023). The concurrent utilisation of sulphur and salicylic acid in a joint application yields synergistic outcomes, encompassing the augmentation of nutrient absorption, fortification against stress, intercommunication of hormones, and promotion of primary branch growth. The plant acquires these effects by combining sulphur and salicylic acid. The thorough comprehension of the fundamental mechanisms offers invaluable insights into agricultural practises, encompassing potential approaches to optimise the development of primary branches and enhance crop productivity in mustard cultivation. There is an optimistic outlook regarding the potential of recent scientific investigations into the intricate molecular mechanisms facilitated by the interplay between sulphur and salicylic acid. These studies hold promise in uncovering novel avenues for enhancing the overall quality of crops, which have yet to be thoroughly explored (Mittal et al., 2022; Mohammadi et al., 2023; Molinari et al., 2023; Moustakas et al., 2022; Mugwanya et al., 2023; Mukarram et al., 2022).

**Table 4.7. Impact of Different Treatments on Number of Primary Branches of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	60 DAS		90 DAS		120 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	2.00 <sup>b</sup> ±0.50	2.16 <sup>a</sup> ±0.28	4.83 <sup>c</sup> ±0.57	5.16 <sup>e</sup> ±0.28	6.00 <sup>d</sup> ±0.50	6.16 <sup>d</sup> ±0.57
<b>T1 (Thiourea-1000 ppm)</b>	2.66 <sup>ab</sup> ±0.28	2.50 <sup>a</sup> ±0.50	7.00 <sup>b</sup> ±0.50	6.50 <sup>de</sup> ±1.00	9.50 <sup>abc</sup> ±0.50	8.83 <sup>c</sup> ±0.76
<b>T2 (Salicylic acid-300 ppm)</b>	2.16 <sup>b</sup> ±0.28	2.16 <sup>a</sup> ±0.28	7.33 <sup>b</sup> ±0.28	7.16 <sup>cde</sup> ±0.76	10.83 <sup>abc</sup> ±0.76	10.00 <sup>bc</sup> ±0.50
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	2.50 <sup>ab</sup> ±0.50	2.83 <sup>a</sup> ±0.28	7.16 <sup>b</sup> ±0.57	7.50 <sup>cd</sup> ±0.50	8.83 <sup>c</sup> ±0.76	9.66 <sup>bc</sup> ±1.04
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	2.33 <sup>ab</sup> ±0.28	2.16 <sup>a</sup> ±0.28	8.16 <sup>ab</sup> ±0.57	8.16 <sup>abcd</sup> ±0.28	9.00 <sup>bc</sup> ±0.50	9.50 <sup>bc</sup> ±0.50
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	2.00 <sup>b</sup> ±0.50	2.16 <sup>a</sup> ±0.28	8.00 <sup>ab</sup> ±0.50	7.83 <sup>abcd</sup> ±0.76	8.83 <sup>c</sup> ±0.76	9.50 <sup>bc</sup> ±0.50
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	3.00 <sup>ab</sup> ±0.50	3.00 <sup>a</sup> ±0.50	8.50 <sup>ab</sup> ±1.00	8.50 <sup>abcd</sup> ±0.50	9.83 <sup>abc</sup> ±0.57	10.00 <sup>bc</sup> ±0.50
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	2.50 <sup>ab</sup> ±0.50	2.66 <sup>a</sup> ±0.28	8.16 <sup>ab</sup> ±1.25	7.66 <sup>bcd</sup> ±0.76	9.33 <sup>abc</sup> ±1.25	8.66 <sup>c</sup> ±0.76
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	3.16 <sup>ab</sup> ±0.57	3.00 <sup>a</sup> ±0.50	10.00 <sup>a</sup> ±0.50	9.83 <sup>ab</sup> ±0.76	11.33 <sup>a</sup> ±0.28	12.00 <sup>a</sup> ±0.50
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	3.66 <sup>a</sup> ±0.28	3.16 <sup>a</sup> ±0.28	8.33 <sup>ab</sup> ±0.28	9.33 <sup>abc</sup> ±1.25	11.00 <sup>ab</sup> ±1.00	11.16 <sup>ab</sup> ±0.76
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	2.66 <sup>ab</sup> ±0.76	2.50 <sup>a</sup> ±0.50	9.00 <sup>ab</sup> ±0.50	8.50 <sup>abcd</sup> ±1.00	10.00 <sup>abc</sup> ±0.50	9.86 <sup>bc</sup> ±0.80
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	3.00 <sup>ab</sup> ±0.50	3.16 <sup>a</sup> ±0.57	9.50 <sup>a</sup> ±1.00	10.00 <sup>a</sup> ±0.50	10.83 <sup>abc</sup> ±0.76	11.00 <sup>ab</sup> ±0.50
<b>CD</b>	0.831		1.216		1.249	
<b>CV</b>	18.487		8.922		7.626	

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.7. Number of primary branches of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Number of Secondary Branches:** The effect of Sulphur and Salicylic acid and their combination on several secondary branches was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 60, 90 and 120 days after sowing (DAS) (Table 4.8 Figure 4.8). In 2021-2022, there was a significant difference in several secondary branches compared to T0 (Control) at 60, 90 and 120 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the number of secondary branches was observed at 60, 90 and 120 DAS. Therefore, at 60 DAS, the percentage increase as compared to T0 was found highest in T6 followed by T11, T9, T8, T3, T10, T2, T5, T7, T1, T4 and the percentage values were 29.82%, 27.27%, 23.07%, 21.56%, 18.36%, 18.36%, 16.66%, 16.66%, 16.66%, 14.89%, 13.04% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T10, T5, T6, T3, T7, T2, T8, T9, T1, T4 and the percentage values were 47.05%, 46.66%, 45.45%, 45.03%, 36.84%, 36.28%, 35.71%, 35.71%, 34.54%, 32.07%, 28.71% respectively. At 120 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T5, T6, T11, T3, T8, T9, T7, T1, T2, T4 and the percentage values were 48.05%, 47.02%, 46.76%, 46.50%, 42.47%, 39.54%, 38.50%, 38.15%, 36.30%, 33.95%, 28.66% respectively. In 2022-2023, there was a significant difference in the number of secondary branches compared to T0 (Control) at 60, 90 and 120 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 60 DAS, the percentage increase as compared to T0 was found to be highest in T9, followed by T11, T6, T8, T3, T7, T10, T1, T5, T2, T4 and the percentage values were 31.66%, 30.50%, 22.64%, 22.64%, 21.15%, 21.15%, 21.15%, 18%, 16.32%, 12.76%, 6.81% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T10, T6, T5, T8, T9, T1, T7, T3, T2, T4 and the percentage values were 45.98%, 43.07%, 42.63%, 39.34%, 34.51%, 34.51%, 32.72%, 32.72%, 30.84%, 30.18%, 28.15% respectively. At 120 DAS, the percentage increase as compared to T0 was found highest in T6 followed by T11, T5, T10, T8, T3, T7, T9, T4, T2, T1 and the percentage values were 50.23%, 49.28%, 47.29%, 43.68%, 40.22%, 39.54%, 39.20%, 37.79%, 35.54%, 34.75%, 33.12% respectively. The secondary branches of the mustard plant (*Brassica juncea* L.) play a vital role in its overall growth, development, and yield. To maximise mustard cultivation practises and improve crop

productivity, it is imperative to understand the significance of secondary branches and their intricate responses to external factors, particularly sulphur (S) and salicylic acid (SA). The significance of secondary branches in the growth and reproductive capacity of the mustard plant primarily stems from their contributions in various ways. The significance of secondary units in plants lies in their function as the initial sites of flower formation (Ullah et al., 2023; Ullah et al., 2022; Verma et al., 2023; Waadt et al., 2022; Wan & Xin, 2022; Wang et al., 2022; Wang et al., 2023; Wang et al., 2023; Wang et al., 2022; Wang & Komatsu, 2022; Wang et al., 2022; Wang et al., 2022; Wang et al., 2022). The likelihood of flowering is directly correlated with the quantity of secondary branches present. A positive correlation exists between the quantity of flowers in bloom and the quantity of seeds produced, thereby serving as a crucial determinant of crop yield. The distinguishing characteristic of the secondary branches of a tree lies in their extensive leaf surfaces, which exhibit a high capacity for solar radiation absorption. As a result of the photosynthetic area's expansion, the photosynthesis rate is enhanced, leading to an augmentation in energy production and an enhancement in nutrient assimilation. The heightened level of photosynthetic activity plays a crucial role in facilitating the plant's ongoing growth and its seeds' maturation. These branches play a crucial role in facilitating the transportation of nutrients throughout the entire plant. The xylem and phloem are vascular tissues that transport water, minerals, and various nutrients from a plant's root system to its leaves and reproductive organs (Sivanesan et al., 2022; Song et al., 2023; Sousa et al., 2022; Spinoso-Castillo & Bello-Bello, 2022). The distribution of nutrients plays a crucial role in facilitating vigorous vegetative growth and ensuring successful seed maturation. The presence of secondary branches enhances the overall structural integrity of the plant. They aid in the dispersion of the plant's weight, thereby mitigating the occurrence of lodging, which refers to the undesirable bending or collapsing of the plant. Ensuring the preservation of structural integrity is of particular significance during the stages of flowering and seed-setting. Sulphur, an essential macronutrient for mustard plants, exerts a notable influence on the growth of secondary branches through various mechanisms. The synthesis of amino acids, specifically cysteine and methionine, significantly relies on the element sulphur's presence. The amino acids discussed are crucial for protein synthesis, integral to developing secondary branches and their subsequent elongation. A subset of amino

acids possessing sulphur atoms can be cofactors for enzymes participating in diverse metabolic pathways. Sulphur indirectly influences secondary components by modulating the synthesis and metabolism of plant hormones, such as auxins and cytokinin's. The optimal quantity of sulphur present in the soil can influence the hormonal balance, a crucial factor in the formation of secondary branches and subsequent growth. The prominent function of salicylic acid in plant defence mechanisms represents just one of the numerous significant impacts that salicylic acid exerts on secondary branches. Salicylic acid has the potential to modulate the expression of genes implicated in growth and development. The genes in question encompass those accountable for the commencement and extension of secondary branches. This process is achieved by activating specific transcription factors, crucial in regulating genes associated with forming secondary branches. Multiple studies have shown that salicylic acid (SA) can enhance cell division and cell expansion in various plant tissues (Takeuchi et al., 2022; Talaat, 2023; Tan et al., 2022; Tariq et al., 2023; Tariq et al., 2022; Todorova et al., 2022; Topcu et al., 2022; Torres & Figueroa, 2023; Tyagi et al., 2022). This characteristic promotes the proliferation of branching and the elongation of secondary branches, thereby facilitating their overall growth and development. Tolerance to stress refers to an individual's ability to withstand and adapt to various environmental stressors. Salicylic acid's stress-induced characteristics (SA) indirectly influence secondary branches, notwithstanding its predominant recognition of its defensive attributes. When plants experience reduced stress levels due to SA-mediated defences, they can allocate a larger proportion of their resources, including energy and nutrients, towards growth-related processes. This encompasses the progression of subsidiary divisions. The combined application of sulphur and salicylic acid in mustard exhibits a synergistic impact on the plant's secondary branches. Sulphur catalyses, facilitating the absorption of vital nutrients such as nitrogen and phosphorus, which play a crucial role in the growth and formation of secondary branches. Nutrient uptake efficiency can be enhanced by applying salicylic acid, resulting in an augmented availability of nutrients to initiate and sustain secondary branch growth (Suliman et al., 2022; Sun et al., 2022; Sun et al., 2023; Sun et al., 2022; Taira & Shiono, 2022). The interaction between salicylic acid and sulphur facilitates the complex hormonal crosstalk. Sulphur has been found to promote the synthesis of auxin, a plant hormone,



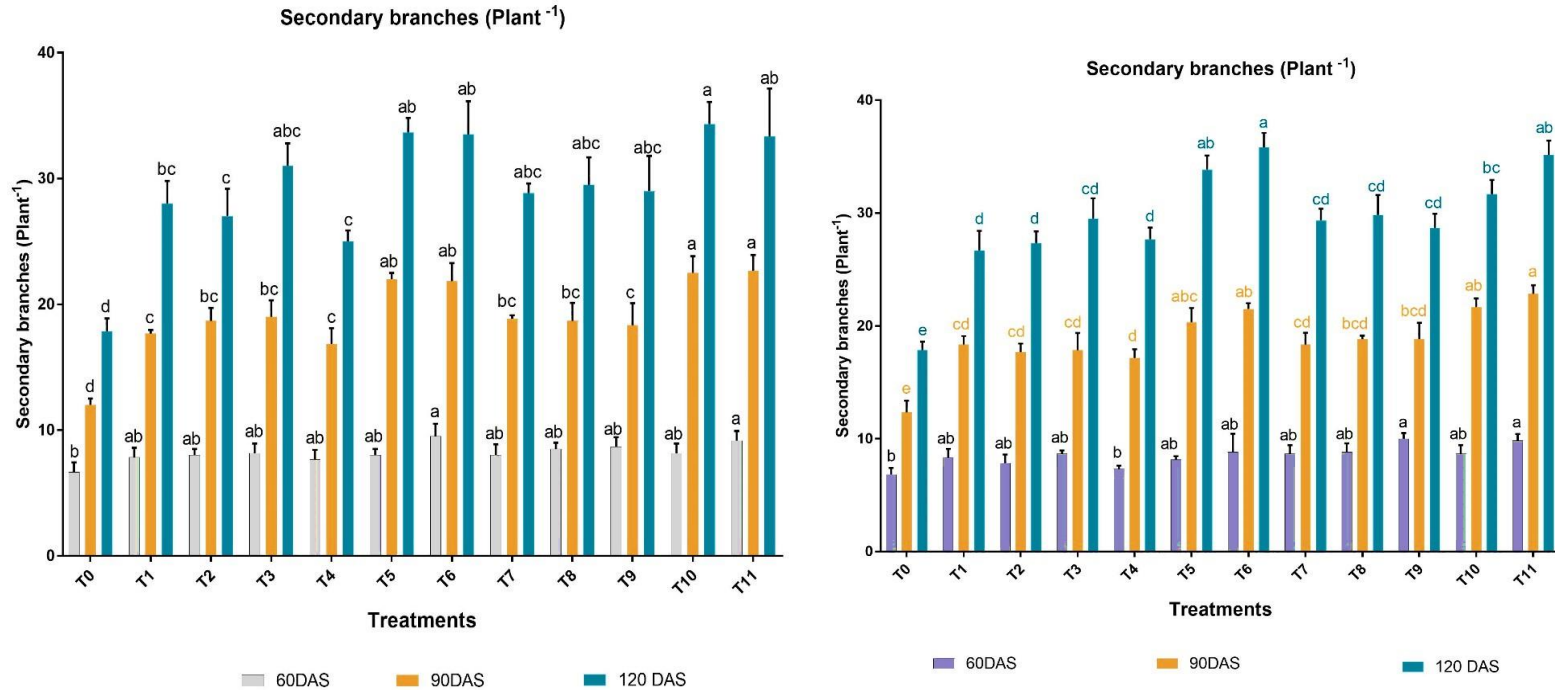
while saline conditions have been observed to enhance the sensitivity and transportation of auxin. The coordinated activity of this process induces cellular division and enlargement, thereby facilitating the development of additional branches. Including salicylic acid (SA) in the growth environment enhances the plant's capacity to withstand stress. In contrast, sulphur guarantees the accessibility of essential constituents required for stress-related compounds, such as antioxidants. The conditions that arise from the synergistic effects of stress resistance and growth promotion are conducive to the proliferation of secondary branches. The secondary branches of a mustard plant play a crucial role in shaping its overall structure and exert substantial influence on its growth, development, and productivity. Sulphur and salicylic acid possess intricate mechanisms that substantially impact the growth of secondary branches. A comprehensive understanding of these mechanisms is imperative to enhance agricultural practises and formulate strategies to promote secondary branch development and augment crop yield in mustard cultivation (Shi et al., 2023; Shi et al., 2022; Si et al., 2023; Singh et al., 2022; Singh & Roychoudhury, 2023; Singh & Nandi, 2022; Singhal et al., 2023).

**Table 4.8. Impact of Different Treatments on Number of Secondary Branches of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	60 DAS		90 DAS		120 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	6.66 <sup>b</sup> ±0.76	6.83 <sup>b</sup> ±0.57	12.00 <sup>d</sup> ±0.50	12.33 <sup>e</sup> ±1.04	17.83 <sup>d</sup> ±1.04	17.83 <sup>e</sup> ±0.76
<b>T1 (Thiourea-1000 ppm)</b>	7.83 <sup>ab</sup> ±0.76	8.33 <sup>ab</sup> ±0.76	17.66 <sup>c</sup> ±0.28	18.33 <sup>cd</sup> ±0.76	28.00 <sup>bc</sup> ±1.80	26.66 <sup>d</sup> ±1.75
<b>T2 (Salicylic acid-300 ppm)</b>	8.00 <sup>ab</sup> ±0.50	7.83 <sup>ab</sup> ±0.76	18.66 <sup>bc</sup> ±1.04	17.66 <sup>cd</sup> ±0.76	27.00 <sup>c</sup> ±2.17	27.33 <sup>d</sup> ±1.04
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	8.16 <sup>ab</sup> ±0.76	8.66 <sup>ab</sup> ±0.28	19.00 <sup>bc</sup> ±1.32	17.83 <sup>cd</sup> ±1.52	31.00 <sup>abc</sup> ±1.80	29.50 <sup>cd</sup> ±1.80
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	7.66 <sup>ab</sup> ±0.76	7.33 <sup>b</sup> ±0.28	16.83 <sup>c</sup> ±1.25	17.16 <sup>d</sup> ±0.76	25.00 <sup>c</sup> ±0.86	27.66 <sup>d</sup> ±1.04
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	8.00 <sup>ab</sup> ±0.50	8.16 <sup>ab</sup> ±0.28	22.00 <sup>ab</sup> ±0.50	20.33 <sup>abc</sup> ±1.25	33.66 <sup>ab</sup> ±1.15	33.83 <sup>ab</sup> ±1.25
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	9.50 <sup>a</sup> ±1.00	8.83 <sup>ab</sup> ±1.60	21.83 <sup>ab</sup> ±1.44	21.50 <sup>ab</sup> ±0.50	33.50 <sup>ab</sup> ±2.64	35.83 <sup>a</sup> ±1.25
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	8.00 <sup>ab</sup> ±0.86	8.66 <sup>ab</sup> ±0.76	18.83 <sup>bc</sup> ±0.28	18.33 <sup>cd</sup> ±1.04	28.83 <sup>abc</sup> ±0.76	29.33 <sup>cd</sup> ±1.04
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	8.50 <sup>ab</sup> ±0.50	8.83 <sup>ab</sup> ±0.76	18.66 <sup>bc</sup> ±1.44	18.83 <sup>bcd</sup> ±0.28	29.50 <sup>abc</sup> ±2.17	29.83 <sup>cd</sup> ±1.75
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	8.66 <sup>ab</sup> ±0.76	10.00 <sup>a</sup> ±0.50	18.33 <sup>c</sup> ±1.75	18.83 <sup>bcd</sup> ±1.44	29.00 <sup>abc</sup> ±2.78	28.66 <sup>cd</sup> ±1.25
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	8.16 <sup>ab</sup> ±0.76	8.66 <sup>ab</sup> ±0.76	22.50 <sup>a</sup> ±1.32	21.66 <sup>ab</sup> ±0.76	34.33 <sup>a</sup> ±1.75	31.66 <sup>bc</sup> ±1.25
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	9.16 <sup>a</sup> ±0.76	9.83 <sup>a</sup> ±0.57	22.66 <sup>a</sup> ±1.25	22.83 <sup>a</sup> ±0.76	33.33 <sup>ab</sup> ±3.81	35.16 <sup>ab</sup> ±1.25
<b>CD</b>	1.254	1.3	2.005	1.704	3.655	1.745
<b>CV</b>	8.976	8.971	6.166	5.316	7.331	3.478

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.8. Number of secondary branches of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm);

**T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).**

**Stem Diameter(mm):** The effect of Sulphur and Salicylic acid and their combination on Stem diameter was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60 and 90 days after sowing (DAS) (Table 4.9, Figure 4.9). In 2021-2022, there was a significant difference in Stem diameter compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the stem diameter was observed at 30, 60 and 90 DAS. Therefore, at 30 DAS, the percentage increase as compared to T0 was found highest in T8 followed by T11, T9, T10, T7, T2, T5, T4, T3, T6, T1 and the percentage values were 48.77%, 44.01%, 40.27%, 37.03%, 29.89%, 27.89%, 27.35%, 17.81%, 17.25%, 13.92%, 9.31% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T8, T10, T7, T5, T3, T2, T9, T1, T6, T4 and the percentage values were 39.44%, 38.30%, 32.78%, 27.11%, 25.45%, 21.70%, 21.13%, 20.29%, 13.69%, 13.28%, 12.84% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T8, T10, T6, T5, T9, T4, T3, T7, T1, T2 and the percentage values were 39.21%, 37.50%, 36.07%, 34.43%, 31.70%, 22.93%, 20.26%, 17.01%, 13.59%, 12.17%, 8.99% respectively. In 2022-2023, there was a significant difference in Stem diameter compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T8, followed by T11, T10, T9, T5, T7, T2, T1, T3, and the percentage values were 36.97%, 34.04%, 32.84%, 25.13%, 16.15%, 14.91%, 13.88%, 11.97%, 3.04% respectively. But in T6 and T4, the percentage decreased compared to T0, and the percentage values were -4.94% and -6.20%. At 60 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T8, T10, T5, T2, T7, T3, T1, T9, T6, T4 and the percentage values were 40.40%, 39.88%, 36.75%, 29.84%, 28.46%, 27.40%, 25.00%, 23.63%, 19.31%, 15.05%, 14.01% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T8, T10, T5, T6, T9, T3, T4, T7, T1, T2 and the percentage values were 38.64%, 37.67%, 35.22%, 30.10%, 23.13%, 20.47%, 18.38%, 16.85%, 15.43%, 12.26%, 9.59% respectively. The impact of stem diameter on various aspects of growth, development, and agricultural productivity in *Brassica juncea* L., commonly known as mustard, has been extensively investigated in

scientific studies. Measuring the stem diameter is a crucial aspect of mustard research. Acquiring knowledge regarding the impact of external factors, such as sulphur (S) and salicylic acid (SA), on this parameter can yield valuable insights for enhancing mustard cultivation practices (Kosakivska et al., 2022; Kour et al., 2023; Kudoyarova, 2022; Kumar & Ohri, 2023; Kurepa & Smalle, 2022; Kurniawan & Chuang, 2022; Kuromori et al., 2022; Lajayer et al., 2022; Li et al., 2022; Li et al., 2022; Li et al., 2022; Li, Ren, et al., 2023). A positive correlation exists between the diameter of a plant's stem and its structural stability and overall strength. A robust stem with substantial diameter becomes increasingly crucial as the plant matures. This will offer essential assistance and mitigate the risk of the stem bending, lodging, or collapsing. Preserving the plant's structural integrity is of utmost importance to shield it from detrimental environmental elements, including wind, rain, and mechanical pressures, which can impede its development and reduce productivity. The stem is the principal conduit for transporting water, minerals, and nutrients from the plant's subterranean root system to its aboveground structures, including the leaves, flowers, and pods. The transportation process takes place in a clockwise direction along the stem. A stem with increased robustness and girth can accommodate a more extensive vascular system, thereby enhancing the efficiency of vital resource distribution. Enhancing the plant's acquisition of essential nutrients and water facilitates optimal growth and reproductive success. The diameter of a plant's stem directly correlates with the allocation of its resources. The allocation of a plant's resources to its vegetative and reproductive components can be influenced by the diameter of its stem (Karaman, 2023; Karamat et al., 2022; Katoch et al., 2022; Kaviani et al., 2023; Kaya et al., 2023; Khalid et al., 2023; Khan et al., 2022, 2023; Khan et al., 2022; Kochanek et al., 2023). The allocation of resources has a notable influence on crop productivity, consequently affecting plants' reproductive capacity in terms of flower and seed production. To enhance crop productivity and optimise resource allocation strategies, it is imperative to acquire a comprehensive understanding of the various factors that impact stem diameter. A stem possessing a significantly greater thickness exhibits an enhanced inherent ability to resist diseases, pests, and environmental stresses. A robust branch can endure physical harm's consequences and serve as a protective barrier against transmitting pathogens. Moreover, the enhanced structural integrity conferred by a thicker stem enables a more

effective weight-bearing capacity for the plant, thus mitigating the risk of lodging even under unfavourable external conditions. The circumference of its stem influences the photosynthetic capacity of a plant indirectly. A stem with greater size and strength can sustain larger foliage, thereby facilitating an augmentation in the quantity of sunlight accessible for photosynthesis. The heightened photosynthetic activity enhances energy production and nutrient assimilation, bolstering the plant's overall health and increasing productivity. The stem diameter of mustard plants can be notably affected by sulphur (S) and salicylic acid (SA), two significant factors in plant physiology. This has the potential to contribute to the optimisation of crop cultivation practices. Sulphur, an essential macronutrient for optimal plant growth, plays a significant role in various aspects of stem development. Sulphur is vital in various amino acids, such as cysteine and methionine. Amino acids play a crucial role as the primary constituents of proteins. Proteins, in turn, assume a crucial function in the progression of stem cells and the phenomenon of stem cell differentiation. Sufficient provision of sulphur is essential for the maintenance of structural integrity and the regulation of stem growth through the process of protein synthesis (Hernández et al., 2022; Hilal et al., 2023; Hong et al., 2022; Huang et al., 2022; Hudeček et al., 2023). Amino acids that possess sulphur are important because they are cofactors for enzymes participating in diverse metabolic pathways. These enzymes influence the physiological mechanisms linked to the differentiation and growth of stem cells. The processes above encompass cellular division, elongation, and lignin synthesis, all influential factors in determining stem diameter. Sulphur indirectly influences stem diameter by affecting the synthesis and metabolism of plant hormones, such as auxins and cytokinin's. The equilibrium of these hormones, crucial for the initiation and growth of stems, can be influenced by the availability of an adequate amount of sulphur. Salicylic acid, a widely recognised compound in plant defence mechanisms, influences stem diameter through diverse mechanisms. The SA can regulate the expression of genes involved in growth and development, including those responsible for initiating and elongating stem growth. This is achieved by activating specific transcription factors, which subsequently govern the expression of genes implicated in stem development, thereby influencing the stem's diameter (Guo et al., 2023; Gupta et al., 2022; Hajiboland et al., 2022; Hartmann et al., 2022; Hernandez-Leon & Valenzuela-Soto, 2022; Hernández et al., 2023). Previous

studies have shown that salicylic acid (SA) is crucial in promoting cell division and cell expansion in various plant tissues, such as the stem. As a result of this particular attribute, the stem experiences an augmentation in its circumference and ability to withstand external forces. The stress-responsive properties of salicylic acid (SA) have an indirect influence on stem diameter. When plants experience reduced stress levels due to SA-mediated defences, they can allocate a larger proportion of their resources, including energy and nutrients, towards growth-related processes. These processes encompass the development of stem cells. The simultaneous application of sulphur and salicylic acid can synergistically impact mustard plants' stem circumference. Sulphur plays a facilitating role in the uptake of crucial nutrients, namely nitrogen and phosphorus, which are vital for the growth and development of the stem. Salicylic acid has the potential to enhance nutrient uptake efficiency, thereby facilitating an augmented nutrient supply that can effectively promote stem initiation and diameter expansion (Huh, 2022; Hui et al., 2022; Huntenburg et al., 2022; Hussein et al., 2023; Iftikhar et al., 2023; Islam et al., 2023; Javadipour et al., 2022; Ji et al., 2022; Jia et al., 2022; Jin et al., 2022; Jofre et al., 2023; Kandhol et al., 2023; Kapoor et al., 2022; Kapoor et al., 2022). The interaction between sulphur and salicylic acid promotes a complex hormonal interaction. It has been observed that sulphur stimulates auxin synthesis, a plant hormone. Conversely, salicylic acid (SA) has been found to augment the transport and sensitivity of auxin. This coordinated process facilitates the growth and elongation of cells, increasing the stem's diameter. It has been observed that the inclusion of salicylic acid (SA) in the growth environment has a positive impact on the ability of plants to withstand and adapt to stressful conditions. Moreover, sulphur is pivotal in facilitating the accessibility of fundamental constituents necessary for forming stress-induced substances, such as antioxidants. The cultivation of stress resistance and the promotion of growth can create conditions that are conducive to an increase in stem diameter. The measurement of stem diameter in mustard research holds great significance as it profoundly influences various aspects of the plant's physiology, including its structural integrity, nutrient transportation, allocation of resources, ability to withstand stress, and capacity for photosynthesis. The utilisation of sulphur and salicylic acid offers the potential for enhancing stem diameter, thereby leading to improved productivity of mustard crops due to their intricate mechanisms. Enhanced



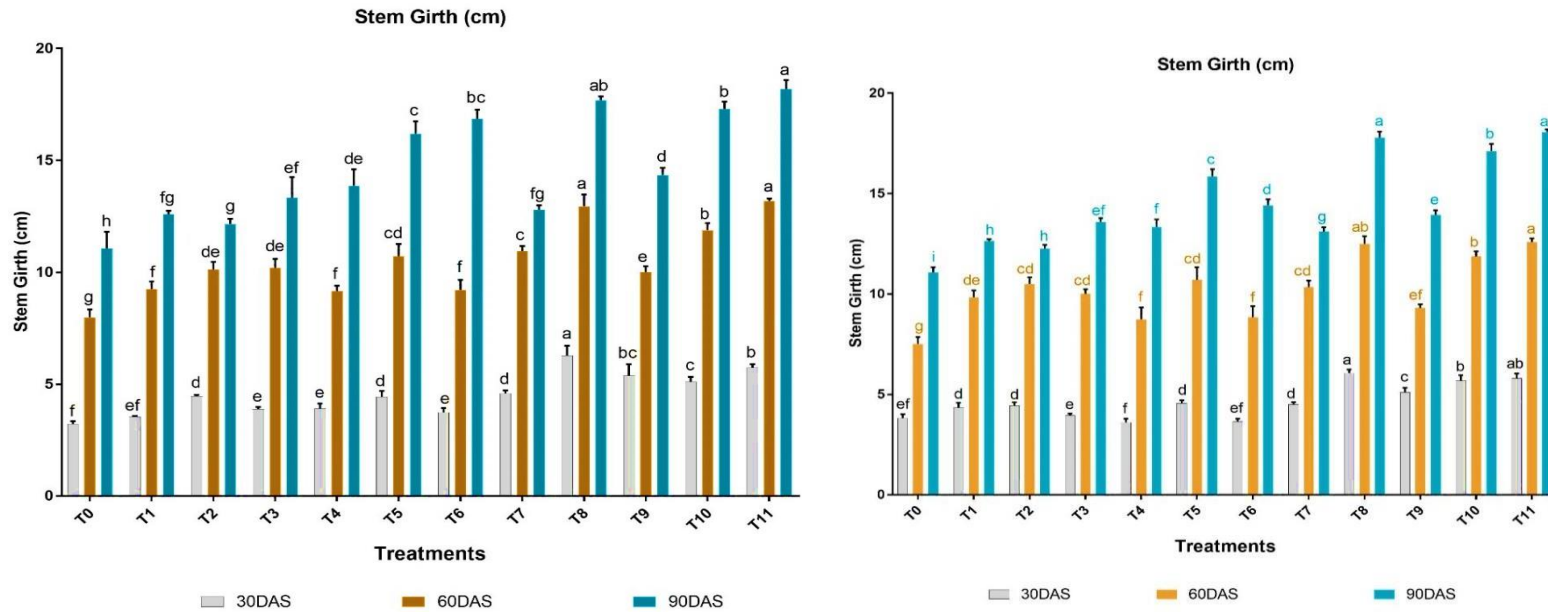
agricultural productivity can be attained by implementing contemporary farming techniques, specifically, those focused on augmenting stem diameter (Dhiman et al., 2022; EL Sabagh et al., 2022; Elnahal et al., 2022; Faisal et al., 2023; Felipez et al., 2022; Feng et al., 2023; Ferreira et al., 2023; Fidler et al., 2022; Fierli et al., 2022; Ganz et al., 2022; Garcya-Laynes et al., 2022; Geng et al., 2022; González-Pérez et al., 2022; Gul et al., 2023; Guo et al., 2023).

**Table 4.9. Impact of Different Treatments on Stem Diameter of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	3.21 <sup>f</sup> ±0.13	3.82 <sup>ef</sup> ±0.19	7.98 <sup>e</sup> ±0.35	7.50 <sup>e</sup> ±0.35	11.05 <sup>h6</sup> ±0.75	11.08 <sup>i</sup> ±0.25
<b>T1 (Thiourea-1000 ppm)</b>	3.54 <sup>ef</sup> ±0.02	4.34 <sup>d</sup> ±0.23	9.25 <sup>f</sup> ±0.33	9.83 <sup>de</sup> ±0.34	12.59 <sup>fg</sup> ±0.16	12.63 <sup>h</sup> ±0.09
<b>T2 (Salicylic acid-300 ppm)</b>	4.45 <sup>d</sup> ±0.06	4.44 <sup>d</sup> ±0.16	10.12 <sup>de</sup> ±0.34	10.49 <sup>cd</sup> ±0.33	12.15 <sup>e</sup> ±0.24	12.26 <sup>h</sup> ±0.18
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	3.88 <sup>e</sup> ±0.09	3.94 <sup>e</sup> ±0.09	10.19 <sup>de</sup> ±0.39	10.01 <sup>cd</sup> ±0.22	13.32 <sup>ef</sup> ±0.92	13.58 <sup>ef</sup> ±0.19
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	3.91 <sup>e</sup> ±0.21	3.60 <sup>f</sup> ±0.19	9.16 <sup>f</sup> ±0.24	8.73 <sup>f</sup> ±0.59	13.86 <sup>de</sup> ±0.73	13.33 <sup>fg</sup> ±0.38
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	4.42 <sup>d</sup> ±0.27	4.56 <sup>d</sup> ±0.14	10.71 <sup>cd</sup> ±0.55	10.70 <sup>c</sup> ±0.63	16.19 <sup>c</sup> ±0.55	15.85 <sup>c</sup> ±0.35
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	3.73 <sup>e</sup> ±0.19	3.64 <sup>ef</sup> ±0.14	9.20 <sup>f</sup> ±0.45	8.83 <sup>f</sup> ±0.56	16.86 <sup>bc</sup> ±0.40	14.42 <sup>d</sup> ±0.29
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	4.58 <sup>d</sup> ±0.13	4.49 <sup>d</sup> ±0.11	10.95 <sup>c</sup> ±0.21	10.34 <sup>cd</sup> ±0.32	12.79 <sup>fg</sup> ±0.19	13.10 <sup>g</sup> ±0.21
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	6.27 <sup>a</sup> ±0.44	6.06 <sup>a</sup> ±0.16	12.94 <sup>a</sup> ±0.53	12.48 <sup>ab</sup> ±0.39	17.69 <sup>ab</sup> ±0.16	17.78 <sup>a</sup> ±0.30
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	5.38 <sup>bc</sup> ±0.50	5.10 <sup>c</sup> ±0.22	10.01 <sup>e</sup> ±0.25	9.30 <sup>ef</sup> ±0.19	14.34 <sup>d</sup> ±0.32	13.93 <sup>c</sup> ±0.23
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	5.10 <sup>c</sup> ±0.22	5.69 <sup>b</sup> ±0.26	11.87 <sup>b</sup> ±0.31	11.87 <sup>b</sup> ±0.25	17.29 <sup>b</sup> ±0.32	17.11 <sup>b</sup> ±0.36
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	5.74 <sup>b</sup> ±0.14	5.79 <sup>ab</sup> ±0.23	13.18 <sup>a</sup> ±0.10	12.59 <sup>a</sup> ±0.17	18.19 <sup>a</sup> ±0.39	18.06 <sup>a</sup> ±0.13
<b>CD</b>	0.435	0.318	0.632	0.618	0.776	0.472
<b>CV</b>	5.652	4.038	3.544	3.546	3.099	1.92

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance

**Figure 4.9. Stem Diameter (mm) of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

#### **4B. Thiourea (sulphur) and salicylic acid-mediated effects on Physiological parameters of Indian mustard grown under the open filed condition**

**Chlorophyll Index:** The effect of Sulphur and Salicylic acid and their combination on the Chlorophyll index was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60 and 90 days after sowing (DAS) (Table 4.10, Figure 4.10). In 2021-2022, there was a significant difference in the Chlorophyll index compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the Chlorophyll index was observed at 30, 60 and 90 DAS. Therefore, at 30 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T4, T1, T8, T2, T3, T7, T11, T5, T9 and the percentage values were 21.57%, 21.07%, 17.97%, 16.70%, 16.51%, 13.50%, 12.02%, 10.12%, 9.91%, 5.13% respectively. But in T6, the percentage decreased compared to T0, and the percentage value was -4.15%. At 60 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T8, T4, T1, T2, T3, T11, T5, T7, T9, T6 and the percentage values were 19.84%, 19.22%, 18.95%, 17.28%, 14.08%, 11.89%, 8.52%, 7.78%, 5.75%, 4.16%, 0.63% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T8, T1, T4, T11, T2, T3, T5, T7, T9, T6 and the percentage values were 23.38%, 20.55%, 19.32%, 16.89%, 13.93%, 13.71%, 13.43%, 12.87%, 11.91%, 6.36%, 4.04% respectively. In 2022-2023, there was a significant difference in the Chlorophyll index compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T4, T1, T8, T2, T3, T11, T9, T7, T5, T6 and the percentage values were 21.78%, 20.36%, 19.91%, 19.06%, 14.94%, 13.52%, 10.03%, 9.61%, 9.32%, 7.70%, 6.87% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T1, T4, T8, T3, T2, T11, T5, T7, T9, T6 and the percentage values were 20.29%, 18.78%, 18.78%, 16.76%, 11.29%, 11.23%, 9.48%, 7.45%, 6.41%, 5.41%, 1.27% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T8, T1, T4, T5, T2, T7, T11, T3, T9, T6 and the percentage values were 21.25%, 21.11%, 19.28%, 17.07%, 16.25%, 15.63%, 13.32%, 10.81%,

10.40%, 7.42%, 1.94% respectively. The study of mustard (*Brassica juncea* L.) extensively utilises the chlorophyll index as a primary measure of plant well-being, photosynthetic efficacy, and nutrient condition. The significance of the chlorophyll index lies in its critical nature (Phokas et al., 2023; Phour & Sindhu, 2022; Poór et al., 2022; Quesada, 2022; Rai & Kaushik, 2023; Raja Gopalan et al., 2022; Ramakrishnan & Zhou, 2022; Rani et al., 2023; Rizvi et al., 2022; Roussos, 2023; Ruidas et al., 2022; Rybczyński et al., 2022; Sachan & Krishna, 2022; Salih, Wu, et al., 2022; Salih, Zhou, et al., 2022). Gaining a comprehensive understanding of the chlorophyll index's importance and the impact of sulphur and salicylic acid on it can yield valuable insights for enhancing mustard cultivation techniques and maximising agricultural output. Evaluating the effects of external factors, such as sulphur (S) and salicylic acid (SA), on mustard plants is important. Chlorophyll is the predominant pigment found in plants, playing a crucial role in facilitating the process of photosynthesis. The chlorophyll index is a quantitative indicator of a plant's photosynthetic capacity, a vital energy production and nutrient absorption process. Elevated photosynthetic activity, quantified by an augmented chlorophyll index, yields greater growth and reproductive vigour. The determination of a plant's nitrogen (N) and magnesium (Mg) levels can be deduced by assessing its chlorophyll index. Insufficient nutrient availability can lead to the degradation of chlorophyll and a reduction in the chlorophyll index. Monitoring the chlorophyll index provides valuable insights into the plant's nutritional needs and overall health. The chlorophyll index can be an early indicator of stress, regardless of whether the stressor is biotic (e.g., pests and diseases) or abiotic (e.g., drought or nutrient deficiencies). A declining chlorophyll index may indicate the necessity for intervention to mitigate plant stress and maintain optimal health (Nasir & Toth, 2022; Negi & Kumar, 2023; Nimsi et al., 2023; Niu & Fu, 2022; Ortiz-García et al., 2022, 2023; Ozturk & Unal, 2023; Paalli et al., 2022; Pal et al., 2023; Parada et al., 2022; Parrey et al., 2023; Patel et al., 2022; Peng et al., 2022; Pérez-Llorca et al., 2023). The chlorophyll index directly correlates with the plant's capacity for growth and the subsequent yield of fruits or vegetables. A high chlorophyll index indicates optimal photosynthetic activity, facilitating vigorous vegetative growth and enhanced reproductive efficacy, ultimately leading to a notable augmentation in crop yield. The chlorophyll index of mustard plants is influenced by various factors, such as sulphur

(S) and salicylic acid (SA), which can be manipulated to enhance agricultural productivity. The process of chlorophyll synthesis depends on the presence of the amino acids cysteine and methionine, which necessitate sulphur as an essential constituent. An adequate quantity of sulphur in the surrounding environment facilitates chlorophyll synthesis, leading to an elevated chlorophyll index and enhanced photosynthetic efficiency. Amino acids containing sulphur are cofactors for enzymes involved in chlorophyll biosynthesis and various other metabolic pathways. Hence, the activation of sulphur-activated enzymes facilitates the maintenance of an elevated chlorophyll index. These enzymes are crucial in facilitating the efficient synthesis and upkeep of chlorophyll molecules. The capacity of sulphur to enhance the absorption and integration of vital nutrients, such as nitrogen and magnesium, can indirectly impact the chlorophyll index. These nutrients play a crucial role in chlorophyll synthesis, and an enhanced accessibility to these nutrients leads to an elevated chlorophyll index (Liu, Li, et al., 2022; Liu, Cui, et al., 2022; Liu, Zong, et al., 2022; Liu, Liu, et al., 2022; Liu, Xiao, et al., 2022; Liu, Meng, et al., 2022; Lombardino et al., 2022; Ma et al., 2022). The involvement of salicylic acid (SA) in plant defence mechanisms can indirectly influence the chlorophyll index by mitigating stress-induced impacts. The degradation of chlorophyll in plants is mitigated when they perceive a decrease in stress levels due to the activation of salicylic acid (SA)-mediated defence mechanisms. This response allows the plant to sustain a higher chlorophyll index. The stress-response properties of SA may lead to an enhancement in photosynthetic efficiency. Salicylic acid (SA) plays a crucial role in facilitating the efficient functioning of chlorophyll molecules. It contributes to an increased chlorophyll index through the mitigation of oxidative stress and the protection of chloroplasts. Consequently, there is an increase in the chlorophyll content. The absorption efficiency of essential nutrients, such as magnesium, which plays a crucial role in the composition of chlorophyll molecules, can be enhanced by using SA. An elevated chlorophyll index is attributed to enhanced chlorophyll synthesis facilitated by heightened magnesium absorption (Mabasa, 2023; Maheshwari et al., 2022; Maia et al., 2022; Makete et al., 2022; Manepalli et al., 2022; Mangena, 2022; Manjunatha et al., 2022; Masmoudi et al., 2023; Megala et al., 2022; Mittal et al., 2022; Mohammadi et al., 2023; Molinari et al., 2023; Moustakas et al., 2022; Mugwanya et al., 2023; Mukarram et al., 2022; Mukhtar et al., 2023; Myers Jr. et al., 2023; Nadeem,

2022; Nam et al., 2023; Napieraj et al., 2023). The chlorophyll index holds significance in mustard research due to its ability to indicate photosynthetic efficiency, nutrient status, stress response, and growth potential. The chlorophyll index in mustard can be positively influenced by the presence of sulphur and salicylic acid due to their mechanisms that enhance chlorophyll synthesis, activate enzymes, facilitate nutrient absorption, and mitigate stress (Li, Luo, et al., 2022; Li, Tong, et al., 2023; Liu et al., 2023; Liu, Wang, et al., 2022). This knowledge forms the foundation for advancing novel agricultural practices and strategies that optimise the chlorophyll index, enhancing crop productivity and overall plant well-being in mustard cultivation. As further investigations are carried out on the precise molecular mechanisms underlying the interactions between sulphur (S) and salicylic acid (SA), it is anticipated that additional avenues for enhancing mustard cultivation through crop improvement will be unveiled (Li, Zhang, et al., 2022; Li, Zheng, et al., 2023; Li et al., 2022; Li, Han, et al., 2022; Li, He, et al., 2023; Li et al., 2022; Li, Han, et al., 2023; Li, Huang, et al., 2022).

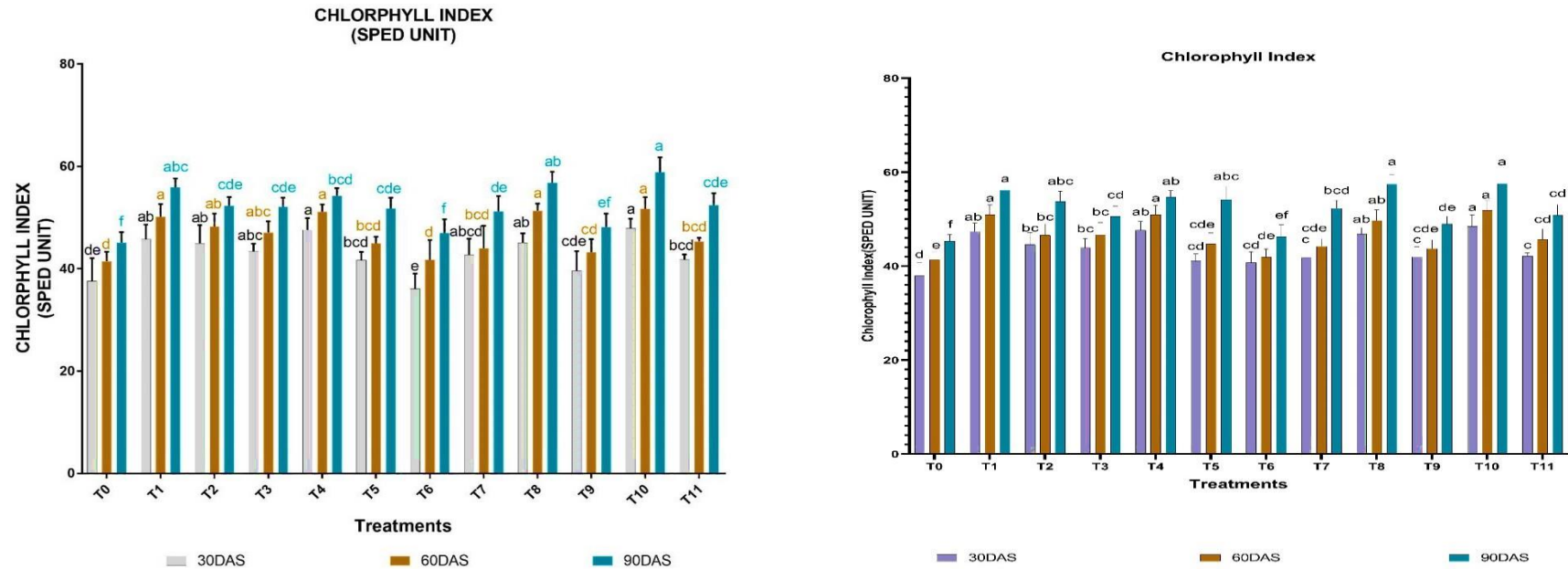
**Table 4.10. Impact of Different Treatments on Chlorophyll Index of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	37.56 <sup>de</sup> ±4.50	37.93 <sup>d</sup> ±2.75	41.46 <sup>d</sup> ±1.87	41.36 <sup>e</sup> ±1.65	45.10 <sup>f</sup> ±2.08	45.33 <sup>f</sup> ±1.35
<b>T1 (Thiourea-1000 ppm)</b>	45.80 <sup>ab</sup> ±2.82	47.36 <sup>ab</sup> ±1.81	50.13 <sup>a</sup> ±2.50	50.93 <sup>a</sup> ±2.08	55.90 <sup>abc</sup> ±1.76	56.16 <sup>a</sup> ±.30
<b>T2 (Salicylic acid-300 ppm)</b>	45.00 <sup>ab</sup> ±3.56	44.60 <sup>bc</sup> ±2.58	48.26 <sup>ab</sup> ±2.55	46.60 <sup>bc</sup> ±2.40	52.26 <sup>cde</sup> ±1.76	53.73 <sup>abc</sup> ±2.21
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	43.43 <sup>abc</sup> ±1.47	43.86 <sup>bc</sup> ±2.02	47.06 <sup>abc</sup> ±2.20	46.63 <sup>bc</sup> ±2.62	52.10 <sup>cde</sup> ±1.83	50.60 <sup>cd</sup> ±2.16
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	47.60 <sup>a</sup> ±2.28	47.63 <sup>ab</sup> ±1.85	51.16 <sup>a</sup> ±1.40	50.93 <sup>a</sup> ±1.96	54.26 <sup>bcd</sup> ±1.50	54.66 <sup>ab</sup> ±1.37
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	41.70 <sup>bcd</sup> ±1.60	41.10 <sup>cd</sup> ±1.49	44.96 <sup>bcd</sup> ±1.30	44.70 <sup>cde</sup> ±2.35	51.76 <sup>cde</sup> ±2.12	54.13 <sup>abc</sup> ±2.83
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	36.06 <sup>e</sup> ±2.97	40.73 <sup>cd</sup> ±2.31	41.73 <sup>d</sup> ±3.88	41.90 <sup>de</sup> ±1.77	47.00 <sup>f</sup> ±2.68	46.23 <sup>ef</sup> ±2.59
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	42.70 <sup>abcd</sup> ±3.19	41.83 <sup>c</sup> ±2.11	44.00 <sup>bcd</sup> ±4.40	44.20 <sup>cde</sup> ±1.60	51.20 <sup>de</sup> ±3.02	52.30 <sup>bcd</sup> ±1.70
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	45.10 <sup>ab</sup> ±1.85	46.86 <sup>ab</sup> ±1.28	51.33 <sup>a</sup> ±1.40	49.70 <sup>ab</sup> ±2.26	56.76 <sup>ab</sup> ±2.21	57.46 <sup>a</sup> ±2.03
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	39.60 <sup>cde</sup> ±3.85	41.96 <sup>c</sup> ±2.13	43.26 <sup>cd</sup> ±2.53	43.73 <sup>cde</sup> ±1.85	48.16 <sup>ef</sup> ±2.60	48.96 <sup>de</sup> ±1.64
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	47.90 <sup>a</sup> ±1.90	48.50 <sup>a</sup> ±2.42	51.73 <sup>a</sup> ±2.23	51.90 <sup>a</sup> ±2.09	58.86 <sup>a</sup> ±2.91	57.56 <sup>a</sup> ±2.66
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	41.80 <sup>bcd</sup> ±.98	42.16 <sup>c</sup> ±.65	45.33 <sup>bcd</sup> ±.75	45.70 <sup>cd</sup> ±2.22	52.40 <sup>cde</sup> ±2.35	50.83 <sup>cd</sup> ±2.25
<b>CD</b>	4.912	3.458	4.366	3.349	3.992	3.552
<b>CV</b>	6.726	4.642	5.485	4.224	4.491	3.983

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance



Figure 4.10. Chlorophyll Index (SPED unit) of Mustard During Rabi 2021-2023 & 2022-23



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Plant fresh weight:** The effect of Sulphur and Salicylic acid and their combination on plant fresh weight was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.11, Figure 4.11) In 2021-2022, there was a significant difference in plant fresh weight compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the plant fresh weight was observed at 30, 60, and 90 DAS. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T6, followed by T11, T10, T2, T3, T5, T9, T4, T8, T1, and the percentage values were 59.13%, 44.70%, 44.36%, 43.61%, 40.53%, 32.93%, 30.95%, 22.75%, 11.47%, 4.79% respectively. But in T7, the percentage decreased compared to T0, and the percentage value was -15.83%. At 60 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T10, T1, T2, T6, T4, T11, T5, T9, T7, T8 and the percentage values were 76.91%, 76.32%, 70.56%, 65.85%, 65.84%, 52.01%, 48.81%, 43.29%, 40.71%, 35.48%, 27.04% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T10, T2, T1, T6, T4, T11, T7, T9, T8, T5 and the percentage values were 77.90%, 77.02%, 68.40%, 67.04%, 61.31%, 51.38%, 46.68%, 40.76%, 34.74%, 28.81%, and 27.44% respectively. In 2022-2023, there was a significant difference in plant fresh weight compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T6, followed by T11, T10, T2, T3, T5, T9, T4, T1, T8, and the percentage values were 56.23%, 45.29%, 44.14%, 40.75%, 37.04%, 33.34%, 32.81%, 27.06%, 8.28%, 6.66% respectively. But in T7, the percentage decreased compared to T0, and the percentage value was -5.62%. At 60 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T3, T1, T6, T2, T11, T4, T9, T5, T7, T8 and the percentage values were 70.66%, 70.43%, 60.82%, 56.40%, 55.06%, 37.61%, 36.97%, 28.56%, 28.47%, 19.92%, 12.13% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T10, T1, T2, T6, T4, T11, T7, T9, T5, T8 and the percentage values were 73.90%, 73.77%, 63.16%, 61.24%, 53.91%, 47.82%, 45.31%, 37.38%, 28.91%, 23.81%, 15.47% respectively. Within agricultural research and crop management, assessing the fresh

weight of mustard (*Brassica juncea* L.) plant tissues, mainly leaves, holds significant relevance. Understanding the importance of new weight and the potential effects of externally applied sulphur (S) and salicylic acid (SA) on mustard leaves is crucial for optimising agricultural practices and enhancing crop productivity. The fresh weight of mustard plants is a reliable indicator of overall growth and development (Wang et al., 2022; F. Wang et al., 2023; Wang et al., 2023; Wang et al., 2022; Wang & Komatsu, 2022; Wang et al., 2022; Wang et al., 2022; Wang et al., 2022; Wante et al., 2022; Waters & Nelson, 2023; Wu et al., 2022; Wurms et al., 2023; Xiao et al., 2022). The phenomenon under consideration is a manifestation of the presence of water and cellular components within plant tissues, which exhibits a strong correlation with the vigour and well-being of the plant. The process of nutrient uptake and assimilation has a significant impact on the overall biomass of a plant's tissues. Plants with robust physiological conditions and active growth can extract essential nutrients from the soil and assimilate them into their biological structures. When a population experiences an increase in fresh weight, it is generally considered a reliable indicator of efficient nutrient uptake and utilisation. The photosynthetic efficiency of mustard leaves exhibits a direct correlation with the rate of weight gain resulting from new biomass production. The observed rise in weight indicates concurrent increases in leaf area, chlorophyll content, and overall photosynthetic activity, resulting in augmented energy production through plant growth. The examination of fluctuations in the fresh weight of a plant can offer valuable insights into its response to environmental stressors (Takeuchi et al., 2022; Talaat, 2023; Tan et al., 2022; Tariq et al., 2023; Tariq et al., 2022; Todorova et al., 2022; Topcu et al., 2022; Torres & Figueroa, 2023; Tyagi et al., 2022; Ullah et al., 2023; Ullah et al., 2022; Verma et al., 2023; Waadt et al., 2022; Wan & Xin, 2022). The new importance of plants may experience a decline when they are exposed to various stressors, including but not limited to drought, pests, diseases, or inadequate nutrient availability. This decrease in fresh weight can be attributed to the plants' necessity to reallocate resources in response to these stress-inducing conditions. Mustard plants necessitate sulphur as a macronutrient for optimal growth and development. The external application of sulphur to the plant can elicit diverse impacts on the plant's fresh weight. Sulphur can enhance a plant's capacity to assimilate crucial nutrients, including nitrogen and phosphorus, which are indispensable for its growth. Enhanced nutrient

availability has the potential to promote nutrient assimilation and overall plant growth, thereby resulting in an augmentation of fresh weight in plants. Sulphur is crucial in synthesising amino acids, particularly cysteine and methionine. These specific amino acids are essential for synthesising proteins, which play a vital role in plants' growth and cellular homeostasis. An augmentation in the accessibility of sulphur may result in an elevation in protein content, thereby contributing to a rise in fresh weight accumulation. The synthesis of secondary metabolites, specifically glucosinolates, exclusive to Brassicaceae plants such as mustard, necessitates the inclusion of sulphur as a constituent. These metabolites can indirectly influence plants' fresh weight by modulating their defence mechanisms and stress responses. Mustard plants can enhance their tolerance towards environmental stressors by activating stress responses facilitated by salicylic acid (SA) (Singh et al., 2022; Singh & Roychoudhury, 2023; Singh & Nandi, 2022; Singhal et al., 2023; Sivanesan et al., 2022; Song et al., 2023; Sousa et al., 2022; Spinoso-Castillo & Bello-Bello, 2022; Suliman et al., 2022; Sun et al., 2022; Sun et al., 2023; Sun et al., 2022; Taira & Shiono, 2022). When stress levels are diminished due to the activation of SA-mediated defence mechanisms, there is a subsequent rise in fresh weight as resources are subsequently reallocated to promote growth and development. Previous studies have shown that applying salicylic acid (SA) can enhance the efficacy of photosynthesis in specific plant species. An augmentation in the fresh weight can be attributed to enhanced photosynthetic activity, leading to an upsurge in energy production and nutrient assimilation. The organisational structure of SA allows for a degree of influence over allocating resources within the plant. The activation of SA-mediated defences decreases stress levels, which provides for allocating extra resources such as energy and nutrients towards growth processes. This ultimately promotes an increase in fresh weight (Sharma & Verma, 2023; Sheikhalipour et al., 2023; Shekhawat et al., 2023; Shi et al., 2023; Shi et al., 2022; Si et al., 2023). The new importance of mustard leaves is a critical parameter that indicates plant growth, nutrient status, photosynthetic efficiency, and responses to environmental stress. Applying sulphur and salicylic acid externally can impact fresh weight by enhancing nutrient uptake, protein synthesis, stress tolerance, and resource allocation. Once a comprehensive understanding of these mechanisms is achieved, developing agricultural practices designed to optimise the fresh weight of mustard becomes possible. This,

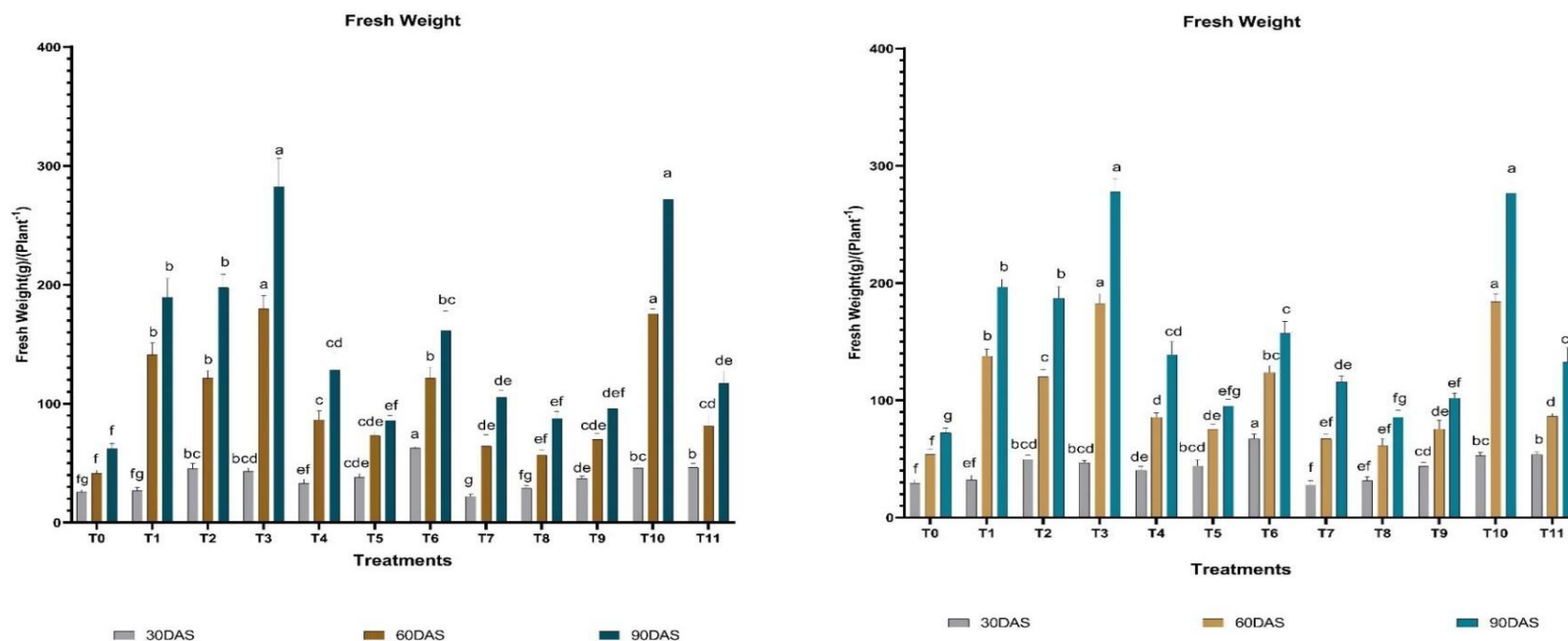
in turn, leads to enhanced crop yield and improved overall quality. Further investigation is necessary to enhance the fresh importance of mustard by refining the application methods and concentrations (Salwan et al., 2023; Samad et al., 2023; Sampedro-Guerrero et al., 2022; Samtani et al., 2022; Shah et al., 2022, 2023; Shang et al., 2022).

**Table 4.11. Impact of Different Treatments on Plant Fresh Weight of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	25.65 <sup>fg</sup> ±2.07	29.48 <sup>f</sup> ±2.79	41.55 <sup>f</sup> ±2.45	54.01 <sup>f</sup> ±4.19	62.47 <sup>f</sup> ±4.14	72.51 <sup>g</sup> ±3.94
<b>T1 (Thiourea-1000 ppm)</b>	26.94 <sup>fg</sup> ±2.74	32.14 <sup>ef</sup> ±4.03	141.15 <sup>b</sup> ±10.20	137.85 <sup>b</sup> ±5.75	189.60 <sup>b</sup> ±15.71	196.85 <sup>b</sup> ±6.52
<b>T2 (Salicylic acid-300 ppm)</b>	45.49 <sup>bc</sup> ±4.19	49.76 <sup>bcd</sup> ±3.64	121.69 <sup>b</sup> ±6.17	120.20 <sup>c</sup> ±6.12	197.71 <sup>b</sup> ±11.20	187.11 <sup>b</sup> ±9.97
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	43.14 <sup>bcd</sup> ±2.94	46.83 <sup>bcd</sup> ±2.03	179.99 <sup>a</sup> ±11.12	182.69 <sup>a</sup> ±8.34	282.75 <sup>a</sup> ±23.57	277.90 <sup>a</sup> ±11.18
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	33.21 <sup>ef</sup> ±3.45	40.42 <sup>de</sup> ±3.34	86.58 <sup>c</sup> ±7.42	85.69 <sup>d</sup> ±3.64	128.50 <sup>cd</sup> ±14.08	138.99 <sup>cd</sup> ±11.21
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	38.25 <sup>cde</sup> ±2.88	44.23 <sup>bcd</sup> ±5.03	73.20 <sup>cde</sup> ±4.22	75.51 <sup>de</sup> ±4.03	86.11 <sup>ef</sup> ±4.13	95.18 <sup>efg</sup> ±5.79
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	62.77 <sup>a</sup> ±1.24	67.36 <sup>a</sup> ±3.89	121.66 <sup>b</sup> ±8.93	123.88 <sup>bc</sup> ±5.51	161.51 <sup>bc</sup> ±16.54	157.35 <sup>c</sup> ±9.92
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	22.14 <sup>g</sup> ±1.76	27.91 <sup>f</sup> ±3.58	64.40 <sup>de</sup> ±9.45	67.44 <sup>ef</sup> ±3.71	105.47 <sup>de</sup> ±6.03	115.81 <sup>de</sup> ±4.93
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	28.98 <sup>fg</sup> ±2.15	31.59 <sup>ef</sup> ±3.20	56.95 <sup>ef</sup> ±4.15	61.47 <sup>ef</sup> ±6.08	87.76 <sup>ef</sup> ±5.65	85.79 <sup>fg</sup> ±5.71
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	37.15 <sup>de</sup> ±2.05	43.88 <sup>cd</sup> ±3.28	70.08 <sup>cde</sup> ±4.73	75.60 <sup>de</sup> ±7.39	95.74 <sup>def</sup> ±5.84	102.02 <sup>ef</sup> ±4.17
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	46.10 <sup>bc</sup> ±2.90	52.79 <sup>bc</sup> ±2.69	175.53 <sup>a</sup> ±4.28	184.14 <sup>a</sup> ±6.44	271.88 <sup>a</sup> ±13.50	276.51 <sup>a</sup> ±9.93
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	46.39 <sup>b</sup> ±3.31	53.90 <sup>b</sup> ±2.08	81.17 <sup>cd</sup> ±9.76	86.57 <sup>d</sup> ±2.41	117.18 <sup>de</sup> ±10.21	132.60 <sup>cd</sup> ±12.56
<b>CD</b>	4.278	5.461	11.692	9.725	20.245	14.906
<b>CV</b>	6.602	7.39	6.781	5.204	7.978	5.708

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

Figure 4.11. Plant Fresh Weight of Mustard During Rabi 2021-2023 & 2022-23



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Plant turgid weight:** The effect of Sulphur and Salicylic acid and their combination on plant turgid weight was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.12, Figure 4.12). In 2021-2022, there was a significant difference in plant turgid weight compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the plant turgid weight was observed at 30, 60, and 90 DAS. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T6 followed by T2, T11, T10, T3, T5, T9, T4, T8, T1 and the percentage values were 55.58%, 38.88%, 38.29%, 37.37%, 32.85%, 28.04%, 25.06%, 20.72%, 2.23%, 0.89% respectively. But in T7, the percentage decreased compared to T0, and the percentage value was -20.46%. At 60 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T10, T1, T2, T6, T4, T11, T5, T9, T7, T8 and the percentage values were 76.18%, 75.73%, 70.23%, 66.34%, 65.16%, 51.95%, 48.11%, 42.29%, 39.09%, 35.02%, 26.12% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T10, T2, T1, T6, T4, T11, T7, T9, T8, T5 and the percentage values were 77.06%, 75.85%, 68.03%, 65.57%, 59.83%, 50.09%, 44.43%, 39%, 33.79%, 28.36%, 24.38% respectively. In 2022-2023, there was a significant difference in plant turgid weight compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found highest in T6 followed by T11, T10, T2, T3, T5, T4, T9, T8, T1 and the percentage values were 56.82%, 44.29%, 43.24%, 39.01%, 35.41%, 32.45%, 31.76%, 31.34%, 8.77%, 8.12% respectively. But in T7, the percentage decreased compared to T0, and the percentage value was -0.55%. At 60 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T3, T1, T6, T2, T4, T11, T9, T5, T7, T8 and the percentage values were 70.16%, 69.11%, 61.05%, 55.27%, 54.29%, 35.26%, 35.24%, 28.84%, 28.43%, 19.66%, 10.29% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T10, T1, T2, T6, T4, T11, T7, T9, T5, T8 and the percentage values were 73.91%, 73.52%, 62.71%, 61.35%, 53.77%, 48.08%, 46.53%, 36.84%, 28.94%, 25.39%, 16.88% respectively. The measurement of turgid weight is a crucial parameter in agricultural research and crop management for



mustard (*Brassica juncea* L.). The phenomenon above demonstrates the capacity of plant cells to retain water and maintain turgor pressure, which is crucial for plants' well-being, development, and adaptive responses to various stressors. In addition, acquiring information regarding the impacts of applying sulphur (S) and salicylic acid (SA) via foliar application on turgid weight can provide valuable perspectives for enhancing agricultural practices and increasing crop productivity. The concept of turgid weight involves the determination of the weight of plant tissue under conditions of complete hydration and turgidity (Zhang et al., 2023; Zhang et al., 2022; Zhang et al., 2022; Zhang et al., 2023; Zhao & Hu, 2023; Zhao et al., 2022; Zhao et al., 2022; Zhao et al., 2022; Zhu et al., 2022; Zhu et al., 2023; Zulfiqar et al., 2022). This indicates that the plant cells are replete with water and apply internal pressure on the cell walls. Maintaining an appropriate level of turgor pressure is imperative for the rigidity of plants, as well as for cell expansion and structural integrity. Turgor pressure is the primary mechanism driving water absorption and movement within plants, functioning as a pressure gradient. The vascular system facilitates the upward trend of water, nutrients, and essential molecules within the plant, enabling their distribution to different plant organs, including the roots, leaves, stems, and reproductive structures. The process above is crucial for the overall functioning of the plant, as well as for the acquisition of nutrients. The cellular turgor pressure plays a vital role in the photosynthesis process. Maintaining structural integrity in chloroplasts within plant cells is essential for facilitating efficient light absorption and gas exchange processes. To attain the highest photosynthesis and energy generation rates, achieving an optimal level of turgor pressure is imperative. Changes in turgor pressure serve as an indicator of a plant's response to environmental stressors. Plants can reduce turgor pressure when exposed to various forms of stress, including drought, salinity, or pathogenic invasion. This phenomenon can lead to the withering of plants and a decrease in their overall growth rate. Mustard plants necessitate sulphur as a macronutrient for optimal growth and development. The utilisation of foliar sulphur can impact turgid weight in various manners, as outlined below: Sulphur enhances the plant's capacity to assimilate crucial nutrients, including nitrogen (N) and phosphorus (P), which are indispensable for maintaining turgor pressure and overall plant well-being. Cysteine and methionine, two specific amino acids, are highly dependent on the presence of the element sulphur.

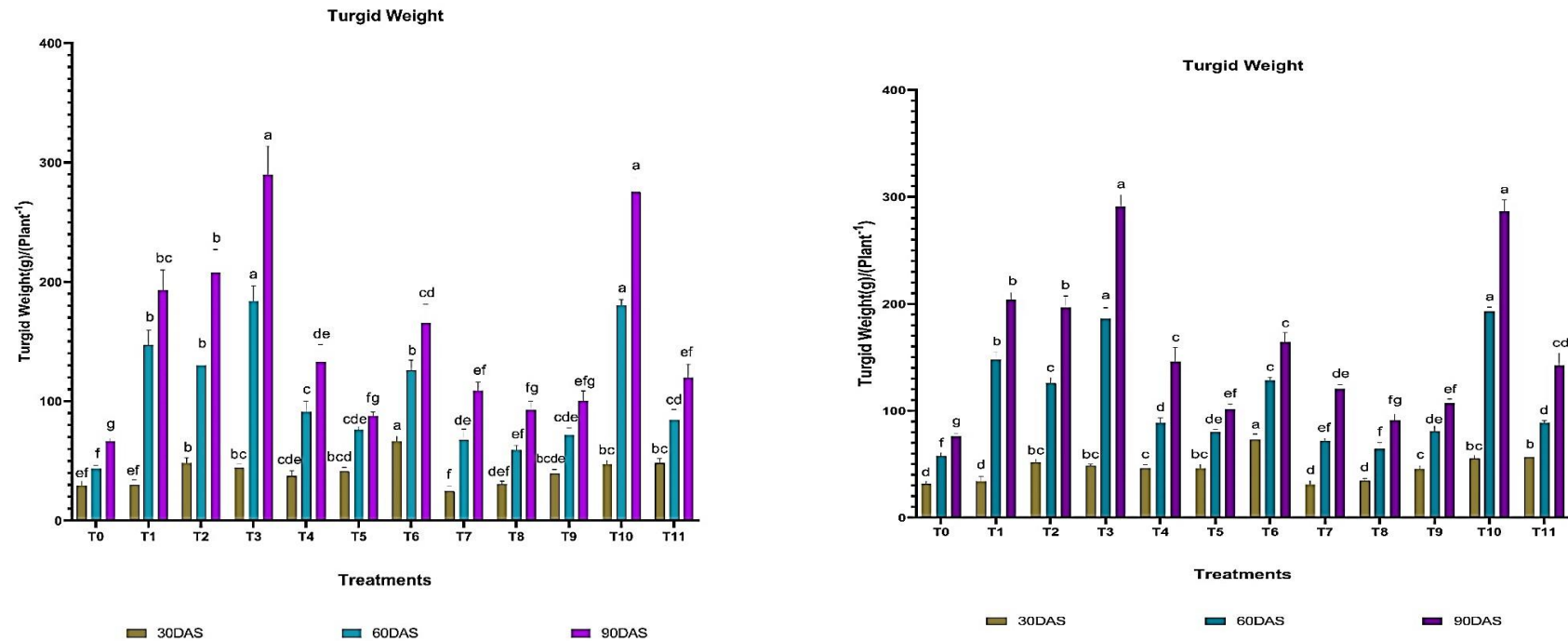
These amino acids are essential for synthesising proteins, crucial in maintaining the osmotic equilibrium within plant cells, thereby influencing turgor pressure. Sulphur can control the concentration of solutes, including ions and sugars, within plant cells, thereby playing a role in osmotic regulation. Sufficient osmotic regulation is imperative for maintaining stable turgor pressure and preventing wilting. Aside from its established role in plant defence mechanisms, salicylic acid has demonstrated its capacity to impact turgid weight. Mustard plants can enhance their resilience against environmental stressors, such as pathogen infections, by activating salicylic acid (SA) as a component of their stress response mechanism. Enhanced stability to stress can facilitate the maintenance of consistent turgor pressure under challenging circumstances. Sulfuric acid (SA) can influence cellular membranes' characteristics, such as their thickness and composition (Yang et al., 2022; Yao et al., 2022; Yin et al., 2023; Yousaf et al., 2022; Yu et al., 2022; Yu et al., 2022; Yu et al., 2023; Yuan et al., 2022; Zahid et al., 2023; Zang et al., 2022). The avoidance of cell collapse and preservation of turgor pressure can be achieved through the optimisation of cell wall structure. The modulation of aquaporins and ion transporters by SA can impact the regulation of water balance in plant cells. The maintenance of turgor pressure can be attributed, at least in part, to these contributing factors. The combined application of sulphur and salicylic acid on mustard plants can enhance turgid weight by facilitating nutrient uptake, promoting protein synthesis, regulating osmotic processes, improving stress tolerance, and enhancing water balance. Maintaining an optimal turgor pressure level is crucial for plants' well-being, growth, and stress response. To improve the productivity and quality of mustard crops, it is imperative to comprehend the underlying mechanisms through which sulphur and salicylic acid influence turgid weight. This understanding can serve as a valuable tool in informing agricultural practices (Xie et al., 2023; Xing et al., 2022; Xu et al., 2023; Xu et al., 2022, 2023; Xu, Cao, et al., 2022; Xu, Zeng, et al., 2022; Yagci & Agar, 2022; Yan et al., 2022; Yang et al., 2023; Yang et al., 2023; Yang & Lee, 2023; Yang et al., 2023).

**Table 4.12. Impact of Different Treatments on Plant Turgid Weight of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	29.62 <sup>ef</sup> ±3.21	31.37 <sup>d</sup> ±2.55	43.81 <sup>f</sup> ±2.23	57.53 <sup>f</sup> ±3.24	66.46 <sup>g</sup> ±2.40	75.94 <sup>g</sup> ±3.07
<b>T1 (Thiourea-1000 ppm)</b>	29.89 <sup>ef</sup> ±4.58	34.15 <sup>d</sup> ±4.53	147.19 <sup>b</sup> ±12.26	147.72 <sup>b</sup> ±6.92	193.07 <sup>bc</sup> ±16.72	203.71 <sup>b</sup> ±6.90
<b>T2 (Salicylic acid-300 ppm)</b>	48.47 <sup>b</sup> ±4.23	51.45 <sup>bc</sup> ±3.31	130.18 <sup>b</sup> ±7.28	125.86 <sup>c</sup> ±4.90	207.88 <sup>b</sup> ±19.10	196.50 <sup>b</sup> ±10.79
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	44.12 <sup>bc</sup> ±3.05	48.58 <sup>bc</sup> ±1.42	184.01 <sup>a</sup> ±12.58	186.29 <sup>a</sup> ±9.95	289.76 <sup>a</sup> ±23.87	291.10 <sup>a</sup> ±10.83
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	37.37 <sup>cde</sup> ±4.42	45.98 <sup>c</sup> ±3.44	91.19 <sup>c</sup> ±8.88	88.87 <sup>d</sup> ±4.55	133.16 <sup>de</sup> ±14.28	146.27 <sup>c</sup> ±12.81
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	41.16 <sup>bcd</sup> ±3.41	46.45 <sup>bc</sup> ±3.50	75.92 <sup>cde</sup> ±2.75	80.39 <sup>de</sup> ±2.21	87.89 <sup>fg</sup> ±3.15	101.80 <sup>ef</sup> ±4.28
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	66.69 <sup>a</sup> ±3.91	72.67 <sup>a</sup> ±5.53	125.78 <sup>b</sup> ±8.55	128.62 <sup>c</sup> ±2.66	165.47 <sup>cd</sup> ±15.73	164.28 <sup>c</sup> ±8.70
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	24.59 <sup>f</sup> ±4.34	31.20 <sup>d</sup> ±3.51	67.43 <sup>de</sup> ±9.07	71.61 <sup>ef</sup> ±2.69	108.96 <sup>ef</sup> ±6.97	120.25 <sup>de</sup> ±4.23
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	30.26 <sup>def</sup> ±2.82	34.39 <sup>d</sup> ±2.48	59.30 <sup>ef</sup> ±3.74	64.13 <sup>f</sup> ±6.04	92.78 <sup>fg</sup> ±7.16	91.37 <sup>fg</sup> ±5.38
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	39.53 <sup>bcd</sup> ±3.46	45.70 <sup>c</sup> ±2.96	71.93 <sup>cde</sup> ±5.89	80.84 <sup>de</sup> ±4.85	100.39 <sup>efg</sup> ±8.14	106.89 <sup>ef</sup> ±4.25
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	47.30 <sup>bc</sup> ±3.31	55.28 <sup>bc</sup> ±3.09	180.52 <sup>a</sup> ±4.81	192.83 <sup>a</sup> ±4.17	275.20 <sup>a</sup> ±11.57	286.84 <sup>a</sup> ±10.30
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	48.00 <sup>bc</sup> ±3.90	56.32 <sup>b</sup> ±3.36	84.45 <sup>cd</sup> ±8.84	88.84 <sup>d</sup> ±2.13	119.61 <sup>ef</sup> ±11.48	142.06 <sup>cd</sup> ±11.96
<b>CD</b>	5.917	5.678	12.775	8.716	22.828	14.73
<b>CV</b>	8.553	7.223	7.129	4.672	8.733	5.382

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.12. Plant Turgid Weight of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm);

**T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).**

**Plant dry weight:** The effect of Sulphur and Salicylic acid and their combination on plant dry weight was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.13, Figure 4.13). In 2021-2022, there was a significant difference in plant dry weight compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the plant dry weight was observed at 30, 60, and 90 DAS. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T6, followed by T2, T10, T11, T3, T5, T8, and T4, and the percentage values were 37.39%, 22.67%, 20.35%, 15.19%, 13.65%, 10.08%, 8.80%, 4.89% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T3, T6, T1, T11, T4, T2, T5, T7, T9, T8 and the percentage values were 43.41%, 41.77%, 32.16%, 27.87%, 25.48%, 24.45%, 22.77%, 14.64%, 10.35%, 3.37%, 3.17% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T10, T6, T1, T11, T2, T4, T7, T5 and the percentage values were 47.73%, 45.60%, 38.69%, 38.41%, 37.92%, 31.17%, 30.36%, 13.14%, 9.64% respectively. But in T8 and T9, the percentage decreased compared to T0; the percentage values were -1.57% and -4.19%. In 2022-2023, there was a significant difference in plant dry weight compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T6, followed by T2, T10, T11, T3, T8, T5, and T4, and the percentage values were 36.54%, 20.77%, 16.19%, 15.03%, 11.27%, 7.02%, 5.69%, 1.83% respectively. But in T1, T7, and T9 percentage decrease as compared to T0 and the percentage values were -7.44%, -13.40%, and -29.90%. At 60 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T3, T6, T1, T11, T4, T2, T5, T7, T9, T8 and the percentage values were 42.18%, 41.25%, 31.31%, 27.40%, 24.54%, 23.79%, 22.05%, 13.62%, 10.03%, 2.49%, 1.92% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T10, T6, T1, T11, T2, T4, T7, T5 and the percentage values were 47.43%, 44.72%, 38.22%, 37.94%, 37.18%, 30.03%, 29.21%, 12.70%, 7.89% respectively. But in T8 and T9, the percentage decreased compared to T0; the percentage values were -3.03% and -5.33%. The assessment of dry

weight in mustard (*Brassica juncea* L.) research and agricultural applications is a pivotal variable with noteworthy implications for comprehending plant development, nutrient allocation, and overall agricultural yield. Moreover, assessing the impacts of sulphur (S) and salicylic acid (SA) administration via foliar spray on the dry weight of mustard demonstrates promise in enhancing agricultural practices to enhance crop productivity and quality (Li, Han, et al., 2023; Li, Huang, et al., 2022; Li, Luo, et al., 2022; Li, Tong, et al., 2023; Liu et al., 2023; Liu, Wang, et al., 2022; Liu, Li, et al., 2022; Liu, Cui, et al., 2022). The assessment of dry weight serves as a precise and definitive approach to quantitatively determine the overall biomass accumulated by mustard plants throughout their complete growth cycle. By eliminating the presence of water, a thorough assessment is conducted to determine the total weight of all constituents of a plant, encompassing leaves, stems, roots, and reproductive organs. The dry weight analysis enhances our understanding of the complex nutrient distribution within the mustard plant. By examining nutrient uptake, translocation, and utilisation efficiency, this comprehensive analysis offers valuable insights into the distribution of nutrients within different parts of the plant. Researchers and farmers can effectively assess the growth rate and overall performance of mustard plants by conscientiously monitoring variations in dry weight over various time intervals. This affords researchers and farmers with the requisite information. The tool is vital for meticulously analysing the effectiveness of diverse agricultural cultivation methods and treatments in various farming practices. Furthermore, the meticulous examination of the results about dry weight serves as a valuable tool for predicting the prospective crop yield (Suliman et al., 2022; L. Sun et al., 2022; P. Sun et al., 2023; Sun et al., 2022). Farmers can extrapolate data from sampled plants to encompass the entire cultivation area to make informed decisions regarding harvest timing and anticipating yield volumes. This facilitates the application of the data across the entire cultivation area. A comprehensive understanding of the dynamics of dry weight in mustard plants is the fundamental basis for implementing sophisticated approaches in nutrient management. Furthermore, it is worth noting that it has practical applications in crop management. This research establishes a solid empirical foundation for identifying imbalances in nutrient levels, enabling targeted interventions to optimise plant well-being and improve yield results. The survival of mustard plants depends on sulphur as a macronutrient, which has been

demonstrated to exert considerable influence on the plants' dry weight through various mechanisms. Sulphur is known to have a significant role in the molecular composition of amino acids such as cysteine and methionine, which are indispensable constituents in the biosynthesis of proteins. Due to the pivotal role of proteins in numerous cellular processes, including growth and biomass accumulation, ample sulphur supply facilitates protein synthesis, thereby leading to an augmentation in dry weight. The significance of sulphur extends beyond its role as a cofactor in enzymes due to its involvement in various metabolic pathways. Activating these enzymes, facilitated by sulphur, initiates a range of processes essential for the plant's growth and development, ultimately influencing the plant's dry weight. Additionally, incorporating sulphur enhances the mustard plant's capacity to assimilate nutrients, specifically vital elements such as nitrogen (N) and phosphorus (P), which are indispensable for the plant's overall development. The influence of sulphur on a plant's dry mass is bolstered by the evidence that these nutrients play a crucial role in biomass accumulation and overall plant vitality. Glucosinolates represent a secondary metabolite that exhibits characteristic properties within the Brassicaceae family, encompassing mustard plants. The presence of sulphur plays a crucial role in facilitating the synthesis of secondary metabolites. These compounds exert an indirect but noticeable impact on the dry weight due to their ability to affect plant growth and biomass distribution. One of the notable effects of salicylic acid on dry weight is its well-established role in the defence mechanisms of plants. The stress-responsive properties of salicylic acid primarily contribute to the enhancement of mustard plants' resistance against pests, diseases, and adverse climatic conditions. The decrease in stress levels, facilitated by the defensive mechanisms mediated by salicylic acid, contributes to a more efficient seed-filling process, ultimately resulting in an augmentation of dry weight. Furthermore, empirical evidence has shown that applying salicylic acid can enhance the rate of photosynthetic activity in specific plant species (Li, Zheng, et al., 2023; Li et al., 2022; Li, Han, et al., 2022; Li, He, et al., 2023; Li et al., 2022). The enhancement above leads to a rise in energy generation, resulting in an expedited biomass accumulation. The efficient allocation of plant resources by salicylic acid provides evidence of the acid's impressive capabilities. Due to the capacity of salicylic acid to mitigate the impacts of stress-induced impediments, a larger allocation of resources, encompassing energy and nutrients,



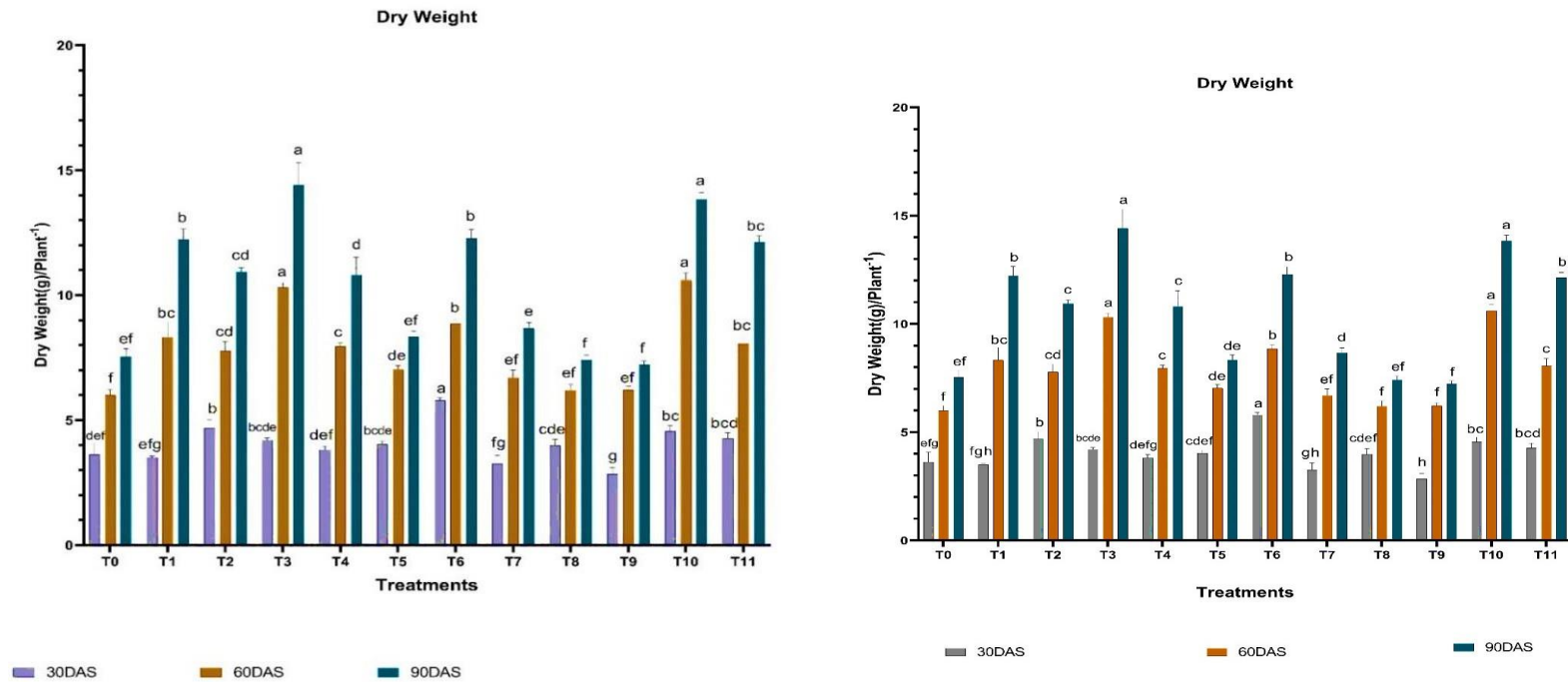
becomes accessible to facilitate the advancement of growth mechanisms. Implementing this strategic allocation is expected to inevitably lead to a rise in the mass of the product when moisture is removed. The significance of the potential synergistic effects resulting from applying sulphur and salicylic acid to the foliar surface of a plant cannot be overstated. The intervention applied to mustard plants results in a synergistic regulation of protein synthesis, enzyme activation, nutrient uptake, stress tolerance, photosynthesis, and resource allocation. In aggregate, these processes exert a substantial influence on the total biomass of the plants. The synergistic interplay of these elements in a mutually advantageous manner provides a potential avenue for altering agricultural methodologies to augment the dry weight of the mustard crop. When utilised as a foliar spray, applying sulphur and salicylic acid exhibits a wide range of effects on dry weight, encompassing protein synthesis, stress alleviation, enzyme stimulation, nutrient control, augmented photosynthetic activity, and resource distribution. A range of mechanisms causes these effects. Future research efforts are expected to enhance the techniques and levels of these applications, leading to advancements in mustard dry weight. Consequently, this will result in improvements in crop yield and quality. As the understanding of this subject matter progresses, additional research efforts are anticipated to enhance the techniques and levels of these implementations (Lajayer et al., 2022; Li et al., 2022; Li et al., 2022; Li et al., 2022; Li, Ren, et al., 2023; Li, Zhang, et al., 2022).

**Table 4.13. Impact of Different Treatments on Plant Dry Weight of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	3.62 <sup>def</sup> ±.44	3.75 <sup>efg</sup> ±.42	6.00 <sup>f</sup> ±.22	6.12 <sup>f</sup> ±.20	7.53 <sup>ef</sup> ±.34	7.69 <sup>ef</sup> ±.33
<b>T1 (Thiourea-1000 ppm)</b>	3.50 <sup>efg</sup> ±.05	3.49 <sup>fgh</sup> ±.13	8.32 <sup>bc</sup> ±.59	8.44 <sup>bc</sup> ±.53	12.22 <sup>b</sup> ±.42	12.40 <sup>b</sup> ±.34
<b>T2 (Salicylic acid-300 ppm)</b>	4.69 <sup>b</sup> ±.33	4.73 <sup>b</sup> ±.24	7.77 <sup>cd</sup> ±.36	7.86 <sup>cd</sup> ±.35	10.94 <sup>cd</sup> ±.16	11.00 <sup>c</sup> ±.13
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	4.20 <sup>bcd</sup> ±.08	4.22 <sup>bcd</sup> ±.04	10.31 <sup>a</sup> ±.18	10.43 <sup>a</sup> ±.12	14.40 <sup>a</sup> ±.88	14.64 <sup>a</sup> ±.80
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	3.81 <sup>def</sup> ±.14	3.82 <sup>defg</sup> ±.08	7.94 <sup>c</sup> ±.16	8.04 <sup>c</sup> ±.14	10.81 <sup>d</sup> ±.71	10.87 <sup>c</sup> ±.55
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	4.03 <sup>bcd</sup> ±.13	3.97 <sup>cdef</sup> ±.05	7.03 <sup>de</sup> ±.16	7.09 <sup>de</sup> ±.07	8.33 <sup>ef</sup> ±.23	8.35 <sup>de</sup> ±.14
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	5.79 <sup>a</sup> ±.11	5.91 <sup>a</sup> ±.12	8.85 <sup>b</sup> ±.19	8.92 <sup>b</sup> ±.15	12.28 <sup>b</sup> ±.35	12.46 <sup>b</sup> ±.28
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	3.26 <sup>fg</sup> ±.32	3.30 <sup>gh</sup> ±.26	6.69 <sup>ef</sup> ±.31	6.81 <sup>ef</sup> ±.28	8.67 <sup>e</sup> ±.23	8.81 <sup>d</sup> ±.23
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	3.97 <sup>cde</sup> ±.26	4.03 <sup>cdef</sup> ±.25	6.20 <sup>ef</sup> ±.24	6.24 <sup>f</sup> ±.29	7.41 <sup>f</sup> ±.18	7.47 <sup>ef</sup> ±.13
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	2.84 <sup>g</sup> ±.24	2.88 <sup>h</sup> ±.21	6.21 <sup>ef</sup> ±.14	6.28 <sup>f</sup> ±.12	7.22 <sup>f</sup> ±.15	7.30 <sup>f</sup> ±.09
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	4.55 <sup>bc</sup> ±.21	4.51 <sup>bc</sup> ±.11	10.61 <sup>a</sup> ±.29	10.59 <sup>a</sup> ±.18	13.84 <sup>a</sup> ±.26	13.92 <sup>a</sup> ±.27
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	4.27 <sup>bcd</sup> ±.22	4.41 <sup>bcd</sup> ±.25	8.05 <sup>bc</sup> ±.34	8.12 <sup>c</sup> ±.30	12.13 <sup>bc</sup> ±.25	12.25 <sup>b</sup> ±.20
<b>CD</b>	0.404	0.355	0.517	0.459	0.649	0.566
<b>CV</b>	5.853	5.089	3.869	3.405	3.629	0.132

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

Figure 4.13. Plant dry weight of Mustard During Rabi 2021-2023 & 2022-23



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea

**(2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).**

### **Relative Water Content (RWC) (%)**

The effect of Sulphur and Salicylic acid and their combination on RWC was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60 and 90 days after sowing (DAS) (Table 4.14, Figure 4.14). In 2021-2022, there was a significant difference in RWC compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the RWC was observed at 30, 60 and 90 DAS. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T3, followed by T10, T11, T8, T6, T9, T2, T5, T7, T1, T4 and the percentage values were 12.91%, 12.62%, 11.85%, 10.78%, 9.35%, 9.35%, 8.81%, 7.83%, 5.41%, 5.16%, 3.13% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T9, T10, T6, T5, T1, T11, T8, T7, T4 and the percentage values were 3.76%, 3.32%, 3.11%, 2.50%, 2.14%, 1.75%, 1.61%, 1.54%, 0.89%, 0.52% respectively. However, in T2, the percentage decreased compared to T0, and the percentage value was -1.00%. At 90 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T1, T11, T5, T3, T6, T7, T4, T9, T2, T8 and the percentage values were 5.56%, 4.98%, 4.70%, 4.62%, 4.34%, 4.27%, 3.46%, 3.07%, 2.02%, 1.97%, 1.07% respectively. 2022-2023, there was a significant difference in RWC compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T2, followed by T3, T9, T11, T10, T5, and T1, and the percentage values were 3.42%, 3.06%, 2.77%, 2.45%, 2.21%, 1.57%, 0.48% respectively. But in T6, T8, T7 and T4 the percentage decrease as compared to T0 and the percentage values were -1.00%, -2.67%, -5.67%, and -7.26% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T11, T4, T6, T8, T10, T2, T7, T5 and the percentage values were 5.01%, 4.24%, 3.14%, 3.06%, 2.41%, 3.25%, 2.20%, 0.49%, 0.23% respectively. But in T1 and T9, the percentage decreased compared to T0, and the percentage values were -0.14% and -0.28%, respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T1 followed by T10, T7, T6, T3, T9, T2 and the percentage values were 1.54%, 1.34%, 1.10%,

0.50%, 0.30%, 0.20%, 0.02% respectively. But in T4, T8, T5 and T11 percentage decrease as compared to T0 and the percentage values were -0.28%, -1.73%, -2.22%, and -2.44% respectively. The measurement of relative water content (RWC) in Mustard (*Brassica juncea* L.) is a crucial physiological parameter with significant implications for plant science and agriculture (Sachan & Krishna, 2022; Salih, Wu, et al., 2022; Salih, Zhou, et al., 2022; Salwan et al., 2023; Samad et al., 2023; Sampetro-Guerrero et al., 2022; Samtani et al., 2022; Shah et al., 2022, 2023). In addition, acquiring an understanding of the potential impacts of sulphur (S) and salicylic acid (SA) foliar spray treatments on the relative water content (RWC) of mustard can yield valuable insights for enhancing agricultural practices and advancing overall crop vitality and productivity. The relative water content (RWC) is a significant physiological parameter utilised to assess the hydration status of plant tissues and their ability to retain water. The provided information elucidates significant insights into the plant's ability to maintain crucial physiological functions, endure environmental pressures, and regulate water homeostasis. The calculation of relative water content (RWC) involves the assessment of the water content of plant tissues relative to their maximum water-holding capacity. The RWC, commonly denoted as a percentage, can be computed using the formula provided in this reference (Rai & Kaushik, 2023; Raja Gopalan et al., 2022; Ramakrishnan & Zhou, 2022; Rani et al., 2023; Rizvi et al., 2022; Roussos, 2023; Ruidas et al., 2022; Rybczyński et al., 2022). The assessment of relative water content (RWC) in mustard and other crops holds considerable significance for various reasons. Measuring plants' relative water content (RWC) is a highly responsive indicator for assessing their response to drought-induced stress. This technology aids researchers and farmers in accurately determining the onset and intensity of water stress, enabling them to implement interventions to mitigate potential reductions in crop yield promptly. Monitoring relative water content (RWC) can yield valuable insights into mustard plants' general well-being and hydration status. A reduction in relative water content (RWC) may indicate insufficient water availability or compromised water absorption capacity, suggesting the need for adjustments in irrigation practices. Measuring a plant's relative water content is a valuable indicator of its ability to endure and recuperate from various environmental stressors, including salinity, drought, and extreme temperatures. Plants belonging to the mustard family that exhibit higher relative water content (RWC)

values tend to demonstrate increased resilience against environmental stressors. The capacity of a plant to assimilate nutrients and distribute them throughout its structure is contingent upon its hydration level. Maintaining optimal RWC levels is crucial for facilitating efficient nutrient absorption, thereby fostering vigorous growth and development. The prediction of potential crop yield can be facilitated by using data on Relative Water Content (RWC). A positive correlation often exists between elevated relative water content (RWC) values and increased yield potential. Higher RWC values provide more water for important physiological processes like photosynthesis and seed maturation. Sulphur is a crucial constituent in synthesising various compounds, encompassing amino acids and proteins, vital in maintaining cellular membranes' structural integrity and hydration. There exists a potential correlation between the availability of sulphur and the enhancement of a plant's capacity to synthesise Osmo protectants and compatible solutes. These substances facilitate water retention by plant cells, thereby contributing to an increased relative water content (RWC). Sulphur also influences the synthesis of sulfur-containing compounds, such as glutathione, an antioxidant. Enhanced antioxidant activity can protect plant cells against oxidative stress resulting from water deficiency, thereby contributing to maintaining relative water content (RWC). Sulfuric acid (SA) is widely recognised for its pivotal involvement in the intricate defence mechanisms employed by plants. The utilisation of salicylic acid (SA) as a foliar spray exhibits promising prospects in activating stress-responsive mechanisms, thereby aiding mustard plants in adapting to the adverse impacts of water stress. Stress tolerance can enhance water retention, increasing relative water content (RWC). Research has shown that salicylic acid (SA) can modulate stomatal behaviour, decreasing transpiration water loss. This phenomenon facilitates a reduction in overall water consumption by the plant, thereby contributing to the maintenance of higher relative water content (RWC) levels. Promoting root growth and development can also be facilitated through pathways mediated by salicylic acid (SA), enhancing the plant's capacity to effectively uptake water and maintain relative water content (RWC). Assessing the relative water content (RWC) in mustard is a significant method for evaluating the plant's hydration status, stress tolerance, and overall health. Applying sulphur and salicylic acid through foliar sprays has yielded advantageous outcomes regarding relative water content (RWC) by enhancing water retention, stress

tolerance, and absorption efficiency. These methods can be effective strategies for optimising crop management to enhance mustard crop health and maximise yields, even when confronted with unfavourable environmental conditions (Paalli et al., 2022; Pal et al., 2023; Parada et al., 2022; Parrey et al., 2023; Patel et al., 2022; Peng et al., 2022; Pérez-Llorca et al., 2023; Phokas et al., 2023; Phour & Sindhu, 2022; Poór et al., 2022; Quesada, 2022).

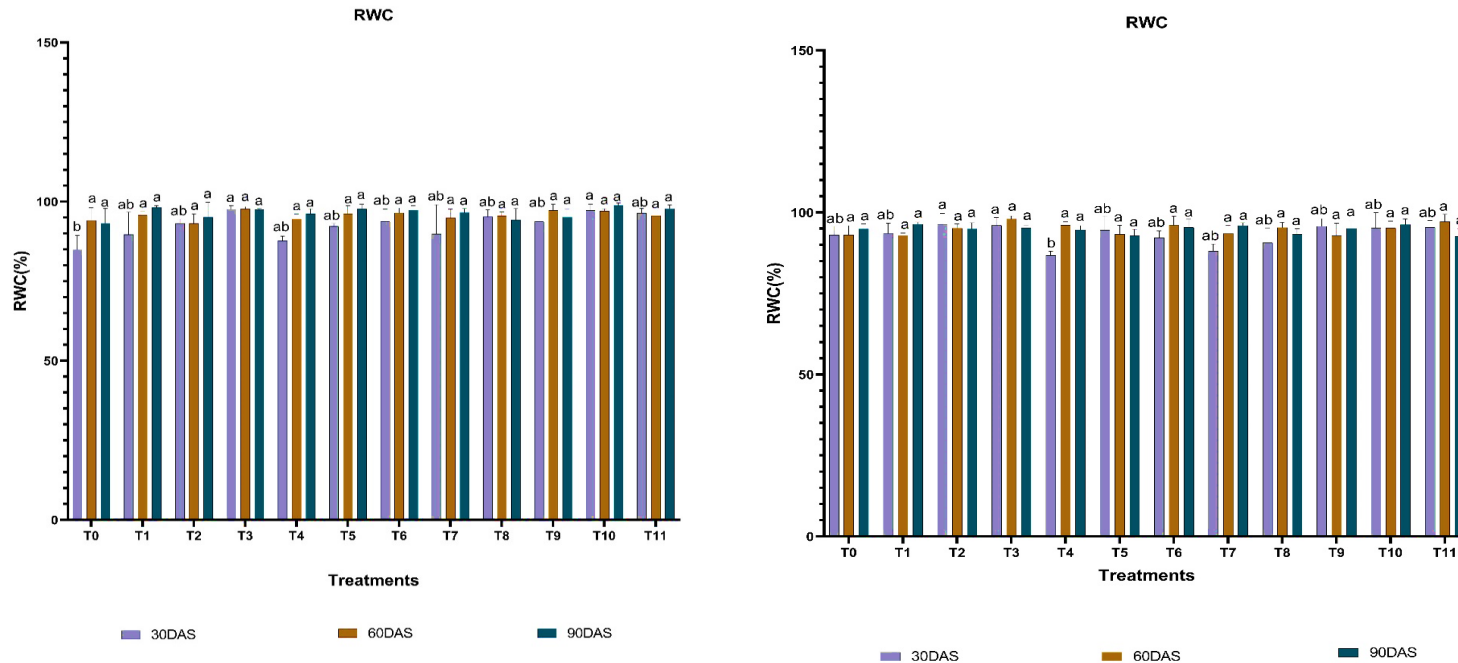


**Table 4.14. Impact of Different Treatments on RWC (%) of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	84.96 <sup>b</sup> ±4.39	93.08 <sup>ab</sup> ±2.63	94.04 <sup>a</sup> ±3.99	93.06 <sup>a</sup> ±2.89	93.21 <sup>a</sup> ±4.68	94.92 <sup>a</sup> ±1.52
<b>T1 (Thiourea-1000 ppm)</b>	89.59 <sup>ab</sup> ±7.12	93.54 <sup>ab</sup> ±3.17	95.71 <sup>a</sup> ±1.20	92.93 <sup>a</sup> ±0.69	98.10 <sup>a</sup> ±0.67	96.41 <sup>a</sup> ±0.62
<b>T2 (Salicylic acid-300 ppm)</b>	93.17 <sup>ab</sup> ±1.30	96.38 <sup>a</sup> ±3.30	93.10 <sup>a</sup> ±2.95	95.16 <sup>a</sup> ±1.28	95.09 <sup>a</sup> ±4.69	94.95 <sup>a</sup> ±1.86
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	97.56 <sup>a</sup> ±1.19	96.03 <sup>a</sup> ±2.34	97.71 <sup>a</sup> ±0.67	97.98 <sup>a</sup> ±0.95	97.44 <sup>a</sup> ±0.33	95.21 <sup>a</sup> ±0.88
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	87.71 <sup>ab</sup> ±1.42	86.77 <sup>b</sup> ±1.25	94.54 <sup>a</sup> ±1.51	96.09 <sup>a</sup> ±1.06	96.16 <sup>a</sup> ±1.55	94.66 <sup>a</sup> ±1.29
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	92.19 <sup>ab</sup> ±1.00	94.56 <sup>ab</sup> ±4.35	96.09 <sup>a</sup> ±2.68	93.28 <sup>a</sup> ±2.76	97.73 <sup>a</sup> ±1.41	92.86 <sup>a</sup> ±1.93
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	93.73 <sup>ab</sup> ±3.95	92.15 <sup>ab</sup> ±2.10	96.45 <sup>a</sup> ±1.53	96.01 <sup>a</sup> ±2.77	97.37 <sup>a</sup> ±1.39	95.41 <sup>a</sup> ±2.53
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	89.82 <sup>ab</sup> ±9.19	88.08 <sup>ab</sup> ±2.17	94.88 <sup>a</sup> ±2.74	93.53 <sup>a</sup> ±2.46	96.55 <sup>a</sup> ±1.25	95.99 <sup>a</sup> ±0.82
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	95.23 <sup>ab</sup> ±2.12	90.66 <sup>ab</sup> ±4.55	95.52 <sup>a</sup> ±1.21	95.37 <sup>a</sup> ±1.58	94.22 <sup>a</sup> ±3.59	93.31 <sup>a</sup> ±1.61
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	93.73 <sup>ab</sup> ±3.60	95.73 <sup>ab</sup> ±2.34	97.27 <sup>a</sup> ±1.87	92.80 <sup>a</sup> ±3.87	95.13 <sup>a</sup> ±2.58	95.12 <sup>a</sup> ±2.32
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	97.24 <sup>a</sup> ±1.90	95.19 <sup>ab</sup> ±4.77	97.06 <sup>a</sup> ±0.65	95.21 <sup>a</sup> ±2.10	98.70 <sup>a</sup> ±0.85	96.22 <sup>a</sup> ±1.80
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	96.39 <sup>ab</sup> ±1.49	95.42 <sup>ab</sup> ±2.15	95.58 <sup>a</sup> ±1.71	97.19 <sup>a</sup> ±2.24	97.81 <sup>a</sup> ±1.13	92.66 <sup>a</sup> ±2.36
<b>CD</b>	6.853	5.404	N/A	3.349	N/A	N/A
<b>CV</b>	4.342	3.405	2.029	2.071	2.436	1.903

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure-4.14. RWC (%) of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Crop Growth Rate( $\text{gm}^{-2}\text{day}^{-1}$ ):** The effect of Sulphur and Salicylic acid and their combination on CGR was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.15, Figure 4.15). In 2021-2022, there was a significant difference in CGR compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the NAR was observed at 30, 60, and 90 DAS. Therefore, at 60-30 DAS, the percentage increase as compared to T0 was found to be highest in T3 followed by T10, T1, T4, T11, T7, T9, T2, T6, T5 and the percentage values were 61.10%, 60.75%, 50.69%, 42.50%, 37.12%, 30.77%, 29.47%, 22.91%, 22.24%, 20.77% respectively. But percentage also decrease in T8 as compared to T0 and the percentage value was -6.89%. At 90-60 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T11, T1, T6, T10, T2, T4, T7 and the percentage values were 62.73%, 62.525, 60.88%, 55.53%, 52.78%, 51.78%, 46.74%, 22.63% respectively. But the percentage also decrease in T5, T8, T9 as compared to T0 and the percentage values were -17.43%, -25.82%, -50.65%. In 2022-2023, there was a significant difference in CGR compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 60-30 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T10, T1, T4, T11, T7, T9, T2, T5, T6 and the percentage values were 61.68%, 60.93%, 51.98%, 43.68%, 35.88%, 32.16%, 30.02%, 23.98%, 23.74%, 21.04% respectively. But percentage also decrease in T8 as compared to T0 and the percentage value was -7.37%. At 90-60 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T11, T1, T6, T10, T2, T4, T7 and the percentage values were 62.73%, 62.01%, 60.38%, 55.65%, 52.80%, 50.00%, 44.58%, 21.76% respectively. But percentage also decrease in T5, T8, T9 and the percentage values were -24.27%, -28.33%, -53.41%. *Brassica juncea*, commonly referred to as Mustard, is a widely cultivated oilseed crop that is highly regarded for its multifaceted applications in culinary and industrial contexts. The enhancement of mustard crop growth and productivity is a significant concern among both farmers and researchers. The utilisation of salicylic acid (SA) and sulphur (S) has garnered significant interest in the field of agriculture owing to their potential effects on crop growth rates. The objective

of this essay is to investigate the impact of salicylic acid and sulphur on the growth rate of mustard crops, examining the scientific research and agricultural implications linked to these treatments (Song et al., 2023; Sousa et al., 2022; Spinoso-Castillo & Bello-Bello, 2022; Suliman et al., 2022; Sun et al., 2022; Sun et al., 2023; Sun et al., 2022; Taira & Shiono, 2022; Takeuchi et al., 2022; Talaat, 2023; Tan et al., 2022; Tariq et al., 2023). The measurement of crop growth rate (CGR) is of great significance in the field of agriculture, as it provides a direct assessment of the rate at which crop biomass increases within a specific time frame. The statement emphasises the importance of a crop's vigour and general well-being, indicating its ability to efficiently utilise resources for the purpose of growth and development. The crop growth rate (CGR) is a crucial factor that has a substantial influence on the final crop yield. As a result, it has become a primary area of interest for academic research and agricultural management approaches. Salicylic acid (SA), an endogenous phytohormone, is extensively acknowledged for its participation in plant defence mechanisms and stress-related reactions. However, recent research has provided insights into the potential of this phenomenon to enhance crop growth. The application of salicylic acid (SA) externally has the potential to elicit various physiological and biochemical reactions in plants. The responses encompass increased rates of photosynthesis, enhanced nutrient uptake, and heightened resistance to abiotic environmental stressors. The effects can have a substantial impact on the crop growth rate (CGR) of mustard plants. Recent research has yielded findings that support the notion that the utilisation of salicylic acid can lead to a notable augmentation of the crop growth rate (CGR) in mustard plants. Salicylic acid (SA) serves as a signalling molecule that triggers the activation of genes associated with growth and development processes. As a result, this phenomenon gives rise to a heightened rate of biomass accumulation and enhanced growth rates in agricultural crops. Furthermore, the utilisation of systemic acquired resistance (SA) augments the crop's capacity to endure unfavourable environmental circumstances, such as water scarcity and the invasion of pathogens, thus alleviating the detrimental impacts of these stressors on the development of plants. Consequently, this phenomenon results in an elevated rate of crop growth and a general augmentation in the crop growth rate (CGR). Sulphur (S) is an essential element that plays a vital role in facilitating crop growth by participating in various physiological processes. Sulphur is an essential element that

serves as a vital component of amino acids, vitamins, and coenzymes involved in diverse metabolic pathways, such as photosynthesis. A deficiency of sulphur in mustard crops has the potential to reduce the CGR because of limitations in the synthesis of chlorophyll and proteins that are crucial for the growth and development of the plant. Prior research has established that the application of sulphur to mustard crops has a notable and beneficial impact on the rate of crop growth. Sulphur is known to have a substantial impact on the facilitation of the synthesis of crucial biomolecules, including cysteine and methionine. These biomolecules are essential to produce chlorophyll and enzymes that play a vital role in the process of photosynthesis. As a result, mustard crops that undergo sulphur treatment exhibit increased levels of chlorophyll content and improved photosynthetic efficiency. These enhancements have a direct impact on the increase in biomass accumulation and the overall rates of growth. Significantly, the simultaneous application of salicylic acid and sulphur exhibits synergistic effects on chlorophyll fluorescence in mustard plants. When used together, these two substances have a synergistic effect that enhances their individual positive impacts on crop development. Salicylic acid (SA) has been found to augment the activation of growth-promoting pathways in crops, thereby enhancing their capacity to efficiently absorb and utilise sulphur. Sulphur is significant in facilitating signalling pathways that are mediated by salicylic acid (SA), thereby enhancing the crop's ability to withstand both biotic and abiotic stresses. This ultimately results in an increased crop growth rate and yield (CGR). Moreover, the impact of salicylic acid and sulphur on the rate of cell growth (CGR) transcends the domain of vegetative growth. The application of these substances to mustard crops has resulted in the augmentation of both flower and seed production, thereby leading to an overall increase in crop yields. The phenomenon in question can have significant economic implications for mustard farmers, resulting in a more profitable harvest due to the increased CGR. Emphasising the environmental benefits of salicylic acid and sulphur in mustard cultivation holds significant importance. Through the enhancement of the CGR and subsequent improvement of photosynthetic activity, these interventions possess the capacity to facilitate carbon sequestration and mitigate the emission of greenhouse gases. Additionally, the potential reduction in the dependence on chemical pesticides and fertilisers in agricultural contexts, facilitated by the increased stress tolerance conferred by salicylic acid (SA)

and sulphur, may promote the adoption of sustainable and environmentally mindful farming practises. The application of salicylic acid and sulphur in mustard crops has a notable impact on the rate of crop growth, leading to increased accumulation of biomass, enhanced photosynthetic efficiency, and an overall improvement in crop productivity. The application of these treatments offers a potentially beneficial strategy for enhancing mustard cultivation, resulting in economic and environmental benefits. The progression of research has yielded valuable resources for farmers and agricultural scientists, enabling them to optimise mustard production and make substantial contributions to global food security. In modern agriculture, it is crucial to accurately measure and efficiently utilise crop growth rates (CGR) to meet the food requirements of a growing global population while also safeguarding our ecological resources. The Crop Growth Rate (CGR) is an essential agricultural metric that plays a critical role in assessing and overseeing crop productivity. The measurement assesses the gradual increase in crop biomass during a specified period, thereby indicating their growth, health, and overall state. The significance of crop growth rate (CGR) in the field of agriculture cannot be overstated, as it has a profound influence on various aspects of crop management, yield prediction, and allocation of resources. The significance of Crop Growth Rate (CGR) in agriculture is primarily attributed to the following factors: The measurement of crop growth rate (CGR) provides valuable insights into the potential yield of a crop. By employing CGR measurements at different stages of growth, farmers and researchers can generate informed predictions about the final yield. The acquisition of this data holds significant importance to efficiently plan harvest logistics, develop marketing strategies, and effectively accomplish food production goals. The evaluation of crop health entails the analysis of variations in the Crop Growth Rate (CGR), which serves as an early indication of factors impacting the overall condition of crops. A decline in Crop Growth Rate (CGR) is indicative of nutrient deficiencies, diseases, or water stress. By early identification of these issues, suitable measures can be implemented to mitigate decreases in crop yield and maintain the overall quality of the harvest. The Crop Growth Rate (CGR) concept holds considerable importance in influencing resource management strategies, including those pertaining to irrigation, fertilisation, and pest control. Understanding the rate at which a crop grows enables the efficient allocation of resources. The implementation of irrigation or

fertilisation practises in excessive amounts possesses the capacity to be both environmentally inefficient and detrimental. On the other hand, inadequate irrigation or fertilisation can lead to reduced agricultural productivity. The attainment of environmental sustainability is accomplished through the implementation of effective strategies for the utilisation of resources, which are informed by comprehensive data on crop growth rate (CGR). Farmers could mitigate potential adverse effects on water bodies and conserve water resources through the implementation of responsible agricultural practises, which involve reducing the excessive utilisation of water and fertilisers. This approach aids in reducing the probability of nutrient runoff, which has the potential to negatively impact aquatic ecosystems. The implementation of this sustainable approach has been shown to significantly decrease the environmental impact linked to agricultural practises. The evaluation of crops' capacity to withstand the impacts of climate change is crucial in ensuring climate resilience. The measurement of crop growth rate (CGR) holds significant importance in this context. The variability in climatic conditions possesses the capacity to impact the trends observed in crop development. The practise of monitoring crop growth and development allows farmers to modify their agricultural methods and make well-informed choices when it comes to selecting crop varieties that are better suited to changing temperature and precipitation patterns. The facilitation of optimising planting dates is enhanced through a comprehensive understanding of crop growth rates (CGR), as it assists in identifying the most advantageous timing for planting. The temporal coordination of agricultural activities holds significant importance, as the act of planting crops either prematurely or belatedly can have detrimental effects on crop yields. The utilisation of CGR data enables the coordination of planting schedules with the inherent growth potential of the crop. Crop Selection in Agricultural Practises: Different types of crops display a wide range of characteristics in terms of their Crop Growth Rate (CGR). Farmers can employ CGR data to make well-informed decisions pertaining to crop selection. This entails considering various factors, including regional climate patterns, availability of resources, and market demands. This practise enables the enhancement of agricultural productivity and profitability. Enhanced productivity pertains to the optimisation or augmentation of the efficiency and efficacy of individuals, teams, or organisations in attaining their objectives. A high crop growth

rate (CGR) is indicative of a strong and efficient growth of crops. Through the implementation of techniques that promote favourable conditions for crop growth and development, such as the adequate supply of nutrients and irrigation, individuals involved in agriculture possess the ability to enhance crop productivity and achieve significantly higher yields. Food security is a condition characterised by universal access to an adequate supply of food that is both safe and nutritious, thereby fulfilling the dietary requirements of all individuals. Ensuring food security is of utmost importance in the context of a growing global population and changing dietary preferences. The topic of interest pertains to the field of research and development. Crop growth rate (CGR) is utilised by researchers as a fundamental parameter in the field of crop modelling and simulations. This technology enables the creation of predictive models that have the capability to forecast crop growth, yield, and responses to variations in environmental conditions. The information provided possesses considerable importance in the advancement of agricultural science and technology. In conclusion, the Crop Growth Rate plays a crucial role in the field of agriculture due to its extensive implications for crop management, resource allocation, and the advancement of sustainable farming practises. The phenomenon holds great significance across multiple domains, encompassing the prediction of agricultural productivity, evaluation of crop vitality, allocation of resources, advancement of environmental sustainability, and reinforcement of climate resilience. The significance of environmental agriculture is underscored by the ongoing development of agriculture, which is propelled by technological advancements and a dedication to sustainability. This approach serves as a crucial means of improving crop yield and mitigating ecological damage. The accurate measurement and efficient utilisation of crop growth rate (CGR) are crucial in modern agricultural practises to meet the needs of feeding a growing global population while also protecting natural resources (Sheikhalipour et al., 2023; Shekhawat et al., 2023; Shi et al., 2023; Shi et al., 2022; Si et al., 2023; Singh et al., 2022; Singh & Roychoudhury, 2023; Singh & Nandi, 2022; Singhal et al., 2023; Sivanesan et al., 2022).

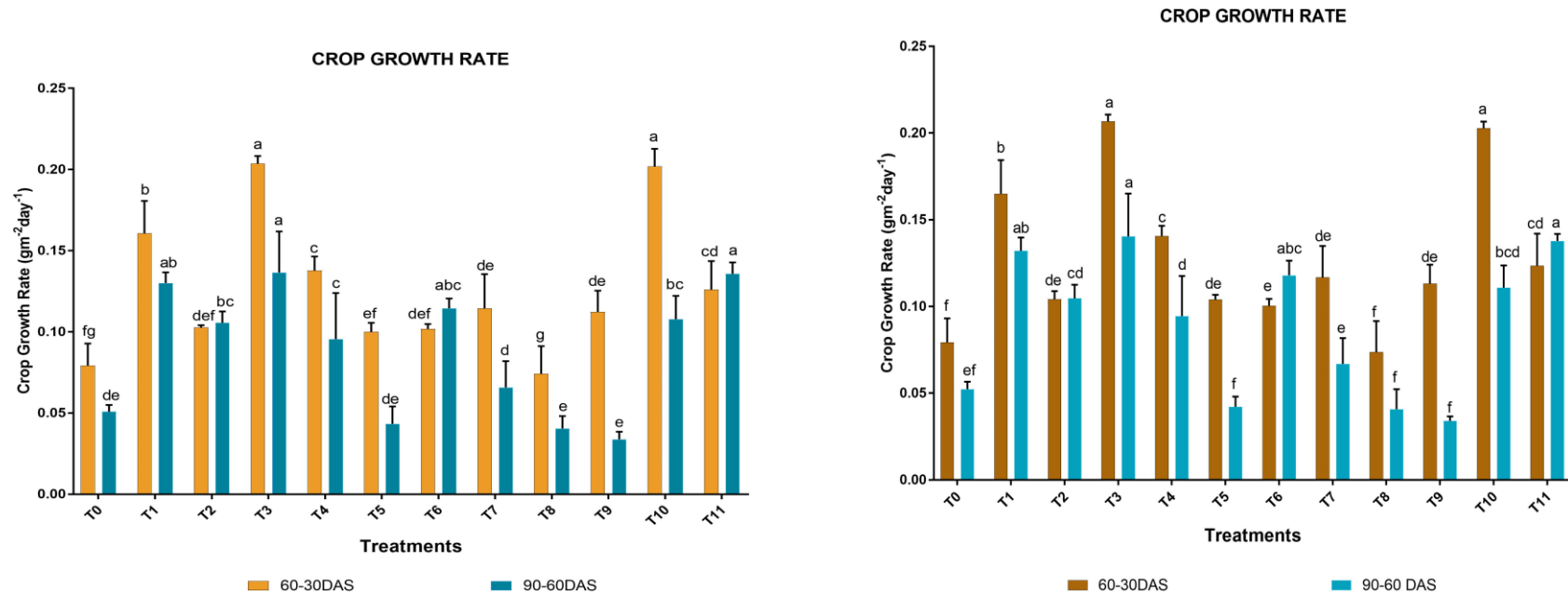


**Table 4.15. Impact of Different Treatments on Crop Growth Rate of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	0.079 <sup>fg</sup> ±0.013	0.079 <sup>f</sup> ±0.013	0.050 <sup>de</sup> ±0.004	0.052 <sup>ef</sup> ±0.004
<b>T1 (Thiourea-1000 ppm)</b>	0.160 <sup>b</sup> ±0.019	0.165 <sup>b</sup> ±0.019	0.130 <sup>ab</sup> ±0.006	0.132 <sup>ab</sup> ±0.007
<b>T2 (Salicylic acid-300 ppm)</b>	0.102 <sup>def</sup> ±0.001	0.104 <sup>de</sup> ±0.004	0.105 <sup>bc</sup> ±0.007	0.104 <sup>cd</sup> ±0.007
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	0.203 <sup>a</sup> ±0.004	0.206 <sup>a</sup> ±0.003	0.136 <sup>a</sup> ±0.025	0.140 <sup>a</sup> ±0.024
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	0.137 <sup>c</sup> ±0.008	0.140 <sup>c</sup> ±0.005	0.095 <sup>c</sup> ±0.028	0.094 <sup>d</sup> ±0.023
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	0.100 <sup>ef</sup> ±0.005	0.103 <sup>de</sup> ±0.002	0.043 <sup>de</sup> ±0.010	0.042 <sup>f</sup> ±0.005
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	0.101 <sup>def</sup> ±0.002	0.100 <sup>e</sup> ±0.004	0.114 <sup>abc</sup> ±0.006	0.118 <sup>abc</sup> ±0.008
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	0.114 <sup>de</sup> ±0.021	0.116 <sup>de</sup> ±0.018	0.065 <sup>d</sup> ±0.016	0.066 <sup>e</sup> ±0.014
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	0.074 <sup>g</sup> ±0.017	0.073 <sup>f</sup> ±0.017	0.040 <sup>e</sup> ±0.007	0.040 <sup>f</sup> ±0.011
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	0.112 <sup>de</sup> ±0.013	0.113 <sup>de</sup> ±0.010	0.033 <sup>e</sup> ±0.004	0.034 <sup>f</sup> ±0.002
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	0.201 <sup>a</sup> ±0.010	0.202 <sup>a</sup> ±0.003	0.107 <sup>bc</sup> ±0.014	0.110 <sup>bcd</sup> ±0.012
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	0.126 <sup>cd</sup> ±0.017	0.123 <sup>cd</sup> ±0.018	0.135 <sup>a</sup> ±0.007	0.137 <sup>a</sup> ±0.004
<b>CD</b>	0.023	0.022	0.023	0.021
<b>CV</b>	10.809	9.906	15.341	13.819

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

Figure 4.15. CGR of Mustard During Rabi 2021-2023 & 2022-23



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

## Relative Growth Rate

The effect of Sulphur and Salicylic acid and their combination on RGR was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.16, Figure 4.16) In 2021-2022, there was a significant difference in RGR compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the RGR was observed at 30, 60, and 90 DAS. Therefore, at 60-30 DAS, the percentage increase as compared to T0 was found to be highest in T3 followed by T10, T1, T4, T11, T7, T9, T2, T6, T5 and the percentage values were 61.10%, 60.75%, 50.69%, 42.49%, 37.12%, 30.77%, 29.47%, 22.91%, 22.24%, 20.77% respectively. But percentage also decrease in T8 as compared to T0 and the percentage value was -6.89%. At 90-60 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T11, T1, T6, T10, T2, T4, T7 and the percentage values were T5, T8, T9 and the percentage values were 62.73%, 62.52%, 60.88%, 55.53%, 52.78%, 51.78%, 46.74%, 22.63% respectively. But percentage also decrease in T5, T8, T9 as compared to T0 and the percentage values were -17.43%, -25.82%, -50.65%. In 2022-2023, there was a significant difference in RGR compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 60-30 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T10, T1, T4, T11, T7, T9, T2, T5, T6 and the percentage values were 61.68%, 60.93%, 51.98%, 43.68%, 35.88%, 32.15%, 30.02%, 23.98%, 23.74%, 21.03% respectively. But the percentage also decrease in T8 as compared to T0 and the percentage value was -7.38%. At 90-60 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T11, T1, T6, T10, T2, T4, T7 and the percentage values were 62.73%, 62.01%, 60.38%, 55.65%, 52.80%, 50.00%, 44.58%, 21.76% respectively. But the percentage also decrease in T5, T8, T9 as compared to T0 and the percentage values were -24.27%, -28.33%, -53.41% respectively. The measurement of Relative Growth Rate (RGR) in mustard crops carries substantial importance to its implications for crop management and agricultural productivity. Several essential factors underscore the relative growth rate (RGR) quantification in mustard crops. The Relative Growth Rate (RGR)

methodology facilitates the prompt assessment of growth patterns in mustard crops (Mabasa, 2023; Maheshwari et al., 2022; Maia et al., 2022; Makete et al., 2022; Manepalli et al., 2022; Mangena, 2022; Manjunatha et al., 2022; Masmoudi et al., 2023; Megala et al., 2022). By monitoring the Relative Growth Rate (RGR), individuals involved in agriculture and scientific investigation can expeditiously identify deviations from expected growth patterns. These deviations may indicate possible issues, such as nutrient deficiencies, stress due to inadequate water supply, or harm inflicted by pests. The growth monitoring process entails the computation of the Relative Growth Rate (RGR), which functions as a quantitative measure of the rate of development in mustard crops. The collection of this data holds significant importance in monitoring the progression of growth at different stages throughout the lifespan of the crop. The Prediction of Crop Yield: Relative Growth Rate (RGR) data can be employed to make predictions regarding the potential yield of mustard crops. Mustard plants that consistently display a high relative growth rate (RGR) are likelier to yield superior outcomes, a critical determinant in oilseed production. Examining resource allocation in mustard crops, encompassing water, nutrients, and photosynthetic products, about their growth and reproductive processes can be elucidated using the Resource Allocation: RGR approach (Liu, Cui, et al., 2022; Liu, Zong, et al., 2022; Liu, Liu, et al., 2022; Liu, Xiao, et al., 2022; Liu, Meng, et al., 2022; Lombardino et al., 2022; Ma et al., 2022). This knowledge enables the enhancement of strategies for managing resources. The effective identification of plant stress can be achieved by utilising the Relative Growth Rate (RGR), a highly responsive indicator. A reduction in relative growth rate (RGR) may indicate crop stress, which can be attributed to various environmental factors, pests, or diseases. Timely identification of this strain allows for prompt intervention and the implementation of appropriate mitigation strategies. Monitoring the Relative Growth Rate (RGR) is of utmost importance in preserving and improving the health and quality of mustard crops. Plants exhibiting vigorous growth and optimal health demonstrate enhanced resistance to diseases and pests, producing superior quality. Using RGR data can improve decision-making processes about irrigation, fertilisation, and pest control, optimising cultivation practices. By modifying these practices, implementing RGR measurements can improve crop health and optimise productivity. The RGR parameter possesses considerable importance within

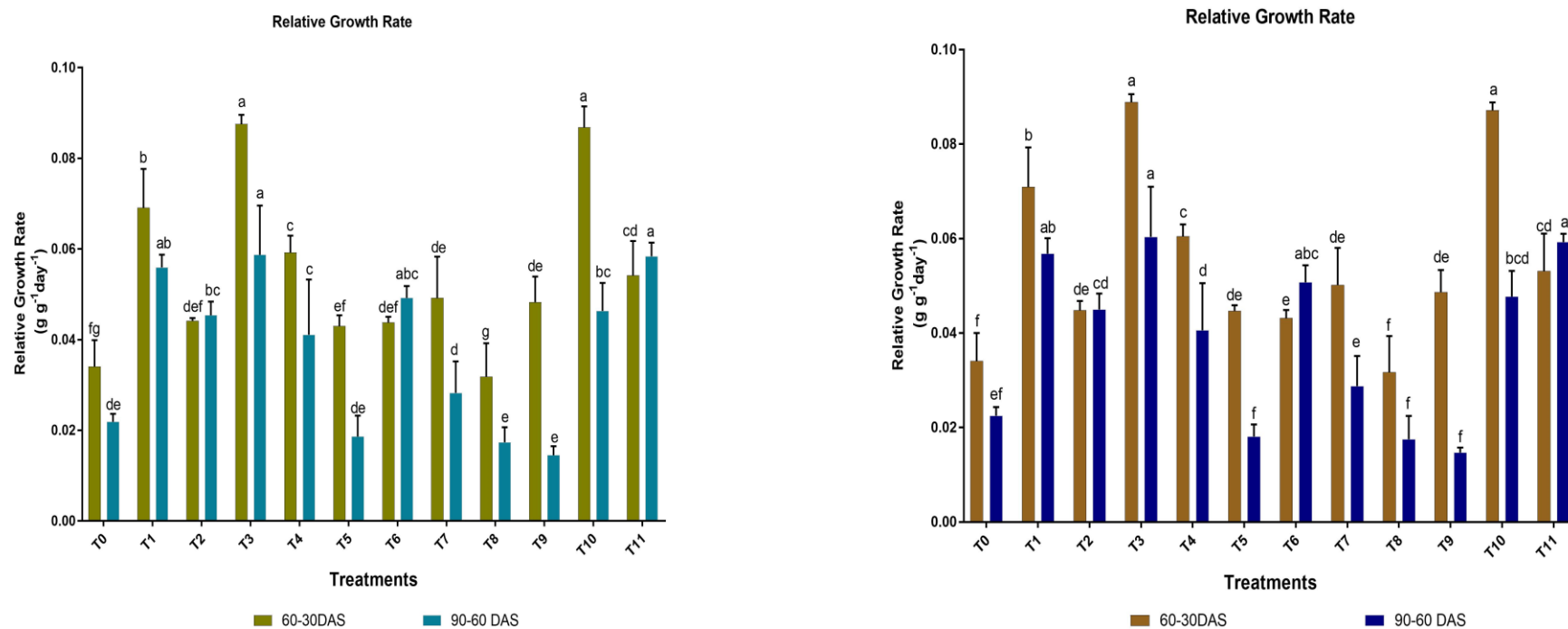
agricultural research and breeding initiatives. This allows researchers to perform comparative analyses on various mustard crop varieties and examine the effects of different treatments, thereby promoting the development of improved crop varieties and agronomic practices (Li, Huang, et al., 2022; Li, Luo, et al., 2022; Li, Tong, et al., 2023; Liu et al., 2023; Liu, Wang, et al., 2022; Liu, Li, et al., 2022). Incorporating RGR (Relative Growth Rate) measurements in mustard crop cultivation can enhance environmental sustainability in the agricultural industry. When plants demonstrate optimal health and efficient growth, they minimise resource utilisation, decreasing wastage and reducing dependence on chemical inputs. In conclusion, evaluating the Relative Growth Rate in mustard crops is fundamental for crop monitoring and management. This technology facilitates farmers and researchers in improving their decision-making processes, efficiently allocating resources, predicting crop yields, and maintaining the overall health and productivity of mustard crops. The factors above hold significant significance within food and oil production, alongside the facilitation of sustainable agricultural methodologies (Li, Zhang, et al., 2022; Li, Zheng, et al., 2023; Li et al., 2022; Li, Han, et al., 2022; Li, He, et al., 2023; Li et al., 2022; Li, Han, et al., 2023).

**Table 4.16. Impact of Different Treatments on Relative Growth Rate of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	0.034 <sup>fg</sup> ±0.005	0.034 <sup>f</sup> ±0.005	0.021 <sup>de</sup> ±0.001	0.022 <sup>ef</sup> ±0.001
<b>T1 (Thiourea-1000 ppm)</b>	0.069 <sup>b</sup> ±0.008	0.070 <sup>b</sup> ±0.008	0.055 <sup>ab</sup> ±0.002	0.056 <sup>ab</sup> ±0.003
<b>T2 (Salicylic acid-300 ppm)</b>	0.044 <sup>def</sup> ±0.000	0.044 <sup>de</sup> ±0.001	0.045 <sup>bc</sup> ±0.003	0.045 <sup>cd</sup> ±0.003
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	0.087 <sup>a</sup> ±0.002	0.088 <sup>a</sup> ±0.001	0.058 <sup>a</sup> ±0.010	0.060 <sup>a</sup> ±0.010
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	0.059 <sup>c</sup> ±0.003	0.060 <sup>c</sup> ±0.002	0.041 <sup>c</sup> ±0.012	0.040 <sup>d</sup> ±0.009
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	0.043 <sup>ef</sup> ±0.002	0.044 <sup>de</sup> ±0.001	0.018 <sup>de</sup> ±0.004	0.018 <sup>f</sup> ±0.002
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	0.043 <sup>def</sup> ±0.001	0.043 <sup>e</sup> ±0.001	0.049 <sup>abc</sup> ±0.002	0.050 <sup>abc</sup> ±0.003
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	0.049 <sup>de</sup> ±0.009	0.050 <sup>de</sup> ±0.007	0.028 <sup>d</sup> ±0.006	0.028 <sup>e</sup> ±0.006
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	0.031 <sup>g</sup> ±0.007	0.031 <sup>f</sup> ±0.007	0.017 <sup>e</sup> ±0.003	0.017 <sup>f</sup> ±0.004
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	0.048 <sup>de</sup> ±0.005	0.048 <sup>de</sup> ±0.004	0.014 <sup>e</sup> ±0.002	0.014 <sup>f</sup> ±0.001
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	0.086 <sup>a</sup> ±0.004	0.087 <sup>a</sup> ±0.001	0.046 <sup>bc</sup> ±0.006	0.047 <sup>bcd</sup> ±0.005
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	0.054 <sup>cd</sup> ±0.007	0.053 <sup>cd</sup> ±0.007	0.058 <sup>a</sup> ±0.003	0.059 <sup>a</sup> ±0.001
<b>CD</b>	0.01	0.009	0.01	0.009
<b>CV</b>	10.809	9.906	15.341	13.819

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.16. RGR ( $\text{g g}^{-1}\text{day}^{-1}$ ) of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm);

**T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).**



**Net Assimilation Rate ( $\text{g m}^{-2} \text{ day}^{-1}$ ):** The effect of Sulphur and Salicylic acid and their combination on NAR was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.17, Figure 4.17). In 2021-2022, there was a significant difference in NAR compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the NAR was observed at 30, 60, and 90 DAS. Therefore, at 60-30 DAS, the percentage increase as compared to T0 was found to be highest in T3 followed by T10, T1, T4, T11, T7, T9, T2, T6, T5 and the percentage values were 61.10%, 60.75%, 50.69%, 42.49%, 37.12%, 30.77%, 29.47%, 22.91%, 22.24%, 20.77% respectively. But percentage also decrease in T8 as compared to control and the percentage value was -6.89%. At 90-60 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T11, T1, T6, T10, T2, T4, T7 and the percentage values were 62.73%, 62.52%, 60.88%, 55.53%, 52.78%, 51.78%, 46.74%, 22.63% respectively. But the percentage also decrease in T5, T8, T9 as compared to T0 and the percentage values were -17.43%, -25.82%, -50.65% respectively. In 2022-2023, there was a significant difference in NAR compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 60-30 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T10, T1, T4, T11, T7, T9, T2, T5, T6 and the percentage values were 61.68%, 60.93%, 51.98%, 43.68%, 35.88%, 32.15%, 30.02%, 23.98%, 23.74%, 21.03% respectively. But percentage also decrease in T8 as compared to T0 and the percentage value was -7.38%. At 90-60 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T11, T1, T6, T10, T2, T4, T7 and the percentage values were 62.73%, 62.01%, 60.38%, 55.65%, 52.80%, 50.00%, 44.58%, 21.76% respectively. But percentage also decrease in T5, T8, T9 as compared to T0 and the percentage values were -24.27%, -28.33%, -53.41%. The Net Assimilation Rate (NAR) is a crucial parameter in the study of plant growth and productivity. The concept of "net primary productivity" pertains to the measurement of the speed at which a plant obtains and retains organic material through the processes of photosynthesis and carbon fixation, while also considering the decrease in biomass resulting from respiration. The application of Net Assimilation Rate (NAR) provides valuable insights into the

efficiency of plants in converting available resources into biomass. This has crucial implications for agricultural productivity, ecological research, and the assessment of plant health. This paper critically analyses the concept of NAR, its significance, the factors influencing it, and its practical applications. Net Assimilation Rate (NAR) is a widely used measure that quantifies the change in a plant's biomass (dry weight) within a specific time and area. It is commonly expressed in units of grammes per square metre per day ( $\text{g/m}^2/\text{day}$ ). The concept of Net Assimilation Rate (NAR) pertains to the assessment and analysis of a plant's capacity to efficiently obtain and utilise vital resources, including light, water, and nutrients, to support its growth and maturation. The term "it" pertains to the overall gain in biomass, considering the energy consumed by the plant for metabolic activities, commonly referred to as respiratory losses. Therefore, the measurement of net assimilation rate (NAR) holds significant significance in evaluating a plant's ability to efficiently convert photosynthetic products into useful biomass. The evaluation of growth: The Net Assimilation Rate (NAR) serves as a reliable metric for quantifying the growth rate of a plant. A high net annual rate (NAR) is indicative of a substantial growth rate, while a low NAR suggests a comparatively sluggish growth rate. The evaluation of the impact of various factors on plant growth can be facilitated by the monitoring of changes in net assimilation rate (NAR) over a period of time. This practise is beneficial for both researchers and farmers. The aforementioned factors encompass a range of elements, including environmental conditions, nutrient availability, and management practises. The prediction of crop yield holds significant importance in the realm of agriculture, and the utilisation of the Normalised Difference Vegetation Index (NDVI) Analysis Ratio (NAR) has emerged as a valuable predictive instrument within this domain. The utilisation of advanced data analysis techniques in the context of NAR facilitates the precise estimation of crop yield. Farmers have the potential to increase their crop yields through the implementation of a methodical approach that involves the assessment of the Net Assimilation Rate (NAR) at different stages of crop growth. This practise allows individuals to collect valuable data pertaining to periods of heightened growth and potential limitations on crop productivity. As a result, farmers are equipped with the necessary knowledge to make informed choices regarding irrigation, fertilisation, and pest management, thereby maximising their agricultural output. The identification of

plant stress can be deduced by examining alterations in the Normalised Difference Vegetation Index (NDVI). Plant stress can be caused by a range of factors, including drought, disease, and nutrient deficiencies. These factors can lead to a decrease in the net assimilation rate (NAR) of plants, as resources that would otherwise be allocated to growth are redirected towards stress-related mechanisms. The timely identification of these alterations can expedite the execution of remedial actions. The Net Primary Productivity to NAR is a significant metric utilised in ecological research to assess the overall productivity of ecosystems. By calculating the Net Assimilation Rate (NAR) for various plant species in an ecosystem, researchers can gain significant insights into resource distribution, competitive interactions, and overall ecosystem health. The practical applications of NAR are as follows: Within the domain of agricultural management, farmers heavily depend on NAR data in order to make informed decisions pertaining to irrigation, fertilisation, and pest control strategies. The assessment of nitrogen assimilation rate (NAR) is of paramount importance in the optimisation of resource allocation and the achievement of maximum crop yields. The assessment of environmental conditions is of utmost importance in the field of ecology, as it allows ecologists to utilise the Naturalness Assessment Rating (NAR) as a means of evaluating the overall health and effectiveness of ecosystems. The utilisation of this assessment tool not only contributes to the conservation of natural habitats, but also enhances their efficient administration. Crop breeding encompasses the intentional endeavours of plant breeders to cultivate crop varieties that demonstrate an increased net assimilation rate (NAR), consequently leading to heightened yield potential and improved efficiency in the utilisation of resources. The stress assessment tool, commonly referred to as NAR, serves as a proactive mechanism for identifying and monitoring stressors that affect plants. This tool facilitates timely interventions to mitigate potential harm. Research: Scientists utilise the Net Assimilation Rate (NAR) as a valuable research tool to examine the impacts of various factors, such as climate change, on plant growth and productivity. The Net Assimilation Rate (NAR) is a fundamental parameter utilised for the assessment of plant growth efficiency and productivity. This study presents noteworthy findings concerning the ability of plants to convert resources into biomass, making it a crucial tool in the fields of agriculture, ecology, and plant physiology. Through the acquisition of knowledge and diligent monitoring of the Net Assimilation Rate (NAR), individuals

involved in research, agriculture, and conservation can make informed decisions that optimise resource utilisation and foster sustainable plant growth. The Brassica spp., commonly known as mustard, is widely acknowledged as a prominent oilseed crop with global significance. It is highly esteemed for its seeds, which possess a substantial amount of oil. The central objectives of agricultural research pertain to the understanding and enhancement of the growth and productivity of mustard plants. The net assimilation rate (NAR) plays a critical role in the determination of biomass accumulation in plants and their potential for yield. In recent times, there has been a growing interest in the study of sulphur (S) and salicylic acid (SA) as they have been identified as important factors that may have a significant impact on the physiology of mustard plants. The present discourse aims to investigate the intricate relationship between these substances and the NAR of mustard plants. Sulphur, often recognised as a secondary macronutrient, plays a vital role in the growth and development of various plant species, including mustard. While the quantity required is smaller than primary macronutrients like nitrogen, phosphorus, and potassium, its role remains equally significant. Sulphur is a fundamental component of various amino acids, vitamins, and enzymes that play crucial roles in a wide range of physiological processes within plant organisms. Sulphur plays a pivotal role in the physiological processes of plants, primarily by actively participating in the intricate process of photosynthesis. Photosynthesis is a crucial biological phenomenon wherein plants utilise light energy and transform it into chemical energy with the aid of numerous enzymes and proteins. Sulphur is significant in the synthesis of essential amino acids, specifically cysteine and methionine, which are fundamental constituents in protein formation. Proteins play a crucial role in the organisation and functioning of chloroplasts, which are responsible for the process of photosynthesis within cells. Hence, a deficiency in sulphur can have a significant effect on the photosynthetic capacity of mustard plants. The correlation between an adequate amount of sulphur and an increased rate of photosynthesis in mustard plants is well-established. Sulphur is a necessary component of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which serves a critical function in the process of photosynthesis by aiding in the crucial step of carbon fixation. The enzyme's activity can be enhanced by supplying an appropriate quantity of sulphur, which consequently increases the rate of

carbon dioxide (CO<sub>2</sub>) fixation and subsequently elevates photosynthetic rates. Moreover, sulphur plays a vital role in the synthesis of chlorophyll, the pigment accountable for the absorption of light energy during the process of photosynthesis. A lack of adequate sulphur availability may lead to the occurrence of chlorosis, which is a physiological anomaly characterised by the yellowing of leaves as a consequence of reduced chlorophyll concentrations. The observation of chlorotic leaves in mustard plants has been found to negatively impact photosynthetic efficiency, thereby affecting the plants' net assimilation rate (NAR). The existing body of literature has provided evidence of a distinct association between the availability of sulphur and the accumulation of biomass in mustard plants. Mustard plants that have received sufficient sulphur treatment exhibit an increase in biomass production as a result of an enhanced photosynthetic rate. The plant's heightened growth potential can be ascribed to its ability to enhance carbohydrate synthesis through the process of photosynthesis. These synthesised carbohydrates are subsequently allocated towards the development of structural components, including stems, leaves, and roots. Moreover, sulphur plays a crucial role in the process of nitrogen metabolism. The process enables the formation of amino acids that contain sulphur, such as cysteine, which can influence the uptake and utilisation of nitrogen in plant organisms. The efficient metabolism of nitrogen, which is facilitated by sulphur, plays a crucial role in the synthesis of proteins and enzymes that are essential for a wide range of cellular processes. Consequently, mustard plants that are adequately supplied with sulphur exhibit an improved capacity to efficiently utilise accessible nitrogen resources, thereby promoting vigorous growth and an elevated net assimilation rate (NAR). Salicylic acid (SA) and sulphur have recently garnered increased attention as factors of interest in agricultural research. Salicylic acid (SA) is an intrinsic phytohormone that assumes a pivotal function in the initiation of defence mechanisms against both biotic and abiotic stressors in plants. While SA is primarily known for its role in plant defence, recent studies have uncovered its participation in the regulation of various physiological processes, including photosynthesis and carbon assimilation. Salicylic acid is commonly associated with the plant's physiological response to diverse stressors. Mustard plants demonstrate an increase in their salicylic acid (SA) concentrations when exposed to different environmental stressors, such as pathogens, drought, or extreme temperatures, as part

of their defence mechanism. Salicylic acid (SA) serves as a signalling molecule, initiating processes that trigger the activation of genes and pathways related to defence mechanisms. However, recent studies have revealed that SA has the potential to enhance the process of photosynthesis and carbon assimilation, even under non-stressful conditions in plants. The augmentation of enzymatic activity in key enzymes involved in photosynthesis, such as RuBisCO, has been observed upon the addition of SA. The augmentation being discussed holds the potential to lead to an increase in carbon fixation, thereby resulting in higher rates of photosynthesis. The utilisation of salicylic acid (SA) has been documented to augment the photosynthetic rate in mustard plants, consequently exerting a significant impact on their net assimilation rate (NAR). When the process of photosynthesis is enhanced, it leads to an augmentation in the synthesis of carbon compounds, thereby resulting in an amplified availability of these compounds for diverse physiological processes within plants. These compounds have the potential to be utilised for immediate energy needs, growth processes, and the accumulation of biomass (Yang et al., 2022; Yao et al., 2022; Yin et al., 2023; Yousaf et al., 2022; Yu et al., 2022; Yu et al., 2022). Moreover, prior research has provided evidence that salicylic acid (SA) has a substantial effect on the distribution of carbon resources within the plant. The process possesses the capacity to enhance the dispersion of carbon towards areas that demonstrate higher growth potential, such as developing foliage and root systems. The implementation of this allocation strategy offers benefits in enhancing the overall biomass and, as a result, the net assimilation rate (NAR) of mustard plants. Notably, the impact of sulphur and salicylic acid (SA) on mustard plants is not independent but exhibits a synergistic relationship. Mustard plants, when subjected to stressful conditions that lead to increased salicylic acid (SA) production, such as pathogen infestation or environmental stressors, can benefit from an adequate supply of sulphur. This additional sulphur can enhance the plant's ability to manage stress and maintain optimal photosynthetic activity. The coalescence of sulphur and salicylic acid (SA) has been observed to induce an augmented defence response and improve the efficiency of photosynthesis. The correlation between sulphur and salicylic acid has a notable impact on the net assimilation rate (NAR) of mustard plants, resulting in enhanced stress tolerance and increased crop yields. Sulphur plays a pivotal role in various physiological processes, including but not limited to photosynthesis and

nitrogen metabolism. Likewise, salicylic acid, which is primarily acknowledged for its role in stress response, also exerts a beneficial impact on photosynthesis and the allocation of carbon. The growth and development of mustard plants can be collectively influenced by the dynamic interaction of these two factors. The presence of a sufficient quantity of sulphur has the potential to positively impact the process of photosynthesis, nitrogen metabolism, and overall growth, resulting in an enhancement of the net assimilation rate (NAR). On the other hand, salicylic acid could augment rates of photosynthesis and optimise the allocation of carbon, especially in instances where the plant is subjected to adverse environmental conditions. The investigation and optimisation of the interaction between sulphur and salicylic acid in mustard cultivation offer a promising strategy for increasing crop yields and supporting the sustainable production of this economically important oilseed crop. Further research is necessary in this field to fully maximise the potential of these factors in enhancing mustard crop productivity, considering both environmental and agronomic factors (Yang et al., 2023; Yang et al., 2023; Yang & Lee, 2023; Yang et al., 2023).

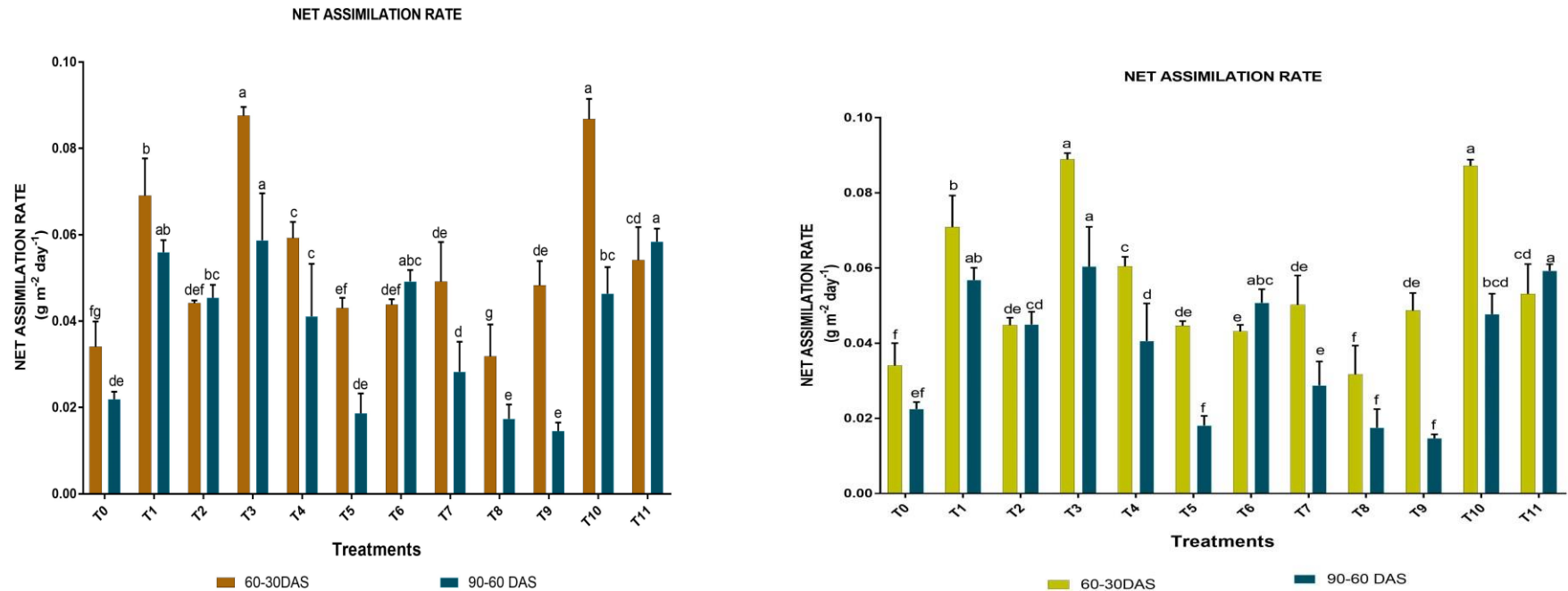
**Table 4.17. Impact of Different Treatments on Net Assimilation Rate of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023
T0 (Control)	0.034 <sup>fg</sup> ±0.005	0.034 <sup>f</sup> ±0.005	0.021 <sup>de</sup> ±0.001	0.022 <sup>ef</sup> ±0.001
T1 (Thiourea-1000 ppm)	0.069 <sup>b</sup> ±0.008	0.070 <sup>b</sup> ±0.008	0.055 <sup>ab</sup> ±0.002	0.056 <sup>ab</sup> ±0.003
T2 (Salicylic acid-300 ppm)	0.044 <sup>def</sup> ±0.000	0.044 <sup>de</sup> ±0.001	0.045 <sup>bc</sup> ±0.003	0.045 <sup>cd</sup> ±0.003
T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]	0.087 <sup>a</sup> ±0.002	0.088 <sup>a</sup> ±0.001	0.058 <sup>a</sup> ±0.010	0.060 <sup>a</sup> ±0.010
T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)	0.059 <sup>c</sup> ±0.003	0.060 <sup>c</sup> ±0.002	0.041 <sup>c</sup> ±0.012	0.040 <sup>d</sup> ±0.009
T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)	0.043 <sup>ef</sup> ±0.002	0.044 <sup>de</sup> ±0.001	0.018 <sup>de</sup> ±0.004	0.018 <sup>f</sup> ±0.002
T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)	0.043 <sup>def</sup> ±0.001	0.043 <sup>e</sup> ±0.001	0.049 <sup>abc</sup> ±0.002	0.050 <sup>abc</sup> ±0.003
T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)	0.049 <sup>de</sup> ±0.009	0.050 <sup>de</sup> ±0.007	0.028 <sup>d</sup> ±0.006	0.028 <sup>e</sup> ±0.006
T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)	0.031 <sup>g</sup> ±0.007	0.031 <sup>f</sup> ±0.007	0.017 <sup>e</sup> ±0.003	0.017 <sup>f</sup> ±0.004
T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)	0.048 <sup>de</sup> ±0.005	0.048 <sup>de</sup> ±0.004	0.014 <sup>e</sup> ±0.002	0.014 <sup>f</sup> ±0.001
T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)	0.086 <sup>a</sup> ±0.004	0.087 <sup>a</sup> ±0.001	0.046 <sup>bc</sup> ±0.006	0.047 <sup>bcd</sup> ±0.005
T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)	0.054 <sup>cd</sup> ±0.007	0.053 <sup>cd</sup> ±0.007	0.058 <sup>a</sup> ±0.003	0.059 <sup>a</sup> ±0.001
CD	0.01	0.009	0.01	0.009
CV	10.809	9.906	15.341	13.819

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.



**Figure 4.17. NAR of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

#### **4C. Thiourea (sulphur) and salicylic acid-mediated effects on yield, yield attributing characters and Biochemical parameters of Indian mustard grown under the open filed condition**

**Silique Number:** The effect of Sulphur and Salicylic acid and their combination on silique number was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 90 and 120 days after sowing (DAS) (Table 4.18, Figure 4.18). In 2021-2022, there was a significant difference in silique number compared to T0 (Control) at 90 and 120 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the silique number was observed at 90 and 120 DAS. Therefore, at 90 DAS, the percentage increase as compared to T0 was found to be highest in T7, followed by T5, T10, T11, T8, T2, T4, T9, T3, T6, T1, and the percentage values were 50%, 49.70%, 48.16%, 46.07%, 45.49%, 43.42%, 42.53%, 37.12%, 34.02%, 31.35%, 16.99% respectively. At 120 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T6, T5, T2, T9, T10, T8, T7, T3, T1, T4 and the percentage values were 53.48%, 49.84%, 47.93%, 39.76%, 37.53%, 34.40%, 27.87%, 25.19%, 24.64%, 22.66%, 19.76% respectively. In 2022-2023, there was a significant difference in Silique number compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 90 DAS, the percentage increase as compared to T0 was found highest in T7 followed by T5, T10, T2, T11, T8, T4, T9, T3, T6, T1 and the percentage values were 51.29%, 49.61%, 46.98%, 46.55%, 45.22%, 44.30%, 39.72%, 37.58%, 34.32%, 31.95%, 17.75% respectively. At 120 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T6, T5, T2, T9, T10, T8, T7, T3, T1, T4 and the percentage values were 52.36%, 47.78%, 46.20%, 39.45%, 37.27%, 30.76%, 28.08%, 24.24%, 22.41%, 21.82%, 18.43% respectively. Studies conducted on *Brassica juncea* L., commonly known as mustard, have revealed that the quantity of silique plays a crucial role in determining the reproductive capacity of plants and, consequently, the yield of crops that can be obtained from these plants. The mustard plant is classified within the Brassica genus and is characterized by elongated seed pods called silique (Maheshwari et al., 2022; Maia et al., 2022; Makete et al., 2022; Manepalli et al., 2022; Mangena, 2022;

Manjunatha et al., 2022; Masmoudi et al., 2023; Megala et al., 2022; Mittal et al., 2022). A comprehensive comprehension of the importance of siliqua number and a thorough exploration of the potential impacts of sulphur (S) and salicylic acid (SA) on this parameter is crucial for enhancing mustard cultivation practises and augmenting crop productivity. A positive correlation can be observed between the quantity of siliqua and the reproductive yield of a plant. The inclusion of numerous seeds within each siliqua enhances the potential for seed yield. A higher siliqua number indicates a greater abundance of sources, which is a critical determinant of crop yield. Using the siliqua number as a metric for evaluating a plant's genetic capacity for seed production is a widely accepted practice. The exploitation of the variability in siliqua number among mustard genotypes or varieties can be utilised by breeding programmes to facilitate the advancement of cultivars with high yield potential. By strategically optimising and manipulating the quantity of siliqua in their crops, growers can enhance the overall crop yield without necessitating an expansion of the planting area. This holds paramount importance in regions characterised by limited arable land. The soil's siliqua content impacts the allocation of resources within the plant. Plants exhibiting a higher siliqua count are inclined to allocate a greater proportion of their resources, including nutrients and energy, towards seed development. This allocation may result in more seeds with extensive and abundant characteristics. Sulphur is a vital nutrient for the mustard plant. Nutrient absorption, specifically for nitrogen (N) and phosphorus (P), is important as it significantly contributes to reproductive growth. An ample amount of sulphur promotes the effective absorption and distribution of nutrients within developing siliques, potentially leading to an augmentation in both the quantity and dimensions of these structures. Plants of the Brassicaceae family, such as mustard, synthesise secondary metabolites, including glucosinolates. Sulphur is a necessary component for this process. These compounds can be chemical defence mechanisms against herbivores and other organisms that consume plants (Liu, Li, et al., 2022; Liu, Cui, et al., 2022; Liu, Zong, et al., 2022; Liu, Liu, et al., 2022; Liu, Xiao, et al., 2022; Liu, Meng, et al., 2022; Lombardino et al., 2022; Ma et al., 2022; Mabasa, 2023). The allocation of resources towards glucosinolate production may indirectly influence siliqua abundance by deterring herbivorous activity. Salicylic acid (SA) is a phytohormone that has garnered significant recognition for its involvement in various plant defence mechanisms.

However, it also plays a role in regulating hormones involved in reproductive processes. Salicylic acid (SA) can modulate the transcriptional activity of genes involved in regulating flower development and initiating fruit formation. The stress-responsive properties of SA can indirectly influence the quantity of siliqua, thereby potentially facilitating the initiation and growth of siliqua through its positive modulation of relevant genes. Activating salicylic acid (SA)-mediated defence mechanisms in plants mitigates the impact of stressors, enabling plants to allocate a greater proportion of their resources, including energy and nutrients, towards reproductive processes. One such reproductive process is the development of siliqua structures. Salicylic acid (SA) can interact with a diverse range of phytohormones, such as auxins and cytokinins, which collectively contribute to regulating flower and fruit development. The intricate interplay between SA and these hormones can impact siliqua's commencement and development, potentially leading to increased siliqua. The simultaneous application of sulphur and salicylic acid in combination can generate synergistic effects on the quantity of siliqua in mustard. Sulphur catalyses the process of nutrient absorption, facilitating the uptake of vital elements such as nitrogen and phosphorus, which play a crucial role in supporting reproductive growth. Salicylic acid can enhance nutrient absorption efficacy, facilitating an augmented nutrient supply to developing siliquas. Sulphur impacts hormones' synthesis and metabolism, whereas SA can modulate hormonal regulation. When the coordination of these activities occurs, it can facilitate an optimal hormonal equilibrium, which is essential for the initiation and progression of siliqua development. The inclusion of salicylic acid (SA) within the plant's growth environment has been observed to enhance the plant's capacity to withstand stress. Sulphur plays a crucial role in facilitating the availability of essential components required for synthesising stress-related compounds, such as antioxidants. The confluence of stress tolerance and growth stimulation facilitates the augmentation of siliqua number. The siliqua number holds significant importance in mustard research due to its direct impact on reproductive output and crop yield. The utilisation of sulphur and salicylic acid presents potential avenues for enhancing the abundance of siliqua in mustard plants due to their distinctive mechanisms of nutrient absorption, hormonal control, allocation of resources, and resilience to stress. These mechanisms involve the uptake of nutrients, the regulation of hormones, and the allocation of resources.

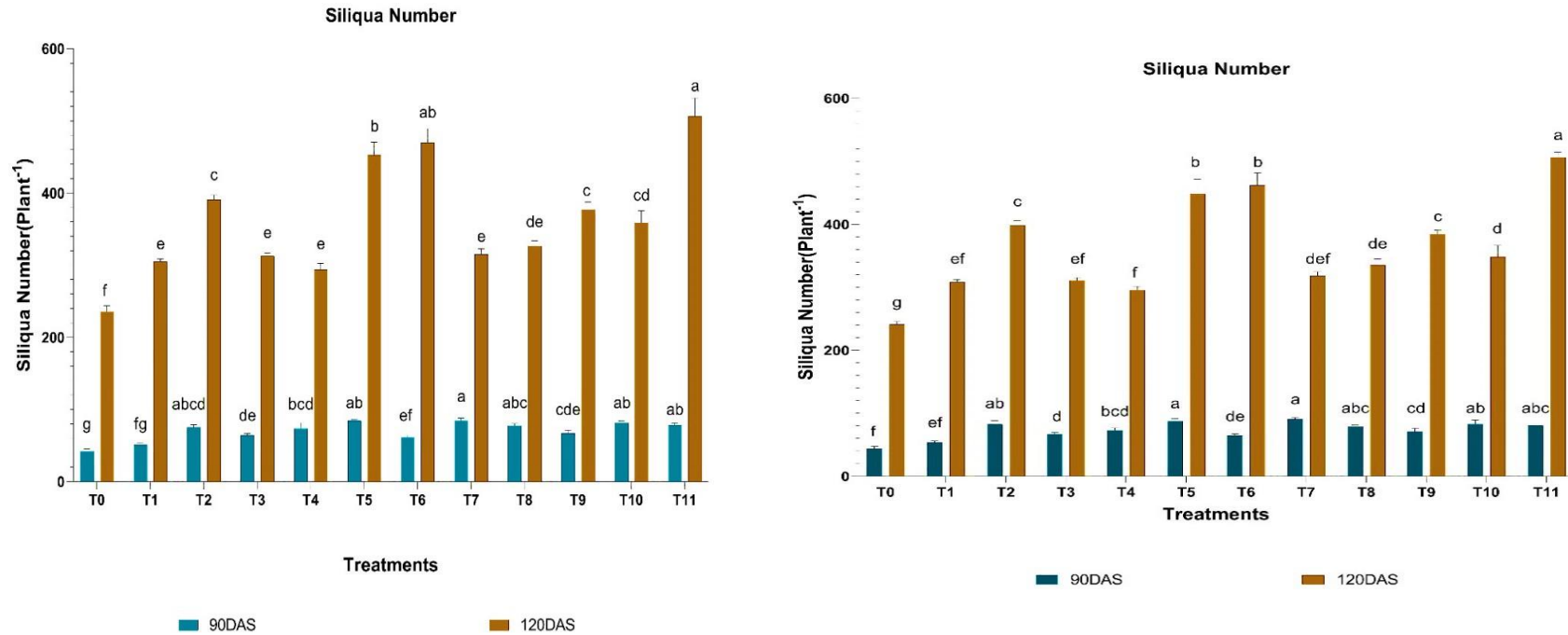
Understanding these mechanisms is crucial for adopting advanced agricultural techniques and potential strategies to increase the yield of siliquas, leading to improved crop productivity and seed yield in mustard farming (Li, Luo, et al., 2022; Li, Tong, et al., 2023; Liu et al., 2023; Liu, Wang, et al., 2022;).

**Table 4.18. Impact of Different Treatments on Siliqua Number of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	90 DAS		120 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	42.33 <sup>e</sup> ±3.32	44.00 <sup>f</sup> ±3.27	235.50 <sup>f</sup> ±8.32	241.16 <sup>g</sup> ±4.50
<b>T1 (Thiourea-1000 ppm)</b>	51.00 <sup>fg</sup> ±2.29	53.50 <sup>ef</sup> ±2.64	304.50 <sup>e</sup> ±4.27	308.50 <sup>ef</sup> ±3.60
<b>T2 (Salicylic acid-300 ppm)</b>	74.83 <sup>abcd</sup> ±4.01	82.33 <sup>ab</sup> ±5.75	391.00 <sup>e</sup> ±6.24	398.33 <sup>e</sup> ±7.25
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	64.16 <sup>de</sup> ±2.56	67.00 <sup>d</sup> ±2.17	312.50 <sup>e</sup> ±4.27	310.83 <sup>ef</sup> ±4.50
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	73.66 <sup>bcd</sup> ±7.81	73.00 <sup>bcd</sup> ±3.90	293.50 <sup>e</sup> ±9.26	295.66 <sup>f</sup> ±5.79
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	84.16 <sup>ab</sup> ±2.25	87.33 <sup>a</sup> ±4.01	452.33 <sup>b</sup> ±18.23	448.33 <sup>b</sup> ±23.30
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	61.66 <sup>ef</sup> ±2.75	64.66 <sup>de</sup> ±2.75	469.50 <sup>ab</sup> ±19.61	461.83 <sup>b</sup> ±19.75
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	84.66 <sup>a</sup> ±3.40	90.33 <sup>a</sup> ±2.56	314.83 <sup>e</sup> ±7.97	318.33 <sup>def</sup> ±6.29
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	77.66 <sup>abc</sup> ±3.01	79.00 <sup>abc</sup> ±2.29	326.50 <sup>de</sup> ±7.26	335.33 <sup>de</sup> ±9.46
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	67.33 <sup>cde</sup> ±3.75	70.50 <sup>cd</sup> ±5.56	377.00 <sup>c</sup> ±10.53	384.50 <sup>c</sup> ±6.38
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	81.66 <sup>ab</sup> ±2.36	83.00 <sup>ab</sup> ±6.06	359.00 <sup>cd</sup> ±16.52	348.33 <sup>d</sup> ±18.05
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	78.50 <sup>ab</sup> ±2.78	80.33 <sup>abc</sup> ±2.46	506.33 <sup>a</sup> ±25.31	506.33 <sup>a</sup> ±8.51
<b>CD</b>	5.966	6.817	21.817	20.552
<b>CV</b>	4.991	5.485	3.435	3.321

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.18. Siliqua Number of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea

**(2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).**



**Siliqua Length-** The effect of Sulphur and Salicylic acid and their combination on siliqua length was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 120 days after sowing (DAS) (Table 4.19, Figure 4.19). In 2021-2022, there was a significant difference in siliqua length compared to T0 (Control) at 120 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the siliqua length was observed at 120 DAS. Therefore, at 120 DAS, the percentage increase as compared to T0 was found highest in T2 followed by T3, T11, T8, T1, T6, T9, T5, T4, T7 and the percentage values were 20.16%, 18.18%, 15.38%, 13.91%, 13.15%, 10.81%, 5.71%, 4.80%, 3.88%, 2.94% respectively. But in T10, the percentage decreased compared to T0, and the percentage value was -1.02%. In 2022-2023, there was a significant difference in Siliqua length compared to T0 (Control) at 120 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 120 DAS, the percentage increase as compared to T0 was found highest in T2 followed by T3, T11, T8, T1, T6, T9, T7, T4, T5 and the percentage values were 21.87%, 18.69%, 18.03%, 15.25%, 13.79%, 13.79%, 5.66%, 4.76%, 2.91%, 1.96% respectively. But, in the T10 treatment, the impact was ineffective, and the percentage value was the same as in T0. In mustard (*Brassica juncea* L.) research, the measurement of siliqua length, expressed in centimetres, holds significant importance as it directly impacts the reproductive success of plants and crop yield. Recognising the importance of siliqua length and investigating the potential influence of sulphur (S) and salicylic acid (SA) on this trait is essential for enhancing mustard cultivation practices and increasing agricultural productivity. The dimension of siliquas plays a pivotal role in determining their capacity to transport seeds (Negi & Kumar, 2023; Nimsi et al., 2023; Niu & Fu, 2022; Ortiz-García et al., 2022, 2023; Ozturk & Unal, 2023; Paalli et al., 2022; Pal et al., 2023; Parada et al., 2022; Parrey et al., 2023; Patel et al., 2022; Peng et al., 2022; Pérez-Llorca et al., 2023). The siliqua's length positively correlates with its inherent capacity, enabling it to accommodate more seeds. Implementing this measure is imperative to substantially enhance the overall seed production, which constitutes a fundamental determinant of crop yield. Moreover, it should be noted that elongated siliquas possess a tendency to accommodate seeds of larger size. This characteristic can be attributed to the augmentation of the seed cavity volume. The larger size of these

seeds confers numerous advantages, including enhanced seedling vigour, increased germination rates, and potentially augmented market value for certain crops. The size of Siliqua can also impact the quality of a seed. Extended siliquas can provide heightened seed protection against various external factors, including pests, diseases, and adverse weather conditions. This consequently leads to the generation of resources of superior quality. Furthermore, the observed differences in siliqua length among various mustard varieties or genotypes present promising opportunities for breeding programmes to produce cultivars with desired seed sizes and quality attributes. Sulphur, an essential macronutrient for mustard plants, influences the length of siliqua. Firstly, it is imperative to acknowledge the pivotal function that it fulfils in facilitating the assimilation of essential nutrients, particularly nitrogen (N), which is indispensable for the comprehensive growth and development of the plant. An adequate amount of sulphur has the potential to enhance the absorption of nutrients, thereby promoting holistic plant growth, including the elongation of siliqua. Furthermore, the synthesis of proteins necessitates the presence of sulphur due to its indispensable role as a constituent of amino acids such as cysteine and methionine, both of which are vital for protein production (Hussein et al., 2023; Iftikhar et al., 2023; Islam et al., 2023; Javadipour et al., 2022; Ji et al., 2022). Proteins are involved in various cellular processes and play a vital role in cell growth and expansion, essential for siliqua elongation. Furthermore, sulphur is essential for the biosynthesis of secondary metabolites, specifically glucosinolates, which are abundantly present in plants belonging to the Brassicaceae family, including mustard. Through their influence on the plant's defence mechanisms, these compounds can indirectly impact the elongation of the siliqua, subsequently resulting in alterations in siliqua development. Despite its primary association with the defensive mechanisms of plants, salicylic acid can potentially influence siliqua length. Salicylic acid (SA) can modulate the transcriptional activity of genes implicated in developmental processes and growth, including those responsible for the elongation of siliqua. The SA can enhance the length of siliquas through its positive regulatory effect on these genes. Moreover, the stress-responsive properties of SA can indirectly influence siliqua length. When plants undergo reduced stress levels due to SA-mediated defences, they can allocate a larger proportion of their resources, including energy and nutrients, towards growth-related processes, which

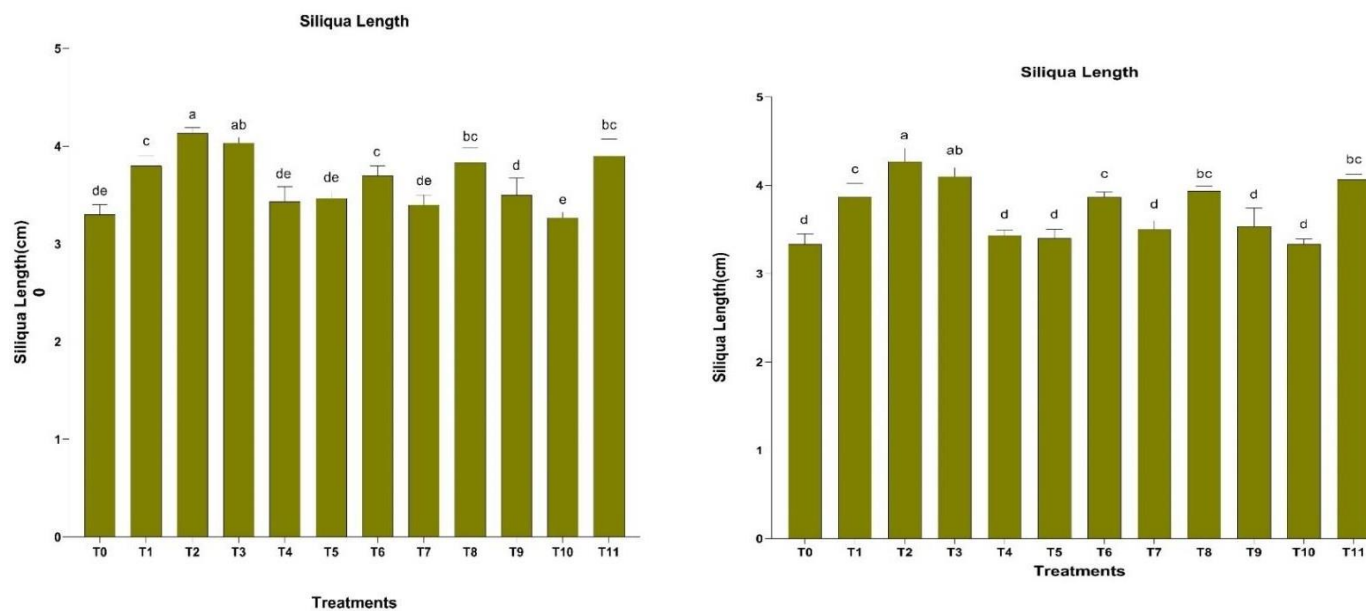
may encompass the formation of siliqua. When co-applied, the combination of sulphur and salicylic acid exhibits the potential to induce a synergistic effect on the elongation of the siliqua in mustard plants. Sulphur makes it easier for organisms to absorb essential nutrients, including nitrogen, for silica crystals' growth. This is complemented by adding salicylic acid, which enhances nutrient absorption efficiency, guaranteeing ample nutrients that can sustain prolonged siliqua development. Sulphur influences the processes of hormone synthesis and metabolism, whereas salicylic acid (SA) is responsible for regulating hormonal regulation. By facilitating the equilibrium of hormones, the harmonious coordination of these processes generates optimal circumstances for the elongation of siliqua. It has been observed that the inclusion of salicylic acid (SA) in the growth medium leads to an improvement in plant stress tolerance. Furthermore, including sulphur in the system guarantees the accessibility of fundamental constituents necessary for forming stress-associated substances, such as antioxidants. The combination of stress resilience and growth promotion factors influences the elongation of siliquas over an extended period. The assessment of siliqua length in mustard research is of considerable significance due to its direct influence on several factors, including seed production, size, quality, and overall crop yield. Sulphur and salicylic acid can elicit a favourable impact on the length of siliqua in mustard plants through their influence on diverse physiological mechanisms, encompassing nutrient absorption, protein production, hormonal control, allocation of resources, and resilience to stress. Enhanced agricultural productivity and improved seed characteristics can be attained by cultivating mustard, provided that the fundamental mechanisms influencing siliqua length are optimised (Mohammadi et al., 2023; Molinari et al., 2023; Moustakas et al., 2022; Mugwanya et al., 2023; Mukarram et al., 2022; Mukhtar et al., 2023; Myers Jr. et al., 2023; Nadeem, 2022; Nam et al., 2023; Napieraj et al., 2023; Nasir & Toth, 2022).

**Table 4.19. Impact of Different Treatments on Siliqua Length of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	120 DAS	
	2021-2022	2022-2023
<b>T0 (Control)</b>	3.30 <sup>de</sup> ±0.10	3.33 <sup>d</sup> ±0.11
<b>T1 (Thiourea-1000 ppm)</b>	3.80 <sup>e</sup> ±0.10	3.86 <sup>e</sup> ±0.15
<b>T2 (Salicylic acid-300 ppm)</b>	4.13 <sup>a</sup> ±0.05	4.26 <sup>a</sup> ±0.15
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	4.03 <sup>ab</sup> ±0.05	4.10 <sup>ab</sup> ±0.10
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	3.43 <sup>de</sup> ±0.15	3.43 <sup>d</sup> ±0.05
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	3.46 <sup>de</sup> ±0.05	3.40 <sup>d</sup> ±0.10
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	3.70 <sup>e</sup> ±0.10	3.86 <sup>e</sup> ±0.05
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	3.40 <sup>de</sup> ±0.10	3.50 <sup>d</sup> ±0.10
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	3.83 <sup>bc</sup> ±0.15	3.93 <sup>bc</sup> ±0.05
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	3.50 <sup>d</sup> ±0.17	3.53 <sup>d</sup> ±0.20
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	3.26 <sup>e</sup> ±0.05	3.33 <sup>d</sup> ±0.05
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	3.90 <sup>bc</sup> ±0.17	4.06 <sup>bc</sup> ±0.05
<b>CD</b>	0.199	0.194
<b>CV</b>	3.200	3.067

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.19. Siliqua Length(cm) of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Membrane Stability Index (%):** The effect of Sulphur and Salicylic acid and their combination on MSI was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.20, Figure 4.20). In 2021-2022, there was a significant difference in MSI compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the MSI was observed at 30, 60, and 90 DAS. Therefore, at 30 DAS, the percentage decrease as compared to T0 was found to be highest in T4 followed by T6, T5, T1, T8, T11, T9, T7, T2, T10, T3 and the percentage values were -17.32%, -23.17%, -35.47%, -56.09%, -73.69%, -84.85%, -118.64%, -128.77%, -147.41%, -159.22%, -176.30% respectively. At 60 DAS, the percentage decrease as compared to T0 was found highest in T4 followed by T5, T6, T7, T11, T1, T8, T10, T2, T9, T3 and the percentage values were -16.33%, -27.94%, -35.33%, -53.30%, -71.03%, -100.03%, -130.23%, -158.64%, -197.68%, -217.89%, -404.51% respectively. At 90 DAS, the percentage decrease as compared to T0 was found to be highest in T9 followed by T10, T4, T3, T7, T8, T2, T1 and the percentage values were -7.17%, -9.33%, -9.63%, -12.70%, -23.93%, -24.77%, -24.99%, -32.74% respectively. But percentage also increase in T5, T11, T6 as compared to T0 and the percentage values were 22.09%, 2.66%, 2.38% respectively. In 2022-2023, there was a significant difference in MSI compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage decrease as compared to T0 was found to be highest in T4, followed by T6, T5, T1, T11, T8, T7, T3, T10, T9, T2 and the percentage values were -32.17%, -33.96%, -47.95%, -52.86%, -59.18%, -96.39%, -148.85%, -149.45%, -157.03%, -199.20%, -305.39% respectively. At 60 DAS, the percentage decrease as compared to T0 was found highest in T4 followed by T5, T6, T9, T11, T10, T8, T1, T3, T7, T2 and the percentage values were -13.58%, -37.59%, -43.88%, -104.00%, -104.90%, -140.22%, -157.20%, -166.33%, -183.76%, -203.34%, -225.73% respectively. At 90 DAS, the percentage decrease as compared to T0 was found to be highest in T6 followed by T4, T2, T3, T11, T10, T9, T7, T8, T1 and the percentage values were -2.63%, -3.45%, -11.28%, -23.63%, -38.91%, -51.00%, -78.93%, -153.87%, -242.81%, -307.80% respectively. But the percentage also increase in T5 as compared to T0 and the percentage value was 30.20%.

The plant species *Brassica juncea* L., commonly known as mustard, possesses a physiological parameter known as the membrane stability index (MSI). This parameter is important in plant research and agriculture due to its profound implications. The Multispecies Symbiotic Index (MSI) measurement in this species holds significant importance (Salih, Wu, et al., 2022; Salih, Zhou, et al., 2022; Salwan et al., 2023; Samad et al., 2023; Sampedro-Guerrero et al., 2022; Samtani et al., 2022; Shah et al., 2022, 2023; Shang et al., 2022; Sharma & Verma, 2023; Sheikhalipour et al., 2023; Shekhawat et al., 2023). Furthermore, acquiring knowledge regarding the potential effects of sulphur (S) and salicylic acid (SA) infused foliar spray applications on the mustard stress index (MSI) can yield valuable insights for enhancing agricultural practises, thereby resulting in heightened crop vigour and productivity. The membrane stability index, also known as electrolyte leakage, is an important parameter that provides insight into the robustness of cell membranes in plant tissues. The previously mentioned phenomenon serves as a complex indicator of the cellular membrane integrity of the plant and its capacity to endure diverse external pressures, encompassing both living organisms and non-living factors. The MSI measurements hold implications that transcend academic interest and encompass practical insights into agricultural practices. The cellular composition of plants involves the presence of fragile membranes that serve the dual purpose of safeguarding the structural integrity of the cells and coordinating a complex array of physiological functions. The structural integrity of plant cells is safeguarded by delicate membranes that envelop them. The quantification of electrolyte loss, specifically the ions potassium (K) and sodium (Na), from plant cells is employed to determine the MSI value, which is subsequently represented as a percentage. Cell membrane leakage occurs when the integrity of the cell membranes is compromised due to damage induced by stress or other causative factors. The MSI assumes a crucial role due to several salient factors that pertain to its domain. Cell membranes that possess high strength and resilience to various stresses can be inferred from a low Membrane Stability Index (MSI), which signifies minimal occurrence of electrolyte leakage. Conversely, elevated MSI values signify a compromised state of membrane integrity, thereby indicating the plant's vulnerability to damage induced by stress. The plasma membrane of plant cells may incur damage when exposed to environmental stresses, such as drought, high temperatures, or

pathogenic attacks. An elevation in the MSI can be a preliminary indicator, alerting researchers to the plant's adaptive response to various stressors. When exposed to environmental stress, a plant reallocates its resources, such as energy and nutrients, towards its repair mechanisms. Monitoring MSI can provide valuable insights for scientists in comprehending the response of plants to stress, as it enables the examination of resource allocation patterns in the face of limited availability. The mustard plant's MSI is significantly impacted by sulphur, a vital constituent that plays a prominent role in the plant's nutrient composition and exerts a substantial influence. Sulphur is a crucial constituent of various amino acids, such as cysteine and methionine, which possess significant significance in the synthesis of proteins. Sulphur also assumes various functions that enhance the plant's resilience against stress. Proteins, in turn, serve both as structural components of cell membranes and as orchestrators of the mechanisms that control cellular repair. The stability of cell membranes is enhanced, and the membrane's mechanical stability index (MSI) is reduced when an adequate supply of sulphur is present. Sulphur compounds can demonstrate antioxidant properties, enabling them to counteract the potentially deleterious reactive oxygen species (ROS) generated in reaction to stress. Sulphur plays an indirect role in preserving membrane integrity and reducing MSI by mitigating the detrimental consequences of oxidative damage. The impact of salicylic acid, a compound widely recognised for its involvement in plant defence mechanisms and stress regulation, extends to the modulation of MSI in mustard. Salicylic acid (SA) as a foliar spray elicits the activation of the plant's stress-responsive mechanisms. Increased SA levels can make a membrane more resistant to the damaging effects of environmental stressors, which in turn lowers the MSI by preventing membrane damage. SA has the potential to positively influence the production of specific structural compounds that enhance the strength of cell membranes (Raja Gopalan et al., 2022; Ramakrishnan & Zhou, 2022; Rani et al., 2023; Rizvi et al., 2022; Roussos, 2023; Ruidas et al., 2022; Rybczyński et al., 2022; Sachan & Krishna, 2022). The reinforcement mentioned above immediately impacts reducing MSI values, a reliable indicator of enhanced membrane stability. The determination of mustard's membrane stability index serves as a valuable tool for comprehending the plant's capacity to withstand stress, its adaptive responses to environmental stimuli, and the allocation of resources within cellular compartments.



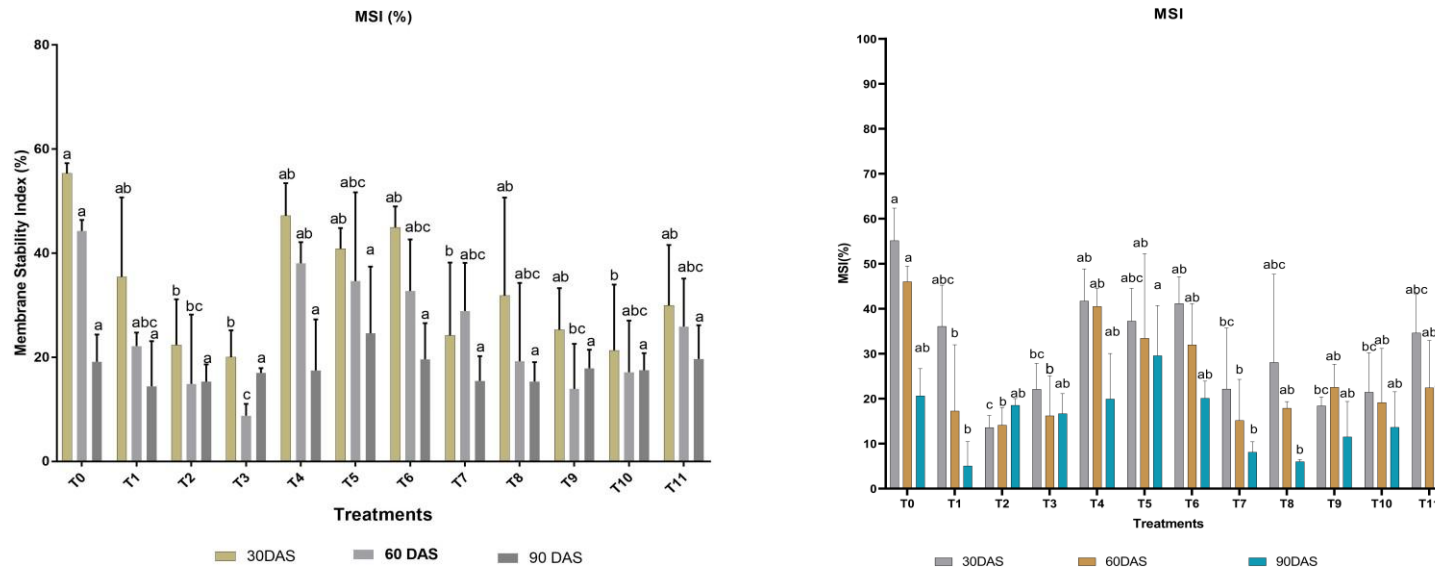
The application of sulphur and salicylic acid as foliar sprays can exert a substantial influence on MSI. This intervention is expected to enhance the structural integrity of cell membranes, mitigate oxidative damage, and augment the plant's capacity to withstand stressors. Implementing these strategies has the potential to enhance the resilience of mustard crops against various threats, leading to improvements in their overall vigour and productivity. Nevertheless, it remains imperative to continually engage in scientific research to refine application protocols and concentrations to optimise outcomes (Phokas et al., 2023; Phour & Sindhu, 2022; Poór et al., 2022; Quesada, 2022; Rai & Kaushik, 2023)

**Table 4.20. Impact of Different Treatments on Membrane Stability Index (%) of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	55.37 <sup>a</sup> ±1.92	55.14 <sup>a</sup> ±7.20	44.29 <sup>a</sup> ±2.11	46.01 <sup>a</sup> ±3.45	19.16 <sup>a</sup> ±5.21	20.64 <sup>ab</sup> ±6.03
<b>T1 (Thiourea-1000 ppm)</b>	35.47 <sup>ab</sup> ±15.25	36.07 <sup>abc</sup> ±9.17	22.14 <sup>abc</sup> ±2.60	17.27 <sup>b</sup> ±14.71	14.43 <sup>a</sup> ±8.67	5.06 <sup>b</sup> ±5.39
<b>T2 (Salicylic acid-300 ppm)</b>	22.38 <sup>b</sup> ±8.77	13.60 <sup>c</sup> ±2.66	14.88 <sup>bc</sup> ±13.31	14.12 <sup>b</sup> ±3.89	15.33 <sup>a</sup> ±3.31	18.54 <sup>ab</sup> ±1.47
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	20.04 <sup>b</sup> ±5.13	22.10 <sup>bc</sup> ±5.76	8.78 <sup>c</sup> ±2.26	16.21 <sup>b</sup> ±8.90	17.00 <sup>a</sup> ±0.91	16.69 <sup>ab</sup> ±4.43
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	47.19 <sup>ab</sup> ±6.27	41.72 <sup>ab</sup> ±7.13	38.07 <sup>ab</sup> ±4.04	40.51 <sup>ab</sup> ±4.05	17.47 <sup>a</sup> ±9.81	19.95 <sup>ab</sup> ±10.11
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	40.87 <sup>ab</sup> ±3.97	37.27 <sup>abc</sup> ±7.26	34.62 <sup>abc</sup> ±17.08	33.44 <sup>ab</sup> ±18.80	24.59 <sup>a</sup> ±12.82	29.57 <sup>a</sup> ±11.15
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	44.95 <sup>ab</sup> ±4.04	41.16 <sup>ab</sup> ±5.96	32.73 <sup>abc</sup> ±9.91	31.98 <sup>ab</sup> ±9.17	19.63 <sup>a</sup> ±6.92	20.11 <sup>ab</sup> ±3.78
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	24.20 <sup>b</sup> ±14.02	22.15 <sup>bc</sup> ±13.57	28.89 <sup>abc</sup> ±9.26	15.17 <sup>b</sup> ±9.12	15.46 <sup>a</sup> ±4.75	8.13 <sup>b</sup> ±2.29
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	31.88 <sup>ab</sup> ±18.81	28.07 <sup>abc</sup> ±19.59	19.24 <sup>abc</sup> ±15.06	17.89 <sup>ab</sup> ±1.46	15.35 <sup>a</sup> ±3.69	6.02 <sup>b</sup> ±0.48
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	25.32 <sup>ab</sup> ±7.95	18.43 <sup>bc</sup> ±1.96	13.93 <sup>bc</sup> ±8.66	22.55 <sup>ab</sup> ±5.10	17.88 <sup>a</sup> ±3.58	11.53 <sup>ab</sup> ±7.85
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	21.36 <sup>b</sup> ±12.62	21.45 <sup>bc</sup> ±8.78	17.12 <sup>abc</sup> ±9.91	19.15 <sup>ab</sup> ±12.01	17.52 <sup>a</sup> ±3.27	13.66 <sup>ab</sup> ±7.87
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	29.95 <sup>ab</sup> ±11.63	34.64 <sup>abc</sup> ±8.52	25.90 <sup>abc</sup> ±9.22	22.45 <sup>ab</sup> ±10.49	19.68 <sup>a</sup> ±6.46	14.85 <sup>ab</sup> ±6.55
<b>CD</b>	17.307	14.02	17.271	16.315	N/A	11.278
<b>CV</b>	30.533	26.548	40.451	38.704	37.878	42.975

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.20. MSI (%) of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Chlorophyll a:** The effect of Sulphur and Salicylic acid and their combination on chlorophyll a was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at 30, 60, and 90 DAS (Table 4.21, Figure 4.21). In 2021-2022, there was a significant difference in chlorophyll compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the chlorophyll a was observed at 30, 60 and 90 DAS. Therefore, at 30 DAS, the percentage increase compared to T0 was the highest in T5, followed by T7 and T11, and the percentage values were 5.55%, 4.01%, and 2.76%, respectively. But in T6, T1, T8, T2, T3, T10, T4, T9 the percentage decrease as compared to T0 and the percentage values were -2.35%, -14.31%, -23.65%, -79.44%, -83.72%, -138.22%, -222.97%, -345.85% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T2, T5, T6, T8, T1, T7, T4 and the percentage values were 39.76%, 26.40%, 20.09%, 14.43%, 13.43%, 13.34%, 6.40%, 5.21% respectively. But in T9, T10, and T3, the percentage decreased compared to T0, and the percentage values were -6.68%, -18.40%, and -28.11%, respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T11, T7, T6, T5, T9, T2 and the percentage values were 18.04%, 13.05%, 12.27%, 6.89%, 5.26%, 2.40%, 0.61% respectively. But in T8, T1, T4, T10 the percentage decrease as compared to T0 and the percentage values were -0.41%, -2.96%, -12.5%, -58.30% respectively. In 2022-2023, there was a significant difference in Chlorophyll compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase compared to T0 was found to be highest in T5, followed by T7, T11, and T6, and the percentage values were 13.19%, 10.75%, 4.91%, and 4.22%, respectively. But in T1, T8, T3, T2, T10, T4, T9 the percentage decrease as compared to T0 and the percentage values were -4.20%, -7.20%, -50.26%, -69.91%, -106.31%, -187.27%, and -330.19% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T2, T5, T6, T1, T8, T4, T7 and the percentage values were 41.42%, 32.75%, 25.87%, 22.12%, 17.82%, 17.76%, 10.84%, 6.84% respectively. But in T9, T10, and T3, the percentage decreased compared to T0, and the percentage values were -3.90%, -12.81%, and -18.29%, respectively. At 90 DAS, the percentage increase as compared to T0 was found

highest in T3 followed by T7, T6, T11, T9, T5, T2, T8 and the percentage values were 15.86%, 14.99%, 8.06%, 6.89%, 6.31%, 5.58%, 5.33%, 4.25% respectively. But in T1, T4, and T10, the percentage decreased as compared to T0, the percentage values were -0.64%, -3.61%, and -51.28%, respectively. The measurement of chlorophyll content in mustard (*Brassica juncea* L.) is of utmost importance in plant research and agriculture. This study offers insights into the plant's photosynthetic activity and overall physiological condition. In addition, acquiring information regarding the potential ramifications of utilising sulphur (S) and salicylic acid (SA) via foliar spray on the concentration of chlorophyll 'a' can provide valuable insights for enhancing agricultural methodologies and augmenting crop productivity (Kurepa & Smalle, 2022; Kurniawan & Chuang, 2022; Kuromori et al., 2022; Lajayer et al., 2022; Li et al., 2022; Li et al., 2022; Li et al., 2022; Li, Ren, et al., 2023; Li, Zhang, et al., 2022). Chlorophyll 'a' is an essential pigment in photosynthesis, which functions to absorb light energy and convert it into chemical energy. The photosynthetic capacity of a plant can be assessed by quantifying its chlorophyll concentration through measurement. The association between elevated levels of chlorophyll and heightened photosynthetic efficiency is a widely observed phenomenon in scientific literature. This correlation ultimately leads to improved growth and increased potential for crop yield. The chlorophyll synthesis is contingent upon essential nutrients: nitrogen (N) and magnesium (Mg). Monitoring the chlorophyll content of a plant is a reliable approach for assessing the plant's nutrient status. The presence of a deviation from the optimal level of chlorophyll may suggest the existence of a nutrient deficiency or imbalance, thereby necessitating prompt corrective measures. Environmental stressors, including drought, nutrient deficiency, and pathogen infestation, may adversely affect chlorophyll concentration. Consequently, quantifying chlorophyll levels can be a proactive mechanism for detecting the physiological alterations induced by plant stress. Plants exhibiting robust growth characteristics tend to possess a greater abundance of the photosynthetic pigment known as chlorophyll (Kaya et al., 2023; Khalid et al., 2023; Khan et al., 2022, 2023; Khan et al., 2022; Kochanek et al., 2023; Kosakivska et al., 2022; Kour et al., 2023; Kudoyarova, 2022; Kumar & Ohri, 2023). Monitoring chlorophyll concentrations within a crop enables a more precise evaluation of the crop's holistic well-being, facilitating informed decision-making regarding plant vitality enhancement.

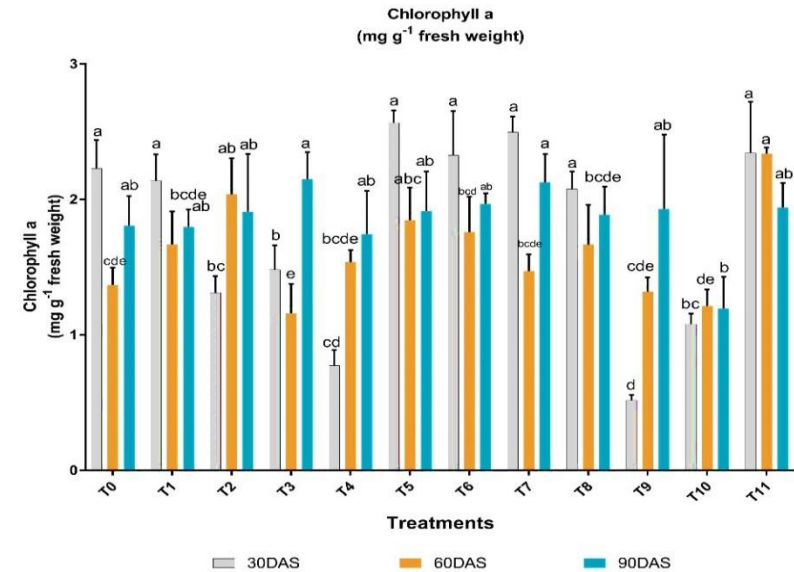
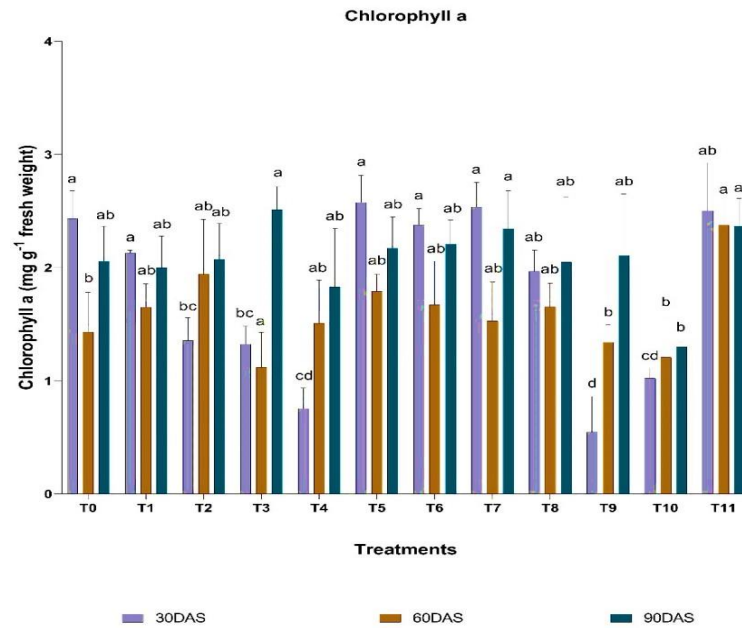
Mustard plants necessitate sulphur as a vital nutrient due to its indispensability in the biosynthesis of amino acids and enzymes that play a crucial role in chlorophyll formation. The utilisation of sulphur as a foliar spray can enhance the availability of this nutrient, thereby facilitating the synthesis of chlorophyll 'a' and resulting in a general augmentation of chlorophyll content. Furthermore, sulphur plays a crucial role in synthesising thylakoid membranes, which serve as the protective housing for chlorophyll 'a' molecules. An adequate quantity of sulphur is imperative for thylakoid membranes' proper formation and functionality, thereby facilitating the efficient execution of photosynthetic processes. The modulation of plant reactions to different stressors can result in alterations to the chlorophyll content of plants. Salicylic acid (SA), primarily recognised for its involvement in plant defence responses, is implicated in this process. When administered as a foliar spray, salicylic acid (SA) can stimulate stress-responsive signalling pathways, enhancing a plant's ability to withstand abiotic stress. The preservation of chlorophyll levels can be achieved by upholding SA-mediated stress tolerance mechanisms, thereby mitigating the adverse impacts of tension on chlorophyll breakdown and degradation. Consequently, there is an enhancement in the efficiency of photosynthesis, accompanied by the preservation of chlorophyll levels. Moreover, previous studies have provided evidence that SA can induce the activation of genes associated with the processes of photosynthesis and biosynthesis of chlorophyll (Jia et al., 2022; Jin et al., 2022; Jofre et al., 2023; Kandhol et al., 2023; Kapoor et al., 2022; Kapoor et al., 2022; Karaman, 2023; Karamat et al., 2022; Katoch et al., 2022; Kaviani et al., 2023). This, in turn, could potentially lead to an augmentation in chlorophyll production. Assessing the photosynthetic activity, plant health, and stress response of mustard can be facilitated by quantifying its chlorophyll content. Using sulphur and salicylic acid as a foliar spray has demonstrated beneficial effects on chlorophyll concentration in plants by enhancing nutrient accessibility, stress resilience, and chlorophyll synthesis. These strategies offer potential approaches to enhance mustard crop productivity, optimise crop management practices, and improve photosynthetic efficiency. To optimise the results, further investigation is necessary to enhance the techniques and concentrations employed in the application process (Huh, 2022; Hui et al., 2022; Huntenburg et al., 2022).

**Table 4.21. Impact of Different Treatments on Chlorophyll a of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	2.43 <sup>a</sup> ±0.24	2.22 <sup>a</sup> ±0.21	1.43 <sup>b</sup> ±0.35	1.37 <sup>cde</sup> ±0.12	2.05 <sup>ab</sup> ±0.30	1.80 <sup>ab</sup> ±0.21
<b>T1 (Thiourea-1000 ppm)</b>	2.12 <sup>a</sup> ±0.02	2.13 <sup>a</sup> ±0.19	1.65 <sup>ab</sup> ±0.20	1.66 <sup>bcde</sup> ±0.24	1.99 <sup>ab</sup> ±0.27	1.79 <sup>ab</sup> ±0.13
<b>T2 (Salicylic acid-300 ppm)</b>	1.35 <sup>bc</sup> ±0.19	1.31 <sup>bc</sup> ±0.12	1.94 <sup>ab</sup> ±0.48	2.03 <sup>ab</sup> ±0.26	2.07 <sup>ab</sup> ±0.32	1.90 <sup>ab</sup> ±0.42
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	1.32 <sup>bc</sup> ±0.15	1.48 <sup>b</sup> ±0.17	1.11 <sup>a</sup> ±0.31	1.15 <sup>e</sup> ±0.21	2.51 <sup>a</sup> ±0.20	2.14 <sup>a</sup> ±0.20
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	0.75 <sup>cd</sup> ±0.18	0.77 <sup>cd</sup> ±0.11	1.50 <sup>ab</sup> ±0.38	1.53 <sup>bcde</sup> ±0.09	1.82 <sup>ab</sup> ±0.51	1.74 <sup>ab</sup> ±0.32
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	2.57 <sup>a</sup> ±0.23	2.56 <sup>a</sup> ±0.08	1.79 <sup>ab</sup> ±0.15	1.84 <sup>abc</sup> ±0.24	2.17 <sup>ab</sup> ±0.27	1.91 <sup>ab</sup> ±0.29
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	2.37 <sup>a</sup> ±0.14	2.32 <sup>a</sup> ±0.32	1.67 <sup>ab</sup> ±0.38	1.75 <sup>bcd</sup> ±0.26	2.20 <sup>ab</sup> ±0.20	1.96 <sup>ab</sup> ±0.08
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	2.53 <sup>a</sup> ±0.21	2.49 <sup>a</sup> ±0.11	1.52 <sup>ab</sup> ±0.34	1.47 <sup>bcde</sup> ±0.12	2.34 <sup>a</sup> ±0.33	2.12 <sup>a</sup> ±0.20
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	1.96 <sup>ab</sup> ±0.18	2.07 <sup>a</sup> ±0.12	1.65 <sup>ab</sup> ±0.20	1.66 <sup>bcde</sup> ±0.29	2.04 <sup>ab</sup> ±0.57	1.88 <sup>ab</sup> ±0.20
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	0.54 <sup>d</sup> ±0.31	0.51 <sup>d</sup> ±0.03	1.34 <sup>b</sup> ±0.15	1.31 <sup>cde</sup> ±0.10	2.10 <sup>ab</sup> ±0.54	1.92 <sup>ab</sup> ±0.54
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	1.02 <sup>cd</sup> ±0.08	1.08 <sup>bc</sup> ±0.07	1.20 <sup>b</sup> ±0.35	1.21 <sup>de</sup> ±0.12	1.29 <sup>b</sup> ±0.04	1.19 <sup>b</sup> ±0.23
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	2.50 <sup>ab</sup> ±0.42	2.34 <sup>a</sup> ±0.37	2.37 <sup>a</sup> ±0.22	2.33 <sup>a</sup> ±0.04	2.36 <sup>a</sup> ±0.24	1.94 <sup>ab</sup> ±0.18
<b>CD</b>	0.382	0.31	0.495	0.304	N/A	0.472
<b>CV</b>	12.497	10.238	18.145	11.05	17.274	14.86

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.21. Chlorophyll a ( $\text{mg g}^{-1}$  fresh weight) of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).



**Chlorophyll b:** The effect of Sulphur and Salicylic acid and their combination on chlorophyll b was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at 30, 60, and 90 DAS (Table 4.22, Figure 4.22). In 2021-2022, there was a significant difference in chlorophyll b compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the chlorophyll b was observed at 30, 60 and 90 DAS. Therefore, at 30 DAS, the percentage increase compared to T0 was highest in T11, and the percentage value was 24.04%. But in T1, T8, T9, T5, T6, T7, T2, T4, T10, and T3 the percentage decrease as compare to T0 and the percentage values were -32.91%, -50.82%, -130.11%, -132.83%, -137.28%, -154.44%, -166.14%, -334.77%, -455.35%, and -774.76% respectively. At 60 DAS, the percentage decrease as compared to T0 was found highest in T5, T11, T7, T6, T1, T8, T3, T10, T2, T9, T4 and the percentage values were -22.28%, -24.42%, -49.03%, -54.63%, -76.46%, -88.02%, -111.20%, -159.39%, -214.31%, -226.74%, -285.33% respectively. At 90 DAS, the percentage increase compared to T0 was highest in T10, followed by T4, T1, and T8; the percentage values were 24.84%, 9.06%, 2.51%, and 0.36%, respectively. But in T2, T9, T5, T6, T7, T11, T3 the percentage decrease as compared to T0 and the percentage values were -0.55%, -2.26%, -5.24%, -7.12%, -14.35%, -15.57%, -24.62% respectively. In 2022-2023, there was a significant difference in Chlorophyll b compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase, compared to T0, was highest in T11, and the percentage value was 17.94%. But in T1, T8, T5, T2, T9, T7, T6, T4, T10, T3 the percentage decrease as compared to T0 and the percentage values were -33.11%, -71.79%, -115.23%, -133.51%, -138.85%, -148.29%, -160.24%, -307.39%, -406.41%, -682.05% respectively. At 60 DAS, the percentage decrease as compared to T0 was found highest in T11, T5, T7, T6, T8, T1, T3, T10, T9, T2, T4 and the percentage values were -22.35%, -28.42%, -49.13%, -64.56%, -70.08%, -73.89%, -103.39%, -131.21%, -217.36%, -270.75%, -275.71% respectively. At 90 DAS, the percentage increase compared to T0 was highest in T3, followed by T9, T11, and T7, and the percentage values were 31.74%, 6.42%, 4.37%, and 2.82%, respectively. But in T5, T2, T6, T4, T1, T10, T8 the percentage decrease as compared to T0 and the percentage values were

-14.41%, -27.23%, -31.27%, -56.53%, -81.32%, -85.66%, -153.48% respectively. Measuring chlorophyll 'b' content in mustard (*Brassica juncea* L.) is crucial in plant research and agriculture. This measurement offers valuable insights into the efficiency of photosynthesis and the overall well-being of the plant. In addition, acquiring information regarding the potential ramifications of utilising sulphur (S) and salicylic acid (SA) via foliar application on the concentration of chlorophyll 'b' can provide valuable insights for enhancing agricultural methodologies and improving crop productivity (Xiao et al., 2022; Xie et al., 2023; Xing et al., 2022; Xu et al., 2023; Xu et al., 2022, 2023; Xu, Cao, et al., 2022; Xu, Zeng, et al., 2022; Yagci & Agar, 2022; Yan et al., 2022; Yang et al., 2023). The efficiency of photosynthesis Chlorophyll 'b' is considered one of the critical pigments involved in the photosynthesis process, alongside chlorophyll 'a'. In the context of harnessing light energy across different segments of the electromagnetic spectrum, it serves as a highly significant constituent. A plant's capacity to utilise a broader spectrum of light for photosynthesis can be assessed by quantifying its chlorophyll b concentration. This measurement is also associated with enhancing the plant's overall photosynthetic efficiency. Chlorophyll 'b' molecules play a crucial role in augmenting the photosynthetic efficiency of plants by enabling the absorption of light energy in wavelengths that are not efficiently captured by chlorophyll 'a' in isolation. This phenomenon broadens the range of light energy harnessed by photosynthesis, thereby enhancing the plant's ability to convert light into chemical energy and organic compounds. The production of chlorophyll 'b' is contingent upon the availability of crucial nutrients, including nitrogen (N) and magnesium (Mg). The assessment of a plant's nutrient status can be facilitated by monitoring the content of chlorophyll 'b'. When the optimal levels are not achieved, it may indicate a potential deficiency or imbalance of nutrients, thereby facilitating the opportunity for timely intervention to rectify the issue. The examination of fluctuations in the concentration of chlorophyll 'b' in plants can yield valuable information regarding the impact of environmental stressors, including but not limited to drought, nutrient deficiency, and pest infestations. Consequently, quantifying chlorophyll 'b' can be valuable in identifying plant responses to stress-induced physiological alterations. Sulphur is a vital nutrient for mustard plants due to its indispensable role in chlorophyll synthesis. Using sulphur as a foliar spray could enhance the availability of this

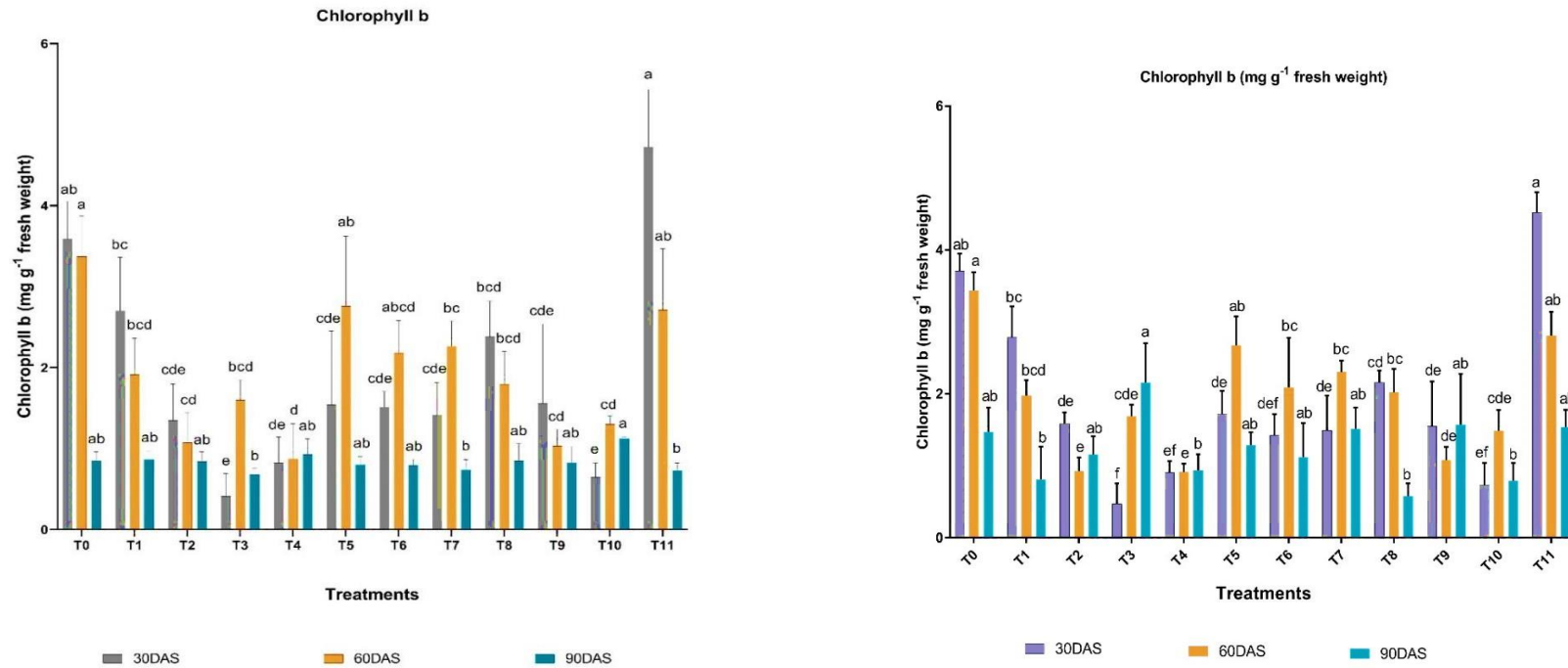
particular nutrient to plants, thereby promoting the synthesis of chlorophyll 'a' and 'b'. The efficient formation of the central magnesium ion ( $Mg^{2+}$ ) in the chlorophyll molecule, essential for synthesising chlorophyll 'b,' necessitates an adequate quantity of sulphur (Wang et al., 2023; Wang & Komatsu, 2022; Wang et al., 2022; Wang et al., 2022; Wang et al., 2022; Wante et al., 2022; Waters & Nelson, 2023; Wu et al., 2022; Wurms et al., 2023). Consequently, the presence of sulphur directly contributes to the synthesis of chlorophyll b, thereby increasing its overall abundance. Salicylic acid (SA) in plants, primarily recognised for its involvement in plant defence mechanisms, can indirectly impact the levels of chlorophyll b in plants by enhancing their stress tolerance. When applied to the plant's foliage, salicylic acid (SA) initiates stress-responsive signalling pathways, aiding mustard plants in adapting to environmental stress factors. The mitigation of stress-induced chlorophyll degradation can be achieved through enhanced stress tolerance, thereby facilitating the preservation of chlorophyll 'b' concentrations. Consequently, there is an observed augmentation in the efficiency of photosynthesis, along with the preservation of chlorophyll 'b' levels. Moreover, previous studies have provided evidence indicating that salicylic acid (SA) can activate specific genes closely linked to photosynthesis and chlorophyll biosynthesis. This activation, in turn, can lead to an augmentation in chlorophyll b synthesis. The assessment of mustard's chlorophyll 'b' concentration is imperative for evaluating its photosynthetic efficacy, overall plant well-being, and ability to cope with stress. Using sulphur and salicylic acid as foliar sprays has yielded advantageous outcomes regarding chlorophyll b levels in plant tissue. This is achieved through enhancing nutrient accessibility, stress resilience, and chlorophyll biosynthesis. Various techniques can be employed to enhance the productivity of mustard crops using improved management practices, increased photosynthetic efficiency, or a combination of both. Further research is necessary to strengthen the efficiency of application procedures and concentrations (Tariq et al., 2022; Todorova et al., 2022; Topcu et al., 2022; Torres & Figueroa, 2023; Tyagi et al., 2022; Ullah et al., 2023; Ullah et al., 2022; Verma et al., 2023; Waadt et al., 2022; Wan & Xin, 2022; Wang et al., 2022).

**Table 4.22. Impact of Different Treatments on Chlorophyll b of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	3.58 <sup>ab</sup> ±0.46	3.71 <sup>ab</sup> ±0.23	3.37 <sup>a</sup> ±0.49	3.43 <sup>a</sup> ±0.25	0.84 <sup>ab</sup> ±0.11	1.47 <sup>ab</sup> ±0.33
<b>T1 (Thiourea-1000 ppm)</b>	2.69 <sup>bc</sup> ±0.66	2.78 <sup>bc</sup> ±0.42	1.91 <sup>bcd</sup> ±0.44	1.97 <sup>bcd</sup> ±0.21	0.86 <sup>ab</sup> ±0.10	0.81 <sup>b</sup> ±0.45
<b>T2 (Salicylic acid-300 ppm)</b>	1.34 <sup>cde</sup> ±0.44	1.58 <sup>de</sup> ±0.15	1.07 <sup>cd</sup> ±0.36	0.92 <sup>e</sup> ±0.18	0.84 <sup>ab</sup> ±0.11	1.15 <sup>ab</sup> ±0.25
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	.41 <sup>e</sup> ±0.28	0.47 <sup>f</sup> ±0.28	1.59 <sup>bcd</sup> ±0.25	1.69 <sup>cde</sup> ±0.15	0.67 <sup>b</sup> ±0.07	2.15 <sup>a</sup> ±0.54
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	.82 <sup>de</sup> ±0.31	0.91 <sup>ef</sup> ±0.15	0.87 <sup>d</sup> ±0.43	0.91 <sup>e</sup> ±0.11	0.92 <sup>ab</sup> ±0.19	0.94 <sup>b</sup> ±0.22
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	1.54 <sup>cde</sup> ±0.91	1.72 <sup>de</sup> ±0.31	2.75 <sup>ab</sup> ±0.86	2.67 <sup>ab</sup> ±0.40	0.80 <sup>ab</sup> ±0.10	1.28 <sup>ab</sup> ±0.18
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	1.51 <sup>cde</sup> ±0.19	1.42 <sup>def</sup> ±0.29	2.18 <sup>abcd</sup> ±0.39	2.08 <sup>bc</sup> ±0.69	0.78 <sup>ab</sup> ±0.07	1.12 <sup>ab</sup> ±0.47
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	1.40 <sup>cde</sup> ±0.40	1.49 <sup>de</sup> ±0.48	2.26 <sup>bc</sup> ±0.31	2.30 <sup>bc</sup> ±0.15	0.73 <sup>b</sup> ±0.12	1.51 <sup>ab</sup> ±0.29
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	2.37 <sup>bcd</sup> ±0.44	2.16 <sup>cd</sup> ±0.16	1.79 <sup>bcd</sup> ±0.40	2.02 <sup>bc</sup> ±0.32	0.84 <sup>ab</sup> ±0.21	0.58 <sup>b</sup> ±0.18
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	1.55 <sup>cde</sup> ±0.97	1.55 <sup>de</sup> ±0.61	1.03 <sup>cd</sup> ±0.20	1.08 <sup>de</sup> ±0.18	0.82 <sup>ab</sup> ±0.20	1.57 <sup>ab</sup> ±0.70
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	0.64 <sup>e</sup> ±0.17	0.73 <sup>ef</sup> ±0.30	1.30 <sup>cd</sup> ±0.10	1.48 <sup>cde</sup> ±0.28	1.12 <sup>a</sup> ±0.01	0.79 <sup>b</sup> ±0.24
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	4.72 <sup>a</sup> ±0.70	4.52 <sup>a</sup> ±0.28	2.71 <sup>ab</sup> ±0.75	2.80 <sup>ab</sup> ±0.33	0.73 <sup>b</sup> ±0.09	1.53 <sup>ab</sup> ±0.24
<b>CD</b>	0.991	0.597	0.823	0.559	N/A	0.649
<b>CV</b>	30.813	18.198	25.326	16.8	15.894	30.595

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.22. Chlorophyll b of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm) + Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Chlorophyll a+b:** The effect of Sulphur and Salicylic acid and their combination on chlorophyll a+b was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at 30, 60, and 90 DAS (Table 4.23, Figure 4.23). In 2021-2022, there was a significant difference in chlorophyll a+b compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the chlorophyll a+b was observed at 30, 60 and 90 DAS. Therefore, at 30 DAS, the percentage decrease as compared to T0 was found highest in T1 followed by T8, T5, T7, T6, T2, T9, T3, T10, T4 and the percentage values were -24.70%, -38.51%, -46.22%, -52.60%, -54.79%, -122.65%, -186.07%, -247.05%, -261.05%, -281.40% respectively. But, in T11, the percentage increased compared to T0, and the percentage value was 16.67%. At 60 DAS, the percentage decrease as compared to T0 was found highest in T5 followed by T6, T7, T1, T8, T2, T3, T10, T4, T9 and the percentage values were -5.60%, -24.66%, -26.68%, -34.84%, -39.37%, -59.22%, -77.01%, -91.48%, -101.43%, -102.40% respectively. But in T11, the percentage increased compared to T0, and the percentage value was 5.55%. At 90 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T11, T7, T6, T5, T9, T2 and the percentage values were 8.97%, 6.30%, 5.89%, 3.20%, 2.42%, 1.09%, 0.27% respectively. In 2022-2023, there was a significant difference in Chlorophyll a+b compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage decrease as compared to T0 was found to be highest in T1, followed by T5, T8, T7, T6, T2, T9, T3, T10, T4 and the percentage values were -20.56%, -38.40%, -40.12%, -48.80%, -58.26%, -104.76%, -186.69%, -203.42%, -227.62%, -252.15% respectively. But, in T11, the percentage increased compared to T0, and the percentage value was 13.49%. At 60 DAS, the percentage decrease as compared to T0 was found highest in T5 followed by T6, T7, T8, T1, T2, T3, T10, T4, T9 and the percentage values were -6.24%, -24.93%, -27.33%, -30.38%, -31.93%, -62.15%, -68.78%, -77.97%, -96.08%, -100.17% respectively. However, in T11, the percentage increased compared to T0, and the percentage value was 6.62%. At 90 DAS, the percentage increase compared to T0 was highest in T3, followed by T7, T9, and T11; the percentage values were 23.82%, 9.93%, 6.36%, and 5.78%, respectively. But in T5, T6, T2, T4, T1, T8, T10 the percentage decrease as compared

to T0 and the percentage values were -2.45%, -6.21%, -6.95%, -22.15%, -25.76%, -32.85%, -65.00% respectively. Quantifying chlorophyll a + b' concentration in mustard (*Brassica juncea* L.) is significant in plant investigation and agricultural practices. This measurement provides valuable insights into the photosynthetic efficiency, the overall health of plants, and the impact of sulphur (S) and salicylic acid (SA) foliar spray treatments on mustard plants (Pérez-Llorca et al., 2023; Phokas et al., 2023; Phour & Sindhu, 2022; Poór et al., 2022; Quesada, 2022; Rai & Kaushik, 2023; Raja Gopalan et al., 2022; Ramakrishnan & Zhou, 2022). The efficacy of photosynthesis Chlorophyll a and chlorophyll b, collectively constituting the principal pigments accountable for photosynthesis, substantially influence the efficiency of photosynthetic activity. Photosynthetic organisms can capture light energy spanning a broad spectrum of wavelengths, enhancing photosynthesis efficiency by optimising available light. The plant's photosynthetic capacity can be quantified by assessing the concentration of chlorophyll a + b' within the plant. Chlorophylls a and b hold significant importance in photosynthesis due to their integral role as essential constituents of the photosystems located within chloroplasts. The initiation of photosynthesis necessitates their presence, as it involves the conversion of carbon dioxide and water into carbohydrates and oxygen through light energy. Fluctuations in the concentration of chlorophyll a+b within a plant can serve as a reliable indicator of alterations in the plant's physiological condition. Various factors, including drought, nutrient deficiency, pest infestations, and disease, can influence the chlorophyll content in plants. Monitoring chlorophyll levels can serve as an effective means of detecting early indications of stress, thereby enabling prompt intervention measures. Chlorophyll synthesis depends on the availability of essential nutrients, such as nitrogen (N) and magnesium (Mg). The quantification of chlorophyll a + b' content provides a means of assessing the plant's nutritional status. Chlorophyll concentrations can serve as an indicator for determining the sufficiency of nutrient presence. Mustard plants necessitate a substantial quantity of sulphur due to its indispensable role as a constituent in chlorophyll synthesis, directly influencing this physiological process. Sulphur is necessary for synthesising magnesium ( $Mg^{2+}$ )-porphyrin complexes, and the active sites of chlorophyll molecules are occupied by magnesium ions ( $Mg^{2+}$ ). An adequate quantity of sulphur is required to facilitate the efficient integration of magnesium ions ( $Mg^{2+}$ ) into chlorophyll molecules, a process

essential for synthesising chlorophyll a + b'. Moreover, sulphur plays a crucial role in synthesising amino acids and proteins, which are fundamental constituents of chlorophyll. Sulphur is vital in synthesising proteins, including the enzymes involved in chlorophyll production. The augmentation of a plant's stress tolerance is associated with the modulation of SA, a compound primarily recognised for its involvement in plant defence mechanisms (Niu & Fu, 2022; Ortiz-García et al., 2022, 2023; Ozturk & Unal, 2023; Paalli et al., 2022; Pal et al., 2023; Parada et al., 2022; Parrey et al., 2023; Patel et al., 2022; Peng et al., 2022). This modulation, in turn, indirectly impacts the chlorophyll a+b content within the plant. The application of SA as a foliar spray induces the activation of stress-responsive pathways in mustard plants, thereby enhancing their ability to tolerate environmental stressors. Plants with an elevated stress tolerance can maintain stable chlorophyll levels in adverse environmental conditions. The degradation of chlorophyll can occur due to stress. Still, stress responses mediated by salicylic acid (SA) can help alleviate this degradation, thus preserving the content of chlorophyll a + b'. Furthermore, it has been determined that SA can effectively modulate the transcription of genes associated with the photosynthesis and biosynthesis of chlorophyll (Mukarram et al., 2022; Mukhtar et al., 2023; Myers Jr. et al., 2023; Nadeem, 2022; Nam et al., 2023; Napieraj et al., 2023; Nasir & Toth, 2022; Negi & Kumar, 2023; Nimsi et al., 2023). There is a potential for an upregulation to enhance mustard plants' capacity to synthesise chlorophyll a and chlorophyll b. Determining chlorophyll a + b content in mustard is imperative for assessing the plant's overall health and response to various stressors, and using sulphur and salicylic acid as foliar spray exhibits promising prospects for enhancing nutrient accessibility, augmenting stress resilience, and promoting chlorophyll synthesis. These strategies can improve crop management practises, increasing mustard crop productivity and optimising photosynthetic efficiency. Further investigation is imperative to refine the application methodologies and concentrations that will yield optimal outcomes (Manepalli et al., 2022; Mangena, 2022; Manjunatha et al., 2022; Masmoudi et al., 2023; Megala et al., 2022; Mittal et al., 2022; Mohammadi et al., 2023; Molinari et al., 2023; Moustakas et al., 2022; Mugwanya et al., 2023).

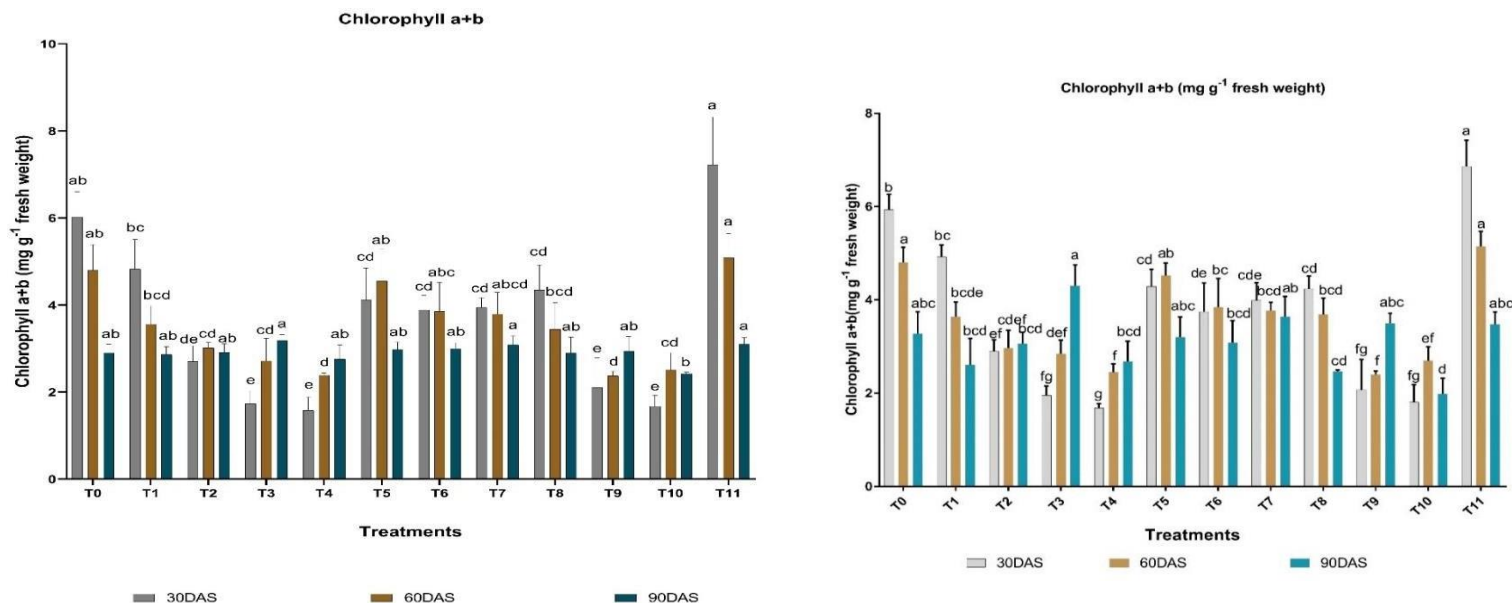


**Table 4.23. Impact of Different Treatments on Chlorophyll a+b of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	6.02 <sup>ab</sup> ±0.58	5.94 <sup>ab</sup> ±0.32	4.80 <sup>ab</sup> ±0.58	4.80 <sup>a</sup> ±0.32	2.90 <sup>a</sup> ±0.19	3.27 <sup>abc</sup> ±0.47
<b>T1 (Thiourea-1000 ppm)</b>	4.82 <sup>bc</sup> ±0.67	4.92 <sup>bc</sup> ±0.25	3.56 <sup>bcd</sup> ±0.42	3.64 <sup>bcd</sup> ±0.31	2.86 <sup>a</sup> ±0.17	2.60 <sup>bcd</sup> ±0.56
<b>T2 (Salicylic acid-300 ppm)</b>	2.70 <sup>de</sup> ±0.36	2.90 <sup>ef</sup> ±0.24	3.01 <sup>cd</sup> ±0.12	2.96 <sup>cdef</sup> ±0.38	2.91 <sup>a</sup> ±0.20	3.06 <sup>bcd</sup> ±0.24
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	1.73 <sup>e</sup> ±0.28	1.95 <sup>fg</sup> ±0.19	2.71 <sup>cd</sup> ±0.52	2.84 <sup>def</sup> ±0.28	3.18 <sup>a</sup> ±0.13	4.30 <sup>a</sup> ±0.45
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	1.57 <sup>e</sup> ±0.30	1.68 <sup>g</sup> ±0.08	2.38 <sup>d</sup> ±0.05	2.45 <sup>f</sup> ±0.17	2.75 <sup>ab</sup> ±0.32	2.68 <sup>bcd</sup> ±0.42
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	4.11 <sup>cd</sup> ±0.73	4.29 <sup>cd</sup> ±0.36	4.54 <sup>ab</sup> ±0.74	4.52 <sup>ab</sup> ±0.26	2.97 <sup>a</sup> ±0.17	3.20 <sup>abc</sup> ±0.43
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	3.88 <sup>cd</sup> ±0.33	3.75 <sup>de</sup> ±0.61	3.85 <sup>abc</sup> ±0.66	3.84 <sup>bc</sup> ±0.61	2.99 <sup>a</sup> ±0.13	3.08 <sup>bcd</sup> ±0.47
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	3.94 <sup>cd</sup> ±0.21	3.99 <sup>cde</sup> ±0.37	3.79 <sup>abcd</sup> ±0.49	3.77 <sup>bcd</sup> ±0.17	3.08 <sup>a</sup> ±0.21	3.64 <sup>ab</sup> ±0.43
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	4.34 <sup>cd</sup> ±0.57	4.23 <sup>cd</sup> ±0.27	3.44 <sup>bcd</sup> ±0.61	3.68 <sup>bcd</sup> ±0.35	2.89 <sup>a</sup> ±0.36	2.46 <sup>cd</sup> ±0.02
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	2.10 <sup>e</sup> ±0.68	2.07 <sup>fg</sup> ±0.65	2.37 <sup>d</sup> ±0.10	2.40 <sup>f</sup> ±0.07	2.93 <sup>a</sup> ±0.34	3.50 <sup>abc</sup> ±0.21
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	1.66 <sup>e</sup> ±0.25	1.81 <sup>fg</sup> ±0.37	2.50 <sup>cd</sup> ±0.39	2.70 <sup>ef</sup> ±0.29	2.42 <sup>b</sup> ±0.02	1.98 <sup>d</sup> ±0.33
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	7.22 <sup>a</sup> ±1.09	6.86 <sup>a</sup> ±0.55	5.08 <sup>a</sup> ±0.56	5.14 <sup>a</sup> ±0.32	3.09 <sup>a</sup> ±0.15	3.48 <sup>abc</sup> ±0.26
<b>CD</b>	0.988	0.683	0.867	0.556	N/A	0.661
<b>CV</b>	15.756	10.824	14.498	9.144	7.789	12.473

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.23. Chlorophyll a+b of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Chlorophyll ab ratio:** The effect of Sulphur and Salicylic acid and their combination on chlorophyll ab ratio was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at 30, 60, and 90 DAS (Table 4.24, Figure 4.24). In 2021-2022, there was a significant difference in chlorophyll ab ratio compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the chlorophyll ab ratio was observed at 30, 60 and 90 DAS. Therefore, at 30 DAS, the percentage decrease as compared to T0 was found to be highest in T3, followed by T5, T7, T10, T6, T2, T4, T8, T1 and the percentage values were 83.70%, 71.87%, 65.04%, 58.11%, 56.71%, 37.89%, 32.38%, 18.53%, 16.65%, respectively. But in T9 and T11, the percentage decreased compared to T0, and the percentage values were -19.77% and -29.10%. At 60 DAS, the percentage increase as compared to T0 was found highest in T4 followed by T2, T9, T11, T8, T10, T1, T6, T5, T3, T7 and the percentage values were 80.22%, 79.78%, 67.96%, 54.31%, 53.82%, 53.50%, 52.46%, 44.31%, 39.17%, 37.86%, 36.51% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T11, T7, T6, T5, T9, T8, T2 and the percentage values were 33.40%, 24.11%, 23.71%, 11.81%, 9.68%, 8.79%, 4.63%, 1.45% respectively. But in T1, T4, and T10 the percentage decrease as compared to T0 and the percentage values were -6.21%, -18.01%, -116.16% respectively. In 2022-2023, there was a significant difference in Chlorophyll ab ratio compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T3, followed by T7, T6, T10, T5, T8, T4, T2, and T1, and the percentage values were 86.07%, 66.56%, 63.50%, 62.53%, 60.37%, 37.50%, 31.42%, 27.25%, 23.41% respectively. But in T11 and T9, the percentage decreased compared to T0, and the percentage values were -16.22% and -67.62%. At 60 DAS, the percentage increase as compared to T0 was found highest in T2 followed by T4, T9, T6, T1, T8, T10, T11, T5, T3, T7 and the percentage values were 82.25%, 76.42%, 68.11%, 57.65%, 53.02%, 52.59%, 52.54%, 52.52%, 43.49%, 41.97%, 37.67% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T8 followed by T1, T6, T4, T2, T10, T9, T5, T7, T11 and the percentage values were 64.94%, 51.95%, 37.15%, 33.64%, 27.55%, 21.31%, 19.95%, 15.22%, 11.90%, 1.73% respectively. But in T3, the

percentage decreased compared to T0, and the percentage value was -20.64%. Quantifying chlorophyll 'a/b ratio' concentration in mustard (*Brassica juncea* L.) is important in plant physiology and agriculture (Ji et al., 2022; Jia et al., 2022; Jin et al., 2022; Jofre et al., 2023; Kandhol et al., 2023; Kapoor et al., 2022; Kapoor et al., 2022; Karaman, 2023; Karamat et al., 2022; Katoch et al., 2022; Kaviani et al., 2023; Kaya et al., 2023; Khalid et al., 2023; Khan et al., 2022, 2023). This study provides significant findings regarding the efficacy of photosynthesis and the potential impacts of sulphur (S) and salicylic acid (SA) foliar spray treatments on mustard crops. The chlorophyll 'a/b ratio' is a valuable indicator used to evaluate the balance between the two primary forms of chlorophyll, 'a' and 'b,' within the photosynthetic apparatus of plants. The above ratio is a metric for evaluating the plant's ability to obtain and utilise light energy during photosynthesis. A diverse range of chlorophyll molecules exists, each exhibiting a distinct light absorption pattern. Chlorophyll 'a' is predominantly accountable for the absorption of light in the red and blue regions of the electromagnetic spectrum, whereas chlorophyll 'b' assumes the responsibility of absorbing light in the red-orange and blue-violet regions. Plants with higher 'a/b ratios' exhibit a greater inclination towards capturing specific wavelengths of light, indicating their enhanced adaptation to the prevailing light conditions. The 'a/b ratio' is a reliable indicator for assessing a plant's response to environmental stresses. The ratio above may be influenced by a range of environmental stressors, including but not limited to drought, nutrient deficiencies, and pathogen attacks. The assessment of a plant's ability to endure stress and maintain photosynthetic efficiency under challenging conditions can be enhanced by monitoring the 'a/b ratio'. The presence of vital nutrients, such as magnesium (Mg) and nitrogen (N), influences the process of chlorophyll synthesis in plants, specifically the production of chlorophylls 'a' and 'b'. The discrepancy in nutrient levels can influence the 'a/b ratio.' Assessing this ratio can be advantageous in ascertaining the presence of an overabundance of a specific nutrient. Sulphur is a crucial constituent in the synthesis of chlorophyll. The maintenance of the magnesium porphyrin ring structure of chlorophyll molecules relies on its crucial role. Sufficient availability of sulphur is necessary for the synthesis of functional chlorophyll molecules. Insufficient sulphur availability can lead to reduced chlorophyll concentrations and an altered ratio of chlorophyll a to chlorophyll b. Furthermore, sulphur is involved in the nitrogen

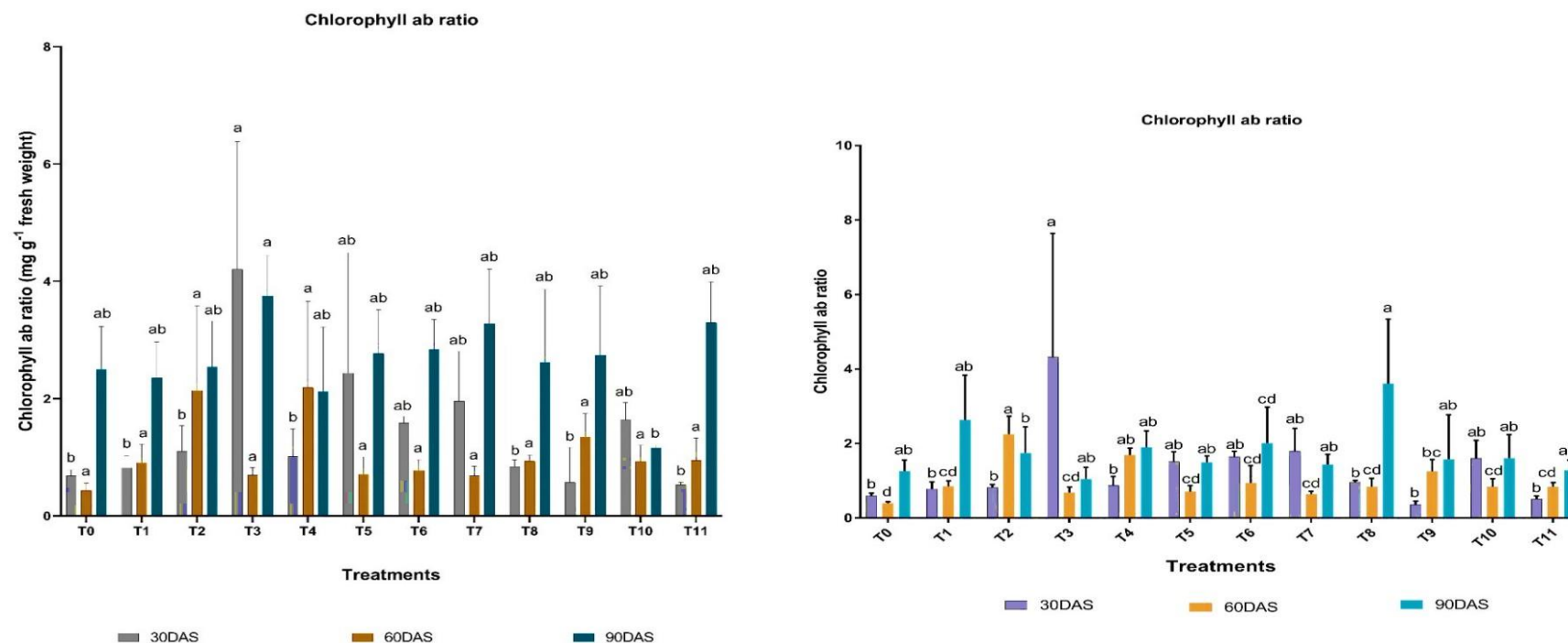
metabolism process, which subsequently exerts an indirect impact on the synthesis of chlorophyll. The synthesis of chlorophyll depends on a consistent availability of essential nutrients, a condition facilitated by sulphur, which promotes the absorption and integration of nitrogen in plants. The impact of SA, a well-known component in plant defence mechanisms, on the "a/b ratio" can be attributed to its ability to enhance a plant's stress tolerance (Hernández et al., 2023; Hernández et al., 2022; Hilal et al., 2023; Hong et al., 2022; Huang et al., 2022; Hudeček et al., 2023; Huh, 2022; Hui et al., 2022; Huntenburg et al., 2022; Hussein et al., 2023; Iftikhar et al., 2023; Islam et al., 2023; Javadipour et al., 2022). When applied to the foliage of mustard plants, salicylic acid (SA) initiates stress-responsive signalling pathways, thereby enhancing the plants' ability to cope with the detrimental impacts of their surroundings. The administration of SA treatment may lead to an enhanced capacity to withstand stress, thereby potentially enabling the maintenance of a stable "a/b ratio" in difficult situations. SA-mediated stress responses prevent the degradation of chlorophyll induced by stress, thereby maintaining the 'a/b ratio.' SA can positively regulate the expression of genes associated with chlorophyll production. This regulation may enhance the synthesis of chlorophyll 'a' and 'b,' thereby promoting an optimal 'a/b ratio' that could confer advantageous effects on the plant (Gul et al., 2023; Guo et al., 2023; Z. Guo et al., 2023; Gupta et al., 2022; Hajiboland et al., 2022; Hartmann et al., 2022; Hernandez-Leon & Valenzuela-Soto, 2022). Determining the chlorophyll 'a/b ratio' content in mustard is imperative for assessing photosynthetic efficiency, plant adaptation to varying light conditions, and responses to stress. Foliar spray applications of sulphur and salicylic acid can enhance the 'a/b ratio' by promoting nutrient availability, stress tolerance, and chlorophyll biosynthesis. These strategies can improve photosynthetic efficiency, optimise crop management practices, and increase mustard crop productivity. Further investigation is necessary to refine the application protocols and concentrations that will yield optimal outcomes (Dhiman et al., 2022; EL Sabagh et al., 2022; Elnahal et al., 2022; Faisal et al., 2023; Felipez et al., 2022; Feng et al., 2023; Ferreira et al., 2023; Fidler et al., 2022; Fierli et al., 2022; Ganz et al., 2022; García-Laynes et al., 2022; Geng et al., 2022; González-Pérez et al., 2022).

**Table 4.24. Impact of Different Treatments on Chlorophyll ab ratio of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	0.68 <sup>b</sup> ±0.10	0.60 <sup>b</sup> ±.06	0.43 <sup>a</sup> ±0.13	0.39 <sup>d</sup> ±0.03	2.50 <sup>ab</sup> ±0.72	1.26 <sup>ab</sup> ±.29
<b>T1 (Thiourea-1000 ppm)</b>	0.82 <sup>b</sup> ±0.20	0.78 <sup>b</sup> ±.18	0.90 <sup>a</sup> ±0.31	0.85 <sup>cd</sup> ±0.15	2.35 <sup>ab</sup> ±0.61	2.63 <sup>ab</sup> ±1.20
<b>T2 (Salicylic acid-300 ppm)</b>	1.10 <sup>b</sup> ±0.43	0.82 <sup>b</sup> ±.07	2.13 <sup>a</sup> ±1.44	2.25 <sup>a</sup> ±0.48	2.53 <sup>ab</sup> ±0.78	1.74 <sup>ab</sup> ±.70
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	4.20 <sup>a</sup> ±2.17	4.32 <sup>a</sup> ±3.31	0.69 <sup>a</sup> ±0.12	0.68 <sup>cd</sup> ±0.14	3.75 <sup>a</sup> ±0.68	1.04 <sup>b</sup> ±.31
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	1.01 <sup>b</sup> ±0.47	0.87 <sup>b</sup> ±.24	2.18 <sup>a</sup> ±1.47	1.69 <sup>ab</sup> ±0.18	2.11 <sup>ab</sup> ±1.10	1.90 <sup>ab</sup> ±.43
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	2.43 <sup>ab</sup> ±2.05	1.51 <sup>ab</sup> ±.26	0.71 <sup>a</sup> ±0.29	0.70 <sup>cd</sup> ±0.17	2.76 <sup>ab</sup> ±0.74	1.49 <sup>ab</sup> ±.17
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	1.58 <sup>ab</sup> ±0.11	1.65 <sup>ab</sup> ±.14	0.77 <sup>a</sup> ±0.18	0.94 <sup>cd</sup> ±0.46	2.83 <sup>ab</sup> ±0.51	2.01 <sup>ab</sup> ±.96
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	1.95 <sup>ab</sup> ±0.84	1.80 <sup>ab</sup> ±.61	0.68 <sup>a</sup> ±0.16	0.64 <sup>cd</sup> ±0.07	3.27 <sup>ab</sup> ±0.93	1.43 <sup>ab</sup> ±.27
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	0.84 <sup>b</sup> ±0.11	0.96 <sup>b</sup> ±.04	0.93 <sup>a</sup> ±0.10	0.84 <sup>cd</sup> ±0.22	2.62 <sup>ab</sup> ±1.24	3.60 <sup>a</sup> ±1.73
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	0.57 <sup>b</sup> ±0.59	0.35 <sup>b</sup> ±.09	1.34 <sup>a</sup> ±0.39	1.25 <sup>bc</sup> ±0.31	2.74 <sup>ab</sup> ±1.17	1.58 <sup>ab</sup> ±1.19
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	1.63 <sup>ab</sup> ±0.30	1.60 <sup>ab</sup> ±.47	0.92 <sup>a</sup> ±0.27	0.84 <sup>cd</sup> ±0.21	1.15 <sup>b</sup> ±0.05	1.60 <sup>ab</sup> ±.63
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	0.53 <sup>b</sup> ±0.04	0.51 <sup>b</sup> ±.07	0.94 <sup>a</sup> ±0.38	0.84 <sup>cd</sup> ±0.11	3.29 <sup>ab</sup> ±0.69	1.28 <sup>ab</sup> ±.27
<b>CD</b>	1.584	1.701	1.058	0.439	N/A	N/A
<b>CV</b>	64.181	75.640	58.687	25.85	31.915	48.109

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.24. Chlorophyll ab ratio of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm);

**T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).**



**Carotenoids:** The effect of Sulphur and Salicylic acid and their combination on carotenoids ratio was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at 30, 60, and 90 DAS (Table 4.25, Figure 4.25). In 2021-2022, there was a significant difference in carotenoids compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the carotenoids was observed at 30, 60 and 90 DAS. Therefore, at 30 DAS, the percentage decrease compared to T0 was highest in T1 and T2, and the percentage values were 8.11% and 1.88%. But in T11, T7, T8, T5, T3, T6, T10, T4, T9 the percentage decrease as compared to T0 and the percentage values were -3.03%, -3.71%, -4.83%, -16.04%, -17.43%, -31.90%, -34.86%, -37.72%, -39.94% respectively. At 60 DAS, the percentage decrease as compared to T0 was found highest in T7 followed by T5, T2, T10, T6, T8, T3, T4, T9, T1 and the percentage values were -21.75%, -22.01%, -24.15%, -31.06%, -36.11%, -37.10%, -41.57%, -78.15%, -85.16%, -109.91% respectively. However, in T11, the percentage increased compared to T0, and the percentage value was 4.87%. At 90 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T7, T9, T11, T5, T2 and the percentage values were 27.83%, 27.52%, 21.72%, 18.94%, 5.25%, 4.99% respectively. But in T6, T1, T4, T8, T10 the percentage decrease as compared to T0 and the percentage values were -8.56%, -13.23%, -15.13%, -44.24%, -85.72% respectively. In 2022-2023, carotenoids significantly differed from T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage decrease as compared to T0 was found to be highest in T2, followed by T7, T11, T8, T3, T5, T10, T6, T9, T4, and the percentage values were -0.37%, -3.37%, -4.32%, -5.57%, -17.07%, -22.48%, -31.17%, -35.87%, -36.57%, -37.52% respectively. However, in T1, the percentage increased compared to T0, and the percentage value was 11.46%. At 60 DAS, the percentage decrease as compared to T0 was found highest in T2 followed by T5, T7, T8, T10, T6, T3, T9, T4, T1 and the percentage values were -18.90%, -21.36%, -25.80%, -30%, -32.05%, -33.25%, -42.33%, -64.32%, -73.59%, -115.86% respectively. However, in T11, the percentage increase compared to T0, and the percentage value was 5.94%. At 90 DAS, the percentage increase as compared to T0 was found highest in T7 followed by T3, T9, T11, T2, T5 and the percentage values were 35.79%, 32.67%,

25.79%, 22.55%, 12.35%, 10.38% respectively. But in T6, T1, T4, T8, T10 the percentage decrease as compared to T0 and the percentage values were -2.90%, -3.23%, -13.10%, -22.60%, -72.31% respectively. Accurate quantification of carotenoid content in mustard (*Brassica juncea* L.) is essential in plant physiology and agriculture. This study offers important insights regarding the health of plants and the potential effects of applying sulphur (S) and salicylic acid (SA) through foliar sprays on mustard crops (Nam et al., 2023; Napieraj et al., 2023; Nasir & Toth, 2022; Negi & Kumar, 2023; Nimsi et al., 2023; Niu & Fu, 2022; Ortiz-García et al., 2022, 2023; Ozturk & Unal, 2023; Paalli et al., 2022; Pal et al., 2023). Mustard is classified as a plant species capable of synthesising carotenoids, a collection of pigments that play a crucial role in numerous physiological processes. The quantification of carotenoids present in a plant can serve as an indicator for assessing the plant's nutritional status and overall well-being. Sufficient levels of carotenoids are imperative for the growth and development of plants. Carotenoids play a crucial role in photosynthesis as they absorb light energy and transfer it to the chlorophyll molecules. Plants' broader light spectrum range enhances the photosynthetic process, leading to an augmented capacity for efficient energy absorption. Carotenoids serve as a protective mechanism for chlorophyll, mitigating the potential degradation that may arise due to the absorption of an excessive quantity of light. Carotenoid pigments serve as antioxidants, protecting plant cells against potential harm induced by reactive oxygen species (ROS) that may arise due to photosynthesis and various metabolic activities (Masmoudi et al., 2023; Megala et al., 2022; Mittal et al., 2022; Mohammadi et al., 2023; Molinari et al., 2023; Moustakas et al., 2022; Mugwanya et al., 2023; Mukarram et al., 2022; Mukhtar et al., 2023; Myers Jr. et al., 2023; Nadeem, 2022). Mustard plants exhibit enhanced tolerance towards oxidative stress when their carotenoid concentrations are maintained at an optimal level. The assessment of alterations in the carotenoid composition of a plant can provide insights into its response to various environmental stressors such as drought, high light intensity, or nutrient imbalances. The assessment of carotenoid levels is beneficial in ascertaining an individual's capacity to adapt to stress. Sulphur is an essential nutrient for mustard plants, and the administration of sulphur in foliar form can influence the concentration of carotenoids. Sulphur is a vital element required for the biosynthesis of amino acids, including cysteine, which serves as a precursor to

glutathione, an indispensable antioxidant. When there is more sulphur available, the plant can make more glutathione. This phenomenon enhances the antioxidant defence mechanism of plants and prevents the oxidation-induced damage of carotenoids (Liu, Xiao, et al., 2022; Liu, Meng, et al., 2022; Lombardino et al., 2022; Ma et al., 2022; Mabasa, 2023; Maheshwari et al., 2022; Maia et al., 2022; Makete et al., 2022; Manepalli et al., 2022; Mangena, 2022; Manjunatha et al., 2022). Moreover, sulphur exerts an influence on both metabolism and nutrient absorption. Magnesium (Mg) plays a crucial role in the composition of chlorophyll molecules. The absorption and utilisation of magnesium in the human body can be enhanced when sulphur levels are optimal. This guarantees adequate levels of chlorophyll to facilitate efficient photosynthesis, thereby indirectly supporting the functioning of carotenoids. The application of SA on plant leaves has the potential to enhance carotenoid content by bolstering stress resistance mechanisms. Research has demonstrated that mustard plants subjected to SA treatment exhibit activation of stress-responsive pathways, thereby improving their ability to cope with diverse environmental stressors. Increased resistance to stress contributes to maintaining carotenoid levels even in trying circumstances. The preservation of carotenoids in their functional state during photosynthesis and other plant processes is facilitated by the role of salicylic acid (SA) as an antioxidant. SA shields carotenoids from oxidative damage inflicted by reactive oxygen species (ROS). There is a potential for SA-mediated pathways to additionally facilitate gene expression within the carotenoid biosynthesis process, thereby augmenting the overall carotenoid content (Li, Luo, et al., 2022; Li, Tong, et al., 2023; Liu et al., 2023; Liu, Wang, et al., 2022; Liu, Li, et al., 2022; Liu, Cui, et al., 2022; Liu, Zong, et al., 2022; Liu, Liu, et al., 2022). Assessing the well-being of a plant, its photosynthetic efficacy, and its stress response can be facilitated by quantifying carotenoid levels in mustard. The application of sulphur and salicylic acid through foliar spray has been found to enhance carotenoid levels in plants by improving nutrient availability, antioxidant defence, stress tolerance, and biosynthesis. These strategies offer the potential to strengthen crop management practises, thereby enhancing the vitality of mustard plants and increasing crop productivity. Further investigation is necessary to optimise the techniques and concentrations for optimal results (Li, Zhang,

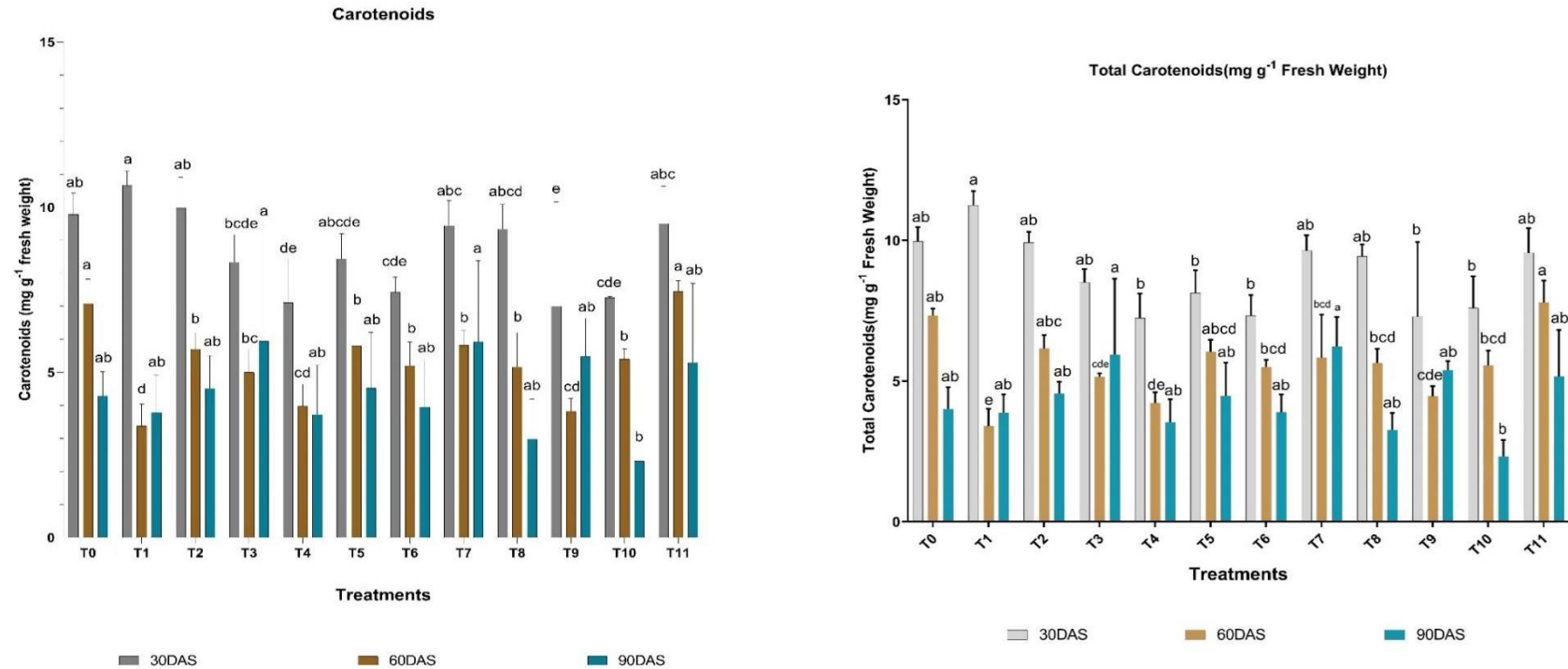
et al., 2022; Li, Zheng, et al., 2023; Li et al., 2022; Li, Han, et al., 2022; Li, He, et al., 2023; Li et al., 2022; Li, Han, et al., 2023; Li, Huang, et al., 2022).

**Table 4.25. Impact of Different Treatments on Carotenoids of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	9.79 <sup>ab</sup> ±0.64	9.96 <sup>ab</sup> ±0.50	7.08 <sup>a</sup> ±0.73	7.33 <sup>ab</sup> ±0.24	4.28 <sup>ab</sup> ±0.73	4.00 <sup>ab</sup> ±0.77
<b>T1 (Thiourea-1000 ppm)</b>	10.65 <sup>a</sup> ±0.43	11.25 <sup>a</sup> ±0.50	3.37 <sup>d</sup> ±0.66	3.39 <sup>e</sup> ±0.62	3.78 <sup>ab</sup> ±1.13	3.87 <sup>ab</sup> ±0.65
<b>T2 (Salicylic acid-300 ppm)</b>	9.97 <sup>ab</sup> ±0.92	9.92 <sup>ab</sup> ±0.38	5.70 <sup>b</sup> ±0.49	6.16 <sup>abc</sup> ±0.46	4.51 <sup>ab</sup> ±0.99	4.56 <sup>ab</sup> ±0.41
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	8.33 <sup>bcde</sup> ±0.82	8.51 <sup>ab</sup> ±0.47	5.00 <sup>bc</sup> ±0.69	5.15 <sup>cde</sup> ±0.13	5.94 <sup>a</sup> ±3.49	5.94 <sup>a</sup> ±2.70
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	7.10 <sup>de</sup> ±1.32	7.24 <sup>b</sup> ±0.86	3.97 <sup>cd</sup> ±0.66	4.22 <sup>de</sup> ±0.37	3.72 <sup>ab</sup> ±1.49	3.53 <sup>ab</sup> ±0.81
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	8.43 <sup>abcde</sup> ±0.76	8.13 <sup>b</sup> ±0.80	5.80 <sup>b</sup> ±1.02	6.04 <sup>abc</sup> ±0.43	4.52 <sup>ab</sup> ±1.68	4.46 <sup>ab</sup> ±1.19
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	7.42 <sup>cde</sup> ±0.46	7.33 <sup>b</sup> ±0.72	5.20 <sup>b</sup> ±0.71	5.50 <sup>bcd</sup> ±0.25	3.95 <sup>ab</sup> ±1.41	3.88 <sup>ab</sup> ±0.63
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	9.43 <sup>abc</sup> ±0.76	9.64 <sup>ab</sup> ±0.54	5.81 <sup>b</sup> ±0.45	5.82 <sup>bcd</sup> ±1.53	5.91 <sup>a</sup> ±2.46	6.23 <sup>a</sup> ±1.05
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	9.33 <sup>abcd</sup> ±0.74	9.43 <sup>ab</sup> ±0.41	5.16 <sup>b</sup> ±1.03	5.63 <sup>bcd</sup> ±0.51	2.97 <sup>ab</sup> ±1.21	3.26 <sup>ab</sup> ±0.61
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	6.99 <sup>e</sup> ±3.16	7.29 <sup>b</sup> ±2.65	3.82 <sup>cd</sup> ±0.38	4.46 <sup>cde</sup> ±0.35	5.47 <sup>ab</sup> ±1.15	5.39 <sup>ab</sup> ±0.31
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	7.25 <sup>cde</sup> ±0.03	7.59 <sup>b</sup> ±1.12	5.40 <sup>b</sup> ±0.30	5.55 <sup>bcd</sup> ±0.52	2.30 <sup>b</sup> ±0.30	2.32 <sup>b</sup> ±0.58
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	9.50 <sup>abc</sup> ±1.13	9.55 <sup>ab</sup> ±0.88	7.44 <sup>a</sup> ±0.33	7.79 <sup>a</sup> ±0.77	5.29 <sup>ab</sup> ±2.40	5.16 <sup>ab</sup> ±1.65
<b>CD</b>	2.066	1.697	1.187	1.04	N/A	1.985
<b>CV</b>	13.952	11.28	13.092	10.921	40.163	26.547

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.25. Carotenoids of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea

**(2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).**

**Total Phenol:** The effect of Sulphur and Salicylic acid and their combination on total phenol was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.26, Figure 4.26). In 2021-2022, there was a significant difference in total phenol compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the total phenol was observed at 30, 60, and 90 DAS. Therefore, at 30 DAS, the percentage increase compared to T0 was highest in T11, followed by T5 T10 and the percentage values were 16.27%, 3.64%, and 1.20% respectively. But in T1, T8, T2, T6, T3, T7, T4, and T9 the percentage decrease as compared to T0 and the percentage values were -0.81%, -3.92%, -9.61%, -12.78%, -19.70%, -32.79%, -34.23%, and -44.44% respectively. At 60 DAS, the percentage increase as compared to T0 was found to be highest in T8, followed by T5, T11, T2, and T3, and the percentage values were 15.84%, 11.89%, 9.49%, 5.81%, 2.65% respectively. But in T10, T1, T7, T6, T9, and T4 the percentage decrease as compared to T0 and the percentage values were -0.72%, -0.86%, -2.80%, -8.07%, -21.04%, -37.54% respectively. At 90 DAS, the percentage increase compared to T0 was highest in T8, followed by T5, T2, and T3, and the percentage values were 9.32%, 8.73%, 7.16%, and 2.64% respectively. But in T7, T6, T11, T10, T9, T4, T1 the percentage decrease as compared to T0 and the percentage values were -2.63%, -10.75%, -18.64%, -28.67%, -40.56%, -43.14%, -61.66% respectively. In 2022-2023, there was a significant difference in total phenol compared to T0 (Control) at 30, 60 and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase compared to T0 was highest in T11, followed by T1, T5, and T10, and the percentage values were 16.45%, 5.46%, 5.09%, and 3.45%, respectively. But in T8, T6, T2, T3, T4, T7, T9 the percentage decrease as compared to T0 and the percentage values were -0.55%, -8.35%, -9.33%, -11.52%, -26.48%, -34.19%, -38.28% respectively. At 60 DAS, the percentage increase compared to T0 was highest in T8, followed by T5, T11, T2, and T3. T10 and the percentage values were 17.11%, 10.23%, 9.06%, 4.35%, 1.95%, and 1.95% respectively. But in T1, T7, T6, T9, T4 the percentage decrease as compared to T0 and the percentage values were -2.63%, -4%, -11.25%, -25.35%, -36.04% respectively. At 90 DAS, the percentage increase compared to T0 was highest in T8, followed by T5,



T2, and T3; the percentage values were 8.01%, 7.21%, 6.04%, and 0.40%, respectively. But in T7, T6, T11, T10, T9, T4, T1 the percentage decrease as compared to T0 and the percentage values were -2.47%, -12.85%, -17.48%, -26.87%, -34.17%, -37.13%, -54.13% respectively. Quantifying the total phenolic content in *Brassica juncea* L., commonly known as the mustard plant, holds significant importance in plant research and agriculture. Furthermore, acquiring a thorough comprehension of the potential ramifications resulting from the utilisation of sulphur (S) and salicylic acid (SA) via foliar spray on the overall phenol content of mustard plants not only contributes to our existing body of knowledge but also offers valuable insights for the enhancement of agricultural methods and the augmentation of the general well-being and productivity of crops. Phenolic compounds are widely recognised for their significant antioxidant capabilities, and assessing a plant's overall phenol content is widely considered a crucial indicator for evaluating the plant's antioxidant capacity. Phenols function as protective agents, effectively safeguarding plant cells against oxidative damage induced by reactive oxygen species (ROS). Reactive oxygen species (ROS) can arise as a byproduct of regular metabolic processes or as a consequence of the perturbations caused by stress. Plants often respond to various environmental stresses, including biotic factors, such as harmful pathogens, and abiotic factors, such as drought or UV radiation, by increasing the production of phenolic compounds. Plants employ this defensive mechanism to counteract these stressors, which can negatively impact their health. The augmentation serves as a conspicuous and pressing indication, suggesting that the plant, akin to a seasoned guardian, is actively reacting to the pressures of difficult conditions and exerting conscientious endeavours to mitigate their adverse effects. Phenolic compounds are crucial in plant defence mechanisms and serve as sentinels and antioxidants. They assume the role of chemical deterrents and serve as formidable obstacles, impeding the intrusion of herbivorous adversaries and toxic pathogens. Hence, the assessment of the overall phenolic content in a plant offers valuable insights into its capacity for adaptation and resistance to potential biotic stressors. The strategic allocation of carbon resources is often required for the biosynthesis of phenolic compounds. This is necessary to ensure the attainment of optimal outcomes. When examined from this perspective, monitoring the overall phenol content assumes a crucial and indispensable obligation. This perspective offers

an opportunity to assess the plant's allocation of scarce resources, with a particular focus on the role of carbon. This phenomenon elucidates the plant's allocation of valuable resources towards fortifying its defence mechanisms, particularly in anticipation of imminent stressors, as evidenced by its proactive behaviour. The synthesis of phenolic compounds, which serve as the basis for the antioxidant defences of mustard plants, is significantly impacted by sulphur. Sulphur is crucial in producing various secondary metabolites, including phenolic compounds. Sulphur has been identified as a significant factor in modulating the synthesis of phenolic compounds in mustard plants. Specifically, amino acids that possess sulphur, such as cysteine, have a significant function as pivotal components in the intricate mechanism of phenolic compound synthesis. One indirect demonstration of sulphur's significant impact is observed in synthesising crucial amino acids required for the production of phenol, a process for which sulphur bears responsibility. Notably, sulphur also assumes a substantial function in regulating the plant's antioxidant capacity (Suliman et al., 2022; Sun et al., 2022; Sun et al., 2023; Sun et al., 2022; Taira & Shiono, 2022; Takeuchi et al., 2022; Talaat, 2023; Tan et al., 2022; Tariq et al., 2023; Tariq et al., 2022). This is achieved through the reduction of oxidative stress and the consequent decrease in the reliance on phenolic compounds for scavenging reactive oxygen species (ROS). Consequently, sulphur exhibits a wide range of effects, potentially resulting in decreased accumulation of phenolic compounds under plant stress conditions. Applying salicylic acid as a foliar spray elicits alterations in the overall phenol content of mustard. The modifications above are instigated due to the role of salicylic acid in coordinating stress responses and triggering defence mechanisms. The renowned capacity of salicylic acid (SA) to induce systemic acquired resistance (SAR) is closely associated with its remarkable propensity to enhance the synthesis of phenolic compounds. The augmentation process is paramount in enhancing the plant's capacity to endure biotic stressors. The application of salicylic acid (SA) has been observed to influence the intricate gene regulation system in plants substantially, stimulating particular genes associated with phenol biosynthesis pathways. The collaborative endeavour leads to a harmonious augmentation in synthesising phenolic compounds within mustard plants, consequently enhancing the plants' capacity to defend against biotic adversaries. Moreover, the consequences of SA broadly influence the distribution of resources within the plant.

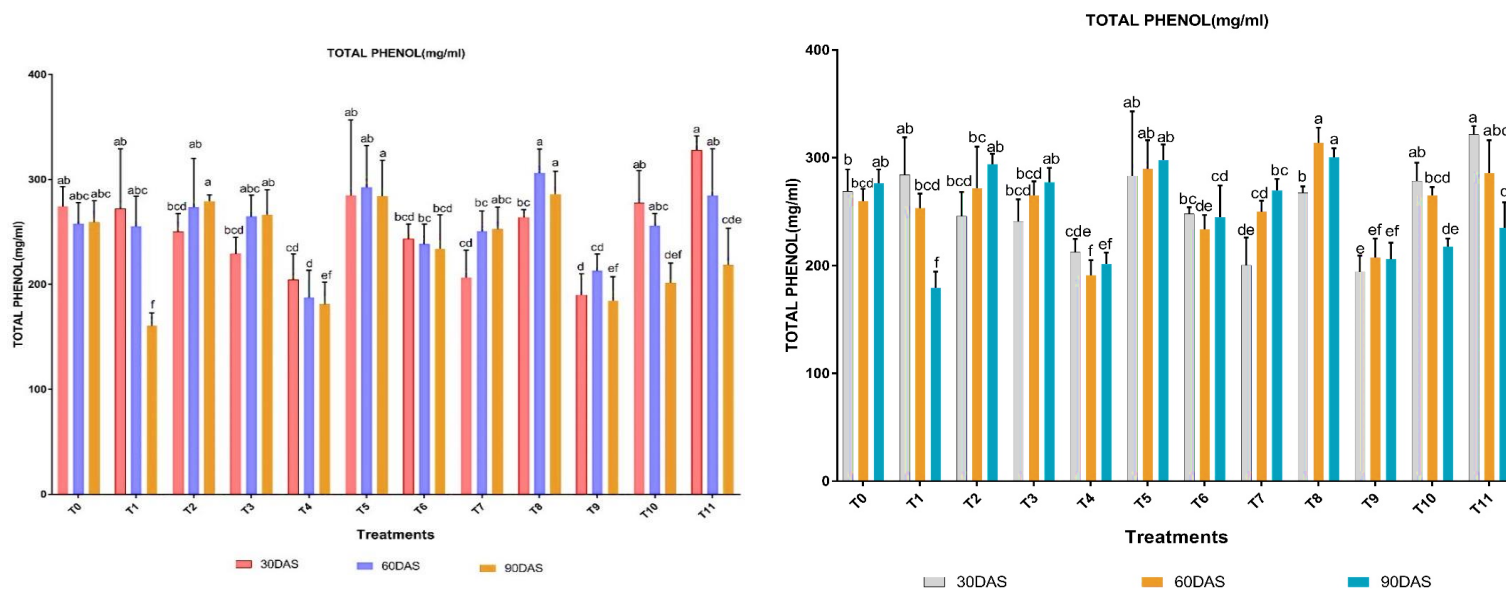
When exposed to systemic acquired resistance (SA), plants activate stress responses by redistributing resources to produce phenolic compounds, including carbon and energy. As the plant endeavours to enhance its resilience against the prevailing challenges, it reallocates its resources, leading to heightened phenol concentrations. Assessing the total phenolic content in mustard is paramount in understanding the plant's antioxidant capacity, stress responses, and defence mechanisms. The enduring influence on the overall phenol content can be observed using foliar sprays containing sulphur and salicylic acid. The process above encompasses the regulation of phenol synthesis, the activation of antioxidant defence mechanisms, and the allocation of resources, leading to the establishment of a highly coordinated and intricate system. These strategies can enhance the resistance of mustard plants to stress and increase crop productivity, particularly in conditions that are known to induce stress (Singh et al., 2022; Singh & Roychoudhury, 2023; Singh & Nandi, 2022; Singhal et al., 2023; Sivanesan et al., 2022; Song et al., 2023; Sousa et al., 2022; Spinoso-Castillo & Bello-Bello, 2022).

**Table 4.26. Impact of Different Treatments on Total Phenol of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	274.44 <sup>ab</sup> ±18.98	268.88 <sup>b</sup> ±20.39	257.77 <sup>abc</sup> ±20.36	260.00 <sup>bcd</sup> ±11.27	259.25 <sup>abc</sup> ±20.67	276.29 <sup>ab</sup> ±12.87
<b>T1 (Thiourea-1000 ppm)</b>	272.22 <sup>ab</sup> ±56.90	284.44 <sup>ab</sup> ±34.55	255.55 <sup>abc</sup> ±28.34	253.33 <sup>bcd</sup> ±13.33	160.37 <sup>f</sup> ±12.48	179.25 <sup>f</sup> ±15.16
<b>T2 (Salicylic acid-300 ppm)</b>	250.37 <sup>bcd</sup> ±16.97	245.92 <sup>bcd</sup> ±22.34	273.70 <sup>ab</sup> ±46.24	271.85 <sup>bc</sup> ±38.49	279.25 <sup>a</sup> ±6.11	294.07 <sup>ab</sup> ±9.70
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	229.25 <sup>bcd</sup> ±15.72	241.11 <sup>bcd</sup> ±20.39	264.81 <sup>abc</sup> ±20.19	265.18 <sup>bcd</sup> ±12.97	266.29 <sup>ab</sup> ±23.94	277.40 <sup>ab</sup> ±13.25
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	204.44 <sup>cd</sup> ±24.57	212.59 <sup>cde</sup> ±12.18	187.40 <sup>d</sup> ±26.06	191.11 <sup>f</sup> ±13.87	181.11 <sup>ef</sup> ±21.19	201.48 <sup>ef</sup> ±10.67
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	284.81 <sup>ab</sup> ±72.01	283.33 <sup>ab</sup> ±59.75	292.59 <sup>ab</sup> ±39.77	289.62 <sup>ab</sup> ±26.67	284.07 <sup>a</sup> ±34.01	297.77 <sup>a</sup> ±14.69
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	243.33 <sup>bcd</sup> ±14.18	248.14 <sup>bc</sup> ±6.11	238.51 <sup>bc</sup> ±18.99	233.70 <sup>de</sup> ±13.34	234.07 <sup>bcd</sup> ±32.32	244.81 <sup>cd</sup> ±29.52
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	206.66 <sup>cd</sup> ±25.84	200.37 <sup>de</sup> ±25.68	250.74 <sup>bc</sup> ±19.31	250.00 <sup>cd</sup> ±10.18	252.59 <sup>abc</sup> ±21.26	269.62 <sup>bc</sup> ±10.79
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	264.07 <sup>bc</sup> ±7.39	267.40 <sup>b</sup> ±6.11	306.29 <sup>a</sup> ±22.69	313.70 <sup>a</sup> ±14.33	285.92 <sup>a</sup> ±21.72	300.37 <sup>a</sup> ±8.48
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	190.00 <sup>d</sup> ±20.00	194.44 <sup>e</sup> ±14.94	212.96 <sup>cd</sup> ±15.96	207.40 <sup>ef</sup> ±17.71	184.44 <sup>ef</sup> ±22.95	205.92 <sup>ef</sup> ±15.32
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	277.77 <sup>ab</sup> ±30.69	278.51 <sup>ab</sup> ±16.86	255.92 <sup>abc</sup> ±11.56	265.18 <sup>bcd</sup> ±7.80	201.48 <sup>def</sup> ±18.73	217.77 <sup>de</sup> ±7.28
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	327.77 <sup>a</sup> ±13.92	321.85 <sup>a</sup> ±7.56	284.81 <sup>ab</sup> ±44.53	285.92 <sup>abc</sup> ±30.35	218.51 <sup>cde</sup> ±35.17	235.18 <sup>d</sup> ±23.42
<b>CD</b>	52.844	42.926	49.936	34.997	42.061	25.265
<b>CV</b>	12.300	9.92	11.412	7.983	10.549	5.93

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.26. Total Phenol of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Flavanols:** The effect of Sulphur and Salicylic acid and their combination on flavanols was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.27, Figure 4.27). 2021-2022, there was a significant difference in flavanols compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the flavanols was observed at 30, 60, and 90 DAS. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T3, followed by T5, T11, T2, T8, T9, T10, T4, and T1, and the percentage values were 30.37%, 26.94%, 25.79%, 24.80%, 20.34%, 19.43%, 14.54%, 12.15%, 0.35% respectively. But in T7 and T6, the percentage decreased compared to T0; the percentage values were -10.16% and -20.00%. At 60 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T11, T5, T2, T9, T8, T10, T4, T1 and the percentage values were 26.91%, 25.67%, 22.87%, 22.28%, 17.25%, 14.96%, 8.08%, 4.65%, 0.96% respectively. But in T6 and T7, the percentage decreased compared to T0, and the percentage values were -7.72% and -20.87%. At 90 DAS, the percentage increase as compared to T0 was found highest in T2 followed by T5, T3, T9, T1, T11, T10 and the percentage values were 23.84%, 22.99%, 11.54%, 11.54%, 6.25%, 1.14%, 0.86% respectively. But in T8, T4, T7, and T6 the percentage decrease as compared to T0 and the percentage values were -5.83%, -8.15%, -8.83%, -27.31% respectively. In 2022-2023, there was a significant difference in flavanols compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T3, followed by T5, T11, T2, T9, T8, T4, and T9, and the percentage values were 26.98%, 23.97%, 23.77%, 16.67%, 16.67%, 13.74%, 11.94%, 11.41% respectively. But in T1, T7, and T6 the percentage decrease as compared to T0 and the percentage values were -5.73%, -8.45%, -20.91% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T5 followed by T11, T3, T9, T2, T8, T10, T4 and the percentage values were 30.47%, 27.42%, 23.17%, 13.93%, 13.22%, 9.48%, 8.16%, 6.25% respectively. But in T1, T6, and T7 the percentage decrease as compared to T0 and the percentage values were -3.27%, -7.51%, and -22.10%. At 90 DAS, the percentage increase as compared to T0 was found highest in T5 followed by T2, T9, T3, T1, T10, T11 and the percentage

values were 26.74%, 24.10%, 16.38%, 14.47%, 9.41%, 3.98%, 2.88% respectively. But in T7, T4, T8, and T6 the percentage decrease as compared to T0 and the percentage values were -4.65%, -4.98%, -9.06%, and -21.66%. The measurement of flavanols in mustard (*Brassica juncea* L.) is a crucial biochemical parameter with substantial implications for plant research and agriculture. In addition, comprehending the potential impacts of sulphur (S) and salicylic acid (SA) foliar spray treatments on flavanol concentrations in mustard presents an avenue for enhancing agricultural methodologies and optimising crop vitality and productivity. Flavanols are a class of polyphenolic compounds recognised for their notable antioxidant properties. Flavanols can be classified as a subgroup within the broader category of flavonoids (Quesada, 2022; Rai & Kaushik, 2023; Raja Gopalan et al., 2022; Ramakrishnan & Zhou, 2022; Rani et al., 2023; Rizvi et al., 2022; Roussos, 2023; Ruidas et al., 2022; Rybczyński et al., 2022). Antioxidants are crucial in safeguarding plant cells against oxidative damage inflicted by reactive oxygen species (ROS), which can arise during stress-induced disruption and routine metabolic activities. Flavanols, due to their exceptional capacity to eliminate free radicals, assume the responsibility of safeguarding the cellular integrity of the plant. Plants often enhance their synthesis of flavonols as a response to diverse environmental stressors, encompassing biotic factors such as pathogens and abiotic factors like drought and intense ultraviolet (UV) radiation. The aforementioned defensive response demonstrates the plant's proactive approach to mitigating the adverse consequences of the stress it is currently undergoing. Detecting heightened flavanol concentrations indicates the plant is actively fortifying its defences against the prevailing environmental pressures. Flavanols actively engage in the plant's defence mechanisms while also serving as antioxidants that provide passive protection to the plant. These compounds often serve as chemical deterrents against herbivores and pathogens, enhancing the plant's defence mechanism against biotic stressors. Therefore, measuring flavanol concentrations in mustard plants is a valuable indicator, providing significant insights into the plant's readiness to confront potential biotic stress factors. The efficient production of flavanols necessitates the meticulous allocation and utilisation of various resources, with carbon being of utmost significance. Consequently, quantifying the flavanol concentration offers insight into the plant's resource allocation strategy. The observation offers valuable insights into the plant's

allocation of resources, particularly carbon, to bolster its defence mechanisms in the face of stress. Sulphur is crucial in synthesising various secondary metabolites, such as flavanols. Sulphur is an essential macronutrient. The presence of adequate sulphur enhances the capacity of mustard plants to synthesise flavanols, thereby reinforcing the plants' antioxidant defence mechanism. The presence of sulphur also affects the synthesis of sulfur-containing amino acids, such as cysteine, which play a crucial role in producing flavanols. Furthermore, the participation of sulphur in the plant's comprehensive antioxidant capacity contributes to alleviating oxidative stress, potentially leading to reduced flavonol accumulation during periods of stress. Applying salicylic acid as a foliar spray on mustard plants elicits a coordinated response in the plant's stress pathways and triggers the activation of its defence mechanisms. Consequently, this leads to alterations in the flavanol composition of the mustard plant. Systemic acquired resistance (SAR), commonly called SA, is widely recognised for its involvement in the process. Systemic acquired resistance (SAR) is a defensive mechanism often accompanied by an augmentation in the production of flavonols. The genes involved in the biosynthesis pathway of flavonols exhibit responsiveness to stimuli mediated by salicylic acid (SA), which functions as a regulatory agent. The orchestral composition induces a heightened production of flavonols in mustard plants, thereby augmenting their resilience against biotic stressors to which they are subjected. Moreover, the impacts of SA can be observed beyond flavonol biosynthesis. This phenomenon influences the allocation of resources within the plant. When subjected to SA-induced stress, the plant undergoes a reconfiguration of its resource allocation strategy, whereby it reallocates its carbon and energy reserves towards the biosynthesis of flavonols (Ortiz-García et al., 2022, 2023; Ozturk & Unal, 2023; Paalli et al., 2022; Pal et al., 2023; Parada et al., 2022; Parrey et al., 2023; Patel et al., 2022; Peng et al., 2022; Pérez-Llorca et al., 2023; Phokas et al., 2023; Phour & Sindhu, 2022; Poór et al., 2022). When the plant encounters different stressors, it activates its defence mechanisms, redistributing resources and increasing flavanol levels. The quantification of flavanol levels in mustard represents a valuable approach for comprehending the inherent antioxidant capacity, stress responses, and defence mechanisms embedded within the plant. Research has demonstrated that applying foliar sprays containing elevated levels of sulphur and salicylic acid can significantly influence the



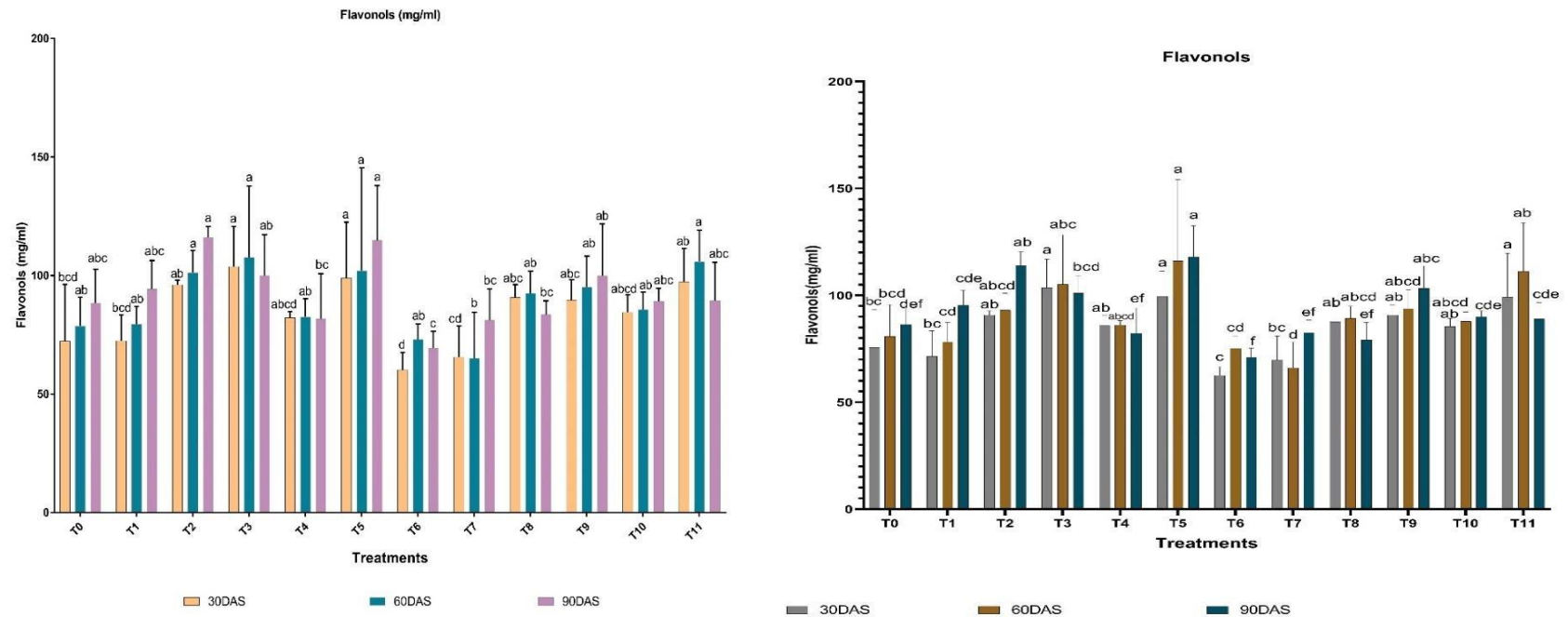
concentration of flavanols. This phenomenon entails orchestrating a harmonious interplay involving the synthesis of flavonols, the activation of antioxidant defence mechanisms, and the allocation of resources. The strategies above possess untapped potential in enhancing the resilience of mustard plants against adverse circumstances and augmenting agricultural productivity, particularly in scenarios where the plants are exposed to demanding environmental conditions. As we advance on this path, it is imperative to prioritise further research endeavours to improve the methodologies and concentrations utilised in applications to attain the intended results (Mugwanya et al., 2023; Mukarram et al., 2022; Mukhtar et al., 2023; Myers Jr. et al., 2023; Nadeem, 2022; Nam et al., 2023; Napieraj et al., 2023; Nasir & Toth, 2022; Negi & Kumar, 2023; Nimsi et al., 2023; Niu & Fu, 2022).

**Table 4.27. Impact of Different Treatments on Flavonols of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	72.28 <sup>ab</sup> ±23.94	75.61 <sup>bc</sup> ±17.59	78.69 <sup>a</sup> ±12.08	80.74 <sup>bcd</sup> ±14.84	88.43 <sup>ab</sup> ±14.27	86.38 <sup>def</sup> ±7.57
<b>T1 (Thiourea-1000 ppm)</b>	72.53 <sup>ab</sup> ±10.85	71.51 <sup>bc</sup> ±11.92	79.46 <sup>a</sup> ±7.57	78.17 <sup>cd</sup> ±9.17	94.33 <sup>ab</sup> ±12.02	95.35 <sup>cde</sup> ±6.93
<b>T2 (Salicylic acid-300 ppm)</b>	96.12 <sup>ab</sup> ±1.93	90.74 <sup>ab</sup> ±1.93	101.25 <sup>a</sup> ±9.40	93.05 <sup>abcd</sup> ±7.85	116.12 <sup>a</sup> ±4.63	113.82 <sup>ab</sup> ±6.54
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	103.82 <sup>a</sup> ±16.96	103.56 <sup>a</sup> ±13.37	107.66 <sup>a</sup> ±30.09	105.10 <sup>abc</sup> ±23.14	99.97 <sup>ab</sup> ±17.30	101.00 <sup>bcd</sup> ±8.32
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	82.28 <sup>ab</sup> ±2.47	85.87 <sup>ab</sup> ±4.63	82.53 <sup>a</sup> ±7.69	86.12 <sup>abcd</sup> ±1.93	81.76 <sup>ab</sup> ±19.04	82.28 <sup>ef</sup> ±11.75
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	98.94 <sup>ab</sup> ±23.58	99.46 <sup>a</sup> ±11.94	102.02 <sup>a</sup> ±43.41	116.12 <sup>a</sup> ±37.96	114.84 <sup>a</sup> ±23.11	117.92 <sup>a</sup> ±14.61
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	60.23 <sup>b</sup> ±7.33	62.53 <sup>c</sup> ±4.07	73.05 <sup>a</sup> ±6.54	75.10 <sup>cd</sup> ±5.77	69.46 <sup>b</sup> ±7.05	71.00 <sup>f</sup> ±4.28
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	65.61 <sup>ab</sup> ±13.12	69.71 <sup>bc</sup> ±11.26	65.10 <sup>a</sup> ±19.48	66.12 <sup>d</sup> ±11.97	81.25 <sup>ab</sup> ±13.15	82.53 <sup>ef</sup> ±5.80
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	90.74 <sup>ab</sup> ±5.45	87.66 <sup>ab</sup> ±6.97	92.53 <sup>a</sup> ±9.32	89.20 <sup>abcd</sup> ±5.67	83.56 <sup>ab</sup> ±5.77	79.20 <sup>ef</sup> ±8.07
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	89.71 <sup>ab</sup> ±8.50	90.74 <sup>ab</sup> ±4.70	95.10 <sup>a</sup> ±13.15	93.82 <sup>abcd</sup> ±8.74	99.97 <sup>ab</sup> ±21.81	103.30 <sup>abc</sup> ±10.40
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	84.58 <sup>ab</sup> ±7.27	85.35 <sup>ab</sup> ±3.87	85.61 <sup>a</sup> ±7.41	87.92 <sup>abcd</sup> ±4.28	89.20 <sup>ab</sup> ±5.40	89.97 <sup>cde</sup> ±2.70
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	97.41 <sup>ab</sup> ±14.02	99.20 <sup>a</sup> ±20.50	105.87 <sup>a</sup> ±13.24	111.25 <sup>ab</sup> ±22.69	89.46 <sup>ab</sup> ±16.15	88.94 <sup>cde</sup> ±7.70
<b>CD</b>	21.151	18.315	N/A	25.89	23.855	13.97
<b>CV</b>	14.683	12.618	18.406	16.837	15.154	8.848

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.27. Flavonols of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Flavonoids:** The effect of Sulphur and Salicylic acid and their combination on flavonoids was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.28, Figure 4.28). In 2021-2022, there was a significant difference in flavonoids compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the flavonoids was observed at 30, 60, and 90 DAS. Therefore, at 30 DAS, the percentage increase compared to T0 was the highest in T10, followed by T7 and T6, and the percentage values were 10.38%, 9.21%, and 1.42%. But in T1, T8, T3, T4, T9, T11, T2, T5 the percentage decrease as compared to T0 and the percentage values were -1.47%, -1.47%, -11.29%, -13.11%, -16.94%, -18.96%, -21.05%, -21.05% respectively. At 60 DAS, the percentage increase as compared to T0 was found to be highest in T5, followed by T7, T1, T6, and T4, and the percentage values were 12.94%, 11.90%, 5.12%, 2.63%, and 1.33% respectively. But in T8, T10, T3, T9, T11, T2 the percentage decrease as compared to T0 and the percentage values were -4.22%, -4.22%, -8.82%, -17.46%, -19.35%, -21.31% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T6 followed by T3, T5, T8, T10, T7, T4, T11 and the percentage values were 25.30%, 17.33%, 11.42%, 8.82%, 8.82%, 6.06%, 3.12%, and 3.12% respectively. But in T1, T2 and T9 the percentage decrease as compared to T0 and the percentage values were -1.63%, -3.33%, -12.72%. In 2022-2023, there was a significant difference in flavonoids compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase compared to T0 was found to be highest in T7 and T10, followed by T8 and T6, and the percentage values were 15.18%, 15.18%, 5.63%, and 2.89%, respectively. In T3, T4, T9, T2, T11, and T5 the percentage decrease as compared to T0 and the percentage values were -4.68%, -8.06%, -8.06%, -13.55%, -15.51%, and -19.64% respectively. But, in T1, the impact of treatment is not effective, and the percentage value was the same as in T0. At 60 DAS, the percentage increase as compared to T0 was found highest in T5 followed by T7, T6, T1, T4, T3, T8, T10 and the percentage values were 25.84%, 24.13%, 20.48%, 17.50%, 16.45%, 10.81%, 8.33%, 2.94% respectively. But in T9, T11 and T2, the percentage decreased as compared to T0, and the percentage values were -3.12%, -3.12%, and -8.19%. At 90

DAS, the percentage increase as compared to T0 was found highest in T6 followed by T3, T8, T11, T5, T10 and the percentage values were 18.98%, 12.32%, 11.11%, 7.24%, 5.88%, 4.47% respectively. In T9, T4, T1, and T7 the percentage decrease as compared to T0 and the percentage values were -6.66%, -8.47%, -10.34%, and -13.87% respectively. However, the T2 impact of treatment was not effective, and the percentage value was the same as T0. The concentrations of flavonoids in Mustard (*Brassica juncea* L.) are a biochemical parameter of great significance, with wide-ranging applications in plant science and agriculture. Furthermore, comprehending the potential impacts of sulphur (S) and salicylic acid (SA) foliar spray treatments on flavonoid concentrations in mustard provides insights into enhancing agricultural practices and improving crops' overall health and productivity. Flavonoids encompass various secondary metabolites renowned for their multifaceted functions within the plant kingdom. These molecules fulfil various functions within the human body, including serving as antioxidants, UV protectors, signalling molecules, and defences against diverse stressors. Assessing a plant's flavonoid composition is important in mustard research and agriculture because it provides crucial insights into its ability to cope with environmental stressors and its integral role in mustard cultivation. Flavonoids exhibit potent antioxidant properties, thereby aiding in safeguarding plant cells against oxidative damage induced by reactive oxygen species (ROS). Reactive oxygen species (ROS) can be generated in response to stress conditions and during various metabolic pathways and have been established to induce detrimental effects on cellular constituents. By quantifying a plant's flavonoid content, researchers can acquire significant knowledge about the plant's capacity to counteract reactive oxygen species (ROS) and maintain the structural stability of its cellular components. Flavonoids serve as a natural defence mechanism in plants, safeguarding them against potential harm caused by solar radiation. Sensitive plant tissues are shielded from the detrimental effects of UV-A, UV-B, and UV-C rays emitted by the sun through absorption, wherein these specific wavelengths of radiation are absorbed. Mustard, a plant commonly exposed to direct sunlight, possesses a significant abundance of flavonoids, crucial in mitigating the adverse effects of UV-induced stress and DNA damage. Flavonoids can act as signalling molecules that facilitate the modulation of plant responses to environmental stimuli. These factors influence processes, including root development,

interactions with symbiotic organisms, and defence mechanisms (Todorova et al., 2022; Topcu et al., 2022; Torres & Figueroa, 2023; Tyagi et al., 2022; Ullah et al., 2023). The measurement of flavonoid content enables researchers to enhance their comprehension of how mustard plants perceive and react to their environment. Numerous studies have provided empirical support for the involvement of flavonoids in the plant kingdom's defence mechanisms against biotic stressors, including herbivores and pathogens. They can deter herbivorous organisms and impede the proliferation of pathogenic microorganisms. The quantification of flavonoids can yield insights into the plant's capacity to resist and combat fungal, bacterial, and other pathogenic threats. The inclusion of sulphur, a vital macronutrient, significantly facilitates the synthesis of flavonoids. Mustard plants exhibit an enhanced ability to synthesise these compounds, thereby bolstering their antioxidant defence mechanism in the presence of sufficient sulphur resources. The involvement of sulphur in synthesising sulfur-containing amino acids, such as cysteine, holds significant importance. The presence of these amino acids is crucial for the biosynthesis of flavonoids. Furthermore, the presence of sulphur in plants has been found to impact their overall antioxidant capacity (Ullah et al., 2022; Verma et al., 2023; Waadt et al., 2022; Wan & Xin, 2022; Wang et al., 2022; Wang et al., 2023; Wang et al., 2023; Wang et al., 2022; Wang & Komatsu, 2022). This, in turn, leads to a decrease in oxidative stress and a reduced need for flavonoids to counteract the harmful effects of reactive oxygen species (ROS). In challenging environmental circumstances, the presence of sulphur ensures the accessibility of essential resources required for synthesising flavonoids, thereby bolstering the plant's defence mechanisms. The flavonoid content of mustard is substantially influenced by applying salicylic acid on the plant's leaves through foliar spraying. Systemic acquired resistance (SAR), commonly referred to as SAR, is a defence mechanism often correlated with the heightened production of flavonoids. Salicylic acid (SA) is a significant participant in the systemic acquired resistance (SAR) field. In mustard plants, salicylic acid (SA) plays a pivotal role in activating genes associated with the biosynthesis pathways of flavonoids, thereby increasing the levels of these compounds. The augmented flavonoid content of the plant leads to the reinforcement of its defences against biotic stressors. Furthermore, the influence of SA extends to allocating resources within the plant. When subjected to salicylic acid (SA), plants undergo a stress response characterised by

redistributing resources, such as carbon and energy, towards synthesising flavonoids. Applying sulphur and salicylic acid onto the foliage can influence the flavonoid content through alterations in synthesis, resource allocation, and stress responses. Implementing these strategies holds considerable promise in augmenting the resilience of mustard crops to environmental stress and bolstering their overall productivity, particularly in challenging environmental contexts. Engaging in continuous research endeavours to refine application techniques and concentrations is imperative to attain the desired outcomes (Taira & Shiono, 2022; Takeuchi et al., 2022; Talaat, 2023; Tan et al., 2022; Tariq et al., 2023; Tariq et al., 2022).

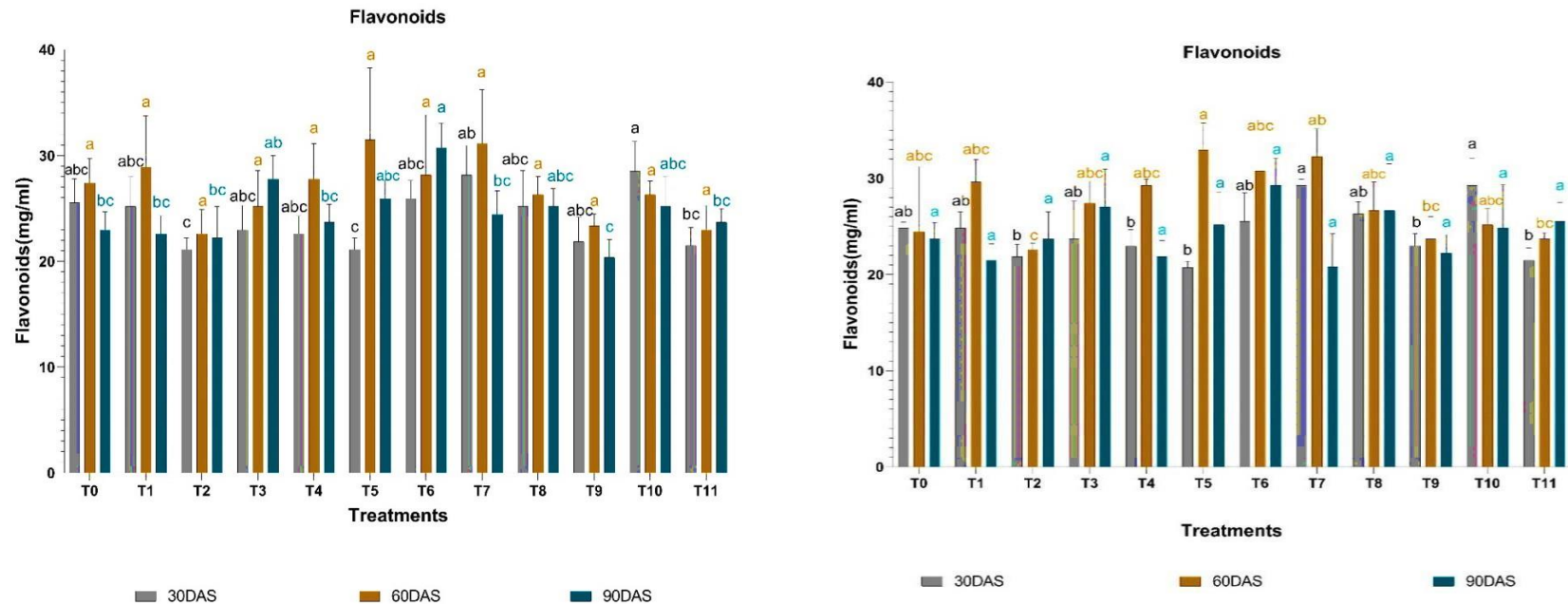
**Table 4.28. Impact of Different Treatments on Flavonoids of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	25.55 <sup>abc</sup> ±2.22	24.81 <sup>ab</sup> ±0.64	27.40 <sup>a</sup> ±2.31	24.44 <sup>abc</sup> ±6.75	22.96 <sup>bc</sup> ±1.69	23.70 <sup>a</sup> ±1.69
<b>T1 (Thiourea-1000 ppm)</b>	25.18 <sup>abc</sup> ±2.79	24.81 <sup>ab</sup> ±1.69	28.88 <sup>a</sup> ±4.84	29.62 <sup>abc</sup> ±2.31	22.59 <sup>bc</sup> ±1.69	21.48 <sup>a</sup> ±1.69
<b>T2 (Salicylic acid-300 ppm)</b>	21.11 <sup>c</sup> ±1.11	21.85 <sup>b</sup> ±1.28	22.59 <sup>a</sup> ±2.31	22.59 <sup>c</sup> ±0.64	22.22 <sup>bc</sup> ±2.93	23.70 <sup>a</sup> ±2.79
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	22.96 <sup>abc</sup> ±2.31	23.70 <sup>ab</sup> ±3.90	25.18 <sup>a</sup> ±3.39	27.40 <sup>abc</sup> ±2.31	27.77 <sup>ab</sup> ±2.22	27.03 <sup>a</sup> ±3.90
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	22.59 <sup>abc</sup> ±1.69	22.96 <sup>b</sup> ±1.69	27.77 <sup>a</sup> ±3.33	29.25 <sup>abc</sup> ±0.64	23.70 <sup>bc</sup> ±1.69	21.85 <sup>a</sup> ±1.69
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	21.11 <sup>c</sup> ±1.11	20.74 <sup>b</sup> ±0.64	31.48 <sup>a</sup> ±6.78	32.96 <sup>a</sup> ±2.79	25.92 <sup>abc</sup> ±1.69	25.18 <sup>a</sup> ±3.39
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	25.92 <sup>abc</sup> ±1.69	25.55 <sup>ab</sup> ±2.93	28.14 <sup>a</sup> ±5.70	30.74 <sup>abc</sup> ±3.57	30.74 <sup>a</sup> ±2.31	29.25 <sup>a</sup> ±2.79
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	28.14 <sup>ab</sup> ±2.79	29.25 <sup>a</sup> ±0.64	31.11 <sup>a</sup> ±5.09	32.22 <sup>ab</sup> ±2.93	24.44 <sup>bc</sup> ±2.22	20.81 <sup>a</sup> ±3.44
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	25.18 <sup>abc</sup> ±3.39	26.29 <sup>ab</sup> ±1.28	26.29 <sup>a</sup> ±1.69	26.66 <sup>abc</sup> ±2.93	25.18 <sup>abc</sup> ±1.69	26.66 <sup>a</sup> ±4.84
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	21.85 <sup>abc</sup> ±2.31	22.96 <sup>b</sup> ±1.28	23.33 <sup>a</sup> ±1.11	23.70 <sup>bc</sup> ±2.31	20.37 <sup>c</sup> ±1.69	22.22 <sup>a</sup> ±1.92
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	28.51 <sup>a</sup> ±2.79	29.25 <sup>a</sup> ±2.79	26.29 <sup>a</sup> ±1.28	25.18 <sup>abc</sup> ±1.69	25.18 <sup>abc</sup> ±2.79	24.81 <sup>a</sup> ±4.49
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	21.48 <sup>bc</sup> ±1.69	21.48 <sup>b</sup> ±1.28	22.96 <sup>a</sup> ±2.31	23.70 <sup>bc</sup> ±0.64	23.70 <sup>bc</sup> ±1.28	25.55 <sup>a</sup> ±1.92
<b>CD</b>	3.85	2.796	N/A	4.987	3.307	4.865
<b>CV</b>	9.36	6.702	14.733	10.69	7.899	11.719

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.



**Figure 4.28. Flavonoids of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Total Soluble Protein:** The effect of Sulphur and Salicylic acid and their combination on total soluble protein was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.29, Figure 4.29). In 2021-2022, there was a significant difference in total soluble protein compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the total soluble protein was observed at 30, 60, and 90 DAS. Therefore, at 30 DAS, the percentage increase compared to T0 was the highest in T10, followed by T11, T8, T7, T5, T4, T1, T9, T3, T2 and the percentage values were 54.99%, 51.61%, 49.46%, 43.60%, 41.89%, 34.25%, 28.48%, 26.25%, 24.96%, 14.30% respectively. But in T6 percentage decrease as compared to T0; and the percentage value was -1.24%. At 60DAS, the percentage increase compared to T0 was the highest in T10, followed by T7, T8, T4, T11, T9, T6, T3, T5, T1, T2 and the percentage values were 54.59%, 52.78%, 49.43%, 46.04%, 45.99%, 40.97%, 39.05%, 38.50%, 36.76%, 27.77%, and 17.02% respectively. At 90DAS, the percentage increase compared to T0 was the highest in T11, followed by T7, T10, T4, T6, T1, T8, T9, T2, T3, T5 and the percentage values were 35.85%, 31.47%, 29.48%, 28.06%, 26.90%, 24.98%, 22.83%, 21.46%, 19.85%, 18.06%, and 17.11% respectively. In 2022-2023, there was a significant difference in total soluble protein compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T10, followed by T8, T11, T7, T5, T4, T1, T3, T9, T2, T6 and the percentage values were 55.20%, 49.78%, 46.48%, 43.87%, 35.99%, 33.92%, 29.18%, 27.90%, 23.12%, 11.62%, and 7.15% respectively. At 60 DAS, the percentage increase as compared to T0 was found to be highest in T10, followed by T8, T4, T7, T11, T9, T3, T5, T6, T1, T2 and the percentage values were 54.40%, 48.64%, 46.18%, 44.51%, 41.74%, 39.56%, 38.50%, 36.00%, 33.49%, 27.21%, and 6.85%. At 90DAS, the percentage increase compared to T0 was the highest in T11, followed by T7, T10, T4, T6, T8, T1, T2, T9, T5, T3 and the percentage values were 40.07%, 37.93%, 35.99%, 33.09%, 27.96%, 27.06%, 26.63%, 25.77%, 25.45%, 22.30%, and 20.35% respectively. Assessing the total soluble protein concentration in mustard plants (*Brassica* spp.) is paramount in understanding the plant's response to various treatments,

including the impact of compounds such as thiourea and salicylic acid. The present study examines the importance of total soluble protein in mustard plants and its correlations with different combinations (Sousa et al., 2022; Spinoso-Castillo & Bello-Bello, 2022; Suliman et al., 2022; Sun et al., 2022; Sun et al., 2023; Sun et al., 2022; Taira & Shiono, 2022; Takeuchi et al., 2022; Talaat, 2023; Tan et al., 2022; Tariq et al., 2023). The Plant Health Indicator is a metric used to assess plants' overall well-being and condition. Quantifying total soluble protein content is crucial to mustard plants' overall health and physiological state. Plants in good health typically demonstrate increased protein levels, a characteristic attributed to their robust growth and maturation processes. Photosynthesis is highly dependent on the existence and proper functioning of proteins, which play a crucial role in the organisation and functioning of photosynthetic pigments and enzymes. Maintaining appropriate protein levels is essential for promoting effective photosynthesis, consequently directly impacting the development and productivity of mustard plants. Proteins play a vital role in facilitating the process of nutrient uptake and transport in plants, thereby contributing significantly to this essential physiological function. Adequate protein levels are crucial for reducing the absorption of essential nutrients from the soil and their subsequent distribution to various plant tissues. The stress response is distinguished by changes in the content of total soluble proteins, which can impact a range of environmental stressors such as drought, salinity, and pathogen attacks. Evaluating protein levels facilitates assessing a plant's ability to withstand stress and adapt to changing environmental conditions (Shekhawat et al., 2023; Shi et al., 2023; Shi et al., 2022; Si et al., 2023; Singh et al., 2022; Singh & Roychoudhury, 2023; Singh & Nandi, 2022; Singhal et al., 2023; Sivanesan et al., 2022; Song et al., 2023). The treatment effects on mustard plants can be efficiently evaluated by employing the total soluble protein content as a biomarker. This research provides significant findings regarding the impact of thiourea and salicylic acid treatments on the physiological mechanisms of plants. The potential of thiourea to enhance plant stress tolerance has been acknowledged. The experimental administration of [specific treatment] to mustard plants has significantly augmented the overall concentration of soluble proteins. The discovery above suggests that applying thiourea can increase the plant's ability to adapt to environmental stressors and maintain protein homeostasis. The potential for enhancing crop yield is contingent upon the

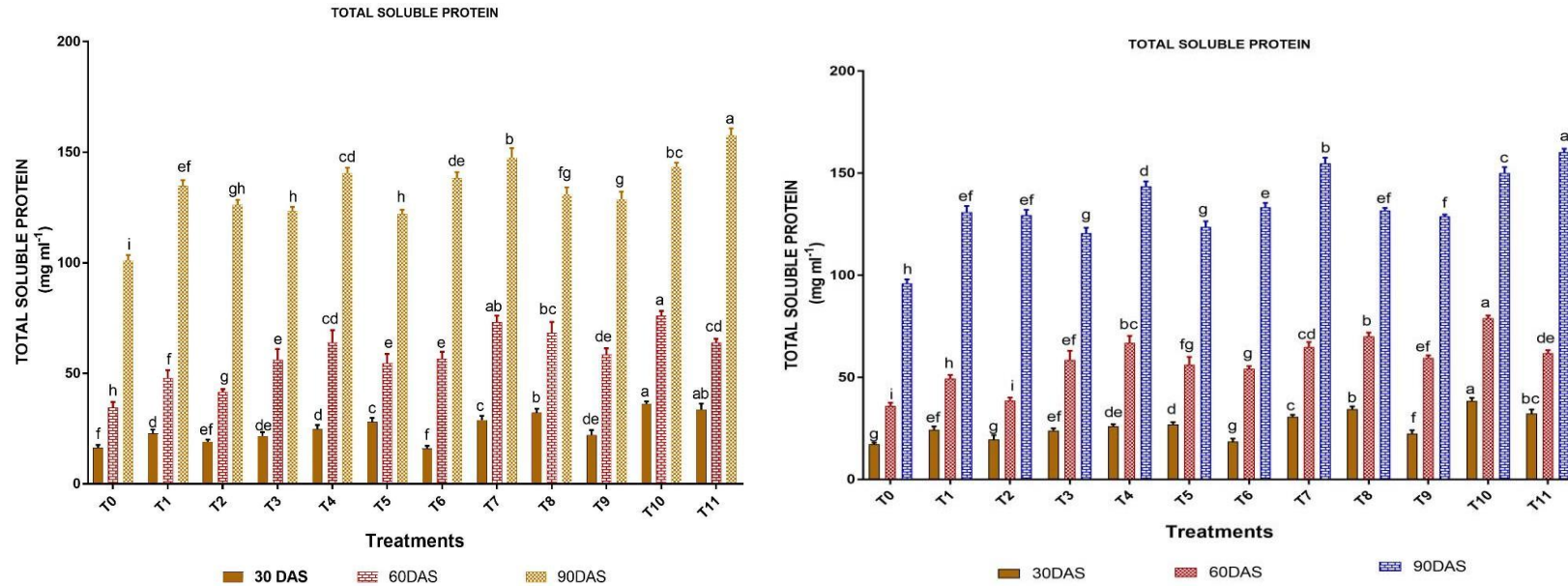
interplay between thiourea and the overall soluble protein content in mustard plants, thus carrying implications for yield improvement. Increased protein levels have the potential to enhance growth, seed yield, and oil quality, therefore assuming a pivotal function in the cultivation of mustard. The initiation of defence mechanisms: Salicylic acid carries a crucial role in the defence mechanisms exhibited by plants. When mustard plants are exposed to this particular treatment, it has the potential to induce the synthesis of pathogenesis-related (PR) proteins, which are a distinct category of soluble proteins (Salwan et al., 2023; Samad et al., 2023; Sampedro-Guerrero et al., 2022; Samtani et al., 2022; Shah et al., 2022, 2023; Shang et al., 2022; Sharma & Verma, 2023; Sheikhalipour et al., 2023). This interaction functions to enhance the plant's ability to withstand pathogens. The Impact on Plant Health: The observed correlation between salicylic acid and total soluble protein levels indicates the plant's physiological response to disease and stress. The presence of increased levels of pathogenesis-related (PR) proteins, a constituent of the total soluble protein fraction, suggests the activation of a defensive response in mustard plants. Measuring the total soluble protein content in mustard plants is a crucial factor that offers valuable information regarding the plant's overall well-being and development (Rai & Kaushik, 2023; Raja Gopalan et al., 2022; Ramakrishnan & Zhou, 2022; Rani et al., 2023; Rizvi et al., 2022; Roussos, 2023; Ruidas et al., 2022; Rybczyński et al., 2022; Sachan & Krishna, 2022; Salih, Wu, et al., 2022; Salih, Zhou, et al., 2022). Furthermore, it is a significant biomarker for assessing the impacts of exogenous interventions on the botanical organism. Examining the relationship between total soluble protein, thiourea, and salicylic acid provides substantial insights into these compounds' influence on mustard plants' physiological processes. Understanding these interactions is crucial for optimising mustard cultivation, enhancing stress tolerance, and improving crop yield and quality (Paalli et al., 2022; Pal et al., 2023; Parada et al., 2022; Parrey et al., 2023; Patel et al., 2022; Peng et al., 2022; Pérez-Llorca et al., 2023; Phokas et al., 2023; Phour & Sindhu, 2022; Poór et al., 2022; Quesada, 2022)

**Table 4.29. Impact of Different Treatments on Total Soluble Protein of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	16.27 <sup>f</sup> ±1.35	17.26 <sup>g</sup> ±.79	34.48 <sup>h</sup> ±2.61	36.00 <sup>i</sup> ±1.56	101.08 <sup>i</sup> ±2.35	96.04 <sup>h</sup> ±1.97
<b>T1 (Thiourea-1000 ppm)</b>	22.75 <sup>d</sup> ±1.79	24.37 <sup>ef</sup> ±1.63	47.75 <sup>f</sup> ±3.67	49.46 <sup>h</sup> ±1.77	134.75 <sup>ef</sup> ±2.56	130.92 <sup>ef</sup> ±3.04
<b>T2 (Salicylic acid-300 ppm)</b>	18.98 <sup>ef</sup> ±0.99	19.53 <sup>g</sup> ±2.22	41.56 <sup>g</sup> ±1.27	38.65 <sup>i</sup> ±1.44	126.13 <sup>gh</sup> ±2.25	129.40 <sup>ef</sup> ±2.70
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	21.68 <sup>de</sup> ±1.71	23.94 <sup>ef</sup> ±1.07	56.07 <sup>e</sup> ±4.83	58.55 <sup>ef</sup> ±4.47	123.37 <sup>h</sup> ±1.87	120.58 <sup>g</sup> ±2.70
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	24.74 <sup>d</sup> ±1.86	26.12 <sup>de</sup> ±0.90	63.91 <sup>cd</sup> ±5.61	66.89 <sup>bc</sup> ±3.42	140.52 <sup>cd</sup> ±2.43	143.55 <sup>d</sup> ±2.41
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	28.00 <sup>c</sup> ±1.78	26.97 <sup>d</sup> ±1.08	54.53 <sup>e</sup> ±4.23	56.26 <sup>g</sup> ±3.70	121.95 <sup>h</sup> ±1.94	123.62 <sup>g</sup> ±2.84
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	16.07 <sup>f</sup> ±1.10	18.59 <sup>g</sup> ±1.38	56.58 <sup>e</sup> ±3.09	54.13 <sup>g</sup> ±1.30	138.28 <sup>de</sup> ±2.67	133.33 <sup>e</sup> ±2.12
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	28.85 <sup>c</sup> ±1.92	30.75 <sup>c</sup> ±0.90	73.04 <sup>ab</sup> ±3.03	64.88 <sup>cd</sup> ±2.42	147.52 <sup>b</sup> ±4.26	154.74 <sup>b</sup> ±2.81
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	32.19 <sup>b</sup> ±1.81	34.38 <sup>b</sup> ±1.38	68.19 <sup>bc</sup> ±5.02	70.11 <sup>b</sup> ±1.76	130.99 <sup>f</sup> ±3.13	131.68 <sup>ef</sup> ±1.30
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	22.06 <sup>de</sup> ±2.23	22.45 <sup>f</sup> ±1.64	58.43 <sup>de</sup> ±2.84	59.57 <sup>ef</sup> ±1.20	128.71 <sup>g</sup> ±3.43	128.85 <sup>f</sup> ±0.93
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	36.15 <sup>a</sup> ±1.15	38.53 <sup>a</sup> ±1.40	75.95 <sup>a</sup> ±2.23	78.95 <sup>a</sup> ±1.37	143.35 <sup>bc</sup> ±1.81	150.07 <sup>c</sup> ±2.90
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	33.62 <sup>ab</sup> ±2.66	32.26 <sup>bc</sup> ±1.98	63.86 <sup>cd</sup> ±1.79	61.80 <sup>de</sup> ±1.51	157.59 <sup>a</sup> ±3.17	160.27 <sup>a</sup> ±1.74
<b>CD</b>	3.096	2.482	6.121	3.847	4.886	4.037
<b>CV</b>	7.234	5.544	6.207	3.896	2.158	1.773

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.29. Total soluble protein of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Total Free Amino Acids:** The effect of Sulphur and Salicylic acid and their combination on total free amino acids was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.30, Figure 4.30). In 2021-2022, there was a significant difference in total free amino acids compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the total free amino acids was observed at 30, 60, and 90 DAS. Therefore, at 30 DAS, the percentage increase compared to T0 was the highest in T11, followed by T9, T3, T10, T8, T1, T2, T7, T6, T4, T5 and the percentage values were 64.22%, 59.82%, 49.66%, 49.23%, 45.85%, 45.53%, 40.41%, 39.76%, 30.36%, 24.32%, and 13.96% respectively. At 60DAS, the percentage increase compared to T0 was the highest in T11, followed by T10, T3, T9, T8, T1, T7, T2, T6, T4, T5 and the percentage values were 60.94%, 56.88%, 55.47%, 53.59%, 47.42%, 43.09%, 36.28%, 32.07%, 27.66%, 19.46%, and 8.25% respectively. At 90DAS, the percentage increase compared to T0 was the highest in T11, followed by T9, T10, T8, T3, T7, T1, T6, T2, T4, T5 and the percentage values were 50.34%, 47.06%, 40.16%, 35.52%, 29.69%, 28.87%, 27.12%, 18.77%, 14.08%, and 1.12% respectively. In 2022-2023, there was a significant difference in total free amino acids compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T11, followed by T8, T10, T3, T9, T2, T4, T6, T7, T1 and the percentage values were 53.47%, 44.90%, 43.94%, 42.87%, 41.43%, 39.83%, 32.49%, 25.58%, 19.87%, and 18.53% respectively. But in T5 percentage decrease as compared to T0 and the percentage value was -13.82%. At 60 DAS, the percentage increase as compared to T0 was found to be highest in T11, followed by T10, T9, T3, T1, T8, T7, T6, T4, T2 and the percentage values were 60.20%, 52.92%, 52.12%, 50.81%, 43.76%, 41.84%, 26.31%, 25.74%, 25.62%, and 13.76% respectively. But in T5 percentage decrease as compared to T0 and the percentage value was -19.92%. At 90DAS, the percentage increase compared to T0 was the highest in T11, followed by T10, T9, T8, T3, T6, T2, T1, T4, T5 and the percentage values were 57.77%, 52.30%, 51.02%, 44.35%, 39.50%, 36.97%, 34.76%, 32.54%, 30.86%, 30.67%, and 2.93% respectively. Free amino acids significantly influence the physiological processes of

mustard plants (*Brassica* spp.). Mustard, a well-known condiment appreciated for its versatile uses and notable nutritional content, relies on various amino acids to support multiple physiological functions (Napieraj et al., 2023; Nasir & Toth, 2022; Negi & Kumar, 2023; Nimsi et al., 2023; Niu & Fu, 2022; Ortiz-García et al., 2022, 2023; Ozturk & Unal, 2023; Paalli et al., 2022). The significance of the interaction between total free amino acids and compounds such as thiourea and salicylic acid in mustard cultivation resides in its influence on plant growth, stress responses, and nutritional quality. This study examines the importance of total free amino acids in mustard plants and explores any potential interactions they may have with thiourea and salicylic acid. Plant protein synthesis depends on utilising free amino acids as essential building blocks. Proteins are paramount in plant physiology as they fulfil crucial functions such as providing structural integrity and enabling defence mechanisms via enzymatic activity. The involvement of amino acids in various metabolic pathways is necessary, as they regulate hormone synthesis, produce phytochemicals, and form other bioactive compounds. The phenomenon above influences mustard plants' growth, development, and stress responses. Nutrient absorption and transportation in plants necessitates the involvement of amino acids. Arbuscular mycorrhizal fungi (AMF) play a pivotal role in facilitating the absorption of essential nutrients from the soil, thereby fostering the overall health and growth of plants. The ability to endure stress: The concentrations of unbound amino acids can experience modifications in reaction to diverse environmental stressors. Mustard plants demonstrate a notable stress tolerance and adaptation capacity, enabling them to effectively withstand and respond to unfavourable environmental conditions, including drought, salinity, and pathogen-induced assaults (Masmoudi et al., 2023; Megala et al., 2022; Mittal et al., 2022; Mohammadi et al., 2023; Molinari et al., 2023; Moustakas et al., 2022; Mugwanya et al., 2023; Mukarram et al., 2022; Mukhtar et al., 2023; Myers Jr. et al., 2023; Nadeem, 2022; Nam et al., 2023). Including free amino acids in mustard leaves and seeds improves their nutritional quality. Incorporating mustard seeds into mustard has been found to substantially impact its flavour, texture, and protein composition, enhancing its nutritional value as a valuable dietary resource. The experimental findings have demonstrated the influence of thiourea on the accumulation of free amino acids in mustard plants. This interaction can potentially increase the concentration of amino acids, thus enhancing the plant's ability



to synthesise proteins. The potential impact of thiourea on the attention of free amino acids in mustard plants has the potential to improve their ability to tolerate stress. Amino acids can serve as Osmo protectants, facilitating plants' ability to acclimate to water stress and salinity conditions. This interaction contributes to enhancing the overall health and growth of the plant. The potential for enhanced nutritional value: Increasing the concentration of free amino acids can improve the nutritional value of mustard. The amino acids under consideration are essential for meeting the dietary needs of humans and exert a substantial influence on the flavour, texture, and overall quality of mustard products. Defence Responses: Salicylic acid is widely acknowledged as a highly effective activator of plant defence mechanisms. The interaction between the substance and free amino acids can lead to the accumulation of defence-related proteins composed of amino acids. This interaction enhances the plant's ability to resist pathogens and adapt to biotic stressors. Metabolic Regulation: The influence of salicylic acid on the metabolic processes of amino acids leads to the regulation of the production of specific amino acids and their allocation within the plant. This interaction has implications for multiple facets of plant physiology, encompassing growth patterns, mechanisms for managing stress, and nutritional composition. The potential impact of the interaction between salicylic acid and free amino acids on the healthy design of mustard plants is noteworthy. Modifications to the structure of amino acids can influence the protein composition and amino acid distribution in mustard products, thus augmenting their nutritional significance as a dietary source. The presence of unbound amino acids in mustard plants is of utmost importance in plant physiology, as it significantly influences various aspects such as plant growth, stress response, and nutritional composition. Understanding the interplay between mustard plants and multiple compounds, such as thiourea and salicylic acid, is crucial for optimising mustard cultivation, enhancing stress resilience, and improving nutritional quality. The interactions above can influence the welfare and growth of plants and the nutritional composition and marketability of mustard products. Through a thorough analysis of these interconnections, it becomes feasible to develop advanced methodologies to optimise the full potential of mustard plants in diverse agricultural and dietary settings (Liu, Xiao, et al., 2022; Liu, Meng, et al., 2022; Lombardino et al., 2022; Ma et al., 2022;

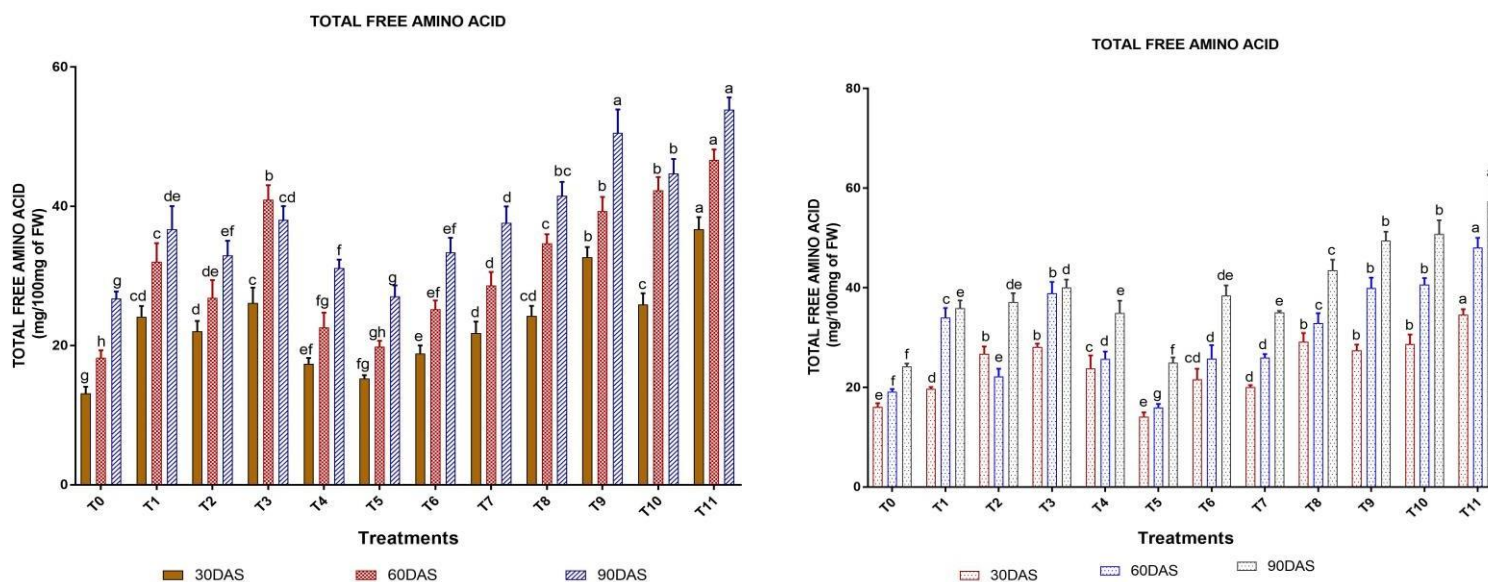
Mabasa, 2023; Maheshwari et al., 2022; Maia et al., 2022; Makete et al., 2022;  
Manepalli et al., 2022; Mangena, 2022; Manjunatha et al., 2022).

**Table 4.30. Impact of Different Treatments on Total Free Amino Acids of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	13.14 <sup>g</sup> ±0.94	16.08 <sup>e</sup> ±0.72	18.23 <sup>h</sup> ±1.08	19.14 <sup>f</sup> ±0.54	26.75 <sup>g</sup> ±1.00	24.23 <sup>f</sup> ±.54
<b>T1 (Thiourea-1000 ppm)</b>	24.12 <sup>cd</sup> ±1.54	19.74 <sup>d</sup> ±0.32	32.03 <sup>c</sup> ±2.67	34.04 <sup>c</sup> ±1.88	36.71 <sup>de</sup> ±3.32	35.92 <sup>e</sup> ±1.53
<b>T2 (Salicylic acid-300 ppm)</b>	22.05 <sup>d</sup> ±1.47	26.73 <sup>b</sup> ±1.48	26.84 <sup>de</sup> ±2.55	22.20 <sup>e</sup> ±1.53	32.94 <sup>ef</sup> ±2.11	37.14 <sup>de</sup> ±1.80
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	26.10 <sup>c</sup> ±2.21	28.15 <sup>b</sup> ±0.62	40.94 <sup>b</sup> ±2.09	38.92 <sup>b</sup> ±2.27	38.05 <sup>cd</sup> ±1.98	40.05 <sup>d</sup> ±1.62
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	17.36 <sup>ef</sup> ±.84	23.82 <sup>c</sup> ±2.60	22.63 <sup>fg</sup> ±2.08	25.74 <sup>d</sup> ±1.45	31.14 <sup>f</sup> ±1.18	34.95 <sup>e</sup> ±2.46
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	15.27 <sup>fg</sup> ±.47	14.13 <sup>e</sup> ±0.84	19.87 <sup>gh</sup> ±0.82	15.96 <sup>g</sup> ±0.72	27.06 <sup>g</sup> ±1.57	24.96 <sup>f</sup> ±1.04
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	18.87 <sup>e</sup> ±1.15	21.61 <sup>cd</sup> ±2.13	25.20 <sup>ef</sup> ±1.27	25.78 <sup>d</sup> ±2.69	33.38 <sup>ef</sup> ±2.10	38.44 <sup>de</sup> ±2.05
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	21.81 <sup>d</sup> ±1.62	20.07 <sup>d</sup> ±0.36	28.61 <sup>d</sup> ±1.94	25.98 <sup>d</sup> ±0.70	37.62 <sup>d</sup> ±2.36	35.04 <sup>e</sup> ±.31
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	24.26 <sup>cd</sup> ±1.43	29.19 <sup>b</sup> ±1.73	34.67 <sup>c</sup> ±1.32	32.92 <sup>c</sup> ±1.98	41.50 <sup>bc</sup> ±2.02	43.54 <sup>c</sup> ±2.10
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	32.70 <sup>b</sup> ±1.45	27.46 <sup>b</sup> ±1.12	39.28 <sup>b</sup> ±2.07	39.98 <sup>b</sup> ±2.04	50.54 <sup>a</sup> ±3.36	49.47 <sup>b</sup> ±1.79
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	25.88 <sup>c</sup> ±1.62	28.69 <sup>b</sup> ±1.92	42.28 <sup>b</sup> ±1.93	40.66 <sup>b</sup> ±1.29	44.72 <sup>b</sup> ±2.07	50.80 <sup>b</sup> ±2.73
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	36.73 <sup>a</sup> ±1.71	34.57 <sup>a</sup> ±1.12	46.68 <sup>a</sup> ±1.48	48.10 <sup>a</sup> ±1.92	53.89 <sup>a</sup> ±1.75	57.38 <sup>a</sup> ±3.82
<b>CD</b>	2.213	2.522	3.289	3.05	3.578	3.357
<b>CV</b>	5.598	6.117	6.139	5.812	5.545	5.008

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.30. Total Free Amino Acids (mg/100mg of fresh weight) of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Total Lipids:** The effect of Sulphur and Salicylic acid and their combination on total lipids was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.31, Figure 4.31). In 2021-2022, there was a significant difference in total lipids compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the total lipids was observed at 30, 60, and 90 DAS. Therefore, at 30 DAS, the percentage increase compared to T0 was the highest in T8, followed by T4, T6, T2, T10, T5, T11, T3, T1, T7, T9 and the percentage values were 36.45%, 32.98%, 32.70%, 32.12%, 31.39%, 25.98%, 24.94%, 23.13%, 22.76%, 18.82%, and 18.62% respectively. At 60DAS, the percentage increase compared to T0 was the highest in T2, followed by T10, T6, T5, T9, T4, T11, T8 and the percentage values were 24.15%, 16.55%, 14.31%, 9.02%, 6.58%, 3.52%, 3.52%, and 2.79% respectively. But in T3, T1, and T7 percentage decrease as compared to T0 and the percentage values were -4.07%, -4.35%, and -17.12%. At 90DAS, the percentage increase compared to T0 was the highest in T2, followed by T5, T10, T4, T11, T1, T7, T6, T3, T9 and the percentage values were 21.13%, 18.28%, 18.09%, 16.78%, 15.61%, 12.13%, 9.72%, 6.70%, 6.21%, and 4.98% respectively. But in T8 percentage decrease as compared to T0 and the percentage value was -1.68%. In 2022-2023, there was a significant difference in total lipids compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T8, followed by T4, T6, T2, T5, T10, T3, T11, T1, T9, T7 and the percentage values were 29.09%, 28.65%, 28.21%, 26.84%, 24.61%, 23.28%, 17.61%, 16.22%, 10.59%, 7.48%, and 6.98% respectively. At 60 DAS, the percentage increase as compared to T0 was found to be highest in T2, followed by T10, T9, T5, T6, T11, T8, T1, T4, T3 and the percentage values were 26.08%, 24.04%, 19.77%, 16.78%, 15.40%, 14.79%, 7.75%, 6.54%, 6.29%, and 3.51% respectively. But in T7 percentage decrease as compared to T0 and the percentage value was -5.30%. At 90DAS, the percentage increase compared to T0 was the highest in T10, followed by T3, T5, T2, T7, T11, T9, T4, T1, T6, and the percentage values were 14.98%, 12.44%, 11.42%, 9.52%, 9.52%, 8.21%, 7.31%, 5.94%, 4.04%, and 3.30% respectively. But in T8 percentage decrease as compared to T0 and the percentage value

was -5.26%. Lipids are integral components of plant cells and play a crucial role in the cultivation of mustard (*Brassica spp.*). These lipids comprise both fats and oils. The lipid composition of mustard plants plays a crucial role in determining the economic value and practicality of mustard crops, given the reputation of these plants for producing seeds that are abundant in oil (Liu, Zong, et al., 2022; Liu, Liu, et al., 2022; Liu, Xiao, et al., 2022; Liu, Meng, et al., 2022; Lombardino et al., 2022; Ma et al., 2022; Mabasa, 2023; Maheshwari et al., 2022). Furthermore, the interaction between overall lipid content and compounds such as thiourea and salicylic acid carries substantial implications for multiple facets of plant physiology, encompassing growth, stress adaptation, and oil composition. The present study investigates the importance of total lipids in mustard plants and their interactions with thiourea and salicylic acid. The topic of discussion pertains to the production of oil. Mustard seeds are predominantly valued for their elevated oil concentration. Oil extraction heavily depends on total lipids as the primary source material, making them essential for the production of mustard oil. This oil exhibits considerable utility within the food industry and is a valuable raw material for biofuel applications. The process of energy storage in plants is facilitated by total lipids, which function as a reservoir for accumulating and storing energy. Lipids undergo the process of catabolism to produce energy in response to metabolic requirements, facilitating various physiological functions such as growth, reproduction, and adaptation to stress. The primary composition of the cell membrane structure consists of lipids, which play a crucial role as fundamental components. Cellulose fibres are paramount in upholding the structural integrity of cells and facilitating the conveyance of vital nutrients and water, thereby substantially contributing to plants' overall health and growth. The transportation of nutrients involves the participation of lipids, vital in transporting nutrients soluble in lipids, such as fat-soluble vitamins and certain phytochemicals. They play a crucial role in transporting these essential compounds throughout the plant. Biosynthesis is essential for producing various compounds, including hormones and signalling molecules, by synthesising total lipids. These compounds play a crucial role in the regulation of plant growth and the response to environmental stimuli. Based on empirical research, thiourea has demonstrated a beneficial influence on the overall lipid composition of mustard plants. This interaction demonstrates the potential to increase oil production obtained from mustard seeds.

Thiourea exhibits the potential to serve as a plant growth regulator through its facilitation of lipid biosynthesis. The documented application of thiourea has shown promise in enhancing plant stress tolerance, which may have implications for lipid content. Thiourea has the potential to alleviate the adverse effects of environmental stressors, such as drought and salinity, thereby assisting mustard plants in maintaining their lipid reserves. Consequently, this phenomenon stimulates robust proliferation and augments the synthesis of oil. The enhancement of oil quality can be achieved through the manipulation of total lipids, as their augmentation can significantly influence the overall quality of the oil. The potential consequence of this phenomenon is a heightened accumulation of oil in mustard seeds, thereby potentially augmenting the oil's quality, flavour, and nutritional composition. Salicylic acid, a pivotal signalling molecule in plant defence mechanisms, can potentially influence lipid modification. The potential ramifications of this phenomenon on the lipid composition of mustard plants may lead to modifications in the oil profile of their seeds. The presence of salicylic acid enables the facilitation of defence mechanisms in plants, thereby triggering stress responses. The plant's capacity to respond to biotic and abiotic stressors may be influenced by its interaction with lipids (Li, Han, et al., 2023; Li, Huang, et al., 2022; Li, Luo, et al., 2022; Li, Tong, et al., 2023; Liu et al., 2023; Liu, Wang, et al., 2022; Liu, Li, et al., 2022; Liu, Cui, et al., 2022). This interaction can potentially influence the plant's utilisation and distribution of lipids. Investigating the potential consequences of the interaction between salicylic acid and total lipids on the quality of oil and its nutritional value has garnered considerable attention. Modifying lipid composition can influence multiple facets of the oil extracted from mustard seeds, encompassing its taste, durability, and nutritional characteristics. Lipids play a multifaceted role in mustard plants, encompassing various functions such as facilitating plant growth, serving as an energy storage mechanism, and contributing to oil production. Understanding the interactions between mustard plants and compounds such as thiourea and salicylic acid is essential for optimising mustard cultivation, improving oil production, and enhancing the plant's stress tolerance. These interactions have the potential to not only affect the lipid content but also influence the quality of oil and the economic value of mustard crops. Through a thorough analysis of these interconnected relationships, it becomes feasible to develop improved methodologies to maximise the full potential of mustard

plants in agriculture and industry (Li et al., 2022; Li et al., 2022; Li, Ren, et al., 2023; Li, Zhang, et al., 2022; Li, Zheng, et al., 2023; Li et al., 2022; Li, Han, et al., 2022; Li, He, et al., 2023; Li et al., 2022).

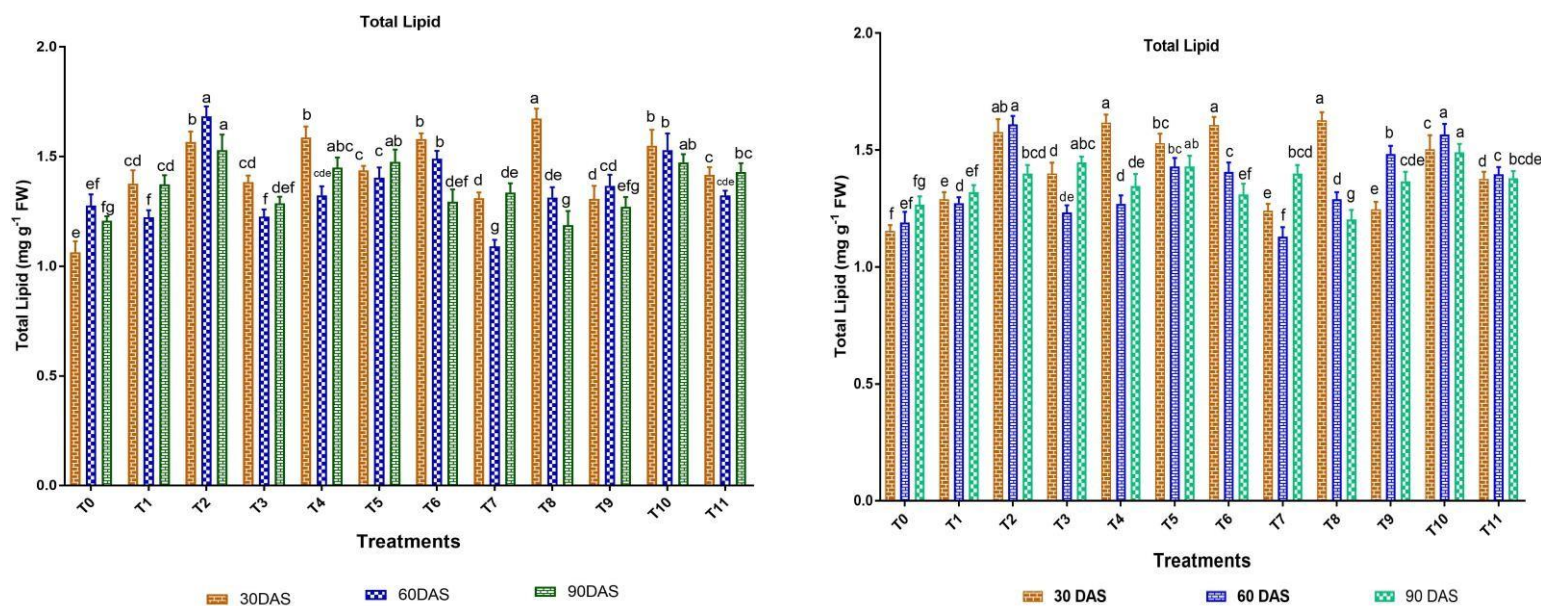


**Table 4.31. Impact of Different Treatments on Total Lipids of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	1.06 <sup>e</sup> ±0.050	1.15 <sup>f</sup> ±0.025	1.27 <sup>ef</sup> ±0.051	1.19 <sup>ef</sup> ±0.045	1.20 <sup>fg</sup> ±0.020	1.26 <sup>fg</sup> ±0.035
<b>T1 (Thiourea-1000 ppm)</b>	1.37 <sup>cd</sup> ±0.061	1.29 <sup>e</sup> ±0.030	1.22 <sup>f</sup> ±0.032	1.27 <sup>d</sup> ±0.025	1.37 <sup>cd</sup> ±0.041	1.32 <sup>ef</sup> ±0.030
<b>T2 (Salicylic acid-300 ppm)</b>	1.56 <sup>b</sup> ±0.047	1.57 <sup>ab</sup> ±0.055	1.68 <sup>a</sup> ±0.045	1.61 <sup>a</sup> ±0.036	1.53 <sup>a</sup> ±0.070	1.40 <sup>bcd</sup> ±0.036
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	1.38 <sup>cd</sup> ±0.030	1.40 <sup>d</sup> ±0.045	1.22 <sup>f</sup> ±0.032	1.23 <sup>de</sup> ±0.030	1.28 <sup>def</sup> ±0.030	1.44 <sup>abc</sup> ±0.025
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	1.58 <sup>b</sup> ±0.050	1.61 <sup>a</sup> ±0.035	1.32 <sup>cde</sup> ±0.041	1.27 <sup>d</sup> ±0.036	1.45 <sup>abc</sup> ±0.045	1.34 <sup>de</sup> ±0.051
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	1.43 <sup>c</sup> ±0.020	1.53 <sup>bc</sup> ±0.040	1.40 <sup>c</sup> ±0.047	1.43 <sup>bc</sup> ±0.036	1.47 <sup>ab</sup> ±0.055	1.43 <sup>ab</sup> ±0.045
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	1.58 <sup>b</sup> ±0.026	1.60 <sup>a</sup> ±0.035	1.49 <sup>b</sup> ±0.036	1.40 <sup>c</sup> ±0.040	1.29 <sup>def</sup> ±0.056	1.31 <sup>ef</sup> ±0.045
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	1.31 <sup>d</sup> ±0.026	1.24 <sup>e</sup> ±0.030	1.09 <sup>g</sup> ±0.030	1.13 <sup>f</sup> ±0.040	1.33 <sup>de</sup> ±0.041	1.40 <sup>bcd</sup> ±0.036
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	1.67 <sup>a</sup> ±0.045	1.62 <sup>a</sup> ±0.035	1.31 <sup>de</sup> ±0.047	1.29 <sup>d</sup> ±0.030	1.18 <sup>g</sup> ±0.065	1.20 <sup>g</sup> ±0.040
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	1.30 <sup>d</sup> ±0.060	1.24 <sup>e</sup> ±0.032	1.36 <sup>cd</sup> ±0.050	1.48 <sup>b</sup> ±0.035	1.27 <sup>efg</sup> ±0.045	1.36 <sup>cde</sup> ±0.040
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	1.55 <sup>b</sup> ±0.072	1.50 <sup>c</sup> ±0.060	1.53 <sup>b</sup> ±0.075	1.56 <sup>a</sup> ±0.045	1.47 <sup>ab</sup> ±0.037	1.49 <sup>a</sup> ±0.036
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	1.41 <sup>c</sup> ±0.035	1.37 <sup>d</sup> ±0.030	1.32 <sup>cde</sup> ±0.020	1.39 <sup>c</sup> ±0.030	1.43 <sup>bc</sup> ±0.040	1.38 <sup>bcd</sup> ±0.030
<b>CD</b>	0.082	0.007	0.077	0.062	0.08	0.06
<b>CV</b>	3.33	2.863	3.324	2.689	3.461	2.595

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

Figure 4.31. Total Lipids (mg g<sup>-1</sup> fresh weight) of Mustard During Rabi 2021-2023 & 2022-23



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Total Soluble Sugar:** The effect of Sulphur and Salicylic acid and their combination on total soluble sugar was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.32, Figure 4.32). In 2021-2022, there was a significant difference in total soluble sugar compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the total soluble sugar was observed at 30, 60, and 90 DAS. Therefore, at 30 DAS, the percentage increase as compared to T0 was found highest in T9 followed by T8, T11, T5, T4, T3, T6, T1, T2, T7 and the percentage values were 60.32%, 57.35%, 47.81%, 47.27%, 45.30%, 42.87%, 34.48%, 31.41%, 31.41%, and 23.77% respectively. But in T10, the percentage decreased compared to T0, and the percentage value was -7.32%. At 60 DAS, the percentage increase as compared to T0 was found highest in T9 followed by T2, T4, T8, T11, T1, T3, T5, T6, T10, T7 and the percentage values were 75.97%, 66.10%, 65.44%, 62.42%, 61.37%, 57.98%, 56.21%, 50.27%, 50.07%, 36.94%, and 36.62% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T9 followed by T2, T3, T5, T4, T1, T10, T8 and the percentage values were 39.76%, 38.01%, 26.79%, 26.62%, 26.12%, 22.35%, 12.34%, 2.44% respectively. But in T11, T6, T7 the percentage decrease as compared to T0 and the percentage values were -1.91%, -11.90%, -39.19% respectively. In 2022-2023, there was a significant difference in total soluble sugar compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T9, followed by T8, T4, T5, T3, T11, T2, T1, T6, T7, T10 and the percentage values were 60.77%, 59.21%, 47.81%, 47.27%, 45.59%, 45.59%, 34.05%, 30.01%, 29.53%, 29.04%, 2.84% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T9 followed by T2, T4, T11, T8, T1, T3, T6, T5, T10, T7 and the percentage values were 74.39%, 65.83%, 65.16%, 63.96%, 62.79%, 55.07%, 54.91%, 51.24%, 51.05%, 37.69%, 30.83% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T2 followed by T9, T3, T4, T5, T1, T10, T8, T11 and the percentage values were 36.13%, 35.88%, 25.24%, 23.37%, 21.77%, 16.55%, 8.65%, 3.70%, 2.87% respectively. But in T6 and T7, the percentage decreased compared to T0, and the percentage values were -7.31% and -

47.67%. The quantification of mustard's overall soluble sugar content (*Brassica juncea* L.) is a crucial biochemical parameter that holds significant importance in plant investigation and agricultural methodologies (Waters & Nelson, 2023; Wu et al., 2022; Wurms et al., 2023; Xiao et al., 2022; Xie et al., 2023; Xing et al., 2022; Xu et al., 2023; Xu et al., 2022, 2023; Xu, Cao, et al., 2022). Furthermore, acquiring information regarding the potential impacts of sulphur (S) and salicylic acid (SA) foliar spray treatments on the overall concentration of soluble sugars in mustard plants provides insights into enhancing agricultural methods and enhancing the overall health and productivity of crops. The quantification of soluble sugars in a plant is a crucial component of its physiological characteristics. The insights gained from this assessment can significantly enhance our understanding of the plant's response to diverse environmental conditions and stressors. The evaluation of total soluble sugar content holds significant importance in mustard research and agriculture, as elucidated in the subsequent sections of this scholarly article. Soluble sugars, including glucose, fructose, and sucrose, play a crucial role in providing energy for the growth and development of plants. They perform a vital function in numerous metabolic processes, including respiration, photosynthesis, and synthesising structural components such as cellulose. The regulation of osmotic balance in plant cells is primarily governed by soluble sugars, which play a significant role in this physiological mechanism (Wang et al., 2022; Wang et al., 2023; Wang et al., 2023; Wang et al., 2022; Wang & Komatsu, 2022; Wang et al., 2022; Wang et al., 2022; Wang et al., 2022; Wante et al., 2022). They play a role in maintaining turgor pressure within the cellular environment, thereby promoting cell rigidity and preventing the occurrence of wilting. The conservation of water and enhanced tolerance to drought conditions are particularly crucial for mustard plants during periods of water stress. Mustard plants are commonly exposed to biotic and abiotic stresses, including pathogen infestations and adverse environmental conditions such as drought, salinity, and extreme temperatures. Plants can accumulate soluble sugars to respond to stressful situations, serving as a stress response mechanism. The assessment of sugar levels can serve as an indicator of the plant's ability to endure stress. Soluble sugars play a vital role in facilitating the adequate transportation of nutrients within plants. The transport of sugars from source tissues, such as leaves, to sink tissues, such as roots and developing seeds, is facilitated by specific molecules that function as

transporters. Efficient nutrient allocation promotes a plant's overall health and productivity. Sulphur is a crucial macronutrient that influences the overall soluble sugar content in mustard through various mechanisms. The synthesis of sulfur-containing amino acids, namely cysteine and methionine, essential for protein biosynthesis, relies on sulphur availability. The photosynthetic process, wherein plants convert sunlight into energy, is greatly facilitated by proteins. An ample supply of sulphur has the potential to induce the process of photosynthesis, thereby enhancing the synthesis of soluble sugars. The involvement of sulphur is crucial for the efficient uptake of vital nutrients such as nitrogen (N) and phosphorus (P) during the assimilation process. Providing these essential nutrients is imperative for both the synthesis of sugar and the overall development of the plant. Compounds that incorporate sulphur possess the capacity to function as antioxidants, thereby affording protection to plant cells against oxidative damage induced by environmental stressors. Sulfur's ability to mitigate stress-induced harm enables the maintenance of elevated sugar levels in unfavourable circumstances. The involvement of systemic acquired resistance (SAR) in the defence mechanisms of plants, particularly in their stress responses, is widely acknowledged. As a part of the plant's natural defence mechanism, SA can instigate the stress-related pathways that ultimately result in the accumulation of soluble sugars. The allocation of resources is one of the areas where SA's influence can be felt within the plant. When salicylic acid (SA) triggers stress responses in plants, it can lead to the allocation of additional resources, such as carbon and energy, towards the synthesis of sugars. This, in turn, leads to elevated levels of soluble sugars. Measuring mustard's total soluble sugar content can yield valuable insights into physiological processes such as energy metabolism, stress responses, osmotic regulation, and nutrient distribution. Applying sulphur and salicylic acid through foliar sprays can impact the concentration of soluble sugars in plants by enhancing photosynthesis, nutrient absorption, stress resistance, and resource allocation. The potential for improving the resilience and productivity of mustard crops, particularly in challenging environmental conditions, can be realised through implementing these strategies. Ongoing research efforts are essential in optimising application techniques and concentrations to achieve desired outcomes (Taira & Shiono, 2022; Takeuchi et al., 2022; Talaat, 2023; Tan et al., 2022; Tariq et al., 2023; Tariq et al., 2022; Todorova et al., 2022; Topcu et al., 2022; Torres & Figueroa,

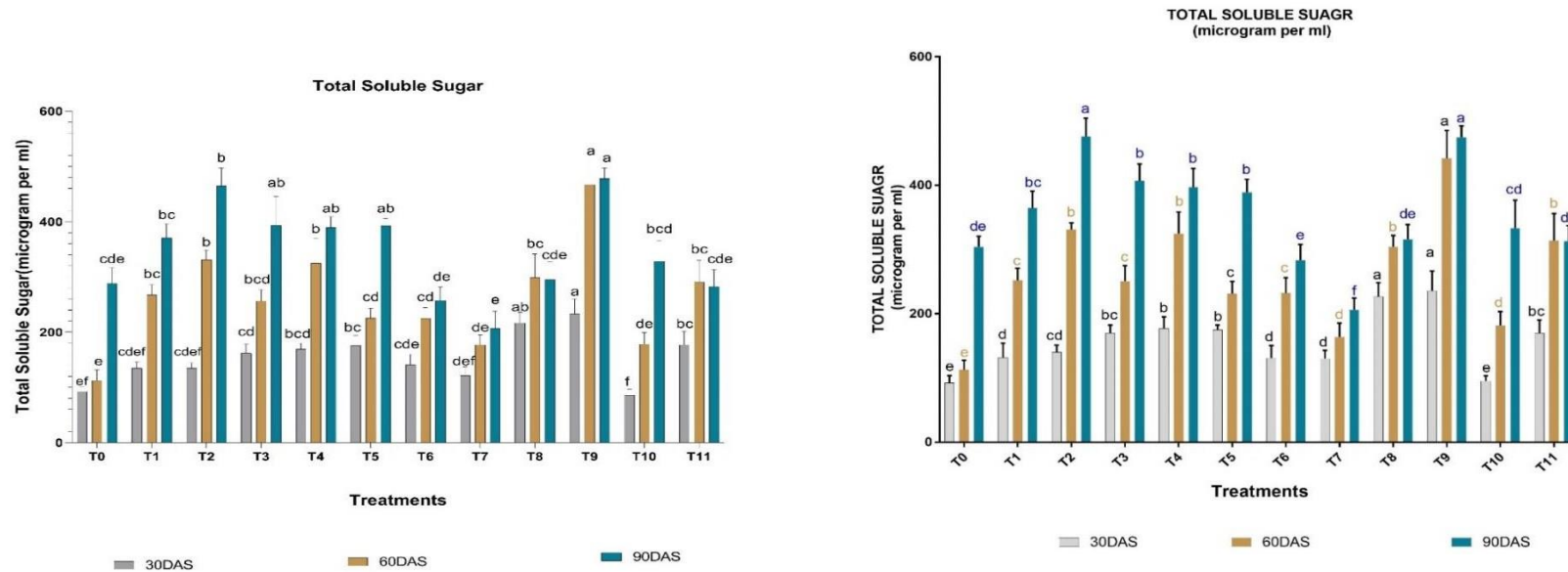
2023; Tyagi et al., 2022; Ullah et al., 2023; Ullah et al., 2022; Verma et al., 2023; Waadt et al., 2022; Wan & Xin, 2022).

**Table 4.32. Impact of Different Treatments on Total Soluble Sugar of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	92.44 <sup>ef</sup> ±8.25	92.44 <sup>e</sup> ±11.25	112.26 <sup>e</sup> ±18.98	113.16 <sup>e</sup> ±14.30	287.93 <sup>cde</sup> ±28.64	304.15 <sup>de</sup> ±16.29
<b>T1 (Thiourea-1000 ppm)</b>	134.78 <sup>cdef</sup> ±10.81	132.08 <sup>d</sup> ±22.12	267.21 <sup>cd</sup> ±18.91	251.90 <sup>e</sup> ±18.98	370.81 <sup>bc</sup> ±25.11	364.51 <sup>bc</sup> ±26.61
<b>T2 (Salicylic acid-300 ppm)</b>	134.78 <sup>cdef</sup> ±9.74	140.18 <sup>cd</sup> ±10.81	331.18 <sup>bc</sup> ±17.58	331.18 <sup>b</sup> ±10.23	464.51 <sup>b</sup> ±32.43	476.22 <sup>a</sup> ±28.38
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	161.81 <sup>cd</sup> ±16.21	169.91 <sup>bc</sup> ±12.38	256.40 <sup>bcd</sup> ±20.40	251.00 <sup>e</sup> ±24.02	393.34 <sup>ab</sup> ±51.94	406.85 <sup>b</sup> ±26.25
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	169.01 <sup>bcd</sup> ±10.92	177.12 <sup>b</sup> ±17.99	324.87 <sup>b</sup> ±44.76	324.87 <sup>b</sup> ±33.79	389.73 <sup>ab</sup> ±18.98	396.94 <sup>b</sup> ±29.23
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	175.32 <sup>bc</sup> ±18.91	175.32 <sup>b</sup> ±7.15	225.77 <sup>cd</sup> ±17.58	231.18 <sup>c</sup> ±18.98	392.44 <sup>ab</sup> ±13.33	388.83 <sup>b</sup> ±20.40
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	141.09 <sup>cde</sup> ±18.98	131.18 <sup>d</sup> ±19.17	224.87 <sup>cd</sup> ±19.17	232.08 <sup>c</sup> ±24.02	257.30 <sup>de</sup> ±24.52	283.43 <sup>e</sup> ±24.32
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	121.27 <sup>def</sup> ±16.21	130.27 <sup>d</sup> ±12.77	177.12 <sup>de</sup> ±17.99	163.61 <sup>d</sup> ±21.84	206.85 <sup>e</sup> ±30.73	205.95 <sup>f</sup> ±18.39
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	216.76 <sup>ab</sup> ±18.98	226.67 <sup>a</sup> ±21.62	298.74 <sup>bc</sup> ±42.24	304.15 <sup>b</sup> ±17.58	295.14 <sup>cde</sup> ±32.24	315.86 <sup>de</sup> ±23.09
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	232.98 <sup>a</sup> ±27.07	235.68 <sup>a</sup> ±30.73	467.2 <sup>a</sup> ±44.65	441.99 <sup>a</sup> ±43.27	478.02 <sup>a</sup> ±18.91	474.42 <sup>a</sup> ±17.99
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	86.13 <sup>f</sup> ±10.81	95.14 <sup>e</sup> ±8.25	178.02 <sup>de</sup> ±21.10	181.63 <sup>d</sup> ±21.67	328.47 <sup>bcd</sup> ±36.49	332.98 <sup>cd</sup> ±43.77
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	177.12 <sup>bc</sup> ±24.52	169.91 <sup>bc</sup> ±20.40	290.63 <sup>bc</sup> ±39.19	314.06 <sup>b</sup> ±41.89	282.53 <sup>cde</sup> ±30.73	313.16 <sup>de</sup> ±24.32
<b>CD</b>	28.909	27.844	51.809	46.516	50.67	42.791
<b>CV</b>	11.042	10.451	11.565	10.428	8.603	7.067

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.32. Total Soluble Sugar of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).



**Total Starch:** The effect of Sulphur and Salicylic acid and their combination on total starch was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.33, Figure 4.33). In 2021-2022, there was a significant difference in total starch compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the total starch was observed at 30, 60, and 90 DAS. Therefore, at 30 DAS, the percentage increase as compared to T0 was found highest in T9 followed by T8, T11, T5, T4, T3, T6, T1, T2, T7 and the percentage values were 60.32%, 57.35%, 47.81%, 47.27%, 45.30%, 42.87%, 34.48%, 31.41%, 31.41%, 23.77% respectively. But in T10, the percentage decreased compared to T0, and the percentage value was -7.32%. At 60 DAS, the percentage increase as compared to T0 was found highest in T9 followed by T2, T4, T8, T11, T1, T3, T5, T6, T10, T7 and the percentage values were 75.97%, 66.10%, 65.44%, 62.42%, 61.37%, 57.98%, 56.21%, 50.27%, 50.07%, 36.94%, 36.62% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T9 followed by T2, T3, T5, T4, T1, T10, T8 and the percentage values were 39.76%, 38.01%, 26.79%, 26.62%, 26.12%, 22.35%, 12.34%, 2.44% respectively. But in T11, T6, T7 the percentage decrease as compared to T0 and the percentage values were -1.91%, -11.90%, -39.19% respectively. In 2022-2023, there was a significant difference in total starch compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T9 followed by T8, T4, T5, T3, T11, T2, T1, T6, T7, T10 and the percentage values were 60.77%, 59.21%, 47.81%, 47.27%, 45.59%, 45.59%, 34.05%, 30.01%, 29.53%, 29.04%, 2.84% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T9 followed by T2, T4, T11, T8, T1, T3, T6, T5, T10, T7 and the percentage values were 74.39%, 65.83%, 65.16%, 63.96%, 62.79%, 55.07%, 54.91%, 51.24%, 51.05%, 37.69%, 30.83% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T2 followed by T9, T3, T4, T5, T1, T10, T8, T11 and the percentage values were 36.13%, 35.88%, 25.24%, 23.37%, 21.77%, 16.55%, 8.65%, 3.70%, 2.87% respectively. But in T6 and T7, the percentage decreased compared to T0, and the percentage values were -7.31% and -47.67%. The assessment of mustard

(*Brassica juncea* L.) starch content is a crucial biochemical parameter with profound implications for plant science and agriculture domains. Furthermore, a comprehensive examination of the potential enduring impacts arising from the application of foliar sprays containing sulphur (S) and salicylic acid (SA) on the overall starch composition of mustard yields substantial and invaluable insights (Song et al., 2023; Sousa et al., 2022; Spinoso-Castillo & Bello-Bello, 2022; Suliman et al., 2022; Sun et al., 2022; Sun et al., 2023; Sun et al., 2022; Taira & Shiono, 2022; Takeuchi et al., 2022; Talaat, 2023; Tan et al., 2022; Tariq et al., 2023; Tariq et al., 2022). The observations above can provide valuable insights for developing strategies to optimise crop management techniques, ultimately improving crop health and productivity. A plant's comprehensive amount of starch represents a multifaceted narrative concerning its carbohydrate storage systems and efficient regulation of energy reserves. The determination of the magnitude of this starch repository holds great importance within the realm of mustard research and agronomy. Starch, a complex polysaccharide of interconnected glucose units, is the principal repository for plant energy reserves. The energy above the reservoir is utilised during diminished photosynthetic activity, such as during nocturnal periods or overcast conditions, characterised by decreased solar radiation availability. The entity above fulfils the role of a primary source from which flora extract the essential nutrients required to sustain their persistent quest for development and metabolic activities. Starch plays a pivotal role in the embryogenesis of seeds. This plays a pivotal role. The function of the endosperm in the embryonic seed is crucial, as it serves as a storage site for energy and carbon, which are essential for the seed's future development (Shekhawat et al., 2023; Shi et al., 2023; Shi et al., 2022; Si et al., 2023; Singh et al., 2022; Singh & Roychoudhury, 2023; Singh & Nandi, 2022; Singhal et al., 2023; Sivanesan et al., 2022). Empirical evidence indicates a positive correlation between a high concentration of starch and the development of seeds characterised by increased size and plumpness. This phenomenon invariably results in an increase in the overall crop yield and an improvement in the crop's qualitative characteristics. Without a doubt, starch plays a pivotal role in determining the resilience of plants. Plants utilise starch as a defensive mechanism to alleviate the detrimental impacts of challenging environmental conditions, such as extended periods of drought, soil salinity, or extreme cold. Using stored starch as a strategic resource is a crucial means for plants to access an easily

accessible energy source, facilitating the maintenance of essential metabolic processes and enhancing their ability to endure adverse environmental circumstances (Salwan et al., 2023; Samad et al., 2023; Sampedro-Guerrero et al., 2022; Samtani et al., 2022; Shah et al., 2022, 2023; Shang et al., 2022; Sharma & Verma, 2023; Sheikhalipour et al., 2023). Fortunately, the narrative does not conclude solely by recounting the chronicles of energy dynamics; instead, it expands to encompass the allocation of resources within the vegetative structure of the plant. When the plant generates excess sugars due to heightened photosynthetic activity, these sugars undergo a precisely coordinated chemical process leading to their conversion into starch. The plant employs a highly discerning approach in distributing its valuable carbon resources, and by comprehending the intricacies of starch dynamics, one can gain comprehensive insights into this phenomenon. The observation of sulphur's role as a significant macronutrient in regulating the overall starch content of mustard is a subject of great interest due to the intricate network of modulations involved. The significant role of sulphur in synthesising sulfur-containing amino acids, such as cysteine and methionine, profoundly influences protein synthesis. The influence above exhibits long-lasting and noteworthy effects. These proteins are essential for regulating photosynthesis, a multifaceted process that converts sunlight into energy necessary for the plant's survival. The ample presence of sulphur enhances the efficiency of the photosynthetic apparatus, thereby facilitating an augmentation in the synthesis of sugar. The sugar is subsequently intended for utilisation in the manufacturing process of starch. Furthermore, due to its antioxidant properties, sulphur safeguards against oxidative damage induced by diverse stressors the body encounters. Despite the challenging and stressful environmental conditions, this multifaceted protective mechanism plays a crucial role in maintaining elevated levels of starch within the plant. When considering the application of foliar sprays, salicylic acid plays a significant role in influencing the overall starch content of mustard plants (Rani et al., 2023; Rizvi et al., 2022; Roussos, 2023; Ruidas et al., 2022; Rybczyński et al., 2022; Sachan & Krishna, 2022; Salih, Wu, et al., 2022; Salih, Zhou, et al., 2022). Salicylic acid (SA) is widely recognised for its prominent role in plant defence mechanisms and ability to respond to stress effectively. It triggers a cascade of interconnected physiological and biochemical processes. The events above ultimately result in the accumulation of starch reserves, orchestrated as a

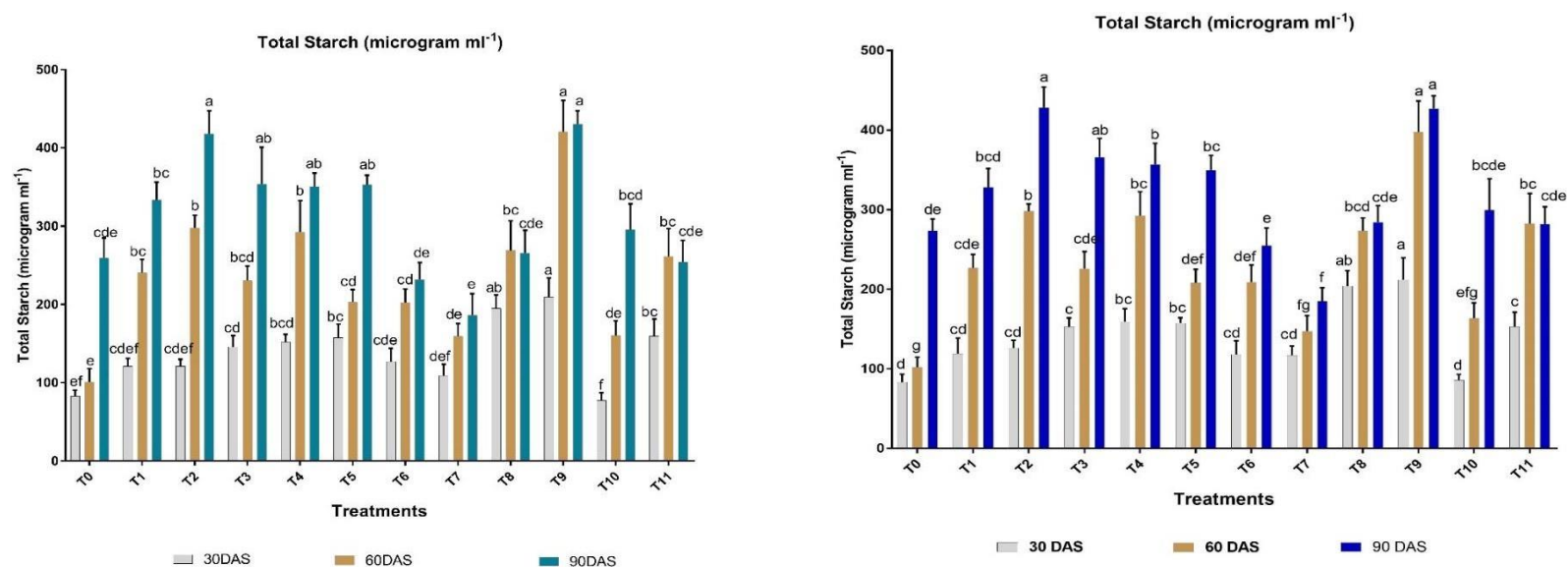
constituent of the plant's defence mechanism. The heightened ability to withstand stress is observed as a manifestation of the enlarged starch storage capacity, serving as evidence of the capabilities of SA. It is noteworthy that SA not only oversees the establishment of a defensive arsenal but also governs the allocation of resources within the lush boundaries of the plant. The skilful manipulation of stress responses confers the ability to allocate resources more effectively, including carbon and energy, to the central hub of starch synthesis, leading to a subsequent rise in starch levels. The measurement of mustard's overall starch content unveils an intricate network, offering valuable insights into energy dynamics, seed development, stress resistance, and resource allocation within the plant. It is conceivable that the levels of starch content can be manipulated by employing foliar sprays containing sulphur and salicylic acid. Implementing these strategies can enhance the resilience and productivity of mustard crops, especially in the face of unpredictable environmental conditions. The strategies above resemble the alchemical process of transforming fundamental elements into gold (Peng et al., 2022; Pérez-Llorca et al., 2023; Phokas et al., 2023; Phour & Sindhu, 2022; Poór et al., 2022; Quesada, 2022; Rai & Kaushik, 2023; Raja Gopalan et al., 2022; Ramakrishnan & Zhou, 2022).

**Table 4.33. Impact of Different Treatments on Total Starch of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	83.19 <sup>ef</sup> ±7.43	83.19 <sup>d</sup> ±10.12	101.03 <sup>e</sup> ±17.08	101.84 <sup>g</sup> ±12.87	259.14 <sup>cde</sup> ±25.78	273.73 <sup>de</sup> ±14.66
<b>T1 (Thiourea-1000 ppm)</b>	121.30 <sup>cdef</sup> ±9.72	118.87 <sup>cd</sup> ±19.91	240.49 <sup>bc</sup> ±17.02	226.71 <sup>cde</sup> ±17.08	333.73 <sup>bc</sup> ±22.60	328.06 <sup>bcd</sup> ±23.95
<b>T2 (Salicylic acid-300 ppm)</b>	121.30 <sup>cdef</sup> ±8.77	126.17 <sup>cd</sup> ±9.72	298.06 <sup>b</sup> ±15.82	298.06 <sup>b</sup> ±9.20	418.06 <sup>a</sup> ±29.18	428.60 <sup>a</sup> ±25.55
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	145.62 <sup>cd</sup> ±14.59	152.92 <sup>c</sup> ±11.14	230.76 <sup>bcd</sup> ±18.36	225.90 <sup>cde</sup> ±21.61	354.00 <sup>ab</sup> ±46.74	366.17 <sup>ab</sup> ±23.62
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	152.11 <sup>bcd</sup> ±9.83	159.41 <sup>bc</sup> ±16.19	292.38 <sup>b</sup> ±40.28	292.38 <sup>bc</sup> ±30.41	350.76 <sup>ab</sup> ±17.08	357.25 <sup>b</sup> ±26.31
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	157.79 <sup>bc</sup> ±17.02	157.79 <sup>bc</sup> ±6.43	203.19 <sup>cd</sup> ±15.82	208.06 <sup>def</sup> ±17.08	353.19 <sup>ab</sup> ±11.99	349.95 <sup>bc</sup> ±18.36
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	126.98 <sup>cde</sup> ±17.08	118.06 <sup>cd</sup> ±17.25	202.38 <sup>cd</sup> ±17.25	208.87 <sup>def</sup> ±21.61	231.57 <sup>de</sup> ±22.07	255.08 <sup>e</sup> ±21.89
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	109.14 <sup>def</sup> ±14.59	117.25 <sup>cd</sup> ±11.49	159.41 <sup>de</sup> ±16.19	147.25 <sup>fg</sup> ±19.66	186.17 <sup>e</sup> ±27.66	185.35 <sup>f</sup> ±16.55
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	195.08 <sup>ab</sup> ±17.08	204.00 <sup>ab</sup> ±19.45	268.87 <sup>bc</sup> ±38.02	273.73 <sup>bcd</sup> ±15.82	265.62 <sup>cde</sup> ±29.01	284.27 <sup>cde</sup> ±20.78
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	209.68 <sup>a</sup> ±24.36	212.11 <sup>a</sup> ±27.66	420.49 <sup>a</sup> ±40.19	397.79 <sup>a</sup> ±38.94	430.22 <sup>a</sup> ±17.02	426.98 <sup>a</sup> ±16.19
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	77.52 <sup>f</sup> ±9.72	85.62 <sup>d</sup> ±7.43	160.22 <sup>de</sup> ±18.99	163.46 <sup>efg</sup> ±19.51	295.62 <sup>bcd</sup> ±32.84	299.68 <sup>bcd</sup> ±39.39
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	159.41 <sup>bc</sup> ±22.07	152.92 <sup>c</sup> ±18.36	261.57 <sup>bc</sup> ±35.27	282.65 <sup>bc</sup> ±37.70	254.27 <sup>cde</sup> ±27.66	281.84 <sup>cde</sup> ±21.89
<b>CD</b>	26.018	25.06	46.628	41.863	45.605	38.513
<b>CV</b>	11.042	10.451	11.565	10.428	8.603	7.067

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.33. Total Starch of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**PAL (Phenylalanine ammonia-lyase):** The effect of Sulphur and Salicylic acid and their combination on PAL was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.34, Figure 4.34). In 2021-2022, there was a significant difference in PAL compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the PAL was observed at 30, 60, and 90 DAS. Therefore, at 30 DAS, the percentage increase as compared to T0 was found highest in T7 followed by T10, T9, T2, T4, T3, T6, T8, T5, T1, T11 and the percentage values were 73.33%, 70.58%, 69.23%, 67.74%, 66.10%, 60.78%, 59.18%, 59.18%, 48.71%, 42.85%, 37.5% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T4 followed by T2, T7, T10, T11, T3, T6, T9, T5, T1, T8 and the percentage values were 73.33%, 72.97%, 71.42%, 71.42%, 68.25%, 66.66%, 60%, 53.48%, 50%, 48.71%, 44.44% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T4 followed by T10, T11, T2, T7, T3, T9, T1, T6, T8, T5 and the percentage values were 70.58%, 68.35%, 67.10%, 66.66%, 59.01%, 55.35%, 53.70%, 46.80%, 46.80%, 46.80%, 32.43%, 28.57% respectively. In 2022-2023, there was a significant difference in PAL compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T7, followed by T10, T2, T9, T4, T3, T8, T6, T5, T1, T11, and the percentage values were 77.02%, 75.36%, 73.01%, 72.58%, 70.17%, 66.66%, 65.99%, 65.30%, 61.36%, 48.48%, 45.16% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T2 and T4 followed by T7, T3, T11, T10, T6, T8, T9, T5, T1 and the percentage values were 71.05%, 71.05%, 69.01%, 68.11%, 68.11%, 67.64%, 54.16%, 52.17%, 51.11%, 48.83%, 37.14% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T4 followed by T11, T2, T10, T7, T3, T9, T1, T6, T8, T5 and the percentage values were 73.73%, 66.66%, 66.23%, 65.78%, 56.66%, 55.93%, 54.38%, 44.68%, 40.90%, 31.57%, 27.77% respectively. Evaluating Phenylalanine Ammonia-Lyase (PAL) activity in *Brassica juncea* L. (mustard) is critical in plant physiology and agriculture. This measurement yields significant insights into the plant's reaction to stress and the potential impacts of sulphur (S) and

salicylic acid (SA) foliar spray treatments on mustard crops—the process of synthesising phenolic compounds. The phenylalanine ammonia-lyase (PAL) is a crucial component of the phenylpropanoid pathway, a metabolic pathway responsible for the biosynthesis of phenolic compounds, including phenolic acids and flavonoids (Wang et al., 2023; Wang et al., 2023; Wang et al., 2022; Wang & Komatsu, 2022; Wang et al., 2022; Wang et al., 2022; Wang et al., 2022; Wante et al., 2022; Waters & Nelson, 2023; Wu et al., 2022; Wurms et al., 2023; Xiao et al., 2022). Phenolic compounds serve various roles within the plant kingdom, encompassing their function as a defensive mechanism against herbivores, herbivore pathogens, and abiotic stressors. The plant's capacity to synthesise these compounds can be deduced based on the quantified level of phenylalanine ammonia-lyase (PAL) activity. The activity of pathogen-associated lipids (PAL) constitutes a crucial component of the plant's defence mechanisms. The expression of phenylalanine ammonia-lyase (PAL) is commonly elevated in plants experiencing biotic or abiotic stress, leading to an augmentation in the synthesis of phenolic compounds. These compounds can act as antioxidants and contribute to the amelioration of stress-induced damage. Monitoring PAL activity offers valuable insights into the response of plants to various abiotic stresses, such as drought, pathogens, and environmental conditions. Changes in phenylalanine ammonia-lyase (PAL) activity levels can offer valuable insights into the extent of stress severity and the plant's capacity to acclimatise to it. The synthesis of phenolic compounds involves the production of cysteine, an amino acid. The presence of sulphur can influence the production of cysteine, thereby impacting the activity of PAL. An ample amount of sulphur can catalyse the synthesis of cysteine, a precursor molecule essential for the formation of glutathione, a potent antioxidant. In periods of heightened stress, an elevated glutathione concentration can safeguard phenolic compounds and PAL from oxidative damage. Sulphur is essential for mustard plants as it is crucial in synthesising secondary metabolites that possess sulphur, including glucosinolates. Sulphur plays a significant role in this particular process. When plants are exposed to diverse stressors, such as herbivores or pathogens, the metabolites produced by the plants may serve a protective role, potentially affecting the activity of phenylalanine ammonia-lyase (PAL). When administered as a foliar spray, salicylic acid (SA) can modulate phenylalanine ammonia-lyase (PAL) activity by regulating the plant's defence



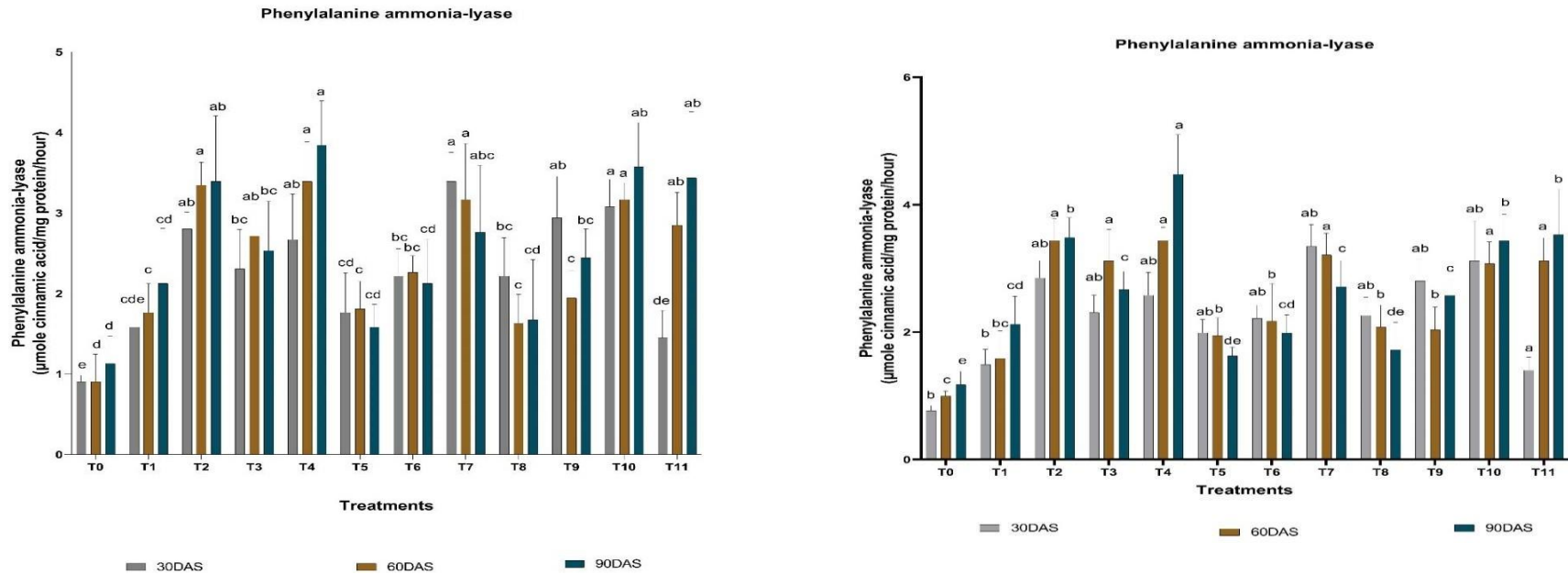
mechanisms. Systemic acquired resistance (SAR) can be elicited against pathogens through the activation of the signalling molecule referred to as salicylic acid (SA). Systemic acquired resistance (SAR) can induce the upregulation of phenylalanine ammonia-lyase (PAL), subsequently resulting in the biosynthesis of phenolic compounds. These processes collectively contribute to the enhancement of plant defence mechanisms. The SA can mitigate oxidative stress and function as an antioxidant, influencing phenylalanine ammonia-lyase (PAL) activity. The ability of stress adaptation (SA) to minimise the detrimental effects of stress can facilitate the maintenance or enhancement of physical activity levels (PAL) in demanding circumstances. The activity of phenylalanine ammonia-lyase (PAL) can be modified by salicylic acid (SA) to contribute to the plant's overall response to environmental challenges. This alteration in PAL activity is part of the plant's stress responses and defence mechanisms. Assessing PAL (phenylalanine ammonia-lyase) activity in mustard plants offers significant insights into their stress responses and ability to synthesise phenolic compounds, which play diverse roles in plant defence mechanisms. The utilisation of sulphur and salicylic acid as foliar spray exhibits the capacity to impact PAL activity through the augmentation of the plant's antioxidant defence mechanisms, synthesis of phenolic compounds, and fortification against the detrimental impacts of environmental stressors. These strategies offer potential avenues for enhancing the mustard plant's disease resistance and overall crop productivity (Todorova et al., 2022; Topcu et al., 2022; Torres & Figueroa, 2023; Tyagi et al., 2022; Ullah et al., 2023; Ullah et al., 2022; Verma et al., 2023; Waadt et al., 2022; Wan & Xin, 2022; Wang et al., 2022).

**Table 4.34. Impact of Different Treatments on PAL (Phenylalanine ammonia-lyase) of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	0.90 <sup>c</sup> ±0.07	0.76 <sup>b</sup> ±0.07	0.90 <sup>d</sup> ±0.34	0.99 <sup>c</sup> ±0.07	1.13 <sup>d</sup> ±0.34	1.17 <sup>e</sup> ±0.20
<b>T1 (Thiourea-1000 ppm)</b>	1.58 <sup>cde</sup> ±0.20	1.49 <sup>b</sup> ±0.23	1.76 <sup>c</sup> ±0.35	1.58 <sup>bc</sup> ±0.43	2.12 <sup>cd</sup> ±0.68	2.12 <sup>cd</sup> ±0.43
<b>T2 (Salicylic acid-300 ppm)</b>	2.80 <sup>ab</sup> ±0.20	2.84 <sup>ab</sup> ±0.27	3.34 <sup>a</sup> ±0.28	3.43 <sup>a</sup> ±0.34	3.39 <sup>ab</sup> ±0.81	3.48 <sup>b</sup> ±0.31
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	2.30 <sup>bc</sup> ±0.48	2.30 <sup>ab</sup> ±0.27	2.71 <sup>ab</sup> ±0.40	3.12 <sup>a</sup> ±0.48	2.53 <sup>bc</sup> ±0.61	2.66 <sup>c</sup> ±0.28
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	2.66 <sup>ab</sup> ±0.56	2.57 <sup>ab</sup> ±0.35	3.39 <sup>a</sup> ±0.48	3.43 <sup>a</sup> ±0.20	3.84 <sup>a</sup> ±0.54	4.47 <sup>a</sup> ±0.62
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	1.76 <sup>cd</sup> ±0.48	1.99 <sup>ab</sup> ±0.20	1.80 <sup>c</sup> ±0.34	1.94 <sup>b</sup> ±0.28	1.58 <sup>cd</sup> ±0.28	1.62 <sup>de</sup> ±0.13
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	2.21 <sup>bc</sup> ±0.34	2.21 <sup>ab</sup> ±0.20	2.26 <sup>bc</sup> ±0.20	2.17 <sup>b</sup> ±0.59	2.12 <sup>cd</sup> ±0.54	1.99 <sup>cd</sup> ±0.28
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	3.39 <sup>a</sup> ±0.35	3.34 <sup>ab</sup> ±0.34	3.16 <sup>a</sup> ±0.69	3.21 <sup>a</sup> ±0.34	2.75 <sup>abc</sup> ±0.82	2.71 <sup>c</sup> ±0.40
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	2.21 <sup>bc</sup> ±0.47	2.26 <sup>ab</sup> ±0.28	1.62 <sup>c</sup> ±0.35	2.08 <sup>b</sup> ±0.34	1.67 <sup>cd</sup> ±0.74	1.71 <sup>de</sup> ±0.43
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	2.93 <sup>ab</sup> ±0.51	2.80 <sup>ab</sup> ±0.34	1.94 <sup>c</sup> ±0.34	2.03 <sup>b</sup> ±0.35	2.44 <sup>bc</sup> ±0.35	2.57 <sup>c</sup> ±0.23
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	3.07 <sup>a</sup> ±0.34	3.12 <sup>ab</sup> ±0.62	3.16 <sup>a</sup> ±0.20	3.07 <sup>a</sup> ±0.34	3.57 <sup>ab</sup> ±0.54	3.43 <sup>b</sup> ±0.41
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	1.44 <sup>de</sup> ±0.34	5.06 <sup>a</sup> ±6.19	2.84 <sup>ab</sup> ±0.40	3.12 <sup>a</sup> ±0.35	3.43 <sup>ab</sup> ±0.81	3.52 <sup>b</sup> ±0.71
<b>CD</b>	0.688	0.495	0.678	0.606	1.105	0.707
<b>CV</b>	17.727	12.835	16.492	14.116	25.4	15.796

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

Figure 4.34. PAL ( $\mu\text{mole cinnamic acid/mg protein/hour}$ ) of Mustard During Rabi 2021-2023 & 2022-23



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Total Free Proline:** The effect of Sulphur and Salicylic acid and their combination on proline was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.35, Figure 4.35). In 2021-2022, there was a significant difference in proline compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the proline was observed at 30, 60, and 90 DAS. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T4, followed by T7, T10, T6, and T11, and the percentage values were 40.88%, 34.52%, 34.36%, 31.25%, 23.41% respectively. But in T9, T1, T2, T8, T3, T5 the percentage decrease as compared to T0 and the percentage values were -3.56%, -20.76%, -44.82%, -56.05%, -167.85%, -191.74% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T7 followed by T10, T4, T6, T9, T11, T1 and the percentage values were 57.65%, 56.47%, 44.33%, 43.52%, 31.91%, 8.24%, 3.71% respectively. But in T2, T5, T8, T3 the percentage decrease as compared to T0 and the percentage values were -24.85%, -38.15%, -97.83%, -105.67% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T7, T4, T11, T8, T5, T9, T1, T6, T3 and the percentage values were 70.28%, 68.35%, 61.28%, 55.50%, 47.71%, 45.59%, 24.04%, 21.83%, 19.48%, 15.69% respectively. But in T2, the percentage decreased compared to T0, and the percentage values were -12.57%. In 2022-2023, there was a significant difference in proline compared to T0 (Control) at 30, 60, and 90 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 30 DAS, the percentage increase as compared to T0 was found to be highest in T4, followed by T7, T11, T6, and T10, and the percentage values were 34.53%, 29.20%, 22.73%, 22.53%, 21.08% respectively. But in T9, T1, T8, T2, T5, T3 percentage decrease as compared to T0 and the percentage values were -23.87%, -58.38%, -81.74%, -89.91%, -157.80%, -169.55% respectively. At 60 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T7, T4, T6, T9, T11 and the percentage values were 59.88%, 59.21%, 52.63%, 46.40%, 37.49%, 16.50% respectively. But in T1, T5, T2, T8, T3 percentage decrease as compared to T0 and the percentage values were -2.08%, -10.55%, -33.72%, -88.80%, -133.96% respectively. At 90 DAS, the percentage increase as compared to T0 was

found highest in T10 followed by T7, T4, T8, T11, T5, T6, T9, T1 and the percentage values were 67.69%, 63.92%, 56.74%, 48.82%, 43.39%, 42.84%, 30.69%, 30.69%, 6.37% respectively. But in T2 and T3, the percentage decreased compared to T0; the percentage values were -9.31% and -31.31%. Measuring total free proline in mustard (*Brassica juncea* L.) is a crucial physiological parameter when investigating plant stress responses and assessing overall plant health. Acquiring knowledge regarding the potential impacts of sulphur (S) and salicylic acid (SA) foliar spray treatments on the overall concentration of free proline in mustard plants can provide valuable insights into strategies for enhancing crop management practices and augmenting the general resilience and productivity of the crop (Singhal et al., 2023; Sivanesan et al., 2022; Song et al., 2023; Sousa et al., 2022; Spinoso-Castillo & Bello-Bello, 2022; Suliman et al., 2022; Sun et al., 2022; Sun et al., 2023; Sun et al., 2022). Plants frequently exhibit proline accumulation as an osmoprotectant when exposed to water stress, salinity, or other environmental stressors. Proline, an amino acid, regulates osmotic balance in plant cells. In periods of heightened stress, this accumulation aids in maintaining cellular turgor pressure by impeding water loss and subsequent dehydration. Quantifying total free proline levels is crucial for assessing the plant's response to stressors. A significant association exists between heightened proline levels and stress factors, indicating the plant's recognition of environmental adversities. Setting proline levels in mustard plants can yield valuable insights into the intensity and duration of their stress. Furthermore, proline exhibits antioxidant characteristics, enabling it to protect plant cells against oxidative harm induced by reactive oxygen species (ROS) generated during periods of plant stress. The presence of proline as an antioxidant plays a crucial role in preserving the integrity of the cell membrane and promoting the plant's overall health under stressful conditions. The potential impact of sulphur on proline levels in mustard can be attributed to its involvement in the metabolism of sulphur-containing amino acids (Shekhawat et al., 2023; B. Shi et al., 2023; Shi et al., 2022; Si et al., 2023; Singh et al., 2022; Singh & Roychoudhury, 2023; Singh & Nandi, 2022). Proline synthesis is contingent upon an adequate quantity of precursor amino acids, such as glutamate. Amino acids, such as cysteine and methionine, which possess sulphur, are essential constituents of the proteins engaged in the metabolic pathways. An ample supply of sulphur can catalyse the biosynthesis of amino acids, including

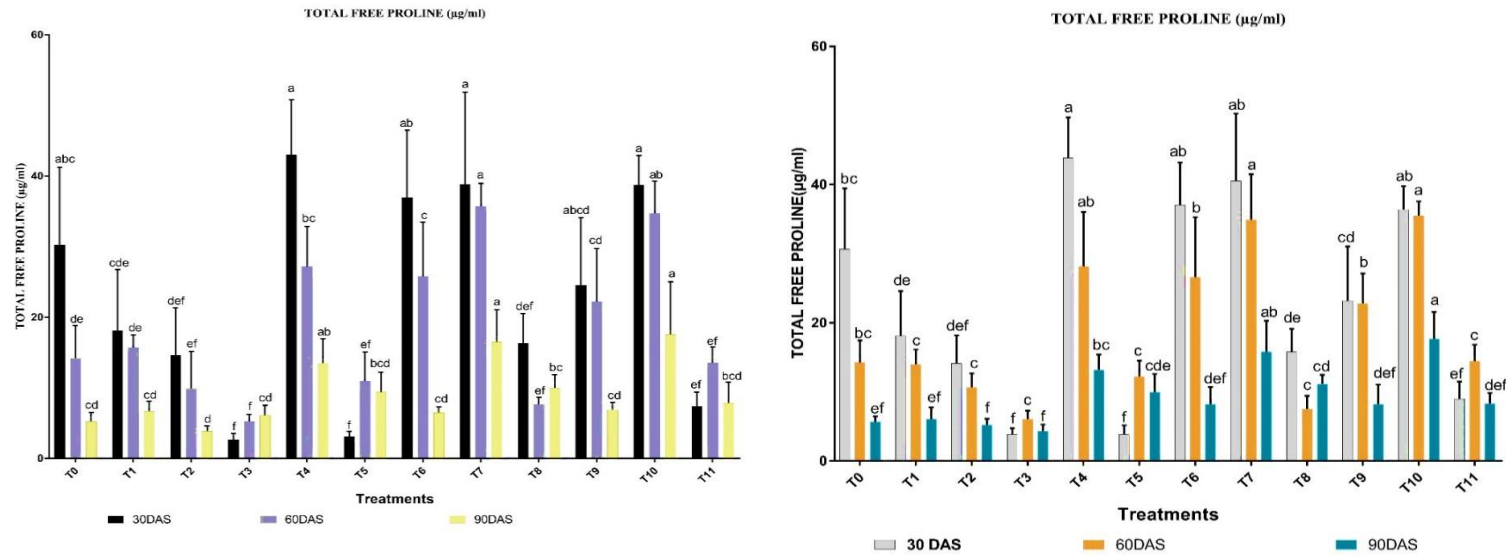
cysteine. Consequently, this process indirectly influences the production of proline. Furthermore, it has been observed that sulphur can act as a catalyst in synthesising secondary metabolites that contain sulphur. A subset of these metabolites has been found to possess antioxidant properties. The metabolites include the capacity to modulate proline levels in mustard plants through their ability to mitigate oxidative stress experienced by these plants. Reducing oxidative stress levels may decrease proline accumulation as a response to stress. The application of SA as a foliar spray can modulate the stress responses of plants, thereby exerting an influence on the proline content in mustard. Systemic acquired resistance (SAR) can be elicited by salicylic acid (SA) as a response to diverse pathogens. The accumulation of proline is often observed as one of the defence mechanisms activated during the plant's stress response, known as systemic acquired resistance (SAR). The SA can regulate the generation of reactive oxygen species (ROS) and modulate the expression of antioxidant enzymes. SA has the potential to influence the levels of proline indirectly (Shah et al., 2022, 2023; Shang et al., 2022; Sharma & Verma, 2023; Sheikhalipour et al., 2023). This is achieved by reducing reactive oxygen species (ROS) levels and enhancing antioxidant capacity. In the event of reduced oxidative stress, it is plausible that the proline accumulation could be diminished, particularly under stress conditions. Mustard plants demonstrate adaptive responses to environmental challenges, wherein stress responses mediated by salicylic acid (SA) may entail alterations in proline metabolism. By quantifying the levels of total free proline in mustard, valuable information can be obtained regarding the plant's capacity to cope with adverse environmental conditions and its corresponding stress responses. Using sulphur and salicylic acid as a foliar spray can influence proline levels by impacting proline synthesis, oxidative stress, and the plant's stress response. Implementing these strategies can enhance the mustard plant's resilience towards focus and augment the overall productivity of the crop, particularly under conditions that are acknowledged to induce stress (Rybczyński et al., 2022; Sachan & Krishna, 2022; Salih, Wu, et al., 2022; Salih, Zhou, et al., 2022; Salwan et al., 2023; Samad et al., 2023; Sampedro-Guerrero et al., 2022; Samtani et al., 2022)

**Table 4.35. Impact of Different Treatments on Total Free Proline of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	30 DAS		60 DAS		90 DAS	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	25.41 <sup>abcde</sup> ±5.13	30.66 <sup>bc</sup> ±8.78	15.12 <sup>bcd</sup> ±3.08	14.24 <sup>c</sup> ±3.20	5.21 <sup>b</sup> ±1.31	5.70 <sup>ef</sup> ±0.77
<b>T1 (Thiourea-1000 ppm)</b>	21.04 <sup>bcde</sup> ±5.69	18.13 <sup>de</sup> ±6.45	15.70 <sup>bcd</sup> ±1.78	13.95 <sup>c</sup> ±2.18	6.67 <sup>b</sup> ±1.43	6.08 <sup>ef</sup> ±1.65
<b>T2 (Salicylic acid-300 ppm)</b>	17.55 <sup>cde</sup> ±4.51	14.15 <sup>def</sup> ±4.04	12.11 <sup>cd</sup> ±2.95	10.65 <sup>c</sup> ±2.03	4.63 <sup>b</sup> ±0.73	5.21 <sup>f</sup> ±0.89
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	9.48 <sup>e</sup> ±1.74	3.85 <sup>f</sup> ±.89	7.35 <sup>d</sup> ±1.87	6.08 <sup>c</sup> ±1.21	6.18 <sup>b</sup> ±1.37	4.34 <sup>f</sup> ±0.93
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	42.99 <sup>a</sup> ±7.82	43.87 <sup>a</sup> ±5.84	27.16 <sup>ab</sup> ±5.68	28.13 <sup>ab</sup> ±7.92	13.47 <sup>ab</sup> ±3.47	13.18 <sup>bc</sup> ±2.22
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	8.71 <sup>e</sup> ±1.02	3.85 <sup>f</sup> ±1.31	10.94 <sup>cd</sup> ±4.13	12.20 <sup>c</sup> ±2.33	9.58 <sup>ab</sup> ±2.51	9.97 <sup>cde</sup> ±2.64
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	36.97 <sup>abc</sup> ±9.49	37.07 <sup>ab</sup> ±6.12	26.77 <sup>ab</sup> ±6.12	26.58 <sup>b</sup> ±8.71	6.47 <sup>b</sup> ±0.84	8.22 <sup>def</sup> ±2.47
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	38.82 <sup>ab</sup> ±13.03	40.56 <sup>ab</sup> ±9.70	35.71 <sup>a</sup> ±3.24	34.93 <sup>a</sup> ±6.58	16.48 <sup>a</sup> ±4.55	15.80 <sup>ab</sup> ±4.51
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	16.28 <sup>de</sup> ±4.24	15.80 <sup>de</sup> ±3.35	7.64 <sup>d</sup> ±1.02	7.54 <sup>c</sup> ±1.89	9.97 <sup>ab</sup> ±1.89	11.14 <sup>cd</sup> ±1.31
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	24.54 <sup>abcde</sup> ±9.53	23.18 <sup>cd</sup> ±7.86	22.21 <sup>bc</sup> ±7.48	22.79 <sup>b</sup> ±4.32	6.86 <sup>b</sup> ±1.05	8.22 <sup>def</sup> ±2.82
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	38.72 <sup>ab</sup> ±4.16	36.39 <sup>ab</sup> ±3.40	34.74 <sup>a</sup> ±4.51	35.51 <sup>a</sup> ±2.04	17.55 <sup>a</sup> ±7.47	17.64 <sup>a</sup> ±3.94
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	33.18 <sup>abcd</sup> ±4.29	9.00 <sup>ef</sup> ±2.47	16.48 <sup>bcd</sup> ±3.53	14.44 <sup>c</sup> ±2.38	11.72 <sup>ab</sup> ±2.33	8.32 <sup>def</sup> ±1.54
<b>CD</b>	11.784	9.573	7.181	6.829	5.379	4.321
<b>CV</b>	26.366	21.207	21.796	20.695	32.977	26.313

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.35. Total Free Proline of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm)



**Economic Yield:** The effect of Sulphur and Salicylic acid and their combination on economic yield was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at harvesting. 2021-2022, there was a significant difference in economic yield compared to T0 (Control) at harvesting (Table 4.36, Figure 4.36). The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the economic yield was observed at harvesting. Therefore, at harvesting, the percentage increase as compared to T0 was found highest in T11 followed by T8, T5, T2, T10, T6, T4, T7, T9, T1 and the percentage values were 25.74%, 19.25%, 18.56%, 16.97%, 15.74%, 15.65%, 11.72%, 11.35%, 9.66%, 7.70% respectively. But in T3, the percentage decreased compared to T0, and the percentage value was -10.23%. 2022-2023, the economic yield significantly differed from T0 (Control) at harvesting. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage increase as compared to T0 was found highest in T11 followed by T6, T5, T8, T2, T7, T1, T4, T9, T10 and the percentage values were 25.91%, 20.13%, 19.90%, 18.91%, 17.27%, 16.91%, 16.28%, 13.21%, 12.09%, 11.21% respectively. But in T3, the percentage decreased compared to T0, and the percentage value was -11.42%. The cultivation of mustard (*Brassica juncea* L.) is deemed crucial as it directly impacts mustard cultivation's profitability and economic sustainability (Singh & Roychoudhury, 2023; Singh & Nandi, 2022; Singhal et al., 2023; Sivanesan et al., 2022; Song et al., 2023; Sousa et al., 2022; Spinoso-Castillo & Bello-Bello, 2022; Suliman et al., 2022). The concept of "economic yield" refers to the quantity and quality of a crop that can be sold and utilised for commercial purposes. The assessment typically involves evaluating various factors, including the marketability of seeds, the content of oil, and the quality of seeds. To enhance financial returns for mustard growers and optimise mustard cultivation practices, it is imperative to comprehend the importance of economic yield. This study explores the potential contributions of sulphur (S) and salicylic acid (SA) in enhancing agricultural productivity (Shah et al., 2022, 2023; Shang et al., 2022; Sharma & Verma, 2023; Sheikhalipour et al., 2023; Shekhawat et al., 2023; Shi et al., 2023; Shi et al., 2022; Si et al., 2023; Singh et al., 2022). The concept of profitability refers to the

ability of a business or organisation to generate financial gains. A mustard farm's economic output significantly influences the farm's financial viability. An augmentation in economic productivity leads to a rise in the quantity of seeds deemed suitable for sale and a corresponding increase in financial gains for agricultural producers. The economic productivity of the mustard crop is a determinant that contributes to the crop's overall worth. Mustard seeds yield an oil that possesses consumable properties and is a fundamental ingredient in diverse sectors, such as the food, pharmaceutical, and cosmetics industries. Consequently, a rise in the economic productivity of the crop leads to a corresponding increase in its overall value. The assessment of mustard's economic yield plays a crucial role in determining the crop's level of competitiveness within the agricultural market. Mustard varieties exhibiting higher yields will likely garner greater interest from potential buyers, potentially leading to elevated market prices and heightened demand. Mustard oil is a crucial component in numerous culinary traditions and domestic kitchens across the globe (Rybczyński et al., 2022; Sachan & Krishna, 2022; Salih, Wu, et al., 2022; Salih, Zhou, et al., 2022; Salwan et al., 2023; Samad et al., 2023; Sampetro-Guerrero et al., 2022; Samtani et al., 2022). Mustard oil is considered a vital culinary ingredient, emphasising the significance of ensuring a consistent availability of this oil to uphold food security. One potential approach to accomplish this objective is to ensure a consistent and significant economic output from the cultivation of mustard. Mustard plants necessitate sulphur as a macronutrient, and the accessibility of sulphur can exert a substantial influence on the economic returns derived from mustard crops. The presence of sulphur is of utmost significance in the oil production process of mustard seeds. The presence of an adequate quantity of sulphur is imperative for the synthesis of oilseeds due to its essential role in the formation of sulphur-containing amino acids. The oil content of mustard seeds can be increased when there is a greater availability of sulphur, potentially leading to a higher economic yield, especially in oilseed varieties. The addition of sulphur can greatly enhance the quality of seeds. This is achieved by augmentation of crucial constituents present in seeds, including oil and protein, thereby enhancing the seeds' nutritional content. The enhanced seed quality of mustard seeds has increased market value, rendering them more attractive to various end-user sectors, such as the edible oil industry. The presence of sulphur enhances the uptake of other vital nutrients, such as nitrogen and phosphorus.

The provision of these essential nutrients is imperative for the optimal development of seeds and the overall growth of plants. The presence of sulphur facilitates enhanced nutrient absorption, notably impacting increasing biomass and seed production. Consequently, this exerts a significant influence on the overall economic yield. Salicylic acid, a compound historically linked to plant defence mechanisms, exhibits the potential to enhance the economic productivity of mustard crops through diverse mechanisms. Salicylic acid (SA) 's stress-responsive properties can enhance mustard plants' resistance to biotic and abiotic stressors. Plants exhibit enhanced allocation of resources towards growth and reproduction under conditions of reduced stress, owing to the activation of salicylic acid (SA)-mediated defence mechanisms. As a result of this phenomenon, the possibility of a rise in seed production and an enhancement in economic yield exists. Research findings have provided evidence that the application of SA positively influences the rates at which seeds undergo germination. A higher rate of germination that is consistent and uniform can result in the production of healthier seedlings. This, in turn, can contribute to enhanced crop establishment and ultimately positively affect economic yield. The application of SA can potentially improve the overall quality of mustard seeds (Poór et al., 2022; Quesada, 2022; Rai & Kaushik, 2023; Raja Gopalan et al., 2022; Ramakrishnan & Zhou, 2022; Rani et al., 2023; Rizvi et al., 2022; Roussos, 2023; Ruidas et al., 2022). The above process can enhance seeds' nutritional composition, amplifying their inherent value for various applications. The enhanced financial gains experienced by farmers due to elevated market prices can be attributed to the enhanced quality of seeds. The economic yield of mustard holds significant importance for mustard growers and the agricultural industry at large. The economic productivity of mustard crops can be substantially affected by the presence of sulphur and salicylic acid, as these substances influence various aspects of the crop's performance. Specifically, they impact the oil content, seed quality, nutrient absorption, disease resistance, stress tolerance, and seed germination of mustard crops (Paalli et al., 2022; Pal et al., 2023; Parada et al., 2022; Parrey et al., 2023; Patel et al., 2022; Peng et al., 2022; Pérez-Llorca et al., 2023; Phokas et al., 2023; Phour & Sindhu, 2022). Understanding these mechanisms holds significant potential for enhancing mustard cultivation and ensuring farmers' financial stability. This knowledge can offer valuable insights for developing more sophisticated agricultural practices and formulating

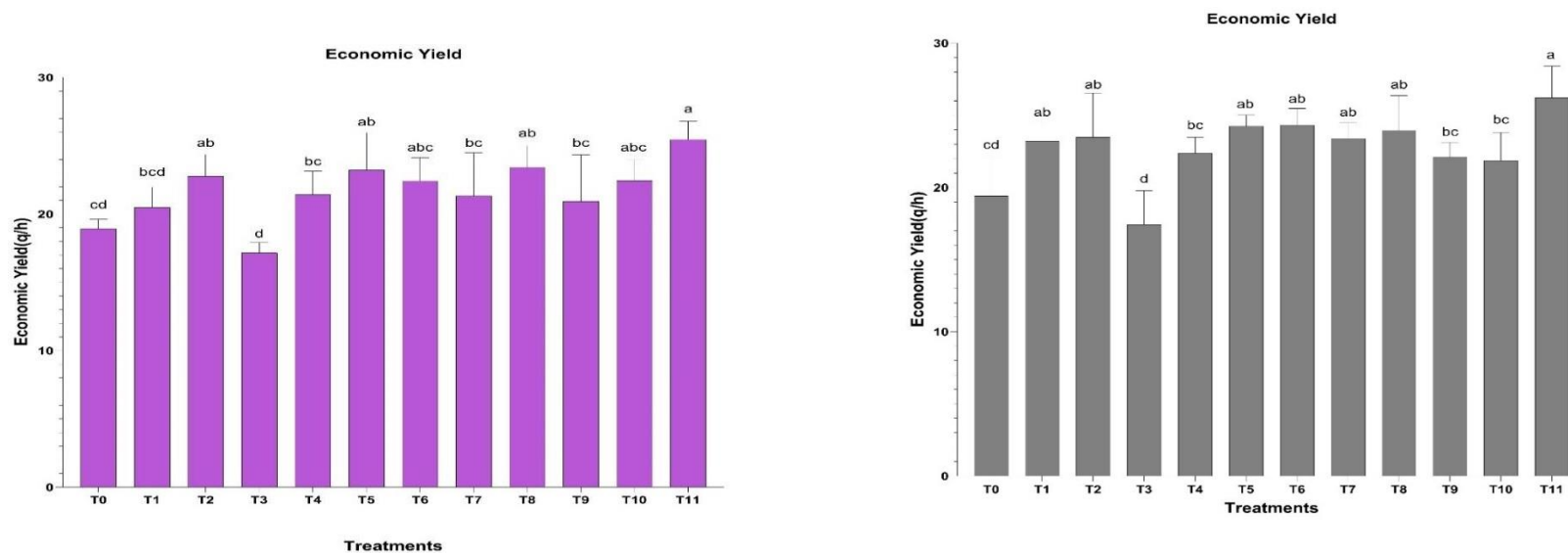
strategies to optimise economic yield. Further investigation into the specific molecular pathways governing interactions between sulphur (S) and salicylic acid (SA) may reveal additional prospects for enhancing crop improvement in mustard cultivation (Nam et al., 2023; Napieraj et al., 2023; Nasir & Toth, 2022; Negi & Kumar, 2023; Nimsi et al., 2023; Niu & Fu, 2022; Ortiz-García et al., 2022, 2023; Ozturk & Unal, 2023)

**Table 4.36. Impact of Different Treatments on Economic Yield, Biological Yield, and Stover Yield of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	Economic Yield		Biological Yield		Stover Yield	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	18.91 <sup>cd</sup> ±0.70	19.41 <sup>cd</sup> ±2.73	56.68 <sup>d</sup> ±2.56	64.17 <sup>ab</sup> ±4.44	37.77 <sup>c</sup> ±2.36	44.75 <sup>a</sup> ±6.53
<b>T1 (Thiourea-1000 ppm)</b>	20.48 <sup>bcd</sup> ±1.47	23.19 <sup>ab</sup> ±1.31	61.74 <sup>bc</sup> ±1.75	65.583 <sup>b</sup> ±8.00	41.25 <sup>bc</sup> ±1.58	42.38 <sup>a</sup> ±7.18
<b>T2 (Salicylic acid-300 ppm)</b>	22.77 <sup>ab</sup> ±1.56	23.47 <sup>ab</sup> ±3.06	66.04 <sup>b</sup> ±1.75	67.35 <sup>ab</sup> ±4.86	43.26 <sup>abc</sup> ±.48	43.88 <sup>a</sup> ±4.82
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	17.15 <sup>d</sup> ±0.76	17.42 <sup>d</sup> ±2.34	62.48 <sup>bc</sup> ±2.57	63.35 <sup>ab</sup> ±3.68	45.33 <sup>ab</sup> ±2.69	45.92 <sup>a</sup> ±1.41
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	21.42 <sup>bc</sup> ±1.71	22.37 <sup>bc</sup> ±1.10	61.26 <sup>c</sup> ±2.03	61.78 <sup>b</sup> ±5.78	39.84 <sup>bc</sup> ±3.48	39.41 <sup>a</sup> ±4.95
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	23.22 <sup>ab</sup> ±2.72	24.24 <sup>ab</sup> ±0.76	65.48 <sup>bc</sup> ±3.30	63.76 <sup>ab</sup> ±2.73	42.26 <sup>abc</sup> ±2.04	39.52 <sup>a</sup> ±2.99
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	22.42 <sup>abc</sup> ±1.69	24.31 <sup>ab</sup> ±1.15	64.51 <sup>bc</sup> ±.68	63.52 <sup>ab</sup> ±4.84	42.08 <sup>abc</sup> ±1.34	39.21 <sup>a</sup> ±3.96
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	21.33 <sup>bc</sup> ±3.16	23.37 <sup>ab</sup> ±1.10	61.25 <sup>c</sup> ±.92	66.45 <sup>ab</sup> ±3.61	39.92 <sup>bc</sup> ±3.12	43.07 <sup>a</sup> ±4.31
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	23.42 <sup>ab</sup> ±1.58	23.95 <sup>ab</sup> ±2.41	64.91 <sup>bc</sup> ±1.83	65.28 <sup>ab</sup> ±6.85	41.48 <sup>bc</sup> ±3.33	41.33 <sup>a</sup> ±7.54
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	20.93 <sup>bc</sup> ±3.40	22.09 <sup>bc</sup> ±1.03	64.22 <sup>bc</sup> ±4.03	63.22 <sup>ab</sup> ±6.33	43.28 <sup>abc</sup> ±6.62	41.13 <sup>a</sup> ±7.34
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	22.44 <sup>abc</sup> ±1.54	21.87 <sup>bc</sup> ±1.93	62.44 <sup>bc</sup> ±1.44	62.35 <sup>b</sup> ±3.73	40.00 <sup>bc</sup> ±1.80	40.48 <sup>a</sup> ±2.74
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	25.46 <sup>a</sup> ±1.31	26.21 <sup>a</sup> ±2.18	72.73 <sup>a</sup> ±1.55	72.97 <sup>a</sup> ±2.15	47.26 <sup>a</sup> ±.91	46.76 <sup>a</sup> ±2.94
<b>CD</b>	3.5	3.279	3.943	N/A	N/A	N/A
<b>CV</b>	9.48	8.49	3.641	7.373	7.258	12.031

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.36. Economic Yield of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

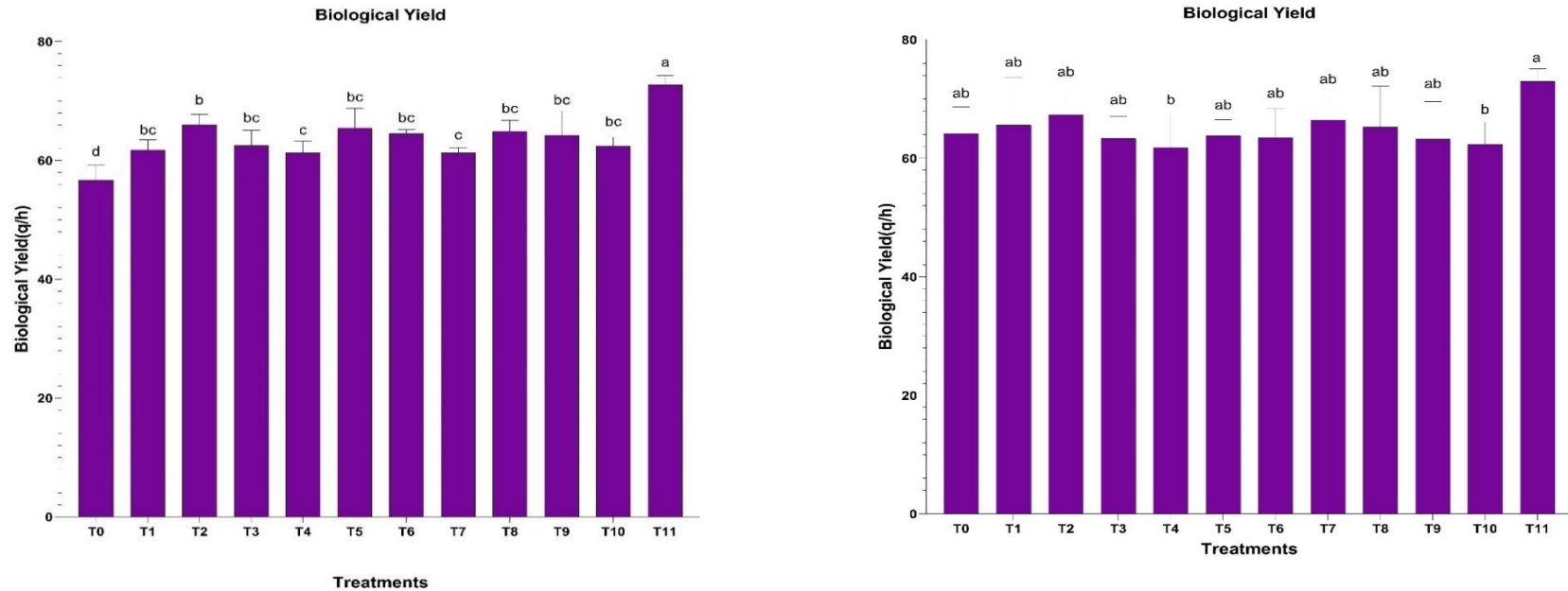
**Biological Yield:** The effect of Sulphur and Salicylic acid and their combination on biological yield was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at harvesting. In 2021-2022, there was a significant difference in biological yield compared to T0 (Control) at harvesting (Table 4.37, Figure 4.37). The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the biological yield was observed at harvesting. Therefore, at harvesting, the percentage increase as compared to T0 was found to be highest in T11, followed by T2, T5, T8, T6, T9, T3, T10, T1, T4, T7 and the percentage values were 22.05%, 14.16%, 13.43%, 12.66%, 12.12%, 11.73%, 9.28%, 9.21%, 8.18%, 7.47%, 7.45% respectively. In 2022-2023, there was a significant difference in biological yield compared to T0 (Control) at harvesting. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage increase compared to T0 was highest in T11, followed by T2, T7, T1, and T8; the percentage values were 12.05%, 4.72%, 3.42%, 2.14%, 1.69% respectively. But in T5, T6, T3, T9, T10, and T4 percentage decrease as compared to T0 and the percentage values were -0.63%, -1.02%, -1.29%, -1.50%, -2.92%, and -3.85% respectively. When engaging in discourse about mustard (*Brassica juncea* L.) research, the phrase "biological yield" denotes the aggregate biomass of the generated plant. This encompasses not solely the photosynthetic components of the botanical organism, such as foliage and stems, but also the generative anatomical features, including siliquas and seeds (Kurniawan & Chuang, 2022; Kuromori et al., 2022; Lajayer et al., 2022; Li et al., 2022; Li et al., 2022; Li et al., 2022; Li, Ren, et al., 2023; Li, Zhang, et al., 2022; Li, Zheng, et al., 2023). To enhance mustard cultivation and increase crop productivity, it is imperative to acquire knowledge regarding the factors that impact biological yield and the potential contributions of sulphur (S) and salicylic acid (SA) in augmenting it. Due to its direct impact on crop yield and overall production, biological yield is essential as a criterion of agricultural output. Various factors, such as genetic traits, environmental conditions, and agronomic practices, can influence the overall biological yield of mustard. The primary goal of mustard cultivation is to optimise both seed and physical products, as these factors significantly impact the overall productivity of the crop. Sulphur, an essential macronutrient for the growth of mustard plants, can substantially influence the biological yield through

various mechanisms. Sulphur assumes a critical function in assimilating nutrients, specifically nitrogen (N), indispensable for the comprehensive development of plants and biomass accrual (Khalid et al., 2023; Khan et al., 2022, 2023; Khan et al., 2022; Kochanek et al., 2023; Kosakivska et al., 2022; Kour et al., 2023; Kudoyarova, 2022; Kumar & Ohri, 2023; Kurepa & Smalle, 2022). The presence of a sufficient quantity of sulphur has the potential to enhance the efficiency of nutrient absorption. Consequently, the above phenomenon would facilitate the enhancement of vigorous vegetative proliferation, encompassing augmented foliar expansion, intensified stem maturation, and heightened root proliferation, culminating in an elevated biological yield. Furthermore, sulphur serves a vital function as a component of amino acids, namely cysteine and methionine, which are indispensable for the mechanism of protein synthesis. Proteins are crucial in various cellular processes, particularly those about plant structure. Enzymes and structural proteins, in particular, are indispensable for plant development and biomass synthesis. Moreover, sulphur-containing amino acids serve as cofactors for enzymes participating in diverse metabolic pathways, exerting additional influence on plant biomass. The modulation of plant hormone synthesis and metabolism, specifically auxins and cytokinins, by sulphur indirectly impacts the biological yield. The hormones being examined are essential for promoting cellular division, cellular expansion, and overall plant growth. The availability of sufficient sulphur can influence the equilibrium of hormones and the subsequent accumulation of vegetative biomass. Salicylic acid, a compound known for its involvement in plant defence mechanisms, has been observed to have the potential to enhance biological yield. The SA can modify the expression of genes implicated in the processes of growth and development. The genes are responsible for cell division, expansion, and biomass accumulation. Due to its capacity to induce cellular proliferation and enlargement across diverse plant tissues, SA may contribute to an augmentation in biomass generation. The stress-responsive properties of SA possess the potential to exert an indirect influence on biological yield. When plants experience reduced stress levels due to the defence mechanisms facilitated by salicylic acid (SA), they may allocate more resources, such as energy and nutrients, towards growth-related processes, including biomass buildup. When sulphur and salicylic acid are combined, they exhibit a synergistic effect that enhances the biological yield of mustard. Sulphur facilitates



plants' uptake of crucial nutrients, such as nitrogen, thereby promoting the growth of vegetative matter and biomass accumulation (Ji et al., 2022; Jia et al., 2022; Jin et al., 2022; Jofre et al., 2023; Kandhol et al., 2023; Kapoor et al., 2022; Kapoor et al., 2022; Karaman, 2023; Karamat et al., 2022; Katoch et al., 2022; Kaviani et al., 2023; Kaya et al., 2023). The efficacy of nutrient uptake by the plant is enhanced by the addition of salicylic acid, thereby stimulating cellular mechanisms that facilitate heightened biomass production. When cultivating mustard, the biological yield assumes significance as it directly influences both the quantity of crop yield and the overall presentation. Mustard plants have the potential to enhance their biological yield through the utilisation of sulphur and salicylic acid, owing to their influence on nutrient uptake, hormonal regulation, protein synthesis, resource allocation, and stress resilience. By comprehending these mechanisms, one can acquire valuable insights that can be utilised in more sophisticated agricultural practices and potential strategies for enhancing biological yield. Consequently, this can increase crop productivity within the context of mustard cultivation. Further investigation into the precise molecular pathways that regulate the interactions between sulphur (S) and salicylic acid (SA) may reveal supplementary avenues for enhancing crop productivity (Huh, 2022; Hui et al., 2022; Huntenburg et al., 2022; Hussein et al., 2023; Iftikhar et al., 2023; Islam et al., 2023; Javadipour et al., 2022).

**Figure 4.37. Biological Yield of Mustard During Rabi 2021-2023 & 2022-23**



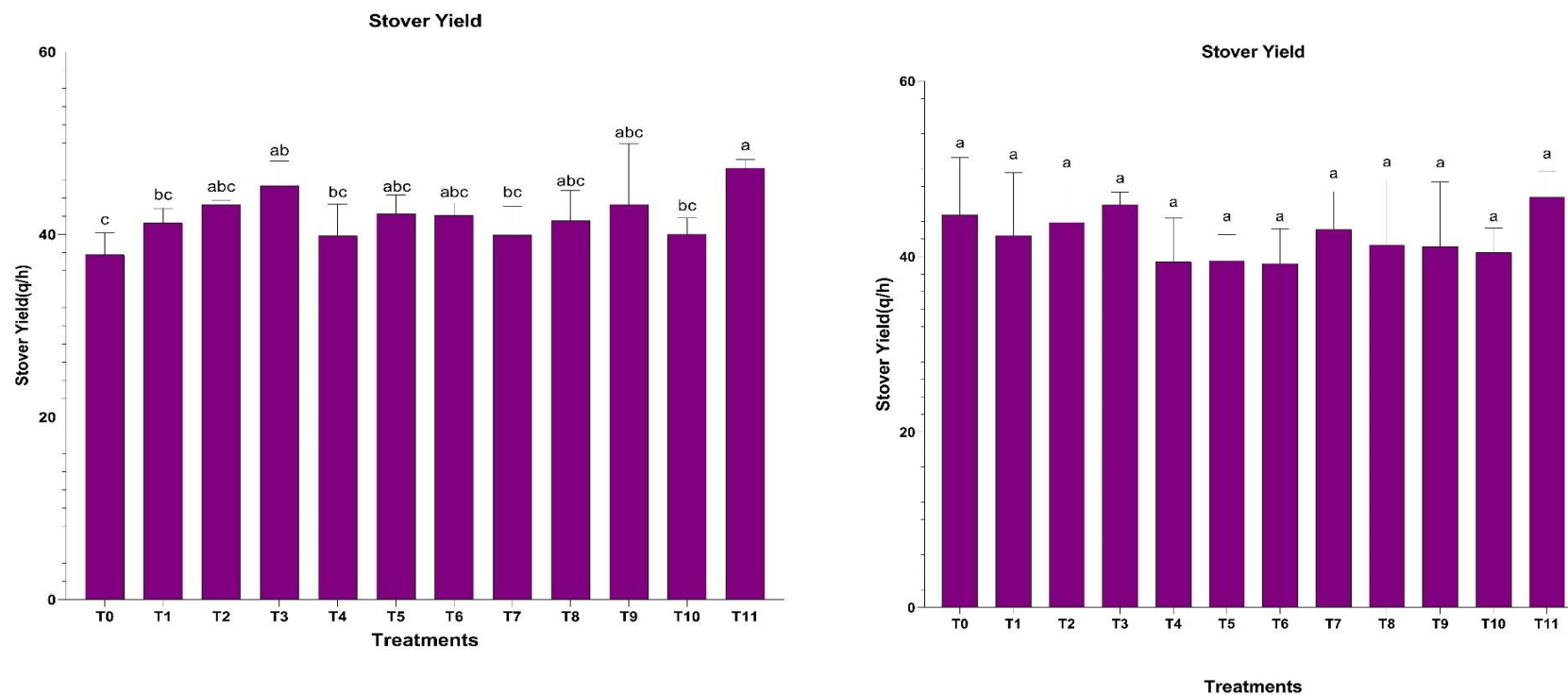
Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Stover Yield:** The effect of Sulphur and Salicylic acid and their combination on stover yield was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at harvesting (Table 4.38, Figure 4.38). In 2021-2022, there was a significant difference in stover yield compared to T0 (Control) at harvesting. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the stover yield was observed at harvesting. Therefore, at harvesting, the percentage increase as compared to T0 was found highest in T11 followed by T3, T9, T2, T5, T6, T8, T1, T10, T7, T4 and the percentage values were 20.07%, 16.66%, 12.73%, 12.68%, 10.62%, 10.24%, 8.94%, 8.42%, 5.55%, 5.37%, and 5.18% respectively. In 2022-2023, there was a significant difference in Stover yield compared to T0 (Control) at harvesting. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage increase compared to T0 was highest in T11 and T3; the percentage values were 4.28% and 2.55%, respectively. But in T2, T7, T1, T8, T9, T10, T5, T4, and T6 the percentage were decrease as compare to T0 and the percentage values were -1.99%, -3.89%, -5.58%, -8.27%, -8.80%, -10.55%, -13.23%, -13.55%, and -14.13% respectively. The stover yield of mustard (*Brassica juncea* L.) holds considerable significance, especially in regions with widespread mustard cultivation. The term "stover" pertains to the aerial portions of a plant, excluding the harvested fruits or seeds. Understanding the importance of stover yield and the potential effects of sulphur (S) and salicylic acid (SA) foliar sprays on mustard stover yield is crucial for optimising agricultural practices and promoting sustainable crop management. Mustard stover consists of vital nutrients and valuable organic matter. After the seed harvesting, the remaining stover can be re-incorporated into the soil as organic mulch or used as livestock fodder (Wang et al., 2022; Wang et al., 2023; Wang et al., 2023; Wang et al., 2022; Wang & Komatsu, 2022; Wang et al., 2022; Wang et al., 2022; Wang et al., 2022; Wang et al., 2022; Wante et al., 2022). Nutrient recycling improves soil fertility and augments the organic carbon content, supporting sustainable agricultural practices. Using straw mulch can function as an organic method for inhibiting the growth of weeds. Mustard stover on the field post-harvest can impede weed growth through the dual mechanisms of light obstruction and physical barrier formation. Mustard stover is crucial in mitigating soil erosion by providing a protective cover for the soil surface, a particularly significant aspect in areas characterised by

sloping topography or high susceptibility to intense precipitation events. Reducing weed competition confers advantages to the mustard crop by preserving soil moisture and nutrients (Topcu et al., 2022; Torres & Figueroa, 2023; Tyagi et al., 2022; Ullah et al., 2023; Ullah et al., 2022; Verma et al., 2023; Waadt et al., 2022; Wan & Xin, 2022). The presence of stover serves as a protective layer, thereby reducing the risk of soil erosion and nutrient loss. The process of stover decomposition contributes organic matter to the soil, thereby enhancing both its structural integrity and water retention capacity. One contributing factor to increased agricultural productivity is the enhanced root growth and nutrient absorption experienced by subsequent crops due to improved soil health. Sulphur is an essential macronutrient for the growth and development of mustard plants. Applying sulphur in foliar form can have various advantages, particularly in stover production. The nutrient content of mustard stover can be enhanced by using a foliar spray containing S. Sulphur, a crucial element in biomass synthesis, as it plays a vital role in producing amino acids and proteins. The increased presence of phosphorus can lead to a higher stover yield due to its positive impact on plant growth. Moreover, sulphur plays a role in enhancing an individual's resilience to stress (Taira & Shiono, 2022; Takeuchi et al., 2022; Talaat, 2023; Tan et al., 2022; Tariq et al., 2023; Tariq et al., 2022; Todorova et al., 2022). Mustard plants with sufficient sulphur exhibit enhanced resilience against environmental stressors, including drought and nutrient deficiencies. Despite challenging growing conditions, it is plausible to achieve a greater stover yield due to the improved stability of the plant. The application of salicylic acid has been observed to positively affect stover yield, as salicylic acid is recognised for its involvement in plant defence mechanisms. The mechanisms above are accountable for this phenomenon. The stress-responsive properties of systemic acquired resistance (SAR) can contribute to the enhanced resistance of mustard plants against diseases and pests. When plants are protected from abiotic stresses through SA-mediated defences, they can allocate a higher proportion of their energy and resources towards stover production. Previous studies have provided evidence to support the notion that SA can enhance photosynthetic efficiency in specific plant species. An augmentation in the photosynthetic rate can lead to an elevation in the biomass quantity generated, encompassing the stover yield (Spinoso-Castillo & Bello-Bello, 2022; Suliman et al., 2022; Sun et al., 2022; Sun et al., 2023; Sun et al., 2022). The

contribution of SA in enhancing the process of photosynthesis has the potential to result in higher amounts of stover. SA has the potential to improve the quality of mustard stover as well. The utilization of SA has been found to promote the growth of plants, leading to enhanced stover quality characterised by improved nutritional content and increased resilience. Consequently, the utilisation of stover becomes a more advantageous asset, either for the enhancement of soil fertility or as a source of sustenance for livestock. The stover yield of mustard is crucial in environmentally sustainable agriculture due to its numerous benefits, such as improving soil quality, suppressing weed growth, and mitigating soil erosion. The potential to enhance mustard stover yield can be attributed to the augmentation of nutrient content, stress tolerance, photosynthesis, and overall plant health by applying foliar sulphur spray and salicylic acid. Implementing these strategies in agricultural practices can enhance stover quantities, yielding benefits for crop management and soil health. Further investigation is necessary to optimise the application techniques and concentrations of sulphur (S) and salicylic acid (SA) to improve mustard stover yield (Singh et al., 2022; Singh & Roychoudhury, 2023; Singh & Nandi, 2022; Singhal et al., 2023; Sivanesan et al., 2022; Song et al., 2023; Sousa et al., 2022).

**Figure 4.38. Stover Yield of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea

**(2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).**

**Harvest Index (HI%):** The effect of Sulphur and Salicylic acid and their combination on harvest index was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at harvesting (Table 4.39, Figure 4.39). In 2021-2022, there was a significant difference in harvest index compared to T0 (Control) at harvesting. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the harvest index was observed at harvesting. Therefore, at harvesting, the percentage increase as compared to T0 was found to be highest in T8, followed by T10, T5, T4, T11, T7, T6, and T2, and the percentage values were 7.62%, 7.11%, 5.71%, 4.70%, 4.61%, 4.11%, 3.90%, 3.12% respectively. But in T1, T9 and T3, the percentage decreased compared to T0, and the percentage values were -0.63%, -1.78%, and -21.48%, respectively. In 2022-2023, there was a significant difference in harvest index (HI) compared to T0 (Control) at harvesting. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage increase as compared to T0 was found highest in T6 followed by T5, T8, T4, T11, T1, T9, T7, T10, T2 and the percentage values were 20.47%, 19.90%, 17.61%, 16.09%, 15.14%, 14.36%, 13.61%, 13.56%, 13.05%, 12.65% respectively. But in T3, the percentage decreased compared to T0, and the percentage value was -11.17%. The Harvest Index (HI) is a crucial parameter in assessing mustard (*Brassica juncea* L.) as a viable agricultural crop, as it plays a pivotal role in evaluating the crop's overall productivity and economic value. Understanding the significance of the Harvest Index and investigating the potential impact of foliar-applied sulphur (S) and salicylic acid (SA) on mustard is crucial for optimising agricultural practices and enhancing crop yield and quality. The Harvest Index quantifies the proportion of the mustard plant's economic yield, typically seeds or grains, about its total above-ground dry matter or biomass. The crop's ability to efficiently convert water, nutrients, and solar energy into valuable harvestable products indicates its performance. A high Harvest Index indicates that a significant proportion of the plant's resources have been allocated towards generating the economic yield, commonly observed as seeds in the case of mustard. This exemplifies the crop's efficient utilisation of available resources to generate valuable commodities. The primary source of income for farmers is derived from the seeds of the mustard plant, owing to their substantial oil and nutrient composition. Mustard is cultivated primarily



for its seeds. A higher Harvest Index signifies a larger proportion of the plant's overall biomass allocated towards seed production, leading to a crop with increased market value. Farmers can derive advantages by monitoring the Harvest Index and striving to enhance it, enabling them to make more informed decisions about resource allocation. In the event of a low Harvest Index, it may be necessary to modify the application of fertilisers, irrigation practises, or other agronomic techniques to enhance seed yield. The consideration of the Harvest Index holds significance for plant breeders. Varieties with a naturally high Harvest Index are desirable due to their greater emphasis on seed production. This particular characteristic can be utilised to enhance the development of mustard cultivars that yield higher quantities of seeds. Mustard plants necessitate sulphur as an essential macronutrient, and the application of sulphur through foliar means can elicit various impacts on the Harvest Index, encompassing the subsequent effects: The presence of sulphur enhances the plant's ability to uptake crucial nutrients, such as nitrogen and phosphorus. These elements are crucial in seeds' germination and the plant's overall physiological development. An adequate supply of nutrients can stimulate seeds' growth and maturation, resulting in a subsequent augmentation of the Harvest Index. Sulphur is a vital constituent of amino acids, specifically cysteine and methionine, which play a crucial role in protein synthesis. Proteins are integral components in various cellular processes, including their involvement in seed formation. An increased accessibility of S may result in enhanced seed quality and a rise in the Harvest Index. Furthermore, sulphur plays a crucial role in enhancing a plant's capacity to withstand and adapt to various forms of stress. Mustard plants exhibit enhanced resilience to environmental stressors, such as drought and nutrient deficiencies, when provided with sufficient quantities of the essential element sulphur. The increased resistance observed in this context possesses the capacity to impact seed yields and improve the Harvest Index positively (Zhang et al., 2022; Zhang et al., 2022; Zhang et al., 2023; Zhao & Hu, 2023; Zhao et al., 2022; Zhao et al., 2022; Zhao et al., 2022; Zhu et al., 2022; Zhu et al., 2023; Zulfiqar et al., 2022). Salicylic acid, renowned for its role in plant defence mechanisms, can exert a favourable influence on the Harvest Index through various mechanisms. SA-mediated defences have been found to protect against a range of diseases and pests in mustard plants, ultimately leading to decreased yield losses. When plants are shielded from these stressors, a greater allocation of

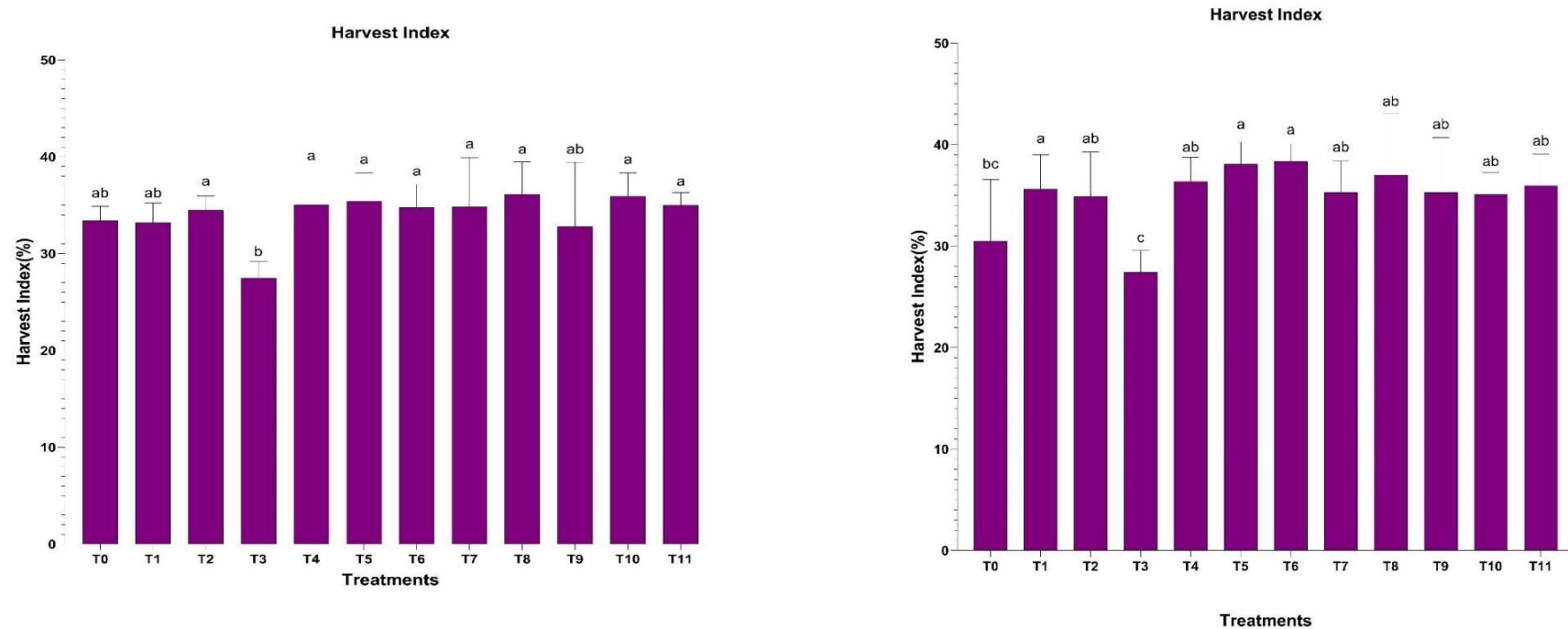
resources can be directed towards seed production, leading to an augmentation in the Harvest Index. Previous studies have provided evidence that applying salicylic acid (SA) can enhance the efficacy of the photosynthetic process in specific plant species. Enhancing the efficiency of photosynthesis has the potential to result in an augmentation of biomass production, thereby facilitating an increase in seed yield. The optimisation of photosynthesis by SA may potentially lead to an increased Harvest Index (Yu et al., 2023; Yuan et al., 2022; Zahid et al., 2023; Zang et al., 2022; Zhang et al., 2023). The supervisory authority (SA) can influence the allocation of resources within the plant. When plants experience reduced stress levels due to activating SA-mediated defence mechanisms, they can allocate more energy and nutrients towards reproductive structures, such as seeds. This ultimately results in an elevation of the Harvest Index. The Harvest Index of mustard is a crucial metric that is an essential indicator of the crop's economic worth and resource utilisation efficiency. The potential impact of applying sulphur and salicylic acid via foliar spray on the Harvest Index is worth considering. This is accomplished through the augmentation of nutrient absorption, the facilitation of protein synthesis, the enhancement of stress tolerance, and the optimisation of resource allocation for seed production. Incorporating these strategies into agricultural practises is promising for enhancing mustard cultivation and crop management, as it can lead to higher seed yields and a more favourable Harvest Index. Further investigation is necessary to optimise S and SA's application techniques and concentrations to maximise the mustard Harvest Index (Yang et al., 2023; Yang & Lee, 2023; Yang et al., 2023; Yang et al., 2022; Yao et al., 2022; Yin et al., 2023; Yousaf et al., 2022; Yu et al., 2022; Yu et al., 2022).

**Table 4.39. Impact of Different Treatments on Harvest Index and Test weight of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	Harvest Index		Test Weight	
	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	33.38 <sup>ab</sup> ±1.47	30.49 <sup>bc</sup> ±6.07	3.55 <sup>c</sup> ±0.06	3.59 <sup>cd</sup> ±0.13
<b>T1 (Thiourea-1000 ppm)</b>	33.17 <sup>ab</sup> ±2.03	35.60 <sup>ab</sup> ±3.37	3.72 <sup>c</sup> ±0.05	3.83 <sup>bc</sup> ±0.22
<b>T2 (Salicylic acid-300 ppm)</b>	34.46 <sup>a</sup> ±1.51	34.90 <sup>ab</sup> ±4.36	4.06 <sup>ab</sup> ±0.05	4.11 <sup>a</sup> ±0.13
<b>T3 (Thiourea-1000 ppm + Salicylic Acid-300 ppm)</b>	27.48 <sup>b</sup> ±1.67	27.42 <sup>c</sup> ±2.12	3.97 <sup>ab</sup> ±0.06	4.01 <sup>ab</sup> ±0.20
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	35.03 <sup>a</sup> ±3.79	36.34 <sup>ab</sup> ±2.39	4.02 <sup>ab</sup> ±9.03	4.06 <sup>ab</sup> ±0.16
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	35.41 <sup>a</sup> ±2.89	38.07 <sup>a</sup> ±2.18	3.55 <sup>c</sup> ±0.03	3.65 <sup>cd</sup> ±0.06
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	34.74 <sup>a</sup> ±2.40	38.34 <sup>a</sup> ±1.71	4.01 <sup>ab</sup> ±0.16	4.09 <sup>a</sup> ±0.08
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	34.82 <sup>ab</sup> ±5.05	35.27 <sup>ab</sup> ±3.13	3.65 <sup>c</sup> ±0.07	3.64 <sup>cd</sup> ±0.13
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	36.14 <sup>a</sup> ±3.32	37.01 <sup>ab</sup> ±6.02	3.58 <sup>c</sup> ±0.07	3.55 <sup>d</sup> ±0.07
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	32.80 <sup>ab</sup> ±6.63	35.29 <sup>ab</sup> ±5.40	3.95 <sup>b</sup> ±0.07	3.98 <sup>ab</sup> ±0.15
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	35.94 <sup>a</sup> ±2.38	35.07 <sup>ab</sup> ±2.15	3.63 <sup>c</sup> ±0.06	3.61 <sup>cd</sup> ±0.07
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	35.00 <sup>a</sup> ±1.29	35.93 <sup>ab</sup> ±3.14	4.17 <sup>a</sup> ±0.04	4.19 <sup>a</sup> ±0.05
<b>CD</b>	N/A	N/A	0.125	0.236
<b>CV</b>	9.987	11.324	1.913	3.591

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.39. Harvest Index of Mustard During Rabi 2021-2023 & 2022-23**



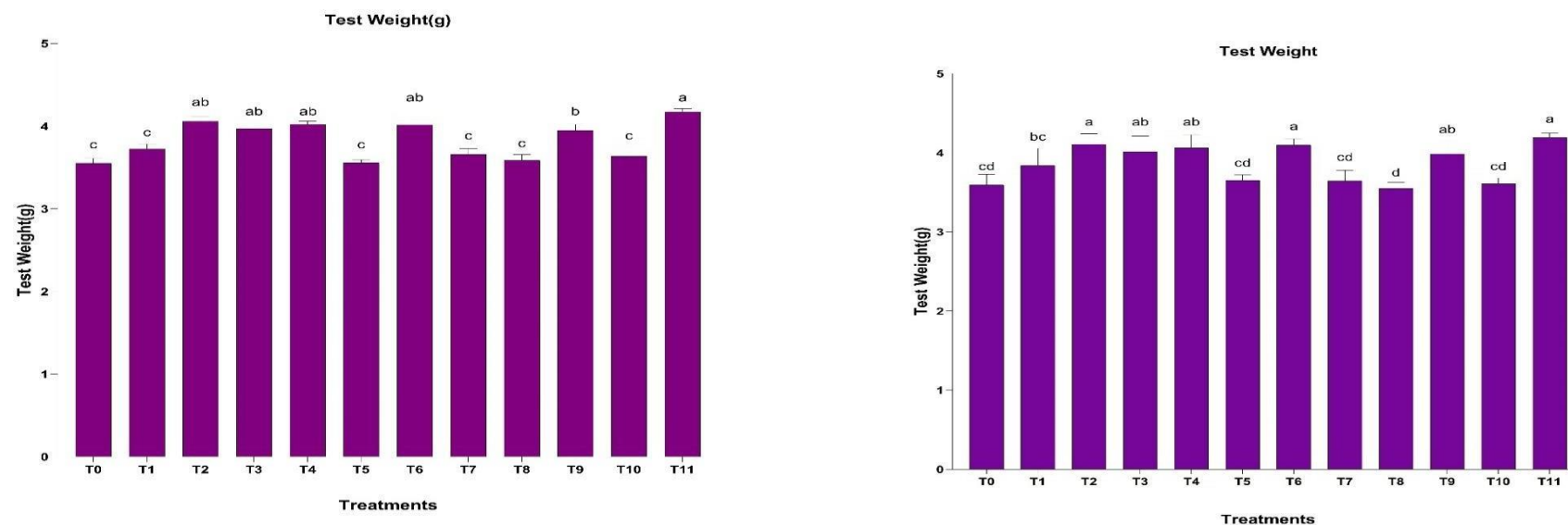
Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Test weight:** The effect of Sulphur and Salicylic acid and their combination on test weight was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at harvesting (Table 4.40, Figure 4.40). In 2021-2022, test weight significantly differed from T0 (Control) at harvesting. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the test weight was observed at harvesting. Therefore, at harvesting, the percentage increase as compared to T0 was found highest in T11 followed by T2, T4, T6, T3, T9, T1, T7, T10, T8, T5 and the percentage values were 14.93%, 12.56%, 11.76%, 11.61%, 10.57%, 10.12%, 4.74%, 2.91%, 2.38%, 1.02%, 0.18% respectively. In 2022-2023, test weight significantly differed from T0 (Control) at harvesting. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage increase as compared to T0 was found highest in T11 followed by T2, T6, T4, T3, T9, T1, T5, T7, T10 and the percentage values were 14.30%, 12.57%, 12.28%, 11.56%, 10.46%, 9.86%, 6.34%, 1.64%, 1.37%, 0.46% respectively. But in T8, the percentage decreased compared to T0, and the percentage value was -1.22%. In agriculture, particularly seed crops such as mustard (*Brassica juncea* L.), the test weight of mustard is a vital parameter that holds considerable agricultural and economic significance. The parameter in question is vulnerable to experiencing substantial effects as a result of the application of sulphur (S) and salicylic acid (SA) via foliar means. The measurement of mustard seed weight is a crucial determinant of seed quality as it provides insights into seed density and overall physical state (Nimsi et al., 2023; Niu & Fu, 2022; Ortiz-García et al., 2022, 2023; Ozturk & Unal, 2023; Paalli et al., 2022; Pal et al., 2023; Parada et al., 2022; Parrey et al., 2023; Patel et al., 2022; Peng et al., 2022). This process is advantageous for the germination of seeds of superior quality, the establishment of vigorous seedlings, and the cultivation of thriving crops. In addition, it is one of the most important factors that decide how much mustard seeds are worth on the market. This phenomenon can be attributed to buyers' preference for elevated test weights, leading to their willingness to offer higher prices for such seeds. Hence, attaining a greater test weight can substantially enhance the economic gains for agricultural producers (Salwan et al., 2023; Samad et al., 2023; Sampedro-Guerrero et al., 2022; Samtani et al., 2022; Shah et al., 2022, 2023). Typically, seeds exhibiting higher test weights are correlated with

increased germination rates and enhanced seedling vigour. The presence of this characteristic is crucial for the successful cultivation of a resilient mustard crop as it guarantees a higher proportion of seeds progressing into viable plants, thereby enhancing the overall crop yield. Moreover, seeds possessing a greater test weight exhibit higher density, reducing the likelihood of sustaining damage during storage or handling procedures. Due to their elevated resistance to mechanical stress, these objects show a reduced chance of experiencing fractures or breakages. In addition to enhancing the overall storability and longevity of the seeds, this practice also mitigates post-harvest losses. Seeds exhibiting a high test weight indicate their well-filled and plump nature, commonly associated with a greater potential yield. More extensive and denser seeds possess more incredible energy and nutrients, facilitating robust initial growth, enhancing crop establishment, and potentially resulting in increased profits. Mustard plants necessitate sulphur as an essential macronutrient, and applying sulphur through the leaves can influence test weight through various mechanisms (Rani et al., 2023; Rizvi et al., 2022; Roussos, 2023; Ruidas et al., 2022; Rybczyński et al., 2022; Sachan & Krishna, 2022; Salih, Wu, et al., 2022; Salih, Zhou, et al., 2022). The inclusion of sulphur enhances the plant's ability to absorb vital nutrients, such as nitrogen (N) and phosphorus (P), which play a crucial role in seed development. The presence of sufficient nutrients can facilitate the process of seed filling and contribute to an increase in test weight. Furthermore, the amino acids cysteine and methionine, which play a crucial role in protein synthesis, necessitate the presence of sulphur as a vital constituent. Increased sulphur availability can lead to improved seed quality and higher test weights. Proteins are crucial in seed development, and sulphur availability can influence this process. The combination of sulphur with denser seeds has the potential to enhance test weights, making it a significant factor contributing to the high value of mustard seeds. The primary value of mustard seeds lies in their oil content. In addition to its widely recognised role in plant defence mechanisms, salicylic acid has been demonstrated to exert a notable influence on test weight. Mustard plants can benefit from activating salicylic acid (SA)-mediated stress responses, enabling them to effectively cope with various environmental stressors, such as pest infestations, diseases, and unfavourable weather conditions (Mukhtar et al., 2023; Myers Jr. et al., 2023; Nadeem, 2022; Nam et al., 2023; Napieraj et al., 2023; Nasir & Toth, 2022; Negi

& Kumar, 2023). Decreased stress levels can result in an augmentation of test weight and a more effective seed-filling process. Furthermore, empirical evidence has shown that SA can enhance the photosynthetic efficiency of specific plant species, potentially resulting in enhanced seed development and increased test weights. Salicylic acid (SA) can also impact the allocation of plant resources (Pérez-Llorca et al., 2023; Phokas et al., 2023; Phour & Sindhu, 2022; Poór et al., 2022; Quesada, 2022; Rai & Kaushik, 2023; Raja Gopalan et al., 2022; Ramakrishnan & Zhou, 2022). Mitigating stress through activating systemic acquired resistance mechanisms enables a more efficient allocation of resources, such as energy and nutrients, towards producing seeds and increasing test weights. The measurement of mustard seed weight is a significant parameter with wide-ranging implications for various factors, encompassing seed quality, seedling vigour, market worth, germination rates, storage, and overall crop productivity. The foliar application of sulphur and salicylic acid shows potential for enhancing test weight by promoting nutrient uptake, protein synthesis, stress tolerance, oil content, and resource allocation to seed filling. Implementing these strategies in agricultural practices may produce mustard seeds of superior quality and enhanced test weights. Implementing this measure would yield advantages for producers of mustard and the broader mustard industry (Mangena, 2022; Manjunatha et al., 2022; Masmoudi et al., 2023; Megala et al., 2022; Mittal et al., 2022; Mohammadi et al., 2023; Molinari et al., 2023; Moustakas et al., 2022; Mugwanya et al., 2023; Mukarram et al., 2022).

**Figure 4.40. Test weight of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).



#### **4D. Thiourea (sulphur) and salicylic acid-mediated effects on yield, yield attributing characters and Biochemical parameters of Indian mustard grown under the open filed condition**

**Oil Content:** The effect of Sulphur and Salicylic acid and their combination on oil content was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.41, Figure 4.41). In 2021-2022, there was a significant difference in oil content compared to T0 (Control) after harvesting. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the oil content was observed after harvesting. Therefore, after harvesting, the percentage increase as compared to T0 was found to be highest in T11, followed by T3, T7, T2, T9, T4, T5, T10, T1, T6, T8, and the percentage values were 28.64%, 22.51%, 22.22%, 21.54%, 21.01%, 19.28%, 17.64%, 16.97%, 12.96%, 12.68%, 12.68% respectively. In 2022-2023, there was a significant difference in oil content compared to T0 (Control) at harvesting. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage increase as compared to T0 was found to be highest in T11, followed by T9, T3, T4, T2, T5, T7, T1, T6, T8, T10, and the percentage values were 35.21%, 29.80%, 28.67%, 27.02%, 26.74%, 26.17%, 25.59%, 23.32%, 22.14%, 21.65%, 20.83% respectively. The measurement of oil content in mustard oil is a crucial parameter that has significant implications for the mustard oil industry and agricultural practices. Furthermore, the enhancement of crop management techniques and the augmentation of mustard oil yield and quality can be facilitated by acquiring knowledge regarding the potential impact of foliar spray applications of sulphur (S) and salicylic acid (SA) on the oil content in mustard. The quantity of oil obtained from mustard seeds directly correlates with the oil content present in mustard oil, commonly expressed as a percentage. The significance of quantifying the amount of oil contained in mustard oil encompasses various dimensions of quality evaluation. The mustard oil's quality and purity assessment is primarily based on its oil content. Typically, an increased oil content is indicative of a greater concentration and superior quality of oil. This is the oil variant that both consumers and industries favour. The extensive utilisation of mustard oil in numerous sectors and culinary contexts renders

it a precious commodity. The oil's composition, specifically its oil content, significantly influences the monetary value of the oil due to its direct impact on the yield achieved during oil extraction and production. Mustard oil is extensively utilised in culinary practices, notably in South Asian cuisine (Kapoor et al., 2022; Karaman, 2023; Karamat et al., 2022; Katoch et al., 2022; Kaviani et al., 2023; Kaya et al., 2023; Khalid et al., 2023; Khan et al., 2022, 2023; Khan et al., 2022). In culinary practises more excellent oil content is deemed advantageous due to its ability to yield a more pronounced and aromatic flavour. The utilisation of technology in various industrial sectors Mustard oil finds extensive utilisation across diverse industrial sectors, encompassing the production of soaps, cosmetics, and biodiesel. To optimise oil extraction efficiency within these industries, it is imperative to increase the oil content. The subsequent mechanisms can be employed to elucidate the impact of sulphur (S) and salicylic acid (SA) on the quantity of oil present in mustard. The Accessibility of Nutrients: Mustard plants necessitate sulphur as a macronutrient for optimal growth, as this element plays an essential role in synthesising lipids and oils. An ample supply of sulphur can catalyse the synthesis of fatty acids, which are the vital constituents of oils. Consequently, there is a potential for an increase in the oil content of mustard seeds. Activating enzymes Sulphur-containing amino acids, such as cysteine and methionine, play a vital role in the enzymatic activation required for lipid and oil synthesis. The augmented accessibility of sulphur in mustard seeds may lead to a subsequent enhancement in oil production, rendering it more efficient. Furthermore, the presence of sulphur affects the oil content and influences the extraction process, thereby impacting the quantity of oil that can be extracted. Mustard seeds with a higher oil content yield a more significant amount of oil when subjected to extraction than seeds with a lower oil content. Consequently, this results in increased aggregate oil production. The physiological reaction to stress is commonly referred to as salicylic acid (SA). The involvement of SA in initiating stress responses in plants is widely recognised. SA-mediated pathways can induce oil accumulation in mustard plants under stress conditions, including pest infestation, disease, or unfavourable environmental circumstances. This phenomenon is integral to the plant's physiological reaction to stress. The lipid and oil synthesis process in mustard seeds can be modulated by salicylic acid (SA), resulting in an augmentation of lipid synthesis. Enlarging seed oil content can be attained by activating

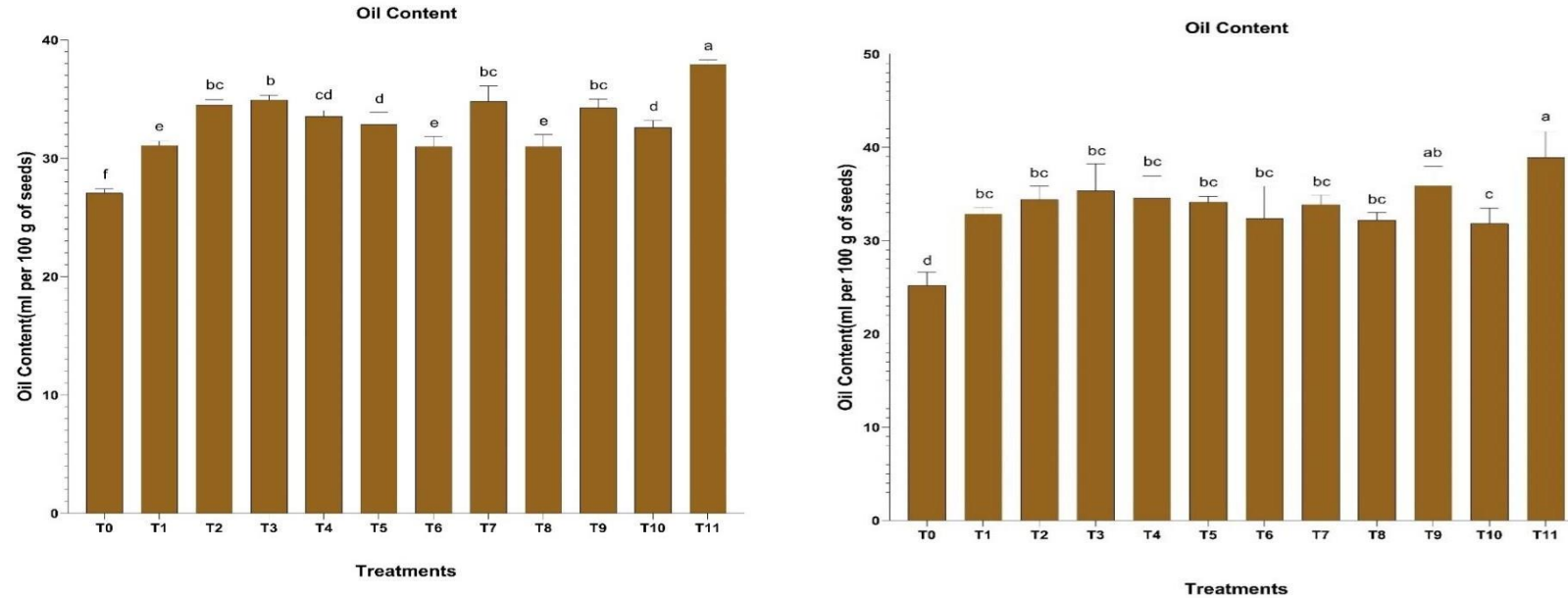
specific genes and enzymes participating in lipid biosynthesis (Jia et al., 2022; Jin et al., 2022; Jofre et al., 2023; Kandhol et al., 2023; Kapoor et al., 2022). Enhanced Seed Production: The involvement of SA in mitigating plant stress may indirectly influence the oil content in seeds. The application of SA can potentially enhance seed development and oil accumulation by facilitating improved plant health and stress tolerance. This phenomenon enhances the plant's capacity to endure unfavourable circumstances. When assessing the quality, economic value, and applicability of mustard oil, its fat content emerges as a critical parameter to be considered. The utilisation of sulphur and salicylic acid as a foliar spray exhibits promising prospects in modifying the oil content of mustard seeds. This is achieved through the stimulation of lipid and oil biosynthesis, enhancement of nutrient accessibility, and induction of stress responses, ultimately leading to a notable augmentation in oil accumulation. These interventions offer prospects for maximising the production of mustard oil, augmenting yields, and improving the oil's quality. Further research is imperative to comprehensively understand the precise mechanisms at play and ascertain the most effective application methods and concentrations, thereby enabling the successful attainment of desired outcomes (Huh, 2022; Hui et al., 2022; Huntenburg et al., 2022; Hussein et al., 2023; Iftikhar et al., 2023; Islam et al., 2023; Javadipour et al., 2022; Ji et al., 2022).

**Table 4.41. Impact of Different Treatments on Oil Content of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	Oil Content		Oil Cake	
	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	27.06 <sup>f</sup> ±0.35	25.20 <sup>d</sup> ±1.41	70.80 <sup>f</sup> ±0.91	71.23 <sup>f</sup> ±1.02
<b>T1 (Thiourea-1000 ppm)</b>	31.10 <sup>e</sup> ±0.36	32.86 <sup>bc</sup> ±0.70	73.13 <sup>e</sup> ±0.80	74.73 <sup>de</sup> ±1.65
<b>T2 (Salicylic acid-300 ppm)</b>	34.50 <sup>bc</sup> ±0.45	34.40 <sup>bc</sup> ±1.45	73.23 <sup>e</sup> ±1.07	74.10 <sup>e</sup> ±0.30
<b>T3 (Thiourea-1000 ppm + Salicylic Acid-300 ppm)</b>	34.93 <sup>b</sup> ±0.40	35.33 <sup>bc</sup> ±2.90	76.66 <sup>cd</sup> ±0.65	76.30 <sup>cd</sup> ±1.75
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	33.53 <sup>cd</sup> ±0.50	34.53 <sup>bc</sup> ±2.37	73.33 <sup>e</sup> ±1.52	75.03 <sup>de</sup> ±0.76
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	32.86 <sup>d</sup> ±1.02	34.13 <sup>bc</sup> ±0.60	74.56 <sup>e</sup> ±1.50	75.36 <sup>de</sup> ±0.81
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	31.00 <sup>e</sup> ±0.86	32.36 <sup>bc</sup> ±3.44	74.40 <sup>e</sup> ±1.21	75.16 <sup>de</sup> ±1.04
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	34.80 <sup>bc</sup> ±1.31	33.86 <sup>bc</sup> ±1.02	78.90 <sup>ab</sup> ±1.01	79.26 <sup>b</sup> ±0.92
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	31.00 <sup>e</sup> ±1.00	32.16 <sup>bc</sup> ±0.85	73.86 <sup>e</sup> ±0.80	73.96 <sup>e</sup> ±1.35
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	34.26 <sup>bc</sup> ±0.73	35.90 <sup>ab</sup> ±2.06	75.00 <sup>de</sup> ±1.00	75.43 <sup>de</sup> ±0.40
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	32.60 <sup>d</sup> ±0.60	31.83 <sup>c</sup> ±1.65	77.33 <sup>bc</sup> ±0.57	77.76 <sup>bc</sup> ±0.73
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	37.93 <sup>a</sup> ±0.40	38.90 <sup>a</sup> ±2.77	79.66 <sup>a</sup> ±1.15	81.20 <sup>a</sup> ±0.62
<b>CD</b>	1.222	3.245	1.871	1.812
<b>CV</b>	2.175	5.692	1.462	1.402

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.41. Oil Content of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

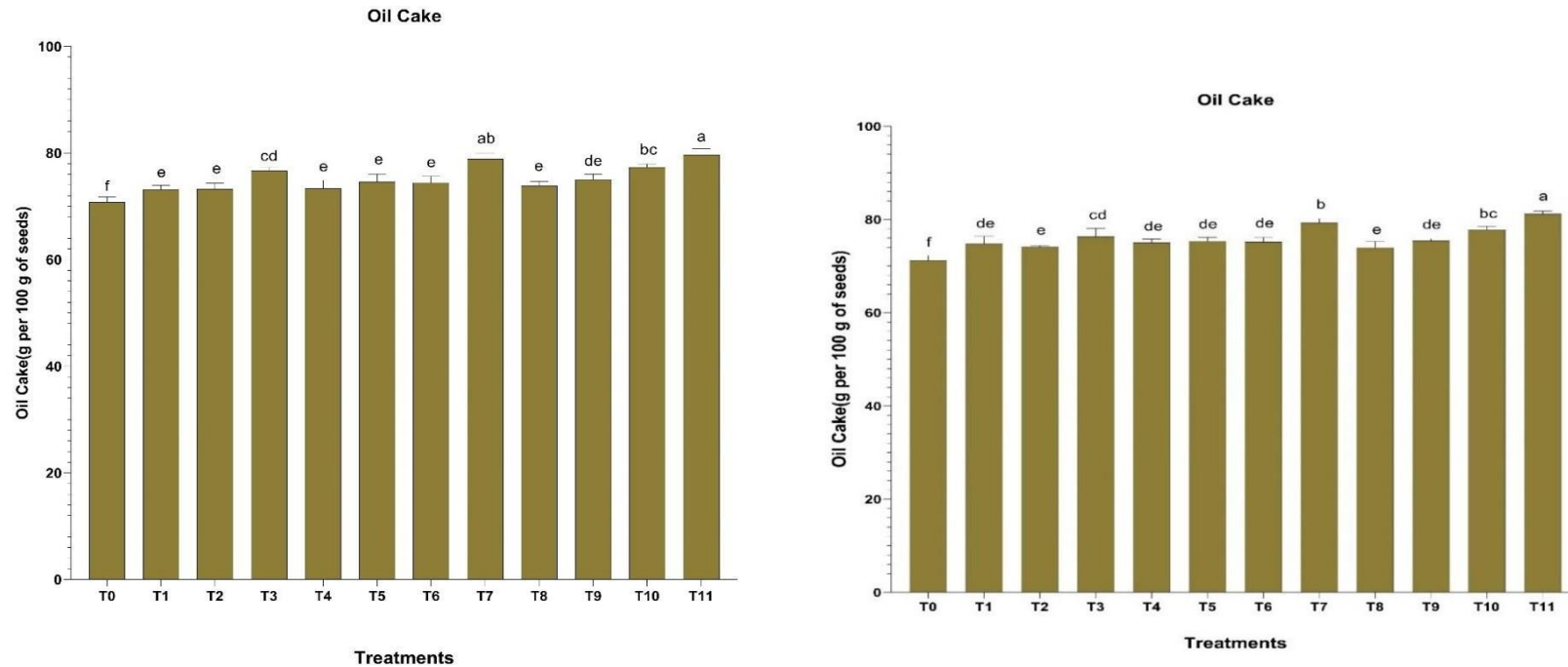
**Oil Cake:** The effect of Sulphur and Salicylic acid and their combination on oil cake was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded after harvesting (Table 4.42, Figure 4.42). In 2021-2022, there was a significant difference in oil cake compared to T0 (Control) after harvesting. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the oil cake was observed after harvesting. Therefore, after harvesting, the percentage increase as compared to T0 was found to be highest in T11, followed by T7, T10, T3, T9, T5, T6, T8, T4, T2, T1 and the percentage values were 11.12%, 10.26%, 8.44%, 7.65%, 5.6%, 5.05%, 4.83%, 4.15%, 3.45%, 3.32%, 3.19% respectively. In 2022-2023, there was a significant difference in oil cake compared to T0 (Control) at harvesting. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage increase as compared to T0 was found highest in T11 followed by T7, T10, T3, T9, T5, T6, T4, T1, T2, T8 and the percentage values were 12.27%, 10.13%, 8.40%, 6.64%, 5.56%, 5.48%, 5.23%, 5.06%, 4.68%, 3.86%, 3.69% respectively. Oil cake is an essential component in both agricultural and industrial sectors, primarily due to its crucial involvement in the production of mustard oil. Additionally, oil cake is closely associated with applying sulphur (S) and salicylic acid (SA) through foliar spray methods. Oil extraction from mustard seeds yields a byproduct called oil cake, also known as oilseed cake or meal. A comprehensive comprehension of the importance and impact of S and SA on the oil cake composition in mustard is imperative. Oil cake is a highly nutritious substance that offers many essential nutrients, such as proteins, carbohydrates, and vital minerals. Due to its substantial nutritional composition, it is frequently employed as an animal feed source. The utilisation of this substance as a dietary supplement is commonly observed in animal husbandry, specifically livestock and poultry. Due to its high nutritional content, it plays a crucial role in animal husbandry by promoting the growth and well-being of animals. The topic of discussion pertains to organic fertilisers. Oil cake is an exceptional organic fertiliser with considerable potential for application in agriculture. The presence of essential nutrients, including nitrogen, phosphorus, and potassium, is imperative for the optimal growth and development of plants (Ullah et al., 2023; Ullah et al., 2022; Verma et al., 2023; Waadt et al., 2022; Wan & Xin, 2022). Integrating oil cake into the soil holds promise

for enhancing soil fertility and nutrient accessibility, thereby resulting in heightened agricultural yield. Oil cake is commonly employed in the manufacturing process of biodiesel. Oil cake is frequently utilised in the production of biodiesel in industrial applications. The residual oil present in the cake can be extracted and subsequently converted into biodiesel, which serves as a renewable and environmentally friendly fuel source. Enhanced Seed Development Sulphur is an essential macronutrient necessary for the survival of mustard plants, playing a crucial role in numerous metabolic processes, including seed production. The utilisation of sulphur as a foliar spray exhibits promising prospects in enhancing the overall well-being of plants and promoting seed development in mustard plants. This, in turn, holds the potential to augment the oil content of the seeds. Consequently, the oil cake derived from these seeds may exhibit a higher proportion of residual oil without complete extraction—the availability of nutrients. The presence of sulphur in the soil enhances the plant's ability to uptake essential nutrients, including nitrogen (N) and phosphorus (P). Providing these nutrients is crucial for the seed's maturation and oil synthesis. The oil content of the sources and the resulting oil cake can be enhanced through the augmentation of nutrient availability via sulphur. The role of salicylic acid (SA) in eliciting diverse stress responses in plants is widely recognised. Salicylic acid (SA)-mediated signalling pathways have been found to induce a range of metabolic processes in mustard plants, including seed development and oil biosynthesis. These pathways are activated in response to stressors such as pests, diseases, or unfavourable environmental conditions (Wang et al., 2022; Wang et al., 2023; Wang et al., 2023; Wang et al., 2022; Wang & Komatsu, 2022). The stressors encompass a range of factors, including both biotic and abiotic elements. As a result of this phenomenon, there exists a potential for an augmentation in the oil concentration of mustard seeds, thereby leading to a corresponding elevation in the oil cake. The impact of salicylic acid (SA) on the synthesis of secondary metabolites in plants has been demonstrated. Despite its primary function as a defence mechanism, it possesses the ability to exert an influence on the chemical composition of seeds. Certain compounds, such as lipids and oils, have the potential to accumulate in the sources as a result of pathways induced by salicylic acid (SA), thereby resulting in increased oil content in the oil cake. Manufacturing mustard oil yields a valuable byproduct called oil cake, which has various applications in producing animal feed, organic fertilizers,

and biodiesel. Foliar spray applications of sulphur and phosphorus have the potential to impact the oil cake content in mustard seeds indirectly. This influence arises from their ability to stimulate seed development, improve nutrient accessibility, and initiate stress responses, resulting in heightened oil accumulation. Various strategies can be employed to augment the nutritional value of oil cake and broaden its practical applications in agriculture and industry (Tariq et al., 2022; Todorova et al., 2022; Topcu et al., 2022; Torres & Figueroa, 2023; Tyagi et al., 2022).



Figure 4.42. Oil Cake of Mustard During Rabi 2021-2023 & 2022-23



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Acid Value:** The effect of Sulphur and Salicylic acid and their combination on Acid value was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.43, Figure 4.43). In 2021-2022, there was a significant difference in proline compared to T0 (Control) after harvesting. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the acid value was observed at harvesting. Therefore, at harvesting, the percentage decrease as compared to T0 was found highest in T11 followed by T4, T2, T1, T6, T3, T10, T7, T8 and the percentage values were -5.73%, -7.61%, -13.97%, -25.44%, -26.94%, -35.03%, -39.93%, -54.18%, -93.60% respectively. But in T9 and T5 percentage increase compared to T0, the percentage values were 20.89% and 11.66%. In 2022-2023, there was a significant difference in acid value compared to T0 (Control) at harvesting. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage decrease as compared to T0 was found highest in T11 followed by T4, T2, T1, T6, T3, T10, T7, T8 and the percentage values were -1.89%, -5.63%, -13.42%, -26.02%, -26.39, -33.02%, -33.02%, -43.66%, -108.21% respectively. But in T9 and T5, the percentage increased compared to T0, and the percentage values were 16.63% and 9.07%. The assessment of the acid value of mustard (*Brassica juncea* L.) is a crucial chemical parameter with substantial implications for plant-related investigations, particularly within the agricultural domain. Furthermore, acquiring knowledge regarding the potential impact of foliar spray applications of sulphur (S) and salicylic acid (SA) on the acid value of mustard can yield valuable insights for enhancing crop management practices, overall crop health, and increasing productivity. The objectives above can be achieved by applying S and SA to the crop. The acid value, a crucial chemical parameter, assesses the stability and quality of edible oils, including mustard oil derived from mustard seeds (Kudoyarova, 2022; Kumar & Ohri, 2023; Kurepa & Smalle, 2022; Kurniawan & Chuang, 2022). This is achieved by calculating the quantity of potassium hydroxide (KOH) in milligrams necessary to neutralise the unbound fatty acids contained within one gramme of fat. The data above is subsequently quantified regarding the concentration of unbound fatty acids in the oil. The acid value is essential for determining the quality of the oil for several reasons. The assessment of quality: the acid value serves as a significant indicator for evaluating oil quality. The

detection of elevated acid values indicates oil degradation or inadequate storage conditions, thereby influencing sensory attributes, including flavour, aroma, and shelf life. The topic of discussion pertains to the stability of storage. Monitoring the acid value is crucial in assessing the enduring storage stability of edible oils. The occurrence of an elevated acid value over some time signifies the event of lipid degradation in oil, leading to its rancidity. This degradation process can be attributed to oil exposure to oxygen, heat, or light. Consumer protection refers to the measures and policies implemented to safeguard the rights and interests of consumers in the marketplace. Maintaining a low acid value is imperative in ensuring the safety of edible oils, such as mustard oil, for human consumption. High acid values can serve as an indicator of the presence of potentially harmful free fatty acids. The subject of interest is the antioxidant activity. Sulfur-containing compounds can demonstrate antioxidant activity, characterised by their capacity to scavenge harmful free radicals and participate in the oxidation of oil lipids. The potential reduction in the acid value of mustard oil may be attributed to the preservative properties of sulphur, which can mitigate oxidative damage induced by free radicals, thereby enhancing its quality and stability. The preservation of oil quality is a critical concern in various industries and sectors. A sufficient quantity of sulphur can enhance a plant's capacity to synthesise antioxidants, thereby facilitating the preservation of oil quality in mustard seeds (Kuromori et al., 2022; Lajayer et al., 2022; Li et al., 2022; Li et al., 2022; Li et al., 2022; Li, Ren, et al., 2023). Improving the quality of the oil may lead to a decrease in the acid value. The Mechanisms of Lipid Oxidation Protection Sulphur is an essential element required for the biosynthesis of various compounds, including glutathione. Glutathione plays a crucial role in protecting lipids from oxidative damage. The presence of lower acid values in mustard oil has been identified as a contributing factor in the reduction of lipid peroxidation. The Concept of Antioxidant Defence: Salicylic acid (SA) can enhance the plant's antioxidant defence mechanisms. When mustard plants are exposed to stressors such as increased temperatures or pathogens, they initiate SA-mediated responses that help reduce oxidative damage to lipids. As a result, there is a possibility of a decrease in the observed acid value of the oil. The induction of stress responses in mustard seeds through applying Salicylic acid has contributed to preserving oil quality. This process effectively reduces the likelihood of oil deterioration and rancidity

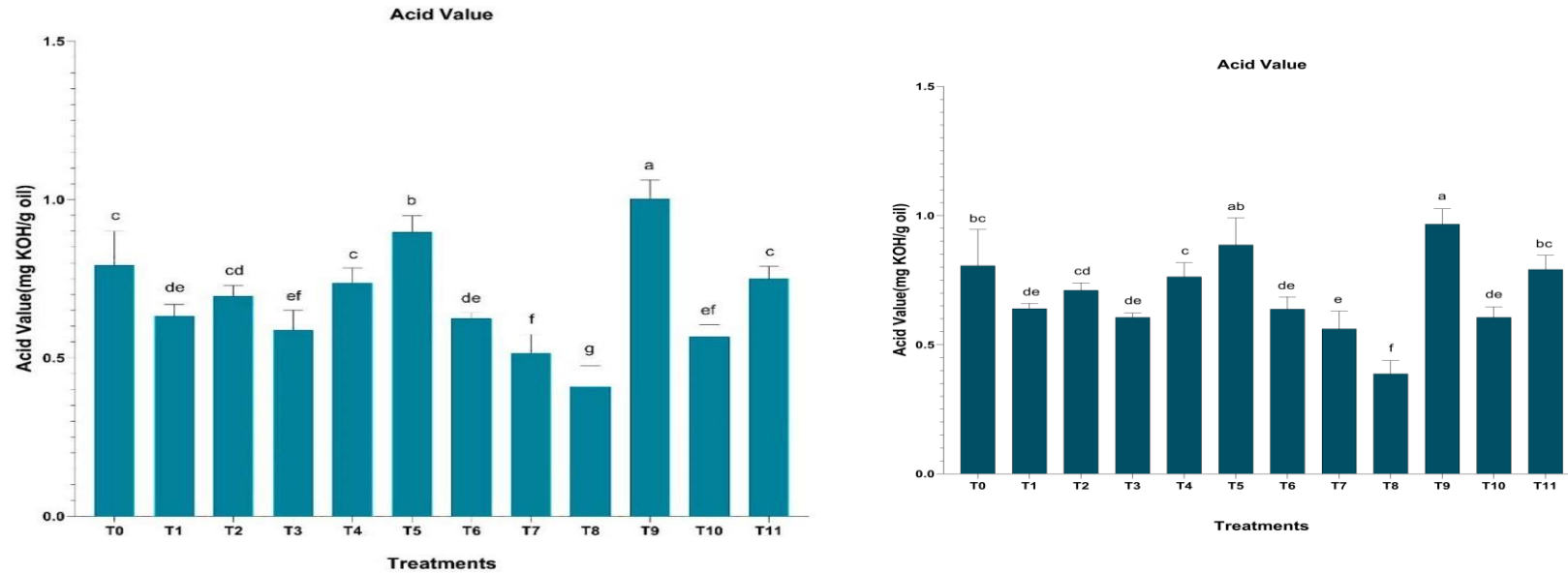
development. Evaluating the acid value in mustard is of utmost importance in assessing the quality and stability of mustard oil derived from mustard seeds. The utilisation of sulphur and salicylic acid as a foliar spray has the potential to influence the acid value through the reduction of oxidative damage, preservation of oil quality, and inhibition of lipid peroxidation. Implementing these strategies holds promise in safeguarding the safety and maintaining the quality of mustard oil. This would yield benefits not only for consumers but also for the mustard industry at large. Fortunately, it is imperative to conduct continuous research to ascertain the optimal application methods and concentrations essential for attaining the desired outcomes (Kochanek et al., 2023; Kosakivska et al., 2022; Kour et al., 2023).

**Table 4.43. Impact of Different Treatments on Acid Value and Iodine Value of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	Acid Value		Iodine Value	
	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	0.79 <sup>c</sup> ±0.10	0.80 <sup>bc</sup> ±0.13	3.08 <sup>a</sup> ±0.57	2.91 <sup>b</sup> ±0.45
<b>T1 (Thiourea-1000 ppm)</b>	0.63 <sup>de</sup> ±0.03	0.63 <sup>de</sup> ±0.02	1.64 <sup>de</sup> ±0.33	1.81 <sup>def</sup> ±0.31
<b>T2 (Salicylic acid-300 ppm)</b>	0.69 <sup>cd</sup> ±0.03	0.71 <sup>cd</sup> ±0.02	2.45 <sup>b</sup> ±0.19	2.28 <sup>cd</sup> ±0.25
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	0.58 <sup>ef</sup> ±0.06	0.60 <sup>de</sup> ±0.01	2.49 <sup>b</sup> ±0.19	2.41 <sup>bc</sup> ±0.12
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	0.73 <sup>c</sup> ±0.04	0.76 <sup>c</sup> ±0.05	1.73 <sup>cde</sup> ±0.19	1.69 <sup>def</sup> ±0.26
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	0.89 <sup>a</sup> ±0.05	0.88 <sup>ab</sup> ±0.10	3.51 <sup>a</sup> ±0.19	3.46 <sup>a</sup> ±0.29
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	0.61 <sup>de</sup> ±0.01	0.63 <sup>de</sup> ±0.04	2.58 <sup>b</sup> ±0.19	2.41 <sup>bc</sup> ±0.25
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	0.51 <sup>f</sup> ±0.05	0.56 <sup>e</sup> ±0.06	2.19 <sup>bc</sup> ±0.26	2.19 <sup>cde</sup> ±0.29
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	0.40 <sup>g</sup> ±0.06	0.38 <sup>f</sup> ±0.05	1.35 <sup>ef</sup> ±0.40	1.31 <sup>fg</sup> ±0.31
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	1.00 <sup>a</sup> ±0.05	0.96 <sup>a</sup> ±0.05	0.93 <sup>f</sup> ±0.14	0.93 <sup>g</sup> ±0.19
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	0.56 <sup>ef</sup> ±0.03	0.60 <sup>de</sup> ±0.04	2.45 <sup>b</sup> ±0.26	2.41 <sup>bc</sup> ±0.21
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	0.74 <sup>c</sup> ±0.03	0.79 <sup>bc</sup> ±0.05	2.15 <sup>bcd</sup> ±0.33	2.11 <sup>cde</sup> ±0.38
<b>CD</b>	0.095	0.114	0.452	0.52
<b>CV</b>	8.117	9.61	11.952	14.084

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.43. Acid Value of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

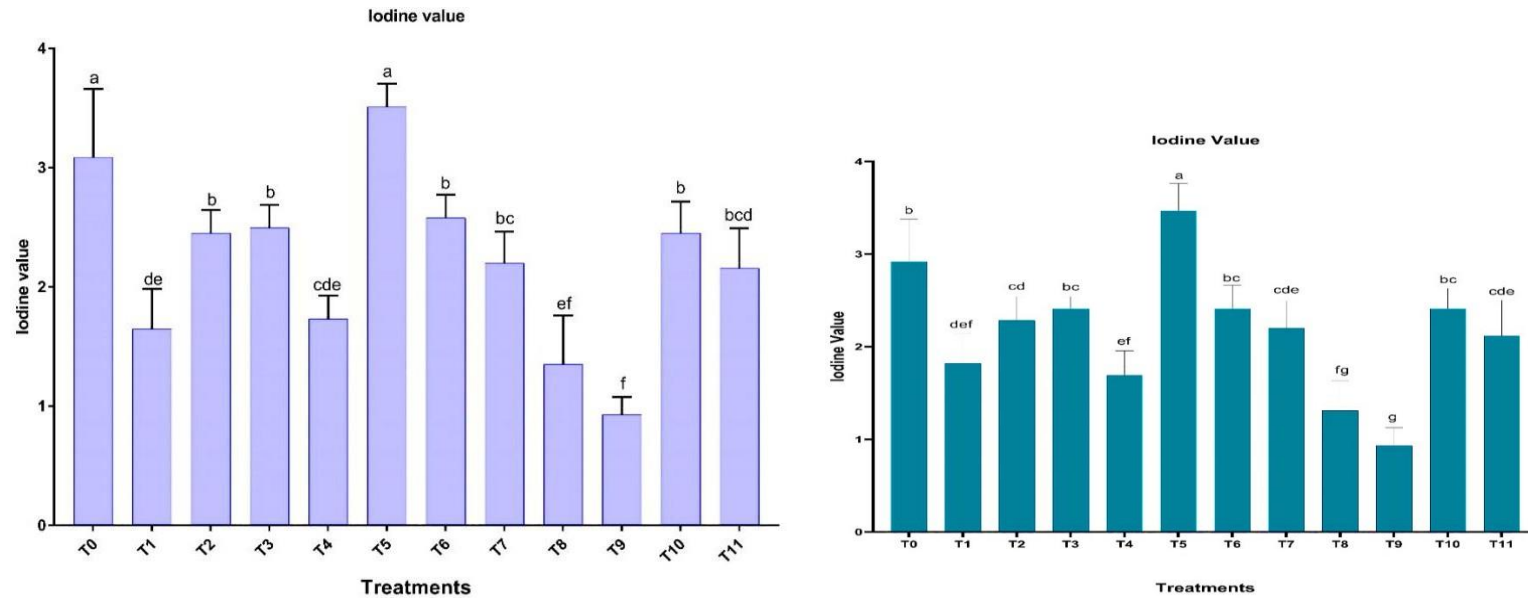
**Iodine Value:** The effect of Sulphur and Salicylic acid and their combination on Iodine value was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded 30, 60, and 90 days after sowing (DAS) (Table 4.44, Figure 4.44). In 2021-2022, there was a significant difference in proline compared to T0 (Control) after harvesting. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the iodine value was observed at harvesting. Therefore, at harvesting, the percentage decrease as compared to T0 was found highest in T6 followed by T3, T2, T10, T7, T11, T4, T1, T8, T9 and the percentage values were -19.67%, -23.73%, -25.87%, -25.87%, -40.39%, -43.14%, -78.10%, -87.20%, -128.20%, -231.88% respectively. However, in T5, the percentage increase compared to T0, and the percentage value was 12.04%. In 2022-2023, there was a significant difference in iodine value compared to T0 (Control) at harvesting. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage decrease as compared to T0 was found highest in T3, T6, T10 followed by T2, T7, T11, T1, T4, T8, T9 and the percentage values were -21.05%, -21.05%, -21.05%, -27.77%, -32.69%, -38%, -60.46%, -72.5%, -122.58%, -213.63% respectively. However, in T5, the percentage increased compared to T0, and the percentage value was 15.85%. Assessing an oil's iodine value is a crucial chemical parameter with substantial implications for discerning the oil's composition, nutritional worth, and appropriateness for diverse applications. This assertion holds particular significance when considering the example of mustard oil. Furthermore, acquiring knowledge regarding the potential impacts of sulphur (S) and salicylic acid (SA) foliar spray applications on the iodine value of mustard oil offers valuable insights into strategies for enhancing agricultural practices and enhancing the overall quality of mustard oil production. Determining unsaturation levels in oil or fat samples can be effectively accomplished using the iodine value, a significant parameter. The provided metric quantifies the iodine absorption capacity of one hundred grammes of the oil, expressed in grammes. The iodine value is a crucial parameter that holds various significant implications. The iodine value is an important parameter that offers valuable insights into the fatty acid composition within the oil. Specifically, it indicates the existence of unsaturated fatty acids, such as oleic acid, linoleic acid, and linolenic acid, along with their respective quantities. The data above is crucial in comprehending the

nutritional composition of the oil, as well as the potential advantages it could offer in terms of one's well-being (Li, Han, et al., 2022; Li, He, et al., 2023; Li et al., 2022). In general, oils with higher iodine values exhibit a greater susceptibility to oxidation and rancidity than oils with lower iodine values. The iodine value has the potential to function as an indicator of the oil's oxidative stability, thereby enabling the assessment of its shelf life and suitability for prolonged storage. Monitoring the iodine value throughout the mustard oil production process is imperative to guarantee uniformity and excellence in the final product. The analysis aids in the identification of discrepancies in the fatty acid composition of the oil, which may have implications for the oil's flavour, scent, and longevity. There is a potential for a substantial reduction in the iodine content of mustard oil when it is adulterated with alternative oils, such as more affordable vegetable oils. The quantification of this parameter can serve as a means to detect cases of adulteration and authenticate the purity of unadulterated mustard oil. The subsequent mechanisms can be employed to elucidate the impact of sulphur (S) and salicylic acid (SA) on the iodine value of mustard oil. Mustard plants require sulphur as an indispensable nutrient because of its central part in producing fatty acids. Sulphur possesses additional significant roles within the plant organism. The fatty acid composition of mustard oil has the potential to change when there is an adequate supply of sulphur available. To provide further clarification, it can promote the amalgamation of diverse unsaturated fatty acids, thereby potentially increasing the iodine value of the oil. A higher iodine value may indicate a higher level of unsaturation; nevertheless, it may also imply an increased susceptibility of the substance to oxidation (Li, Han, et al., 2023; Li, Huang, et al., 2022; Li, Luo, et al., 2022; Li, Tong, et al., 2023). Due to the potential of sulphur to enhance antioxidant activity, the oxidative impact of higher unsaturation could be mitigated, thereby maintaining the stability of the oil. The involvement of salicylic acid (SA) in the plant stress response is widely recognised. Mustard plants may exhibit stress responses through SA-mediated pathways when exposed to diverse stressors, potentially influencing the fatty acid composition of their oil. As a consequence, the iodine's value may change. The potential exists for the involvement of salicylic acid (SA) in mitigating oxidative stress in plants, which may indirectly influence the iodine content. The potential benefit of SA in preserving the unsaturation levels of oil lies in its ability to mitigate oxidative damage



to fatty acids. Assessing the iodine value of mustard oil holds significance in determining its oxidative stability, fatty acid composition, and overall quality. Using sulphur and salicylic acid as a foliar spray can modify the iodine value of oil by altering its fatty acid composition and oxidative characteristics. These interventions present promising opportunities for enhancing mustard oil production while upholding a superior quality standard (L. Li, Zhang, et al., 2022; Li, Zheng, et al., 2023; Li et al., 2022).

**Figure 4.44. Iodine value of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**P-anisidine Value:** The effect of Sulphur and Salicylic acid and their combination on P-anisidine value was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at harvesting (Table 4.45, Figure 4.45). In 2021-2022, there was a significant difference in proline compared to T0 (Control) after harvesting. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the P-anisidine value was observed at harvesting. Therefore, at harvesting, the percentage increase as compared to T0 was found to be highest in T2, followed by T4, T7, T6, and T10, and the percentage values were 46.69%, 30.45%, 17.96%, 12.73%, 12.73% respectively. But in T3, T9, T5, T11, T1, T8 percentage decrease as compared to T0 and the percentage values were -7.87%, -7.87%, -17.09%, -17.09%, -77.92%, -140.35% respectively. In 2022-2023, there was a significant difference in P-anisidine value compared to T0 (Control) at harvesting. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage increase as compared to T0 was found to be highest in T2, followed by T7, T10, T9, T5, T11, T4, T1, and T6, and the percentage values were 59.23%, 40.60%, 40.60%, 25.47%, 20.40%, 20.40%, 14.59%, 7.87%, 7.87% respectively. Although the T8 percentage decreased compared to T0, the percentage value was -20.61%. But, in T3, the treatment's impact was ineffective, and the percentage value was the same as in T0. The evaluation of mustard oil quality and stability is significantly impacted by determining the P-anisidine value, a critical chemical parameter for assessing mustard oil obtained from mustard seeds. To optimise crop vitality and yield to produce premium mustard oil, it is essential to acknowledge the potential impact of foliar spray applications of sulphur (S) and salicylic acid (SA) on the P-anisidine value in mustard (Kochanek et al., 2023; Kosakivska et al., 2022; Kour et al., 2023; Kudoyarova, 2022; Kumar & Ohri, 2023; Kurepa & Smalle, 2022; Kurniawan & Chuang, 2022; Kuromori et al., 2022). During the lipid oxidation process in edible oils, such as mustard oil, the formation of secondary oxidation products, predominantly aldehydes, occurs. The P-anisidine value is utilised to quantify the presence of secondary oxidation products. The significance of P-anisidine in mustard oil is multifaceted. Assessing the freshness and overall quality of edible oils often relies on the significant parameter known as the P-anisidine value. Elevated levels of P-anisidine in oil indicate oxidation, resulting in adverse consequences for the oil's

sensory attributes, including flavour, odour, and overall quality. Monitoring the P-anisidine value is crucial for determining the shelf life of opened mustard oil. Over time, an upward trend in the P-anisidine value suggests the oil's potential to undergo rancidity, rendering it unsuitable for human consumption due to safety concerns. The safety of mustard oil for human consumption is contingent upon its ability to maintain low levels of P-anisidine (Karaman, 2023; Karamat et al., 2022; Katoch et al., 2022; Kaviani et al., 2023; Kaya et al., 2023; Khalid et al., 2023; Khan et al., 2022, 2023; Khan et al., 2022). Elevated levels of P-anisidine may indicate the existence of oxidation byproducts that have the potential to compromise the integrity of the oil, thereby posing a potential hazard. The subsequent array of mechanisms can be employed to elucidate the underlying reasons for the impact of sulphur and salicylic acid on the P-anisidine value of mustard oil. Sulphur-containing compounds, such as glutathione, play a crucial role in facilitating the antioxidant defence system inherent in plants. Sulphur possesses antioxidant properties due to its ability to effectively counteract potentially harmful free radicals and safeguard lipids from oxidative degradation. Mustard plants with an adequate supply of sulphur can generate more antioxidants (Hussein et al., 2023; Iftikhar et al., 2023; Islam et al., 2023; Javadipour et al., 2022; Ji et al., 2022). This enhanced antioxidant production has the potential to impede the formation of aldehydes, subsequently leading to a reduction in P-anisidine levels within the oil they produce. Mustard seeds possess sulphur, a crucial element for synthesising antioxidants and various other compounds that contribute to preserving the oil's superior quality within the seeds. The P-anisidine content in mustard oil can decrease due to improved quality. It is well known that SA can reduce the effects of oxidative stress on plant life. When mustard plants are exposed to various stressors, such as elevated temperatures or pathogens, activating SA-mediated responses may decrease lipid oxidation and aldehydes' formation. Consequently, the concentration of P-anisidine levels in mustard oil may decrease. The application of SA has the potential to elicit stress reactions in mustard seeds, which in turn can contribute to the preservation of oil quality. This is achieved by reducing the probability of oil degradation and the subsequent increase in P-anisidine levels (Jin et al., 2022; Jofre et al., 2023; Kandhol et al., 2023; Kapoor et al., 2022; Kapoor et al., 2022). Assessing the P-anisidine value in mustard oil holds significant importance in evaluating the oil's

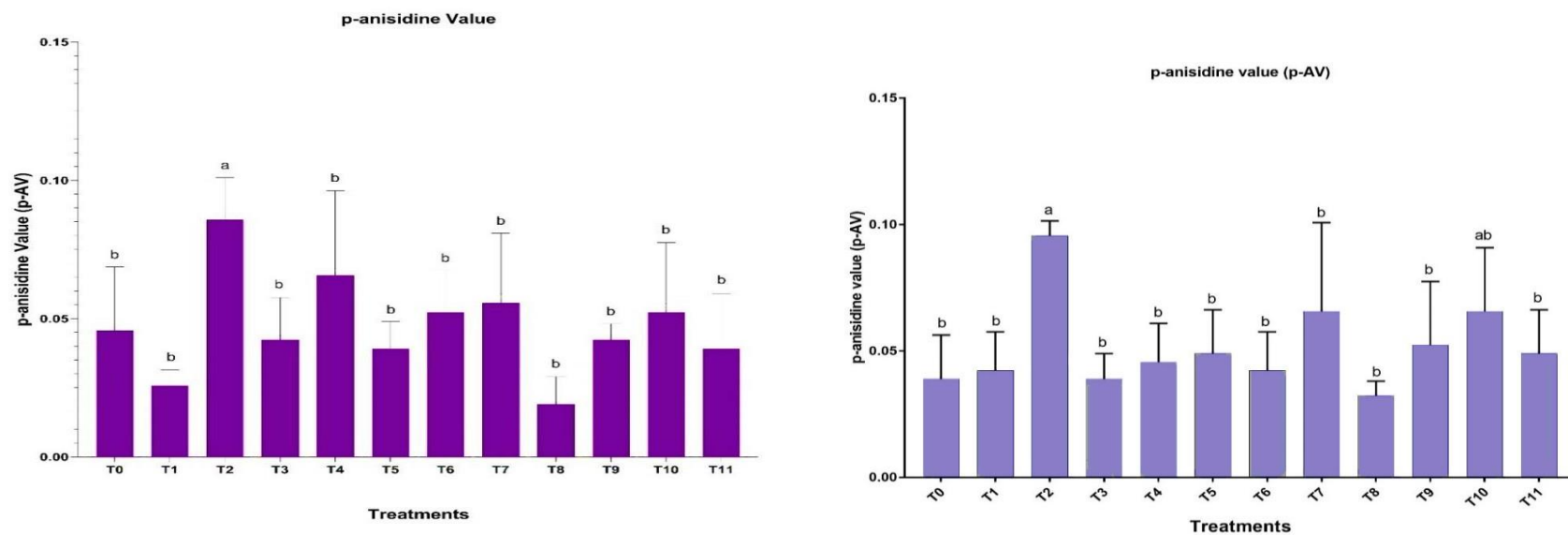
quality and safety. Using sulphur and salicylic acid as a foliar spray can yield notable outcomes on the P-anisidine value through reducing oxidative damage, preserving oil quality, and inhibiting aldehyde formation. Implementing these strategies for protecting the quality and safety of mustard oil holds significant benefits for both consumers and the mustard oil industry (Hernández et al., 2022; Hilal et al., 2023; Hong et al., 2022; Huang et al., 2022; Hudeček et al., 2023; Huh, 2022; Hui et al., 2022; Huntensburg et al., 2022).

**Table 4.45. Impact of Different Treatments on P-anisidine Value and Peroxide Value of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	P-anisidine value		Peroxide Value	
	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	0.045 <sup>b</sup> ±0.023	0.032 <sup>b</sup> ±0.025	0.73 <sup>cde</sup> ±0.30	0.60 <sup>bcd</sup> ±0.20
<b>T1 (Thiourea-1000 ppm)</b>	0.032 <sup>b</sup> ±0.005	0.032 <sup>b</sup> ±0.025	0.40 <sup>de</sup> ±0.20	0.73 <sup>bcd</sup> ±0.41
<b>T2 (Salicylic acid-300 ppm)</b>	0.129 <sup>a</sup> ±0.017	0.109 <sup>a</sup> ±0.020	0.86 <sup>bcd</sup> ±0.11	1.06 <sup>abc</sup> ±0.41
<b>T3 (Thiourea-1000 ppm + Salicylic Acid-300 ppm)</b>	0.032 <sup>b</sup> ±0.025	0.039 <sup>b</sup> ±0.010	1.06 <sup>bc</sup> ±0.30	0.80 <sup>bcd</sup> ±0.34
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	0.062 <sup>b</sup> ±0.055	0.045 <sup>b</sup> ±0.015	0.93 <sup>bcd</sup> ±0.30	1.06 <sup>abc</sup> ±0.50
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	0.032 <sup>b</sup> ±0.015	0.049 <sup>b</sup> ±0.017	0.26 <sup>e</sup> ±0.11	0.33 <sup>d</sup> ±0.23
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	0.042 <sup>b</sup> ±0.030	0.042 <sup>b</sup> ±0.015	1.40 <sup>ab</sup> ±0.40	1.26 <sup>ab</sup> ±0.41
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	0.055 <sup>b</sup> ±0.025	0.065 <sup>b</sup> ±0.035	0.66 <sup>cde</sup> ±0.30	0.60 <sup>bcd</sup> ±0.40
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	0.019 <sup>b</sup> ±0.010	0.032 <sup>b</sup> ±0.005	1.60 <sup>a</sup> ±0.40	1.53 <sup>a</sup> ±0.30
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	0.032 <sup>b</sup> ±0.020	0.039 <sup>b</sup> ±0.036	0.86 <sup>bcd</sup> ±0.30	1.00 <sup>abcd</sup> ±0.20
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	0.052 <sup>b</sup> ±0.025	0.072 <sup>ab</sup> ±0.035	0.43 <sup>de</sup> ±0.15	0.40 <sup>cd</sup> ±0.20
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	0.032 <sup>b</sup> ±0.023	0.049 <sup>b</sup> ±0.017	0.93 <sup>bcd</sup> ±0.41	1.20 <sup>ab</sup> ±0.40
<b>CD</b>	0.031	0.033	0.481	0.518
<b>CV</b>	38.431	37.450	33.348	34.417

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.45. P-anisidine Value of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Peroxide Value:** The effect of Sulphur and Salicylic acid and their combination on peroxide value was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at harvesting (Table 4.46, Figure 4.46). In 2021-2022, there was a significant difference in peroxide value compared to T0 (Control) after harvesting. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the peroxide value was observed at harvesting. Therefore, at harvesting, the percentage increase as compared to T0 was found to be highest in T8, followed by T6, T3, T4, T11, T2, and T9, and the percentage values were 54.16%, 47.61%, 31.25%, 21.42%, 21.42%, 15.38%, 15.38% respectively. But in T7, T10, T1, T5 percentage decrease as compared to T0 and the percentage values were -9.99%, -69.23%, -83.33%, -175% respectively. In 2022-2023, there was a significant difference in peroxide value compared to T0 (Control) at harvesting. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage increase as compared to T0 was found to be highest in T8, followed by T6, T11, T2, T4, T9, T3, and T1, and the percentage values were 60.86%, 52.63%, 50%, 43.75%, 43.75%, 40%, 25%, 18.18% respectively. But in T10 and T5, the percentage decreased compared to T0, and the percentage values were -50% and -80%. The peroxide value of mustard oil is a crucial chemical parameter to assess the product's freshness, safety, and shelf life after its extraction from mustard seeds. To enhance crop management strategies, enhance the general well-being of crops, and ensure the production of mustard oil of superior quality, it is imperative to comprehend the potential influence of foliar spray applications of sulphur (S) and salicylic acid (SA) on the peroxide value in mustard. Determining peroxide value in edible oils, such as mustard oil, is crucial for assessing the extent of lipid oxidation or rancidity within the oil. Peroxides represent the principal outcomes of lipid oxidation, and this assay aims to quantify the concentration of these peroxides within the oil sample. Several reasons highlight the significance of the peroxide value in mustard oil. The assessment of the freshness and quality of edible oils relies significantly on the peroxide value, which is a crucial parameter. The detection of increased peroxide levels indicates oxidative degradation, adversely affecting the oil's sensory attributes, such as flavour, aroma, and overall quality. Monitoring the peroxide value is essential for estimating the shelf life of mustard oil. As time progresses, an upward trend in peroxide

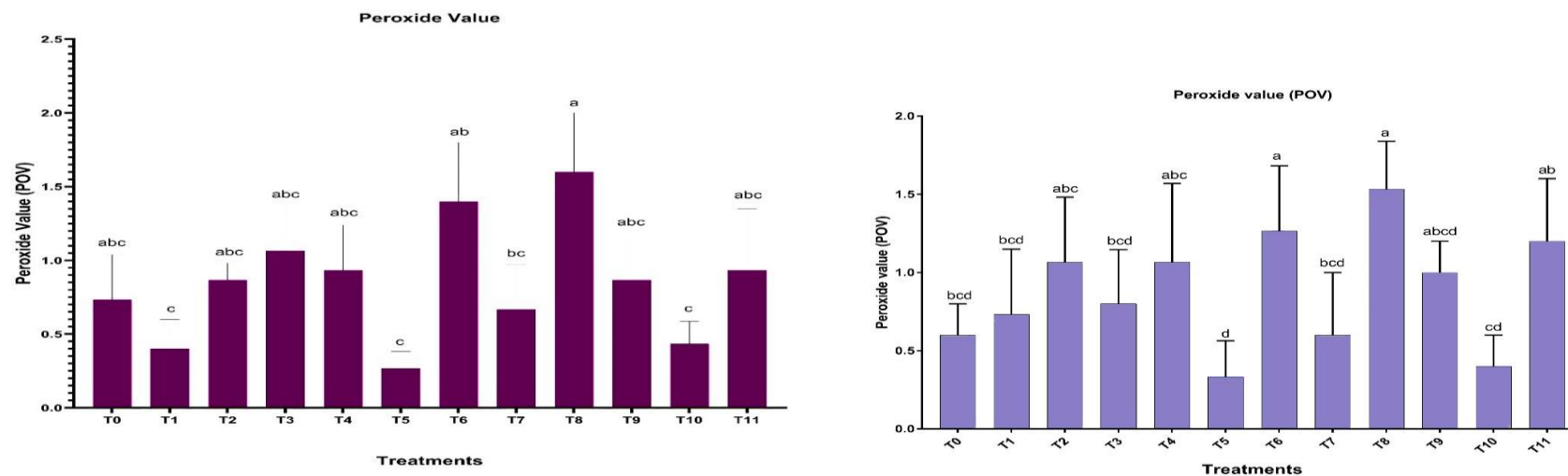


indicates that the oil may have undergone rancidity and is unsuitable for consumption. Mustard oil must uphold minimal peroxide value to be deemed fit for human consumption. Elevated levels of peroxide may serve as an indicator of the existence of potentially hazardous oxidation byproducts, which have the potential to compromise the integrity of the oil (Sun et al., 2022; Sun et al., 2023; Sun et al., 2022; Taira & Shiono, 2022; Takeuchi et al., 2022; Talaat, 2023; Tan et al., 2022). The subsequent array of mechanisms can be employed to elucidate the impact of sulphur and salicylic acid on the peroxide value of mustard oil. Sulphur-containing compounds, such as glutathione, play a vital role in the antioxidant defence system of plants. Sulphur possesses antioxidant properties because it can counteract potentially harmful free radicals and safeguard lipids against oxidative degradation. The provision of sufficient sulphur in mustard plants has been found to promote enhanced synthesis of antioxidants. The potential of these antioxidants lies in their ability to mitigate lipid oxidation in mustard oil, thereby reducing peroxide values.

Mustard seeds possess a high concentration of sulphur, which serves a crucial function in maintaining the quality of oil by facilitating the production of antioxidants and other compounds that aid in preserving oil quality (Song et al., 2023; Sousa et al., 2022; Spinoso-Castillo & Bello-Bello, 2022; Suliman et al., 2022). It is possible to observe a reduction in the peroxide levels of mustard oil due to enhancements in oil quality. In plants, SA is thought to play a role in reducing the effects of oxidative stress. When exposed to environmental stressors like elevated temperatures or pathogens, SA-mediated responses can mitigate lipid oxidation and peroxide formation in mustard plants. Consequently, the levels of peroxide in mustard oil may be reduced. The protection of oil quality in mustard seeds can be achieved through the induction of stress responses by salicylic acid (SA), which reduces the probability of oil degradation and subsequent elevation of peroxide values. Measuring peroxide value in mustard oil is imperative for assessing its quality and safety. Applying sulphur and salicylic acid through foliar spray can influence the peroxide value by mitigating oxidative damage, preserving oil quality, and offering defence against lipid oxidation. These strategies demonstrate significant potential in maintaining mustard oil quality and safety regulations, thus benefiting consumers and the mustard industry (Singh et al., 2022;

Singh & Roychoudhury, 2023; Singh & Nandi, 2022; Singhal et al., 2023; Sivanesan et al., 2022)

**Figure 4.46. Peroxide Value of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Totox Value:** The impact of Sulphur, Salicylic acid, and their combined application on the totox value was investigated in the RH725 variety of Indian Mustard during the growing seasons of 2021-2022 and 2022-2023. Data were meticulously recorded at the time of harvesting (Table 4.47, Figure 4.47). In 2021-2022, a statistically significant difference in totox value emerged compared to the control group (T0) after harvest. To quantify these differences, percentage increases were calculated relative to T0. The observed pattern of percentage increase in totox value at harvest revealed that T8 exhibited the highest increase, followed by T6, T3, T4, T11, T2, and T9, with percentage values of 53.01%, 46.97%, 30.48%, 21.73%, 20.64%, 16.85%, and 14.83%, respectively. Conversely, T7, T10, T1, and T5 displayed a percentage decrease compared to T0, with values of -8.87%, -64.56%, -83.26%, and -164.24%, respectively. In the subsequent year, 2022-2023, a notable variation in totox value compared to the control (T0) was again observed at harvest time. Similar to the previous year, percentage increases were calculated relative to T0. Consequently, at harvest, the highest percentage increase compared to T0 was recorded in T8, followed by T6, T11, T2, T4, T9, T3, T1, and T7, with percentage values of 60.01%, 51.89%, 49.40%, 44.41%, 43.13%, 39.62%, 24.40%, 17.89%, and 2.10%, respectively. However, T10 and T5 exhibited a percentage decrease compared to T0, with values of -43.12% and -73.12%, respectively. The evaluation of the Totox value in oil, particularly in the context of mustard oil, is a noteworthy chemical parameter that holds considerable implications for assessing the freshness, quality, and suitability of the oil for various culinary and industrial applications and in addition, acquiring knowledge regarding the potential impacts of Sulphur (S) and salicylic acid (SA) foliar spray treatments on the Totox value of mustard oil yields valuable insights for enhancing agricultural practices and optimising mustard oil production quality. Mustard oil falls under the classification of edible oils, and the Totox value is a crucial measure for assessing the oxidative stability and overall freshness of such oils (Maia et al., 2022; Makete et al., 2022; Manepalli et al., 2022; Mangena, 2022; Manjunatha et al., 2022; Masmoudi et al., 2023; Megala et al., 2022; Mittal et al., 2022). To achieve this objective, the peroxide value (PV) and the anisidine value (AV) are employed and inserted into the subsequent mathematical expression:  $Totox = (2 * AV) + PV$ . The Totox value holds significant implications, encompassing the following aspects: Assessment of the Novelty of the

Oil: The Totox value is a fundamental indicator of the oil's vulnerability to oxidation and its state of freshness. Fresher oils are typically characterised by lower Totox values, which indicate minimal oxidation and the absence of rancidity and undesirable flavours in the oil (Paalli et al., 2022; Pal et al., 2023; Parada et al., 2022; Parrey et al., 2023; Patel et al., 2022; Peng et al., 2022). The assessment of oxidative degradation can be accomplished by quantifying the Totox value, which indicates the extent to which an oil has experienced oxidative degradation. The observation of elevated Totox values suggests the occurrence of substantial oxidation, which can detrimentally affect the sensory attributes, such as flavour and aroma, as well as the nutritional composition of the oil. Including the Totox value measurement in this section indicates its significance as a crucial aspect of quality control for mustard oil manufacturers. This process aids in guaranteeing that the oil produced meets the required freshness and quality criteria, thereby satisfying consumer expectations. An assessment of the longevity of oil's storage period. Both consumers and producers can perform the estimation of the remaining useful life of oil through the calculation of its Totox value. A direct correlation exists between the Totox value and the oil's shelf life, whereby a lower Totox value signifies an extended duration of quality preservation. The present study explores the potential mechanisms underlying the influence of sulphur (S) and salicylic acid (SA) on the Totox value of mustard oil. Properties in the Battle Against Oxidants: The antioxidant capacity of mustard plants is substantially enhanced by incorporating Sulphur into their soil (Nam et al., 2023; Napieraj et al., 2023; Nasir & Toth, 2022; Negi & Kumar, 2023; Nimsi et al., 2023; Niu & Fu, 2022; Ortiz-García et al., 2022, 2023; Ozturk & Unal, 2023). The potential for influencing the oil's oxidative stability can arise directly or indirectly. A sufficient amount of sulphur can stimulate the plant to enhance its production of sulfur-containing antioxidants, such as glutathione. These antioxidants are crucial in inhibiting oil oxidation, offering valuable preventive benefits. The capacity of sulphur to minimise the generation of harmful free radicals and reactive oxygen species (ROS) renders it a potent agent in mitigating the oxidative deterioration of mustard oil. The reduction of both peroxide value (PV) and toxin value may occur due to the antioxidant action. The induction of stress responses in plants by salicylic acid (SA) is widely recognised in the scientific community (Mohammadi et al., 2023; Molinari et al., 2023; Moustakas et al., 2022; Mugwanya et al., 2023;

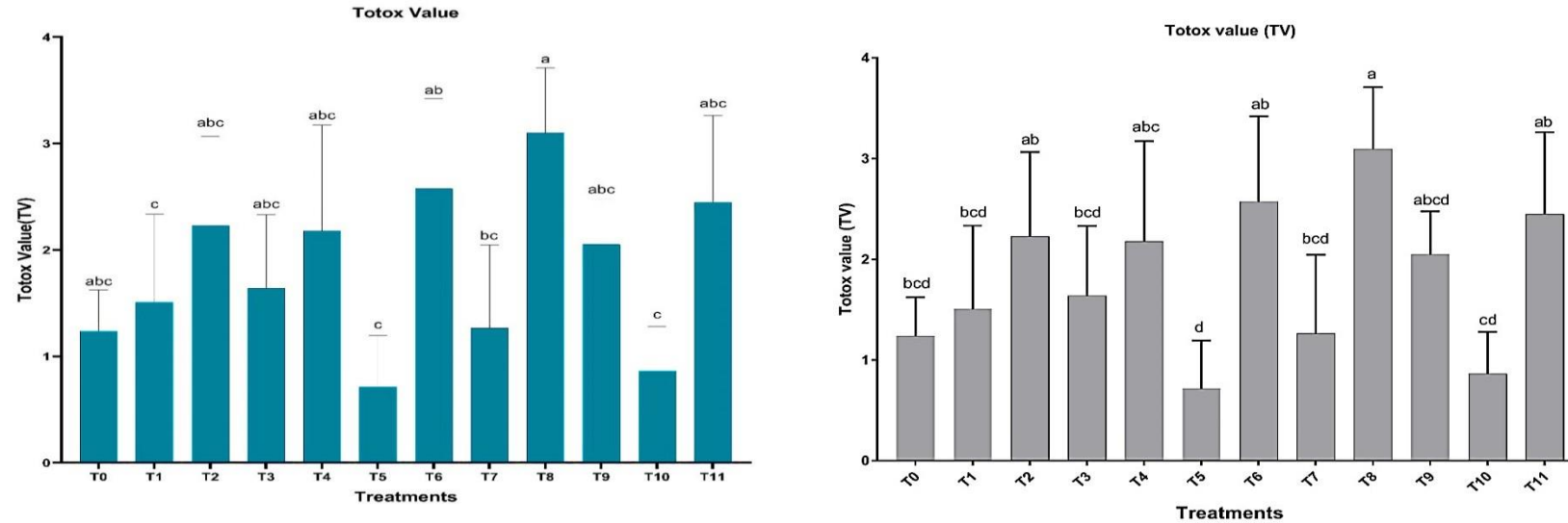
Mukarram et al., 2022; Mukhtar et al., 2023; Myers Jr. et al., 2023; Nadeem, 2022). When mustard plants are exposed to different stressors, the pathways mediated by salicylic acid (SA) within their cells can trigger defence mechanisms that inhibit oil oxidation. This phenomenon can lead to a decline in anisidine values (AV), consequently decreasing the Totox value. The involvement of salicylic acid (SA) in alleviating oxidative stress in plants may indirectly influence the Totox value, thus playing a role in maintaining oil quality. SA have the potential to effectively preserve the freshness and quality of oil by mitigating the detrimental effects of oxidation on its constituents. The assessment of mustard oil's freshness, oxidative stability, and overall quality necessitates careful consideration of the Totox value, which holds significant importance. The potential impact of applying sulphur and salicylic acid via foliar spray on the Totox value lies in its ability to mitigate oxidative damage and enhance the antioxidant capacity of the oil. These interventions offer the potential for augmenting mustard oil production and implementing measures for quality control, consequently creating fresher and superior-quality oil with an extended shelf life (Liu, Xiao, et al., 2022; Liu, Meng, et al., 2022; Lombardino et al., 2022; Ma et al., 2022; Mabasa, 2023; Maheshwari et al., 2022).

**Table 4.47. Impact of Different Treatments on Totox Value of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	Totox Value		Saponification Value		Refractive Index	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	1.51 <sup>abc</sup> ±0.59	1.24 <sup>bcd</sup> ±0.40	13.83 <sup>b</sup> ±1.16	13.65 <sup>bc</sup> ±2.87	1.73 <sup>bc</sup> ±0.15	1.43 <sup>bcde</sup> ±0.25
<b>T1 (Thiourea-1000 ppm)</b>	0.82 <sup>c</sup> ±0.40	1.49 <sup>bcd</sup> ±0.82	10.09 <sup>c</sup> ±.97	10.28 <sup>de</sup> ±1.16	1.76 <sup>b</sup> ±0.15	1.80 <sup>abc</sup> ±0.10
<b>T2 (Salicylic acid-300 ppm)</b>	1.81 <sup>abc</sup> ±0.21	2.26 <sup>ab</sup> ±0.82	2.99 <sup>e</sup> ±1.41	4.48 <sup>gh</sup> ±1.48	1.40 <sup>bcde</sup> ±0.30	1.20 <sup>de</sup> ±0.40
<b>T3 (Thiourea-1000 ppm + Salicylic Acid-300 ppm)</b>	2.17 <sup>abc</sup> ±0.62	1.63 <sup>bcd</sup> ±0.69	16.08 <sup>a</sup> ±.64	15.89 <sup>ab</sup> ±1.16	1.23 <sup>def</sup> ±0.35	1.20 <sup>de</sup> ±0.36
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	1.93 <sup>abc</sup> ±0.58	2.19 <sup>abc</sup> ±0.97	7.66 <sup>d</sup> ±1.16	8.41 <sup>ef</sup> ±1.12	1.36 <sup>bcde</sup> ±0.25	1.50 <sup>bcd</sup> ±0.30
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	0.57 <sup>c</sup> ±0.22	0.69 <sup>d</sup> ±0.47	17.39 <sup>a</sup> ±1.48	16.83 <sup>a</sup> ±2.02	0.96 <sup>ef</sup> ±0.30	0.86 <sup>e</sup> ±0.25
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	2.85 <sup>ab</sup> ±0.81	2.57 <sup>ab</sup> ±0.85	7.66 <sup>d</sup> ±1.16	8.60 <sup>ef</sup> ±0.85	1.56 <sup>bcd</sup> ±0.05	1.40 <sup>bcde</sup> ±0.55
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	1.38 <sup>bc</sup> ±0.62	1.25 <sup>bcd</sup> ±0.78	3.36 <sup>e</sup> ±1.48	2.43 <sup>h</sup> ±0.85	0.86 <sup>f</sup> ±0.32	1.30 <sup>cde</sup> ±0.20
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	3.21 <sup>a</sup> ±0.79	3.08 <sup>a</sup> ±0.62	13.09 <sup>b</sup> ±2.26	12.71 <sup>cd</sup> ±1.41	2.26 <sup>a</sup> ±0.25	2.10 <sup>a</sup> ±0.26
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	1.77 <sup>abc</sup> ±0.60	2.03 <sup>abcd</sup> ±0.40	2.43 <sup>e</sup> ±.85	3.36 <sup>h</sup> ±2.02	1.16 <sup>def</sup> ±0.30	1.26 <sup>cde</sup> ±0.25
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	0.91 <sup>c</sup> ±0.28	0.85 <sup>cd</sup> ±0.41	11.96 <sup>bc</sup> ±1.29	12.34 <sup>cd</sup> ±2.44	1.73 <sup>bc</sup> ±0.23	1.90 <sup>ab</sup> ±0.26
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	1.90 <sup>abc</sup> ±0.81	2.43 <sup>ab</sup> ±0.82	7.10 <sup>d</sup> ±.85	7.10 <sup>fg</sup> ±1.16	1.86 <sup>ab</sup> ±0.35	1.76 <sup>abcd</sup> ±0.25
<b>CD</b>	0.959	1.042	2.457	2.957	0.431	0.532
<b>CV</b>	32.317	33.643	14.124	17.932	16.937	21.135

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.47. Totox Value of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

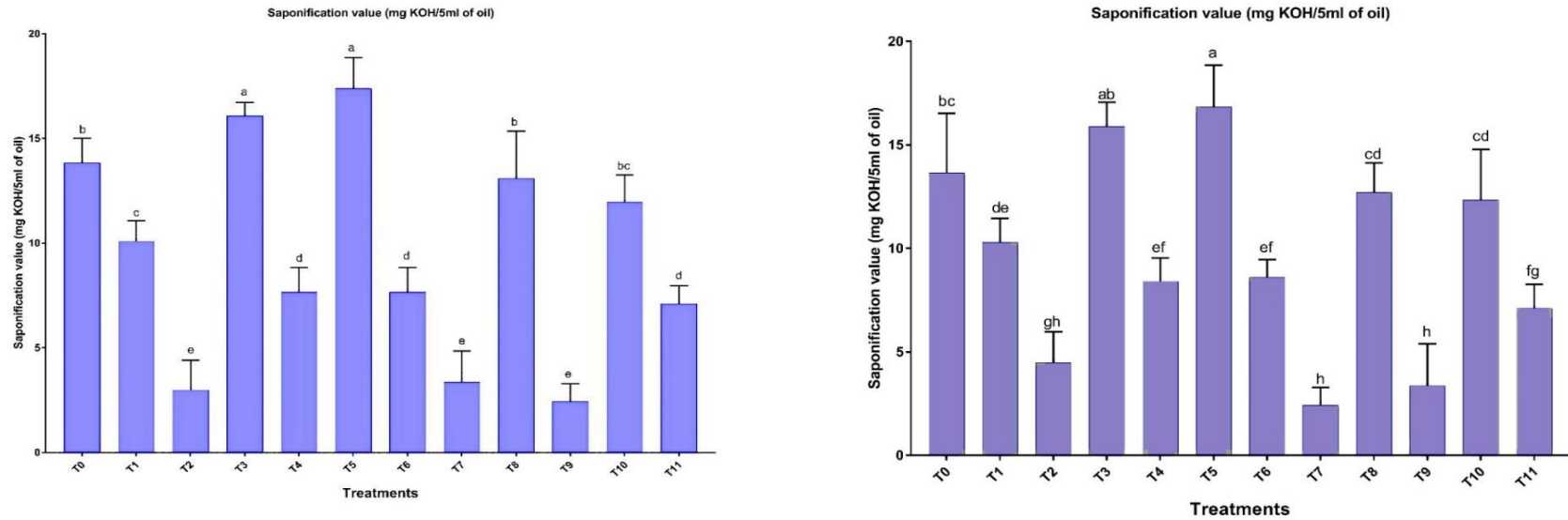


**Saponification Value:** The impact of Sulphur, Salicylic acid, and their combined application on the saponification value was meticulously investigated in the RH725 variety of Indian Mustard over the 2021-2022 and 2022-2023 agricultural seasons. Thorough data collection took place at the time of harvest (Table 4.48, Figure 4.48). In 2021-2022, a notable discrepancy in saponification value was evident compared to the control group (T0) after the harvest, signifying statistical significance. Percentage increases were calculated, offering insight into the patterns of change. Consequently, at harvest, the most substantial percentage decrease relative to T0 was observed in T8, followed by T10, T1, T4, T6, T11, T7, T2, and T9, with percentage values of -5.71%, -15.62%, -37.03%, -80.48%, -80.48%, -94.73%, -311.11%, -362.5%, and -469.23%, respectively. Conversely, T5 and T3 displayed percentage increases relative to T0, with values of 20.43% and 13.95%, respectively. In 2022-2023, a significant discrepancy in saponification value compared to the control (T0) was again observed at harvest time. Percentage increases were calculated as before. Consequently, at harvest, the most significant percentage decrease relative to T0 was noted in T8, followed by T10, T1, T6, T4, T11, T2, T9, and T7, with percentage values of -7.35%, -10.60%, -32.72%, -58.69%, -62.22%, -92.10%, -204.16%, -305.55%, and -461.53%, respectively. Conversely, T5 and T3 exhibited percentage increases relative to T0, with 18.88% and 14.11% values, respectively. As an exemplar case, evaluating the saponification value in mustard oil carries significant significance as a fundamental chemical parameter for assessing the oil's quality, purity, and appropriateness for diverse applications in both industrial and culinary domains. Furthermore, through the examination of the potential impacts associated with the utilisation of foliar spray applications containing sulphur (S) and salicylic acid (SA) on the saponification value of mustard oil, valuable knowledge can be acquired regarding the potential approaches for improving agricultural practises and enhancing the overall standard of mustard oil production. The saponification value serves as an indicator of the mean molecular weight of the fatty acids that are contained within a specific oil or fat specimen (Xiao et al., 2022; Xie et al., 2023; Xing et al., 2022; Xu et al., 2023; Xu et al., 2022, 2023; Xu, Cao, et al., 2022; Xu, Zeng, et al., 2022; Yagci & Agar, 2022; Yan et al., 2022; Yang et al., 2023). Determining the saponification value involves the quantification of potassium hydroxide (KOH) in milligrams required for the saponification of one gramme of the

oil or fat. The saponification value is essential in mustard oil, encompassing various highly significant factors. The saponification value is a crucial parameter utilised for thoroughly evaluating the authenticity and excellence of vegetable oils, with particular emphasis on mustard oil. This metric has been in existence for a considerable duration. The efficacy of this metric becomes increasingly evident when assessing the potential adulteration of the oil with other substances or lower-grade oils. This practice aids in verifying the authenticity of the oil and its compliance with rigorous quality criteria. Moreover, it should be noted that the saponification values of various types of oils and fats exhibit inherent differences. Given these circumstances, the determination of the saponification value holds significant significance in ascertaining the botanical source of the oil and confirming its authenticity as a genuine, unaltered specimen of mustard oil (Wang et al., 2022; Wang & Komatsu, 2022; Wang et al., 2022; Wang et al., 2022; Wang et al., 2022; Wante et al., 2022; Waters & Nelson, 2023; Wu et al., 2022; Wurms et al., 2023). Furthermore, the saponification value offers crucial insights into the fatty acid composition of the oil, enabling the evaluation of its nutritional characteristics and potential applications in culinary and occupational settings. The saponification value of mustard oil can be influenced by various intricate mechanisms associated with sulphur and salicylic acid effects. These mechanisms can be summarised as follows: The synthesis of fatty acids is a multifaceted biological process critically dependent on sulphur, an indispensable nutrient for mustard plants. Adding an appropriate quantity of sulphur to mustard seeds can stimulate the synthesis of fatty acids in the roots, resulting in an augmented saponification value in the resulting oil. Mustard seeds possess a sulphur compound that acts as a catalyst for synthesising high-quality crude. In most instances, oils of superior quality will exhibit a saponification value notably greater than oils of the lower rate. This phenomenon can be attributed to the fact that oils of higher quality show a substantially higher concentration of fatty acids. Mustard seeds heavily depend on salicylic acid (SA) to uphold the structural integrity of their fatty acids (Todorova et al., 2022; Topcu et al., 2022; Torres & Figueroa, 2023; Tyagi et al., 2022; Ullah et al., 2023). SA is widely recognised for its efficacy in alleviating stress-induced plant responses. As a consequence, the production of oil inevitably leads to an elevation in the saponification value. The regulation of stress responses induced by salicylic acid (SA) plays a crucial role in preserving oil quality in mustard seeds. It

is worth noting that an elevated saponification value is commonly associated with enhanced oil quality (Ullah et al., 2022; Verma et al., 2023; Waadt et al., 2022; Wan & Xin, 2022; Wang et al., 2022; Wang et al., 2023; Wang et al., 2023). The assessment of the saponification value holds significant importance in evaluating the purity, excellence, and adaptability of mustard oil. The application of foliar sprays containing sulphur and salicylic acid can influence saponification value by facilitating enhanced oil production characterised by elevated levels of fatty acids. This objective can be achieved through the utilisation of foliar spray. The successful execution of these strategic interventions has the potential to significantly improve the quality and authenticity of mustard oil while also offering substantial benefits to consumers and the broader mustard oil industry (Sun et al., 2022; Taira & Shiono, 2022; Takeuchi et al., 2022; Talaat, 2023; Tan et al., 2022; Tariq et al., 2023; Tariq et al., 2022).

**Figure 4.48. Saponification Value of Mustard During Rabi 2021-2023 & 2022-23**



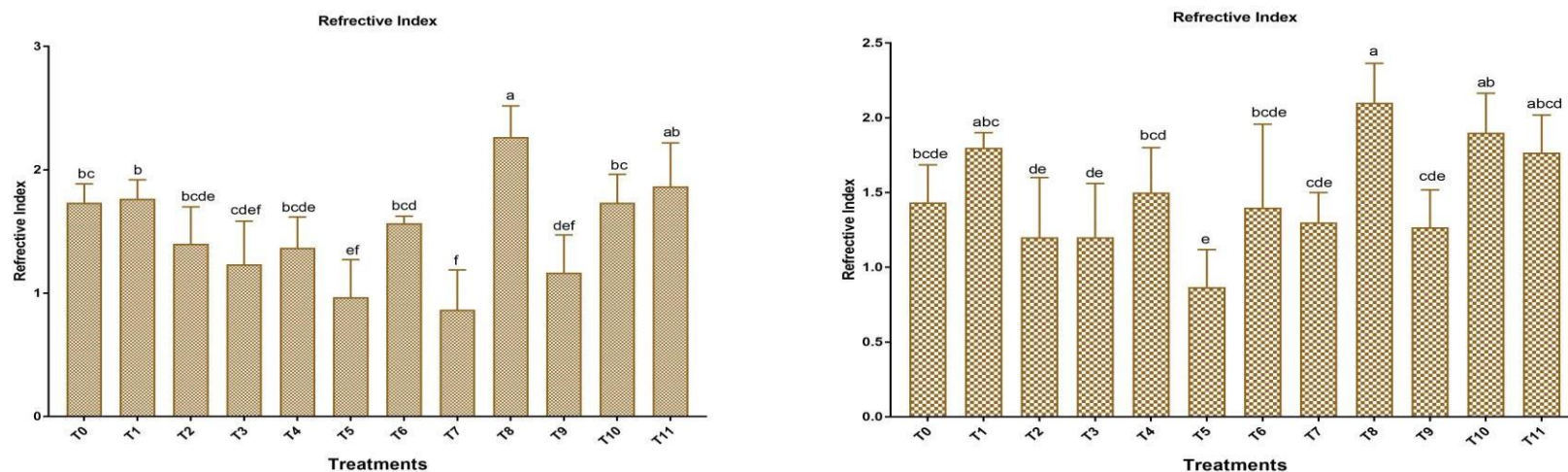
Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Refractive Index:** The effect of Sulphur and Salicylic acid and their combination on refractive index was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at harvesting (Table 4.49, Figure 4.49). In 2021-2022, there was a significant difference in refractive index compared to T0 (Control) at after harvesting. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the refractive index was observed at harvesting. Therefore, at harvesting, the percentage decrease as compared to T0 was found highest in T8 followed by T11, T1 and the percentage values were 23.52%, 7.14%, 1.88% respectively. But in T6, T2, T4, T3, T9, T5, T7 percentage decrease as compared to T0 and the percentage values were -10.63%, -23.80%, -26.82%, -40.54%, -48.57%, -79.31%, -99.99% respectively. And in T10 percentage value is same as T0. In 2022-2023, there was a significant difference in refractive index compared to T0 (Control) at harvesting. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage increase as compared to T0 was found highest in T8 followed by T10, T1, T11, T4 and the percentage values were 31.74%, 24.56%, 20.37%, 18.86%, and 4.44% respectively. But in T6, T7, T9, T2, T3, T5 percentage decrease as compared to T0 and the percentage values were -2.38%, -10.25%, -13.15%, -19.44%, -19.44%, -65.38% respectively. The refractive index is a crucial parameter that measures the extent of light refraction as it passes through a medium. It plays a significant role in various fields, including food science and agriculture. The mustard oil quality and purity assessment relies heavily on the refractive index during mustard cultivation and extraction. Additionally, it is crucial to understand the relationship between the refractive index of mustard oil and various substances like thiourea and salicylic acid to optimise oil production, ensure product quality, and explore potential applications in plant stress responses. This article investigates the importance of the refractive index in mustard oil and its interactions with thiourea and salicylic acid (Kudoyarova, 2022; Kumar & Ohri, 2023; Kurepa & Smalle, 2022; Kurniawan & Chuang, 2022; Kuromori et al., 2022; Lajayer et al., 2022; Li et al., 2022; Li et al., 2022). The Importance of Refractive Index in Mustard Oil: Evaluation of Purity and Quality. The refractive index serves as an indicator of the purity and quality of mustard oil. This analysis provides valuable insights into the oil's composition, including its fat content and the presence of impurities. The identification

of adulteration in mustard oil holds significant significance owing to its susceptibility to blending with alternative oils, which may potentially undermine its overall integrity. The identification of adulteration can be facilitated by utilising the refractive index, which involves comparing the measured value with established standard references. The process of ascertaining the composition of oil entails the analysis of its refractive index, which indicates the oil's chemical constitution, specifically the presence of constituents such as fatty acids and triglycerides. The characterisation of the triglyceride profile of mustard oil holds considerable significance. The topic of discussion pertains to oil authentication. The refractive index analysis is a dependable approach used to authenticate mustard oil, guaranteeing its adherence to the expected standards of quality and composition consumers establish (Kaviani et al., 2023; Kaya et al., 2023; Khalid et al., 2023; Khan et al., 2022, 2023; Khan et al., 2022; Kochanek et al., 2023; Kosakivska et al., 2022; Kour et al., 2023). The current study examines the interplay between thiourea and a particular compound. Improving the Quality of Oil: Scientific inquiry has been dedicated to investigating the potential of thiourea in enhancing the quality of edible oils. The utilisation of mustard oil in the production process has the potential to impact the refractive index of the oil, leading to an improvement in the overall quality of the oil. This interaction suggests that using thiourea holds promise in reducing impurities and enhancing the overall purity of the oil. The potential for detecting adulteration in mustard oil lies in utilising the interaction between thiourea and the refractive index (Huang et al., 2022; Hudeček et al., 2023; Huh, 2022; Hui et al., 2022; Huntenburg et al., 2022). The utilisation of thiourea may improve the consistency of the refractive index in the presence of adulterants, thus aiding in identifying impurities. This, in turn, can contribute to a better understanding of the physiological and molecular mechanisms that govern the stress response in mustard plants. Thiourea is acknowledged for its capacity to augment plant stress tolerance. This phenomenon exerts a direct influence on the physiological processes of plants, thereby potentially exerting an indirect influence on the overall quality of the mustard oil that is generated. The enhanced stress tolerance of mustard plants may be responsible for the potential improvement in oil quality. The primary objective of this study is to examine the interplay between salicylic acid and various other compounds. Oil quality modification Salicylic acid, a phytohormone implicated in plant signalling cascades, can modulate

the refractive index of mustard oil. This interaction can potentially cause changes in the quality of the oil (Jin et al., 2022; Jofre et al., 2023; Kandhol et al., 2023; Kapoor et al., 2022; Kapoor et al., 2022; Karaman, 2023; Karamat et al., 2022; Katoch et al., 2022). The potential influence of salicylic acid on the chemical composition of adipose tissue can result in alterations in taste, durability, and storage duration. The topic of inquiry pertains to the responses plants exhibit when subjected to stress. The observation of salicylic acid's participation in plant defence mechanisms and stress responses has been documented. When mustard plants undergo this particular treatment, there exists the potential to alter the physiological characteristics of the plant, consequently impacting the refractive index of the oil. This phenomenon can potentially induce modifications in the chemical makeup of oil. The potential impact of the interaction between salicylic acid and its refractive index on the nutritional composition of mustard oil is worth considering. Oil's nutritional properties can be influenced by variations in its composition, which can affect its desirability as a potential dietary resource. The assessment of the refractive index of mustard oil holds considerable importance in evaluating its quality, purity, and chemical composition. A thorough understanding of the relationship between the refractive index of mustard oil and compounds like thiourea and salicylic acid is crucial for improving oil production, ensuring high-quality products, and exploring potential applications in plant stress responses (Hussein et al., 2023; Iftikhar et al., 2023; Islam et al., 2023; Javadipour et al., 2022; Ji et al., 2022; Jia et al., 2022). The interactions above can influence the overall quality of oil and the nutritional composition and market attractiveness of mustard oil products. Through analysing these correlations, researchers and producers can develop improved approaches to create higher-quality mustard oil that can be used for both culinary and potential medicinal applications (Hernandez-Leon & Valenzuela-Soto, 2022; Hernández et al., 2023; Hernández et al., 2022; Hilal et al., 2023; Hong et al., 2022).

**Figure- 4.49. Refractive Index of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).



**Oil density:** The effect of Sulphur and Salicylic acid and their combination on oil density was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at harvesting. In 2021-2022, oil density significantly differed from T0 (Control) after harvesting (Table 4.50, Figure 4.50). The percentage increase was calculated by comparing all the treatments with T0. Thus, the percentage increase in the oil density pattern was observed at harvesting. Therefore, at harvesting, the percentage decrease as compared to T0 was found to be highest in T7, followed by T10, T3, T2, T5, T1, T9, and T6, and the percentage values were -0.09%, -0.18%, -0.22%, -0.26%, -0.28%, -0.44%, -0.90%, -0.91% respectively. But in T11, T8 and T4 percentage increased compared to T0; the percentage values were 0.74%, 0.23% and 0.07%. In 2022-2023, oil density significantly differed from T0 (Control) at harvesting. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage increase as compared to T0 was found to be highest in T8, followed by T11, T2, T4, T10, T1, T5, and T3, and the percentage values were 1.43%, 1.42%, 0.94%, 0.87%, 0.72%, 0.69%, 0.69%, 0.06% respectively. But in T9, T6, T7 percentage decrease as compared to T0 and the percentage values were -0.04%, -0.11%, 0.33%. The evaluation of oil quality and agricultural practices relies on the density of mustard oil and the potential effects of foliar spray applications of sulphur (S) and salicylic acid (SA) on mustard oil density. Determining oil density, known as oil-specific gravity, is a fundamental physical characteristic vital in evaluating the quality and purity of edible oils, such as mustard oil. The method involves comparing the density of the oil to that of the water to ascertain their relative proportions (Parada et al., 2022; Parrey et al., 2023; Patel et al., 2022; Peng et al., 2022; Pérez-Llorca et al., 2023; Phokas et al., 2023). The significance of oil density in mustard oil encompasses various dimensions of quality evaluation: The determination of oil density is a significant quality parameter for assessing mustard oil's overall quality and purity. The distinct density values of different edible oils can be attributed to the variations in their chemical compositions. The measurement of oil density plays a crucial role in determining the authenticity and purity of mustard oil, as the addition of lower quality or lower cost oils can modify its density (Samtani et al., 2022; Shah et al., 2022, 2023; Shang et al., 2022; Sharma & Verma, 2023; Sheikhalipour et al., 2023; Shekhawat et al., 2023; Shi et al., 2023; Shi et al., 2022; Si et al., 2023; Singh et al., 2022). The

identification and recognition of adulteration There is a prevalent apprehension regarding the adulteration of mustard oil by adding alternative oils, such as soybean or palm oil. When comparing pure mustard oil with adulterants, it is observed that the latter often exhibits notable variations in density values. Monitoring the temporal variations in oil density can serve as a valuable approach for detecting cases of adulteration and guaranteeing the authenticity of mustard oil provided to consumers.

The manufacturing and processing procedures: Maintaining a consistent oil density is crucial in the industrial manufacturing process of mustard oil, as it is imperative for ensuring the quality of the final product and compliance with relevant regulatory guidelines. Variations in the density of the oil could potentially affect the product's performance across various applications, such as cooking and food processing. The subsequent array of mechanisms can elucidate the underlying reasons for the observed impact of foliar spray applications of sulphur and sulfuric acid on the oil density of mustard (Ruidas et al., 2022; Rybczyński et al., 2022; Sachan & Krishna, 2022; Salih, Wu, et al., 2022; Salih, Zhou, et al., 2022; Salwan et al., 2023; Samad et al., 2023; Sampedro-Guerrero et al., 2022).

The Composition of Fatty Acids: Sulphur is an indispensable nutrient for mustard plants, as it fulfils a crucial role in producing fatty acids, vital for their growth and development. Edible oils, such as mustard oil, predominantly comprise fatty acids as their principal constituent. An adequate amount of sulphur can potentially influence the fatty acid composition of mustard oil, consequently impacting its density. Oil density variations may occur due to shifts in the proportion of individual fatty acids. Sulphur is an additional element that plays a role in enhancing the overall quality of mustard oil. Adding sulphur-containing compounds to the oil can improve its stability and increase its oxidation resistance. It is conceivable that an enhancement in the quality of oil may result in a consequential impact on its density, as new, high-quality oil often exhibits several discernible attributes. The role of salicylic acid (SA) in eliciting diverse stress responses in plants is widely recognised. SA-mediated pathways can impact diverse metabolic processes, including oil biosynthesis, in mustard plants under various stressors like pests, diseases, or unfavourable environmental conditions. The potential impact of stress on the chemical composition and overall quality of oil may lead to alterations in its density. The effect of salicylic acid (SA) on the synthesis of secondary metabolites in plants, mainly those

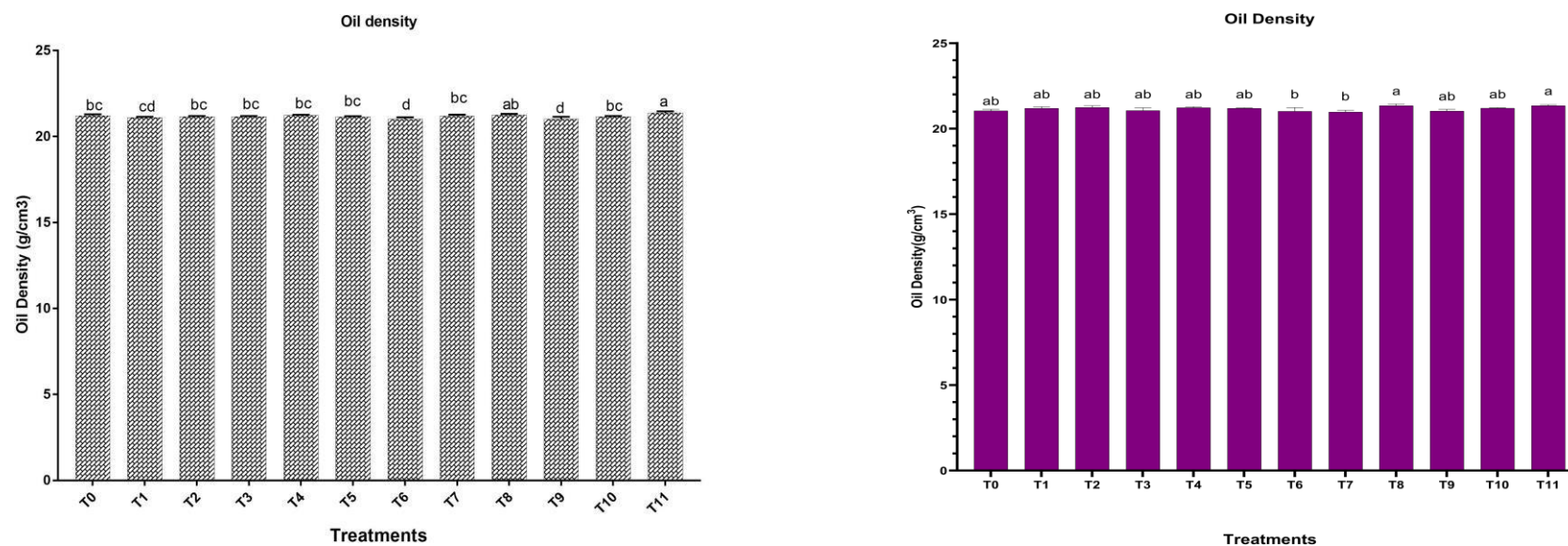
present in oils, is a subject of interest (Phour & Sindhu, 2022; Poór et al., 2022; Quesada, 2022; Rai & Kaushik, 2023; Raja Gopalan et al., 2022; Ramakrishnan & Zhou, 2022; Rani et al., 2023; Rizvi et al., 2022; Roussos, 2023). Despite its primary role as a defensive mechanism, SA can also influence the chemical composition of mustard oil. There is a potential for SA-induced pathways to induce alterations in specific fatty acids or other oil constituents, thereby affecting its density. The assessment of mustard oil quality and the identification of adulteration heavily rely on considering oil density as a crucial parameter. There is a potential for foliar spray applications of sulfuric acid and sulphuric acid to indirectly influence oil density due to their effects on fatty acid synthesis and stress responses in mustard plants. These effects can potentially impact the composition and quality of oil. These interventions present potential strategies for enhancing the quality and genuineness of mustard oil (Nam et al., 2023; Napieraj et al., 2023; Nasir & Toth, 2022; Negi & Kumar, 2023; Nimsi et al., 2023; Niu & Fu, 2022; Ortiz-García et al., 2022, 2023; Ozturk & Unal, 2023; Paalli et al., 2022; Pal et al., 2023).

**Table 4.50. Impact of Different Treatments on Oil Density of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	Oil density		Oil Viscosity	
	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	21.21 <sup>bc</sup> ±0.080	21.04 <sup>ab</sup> ±0.090	239.05 <sup>h</sup> ±6.75	259.62 <sup>d</sup> ±9.08
<b>T1 (Thiourea-1000 ppm)</b>	21.11 <sup>cd</sup> ±0.035	21.19 <sup>ab</sup> ±0.095	258.95 <sup>g</sup> ±6.30	272.93 <sup>cd</sup> ±7.07
<b>T2 (Salicylic acid-300 ppm)</b>	21.15 <sup>bc</sup> ±0.040	21.24 <sup>ab</sup> ±0.106	278.16 <sup>de</sup> ±2.36	297.23 <sup>bc</sup> ±7.82
<b>T3 (Thiourea-1000 ppm + Salicylic Acid-300 ppm)</b>	21.16 <sup>bc</sup> ±0.030	21.05 <sup>ab</sup> ±0.164	256.71 <sup>g</sup> ±1.84	264.66 <sup>d</sup> ±2.76
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	21.22 <sup>bc</sup> ±0.035	21.23 <sup>ab</sup> ±0.052	266.61 <sup>f</sup> ±3.85	257.17 <sup>d</sup> ±8.70
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	21.15 <sup>bc</sup> ±0.036	21.19 <sup>ab</sup> ±0.030	257.53 <sup>g</sup> ±1.81	261.05 <sup>d</sup> ±4.14
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	21.01 <sup>d</sup> ±0.100	21.02 <sup>b</sup> ±0.211	281.48 <sup>de</sup> ±4.38	292.59 <sup>bc</sup> ±8.43
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	21.19 <sup>bc</sup> ±0.085	20.97 <sup>b</sup> ±0.110	275.74 <sup>e</sup> ±1.68	276.10 <sup>cd</sup> ±12.80
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	21.26 <sup>ab</sup> ±0.055	21.35 <sup>a</sup> ±0.095	298.20 <sup>c</sup> ±2.99	303.11 <sup>b</sup> ±7.39
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	21.02 <sup>d</sup> ±0.130	21.03 <sup>ab</sup> ±0.105	355.82 <sup>b</sup> ±7.98	349.18 <sup>a</sup> ±16.58
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	21.17 <sup>bc</sup> ±0.026	21.19 <sup>ab</sup> ±0.030	285.79 <sup>d</sup> ±3.38	257.32 <sup>d</sup> ±5.32
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	21.37 <sup>a</sup> ±0.091	21.34 <sup>a</sup> ±0.056	385.02 <sup>a</sup> ±5.34	373.85 <sup>a</sup> ±3.68
<b>CD</b>	0.129	0.17	8.005	14.541
<b>CV</b>	0.358	0.472	1.639	2.955

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.50. Oil Density of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

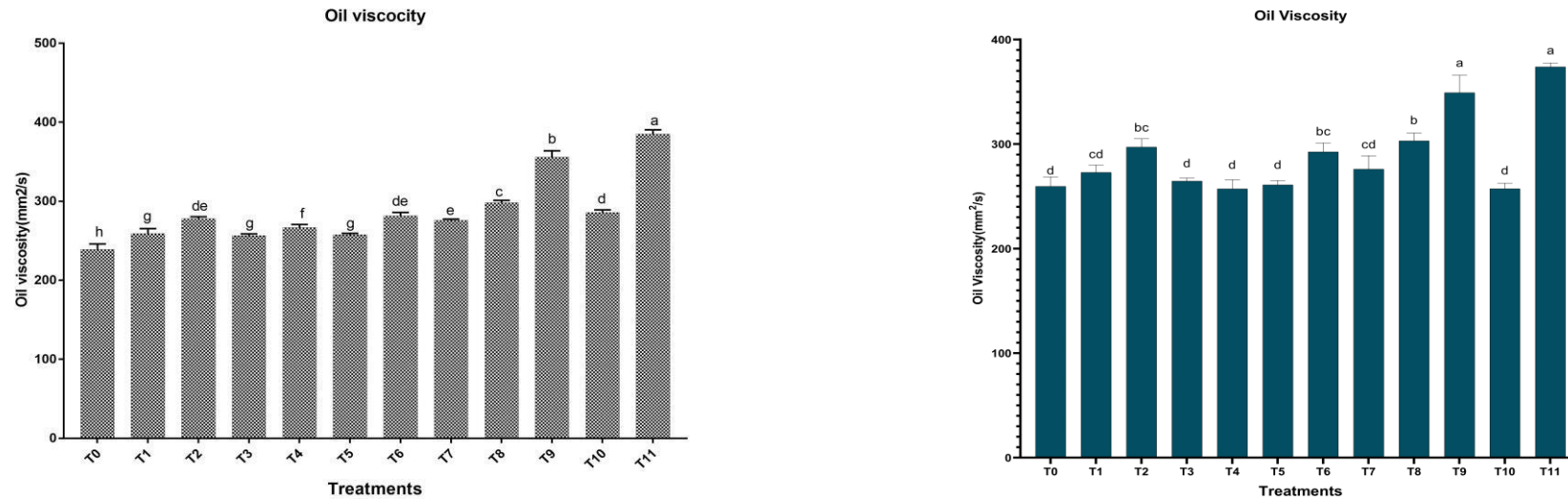
**Oil Viscosity:** The effect of Sulphur and Salicylic acid and their combination on oil viscosity was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at harvesting. In 2021-2022, there was a significant difference in oil viscosity compared to T0 (Control) after harvesting (Table 4.51, Figure 4.51). The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the oil viscosity was observed at harvesting. Therefore, at harvesting, the percentage decrease as compared to T0 was found highest in T11 followed by T9, T8, T10, T6, T2, T7, T4, T1, T5, T3 and the percentage values were 37.91%, 32.81%, 19.83%, 16.35%, 15.07%, 14.06%, 13.30%, 10.33%, 7.68%, 7.17%, 6.87% respectively. In 2022-2023, there was a significant difference in oil viscosity compared to T0 (Control) at harvesting. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage increase as compared to T0 was found to be highest in T11, followed by T9, T8, T2, T6, T7, T1, T3, and T5, and the percentage values were 30.55%, 25.64%, 14.34%, 12.65%, 11.26%, 5.97%, 4.87%, 1.90%, 0.54% respectively. But in T10 and T4, the percentage decreased compared to T0; the percentage values were -0.89% and -0.95%. To gain insight into mustard oil's physical properties and quality, exploring the significance of oil viscosity within this domain is imperative. Additionally, it is crucial to consider the potential impact of sulphur (S) and salicylic acid (SA) foliar spray applications on the viscosity of mustard oil. The subsequent discourse elucidates their significance, accompanied by an exploration of the potential ramifications they may engender. The thickness of oil, which refers to its internal friction, is a significant factor influencing its behaviour and flow characteristics across diverse environments. The subsequent cooking and culinary applications underscore the importance of oil viscosity in mustard oil. Mustard oil is commonly employed in the culinary realm for food preparation across various cuisines. The ingredient's thickness influences its texture and interaction with other constituents during cooking (Waters & Nelson, 2023; Wu et al., 2022; Wurms et al., 2023; Xiao et al., 2022; Xie et al., 2023; Xing et al., 2022; Xu et al., 2023; Xu et al., 2022, 2023; Xu, Cao, et al., 2022). The optimal viscosity of the oil ensures its ability to adhere to food items, distribute uniformly, and impart the desired texture to the dishes. Consumer Preferences: Consumers often exhibit preferences for specific oil viscosities, influenced by their adherence to culinary

traditions and personal inclinations. The preferences above can be fulfilled, and the culinary experience can be enhanced by utilising mustard oil with suitable viscosity. The food industry employs mustard oil in the processing and manufacturing a diverse range of food products. A comprehensive comprehension of the density of a substance and the ability to regulate it is imperative for attaining consistent product quality and facilitating seamless processing. There is a potential for foliar spray applications of sulphur and sulfuric acid to influence the viscosity of mustard oil indirectly. This influence is predominantly linked to these applications' effects on plant growth, oil composition, and quality. The design of fatty acids in mustard plants necessitates the presence of sulphur, an essential nutrient and a crucial constituent in synthesising fatty acids. The proportion of fatty acids in the substance significantly influences the viscosity of mustard oil. The types of fatty acids found in oil can be affected, as well as the proportions of those acids, by sulphur availability (Wang et al., 2023; Wang et al., 2023; Wang et al., 2022; Wang & Komatsu, 2022; Wang et al., 2022; Wang et al., 2022; Wang et al., 2022; Wante et al., 2022). The texture of mustard oil may vary based on the composition of its fatty acids. The addition of sulphur, particularly in the form of mustard oil, has the potential to enhance the quality of the oil. The enhancement of oil quality can lead to specific modifications in its texture and thickness, thereby influencing its viscosity. It is plausible that fresher, higher-quality oil may exhibit discernible variations in viscosity attributes compared to older, lower-quality oil. The role of salicylic acid (SA) in eliciting diverse stress responses in plants is widely recognised. SA-mediated pathways can potentially impact diverse metabolic processes, including oil biosynthesis, in mustard plants under various stressors, such as pests, diseases, or unfavourable environmental conditions. The potential impact of stress on the composition and quality of oil may lead to a consequential alteration in its viscosity (Torres & Figueroa, 2023; Tyagi et al., 2022; Ullah et al., 2023; Ullah et al., 2022; Verma et al., 2023; Waadt et al., 2022; Wan & Xin, 2022; Wang et al., 2022). The influence of salicylic acid (SA) on the production of secondary metabolites in plants, mainly those present in oils, is a topic of interest. Despite its primary purpose being a defensive mechanism, SA can also influence the chemical composition of mustard oil. The alteration of specific constituents within oil may occur due to the effect of self-assembly-induced pathways, subsequently leading to modifications in the oil's

viscosity. Determining mustard oil's suitability for industrial or culinary purposes primarily hinges on assessing its density, which is the most crucial factor—using S and SA as foliar spray exhibits the possibility of exerting an indirect influence on oil viscosity by modulating fatty acid synthesis and stress responses in mustard plants. This phenomenon can lead to a modification in the composition and quality of the oil, subsequently influencing its viscosity. These interventions offer avenues for enhancing mustard oil's overall quality and characteristics (Suliman et al., 2022; Sun et al., 2022; Sun et al., 2023; Sun et al., 2022; Taira & Shiono, 2022; Takeuchi et al., 2022; Talaat, 2023; Tan et al., 2022; Tariq et al., 2023; Tariq et al., 2022; Todorova et al., 2022; Topcu et al., 2022).



**Figure 4.51. Oil Viscosity of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

#### **4E. Thiourea (sulphur) and salicylic acid-mediated effects on Soil parameters of Indian mustard grown under the open filed condition**

**Soil pH:** The effect of Sulphur and Salicylic acid and their combination on soil pH was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at 60 days after sowing (DAS) (Table 4.52, Figure 4.52). In 2021-2022, there was a significant difference in soil pH compared to T0 (Control) at 60 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the soil pH was observed at 60 DAS. Therefore, At 60 DAS the percentage increase as compared to T0 was found highest in T5 followed by T1, T8 and the percentage values were 6.48%, 5.41%, and 1.04% respectively. But the percentage also decrease in T4, T2, T10, T7, T3, T6, T11, T9 as compare to T0 and the percentage values were -0.30%, -1.79%, -2.80%, -3.04%, -3.79%, -5.53%, -8.69%, -10.46% respectively. In 2022-2023, there was a significant difference in soil pH compared to T0 (Control) at 60 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 60 DAS, the percentage increase as compared to T0 was found highest in T5 followed by T1, T4 and the percentage values were 4.24%, 3.84%, and 0.59% respectively. But the percentage also decrease in T8, T2, T7, T3, T6, T10, T11, T9 as compared to T0 and the percentage values were -0.21%, -4.35%, -5.39%, -5.63%, -6.40%, -7.58%, -8.33%, -11.66% respectively. Measuring soil pH is paramount in agriculture, specifically cultivating mustard plants (*Brassica* spp.). The mustard plant is recognised for its versatility and substantial economic importance in agriculture. It is particularly esteemed for its oil-rich seeds and many practical uses. Measuring soil pH is critical in determining mustard crops' growth, nutrient availability, and productivity (Xu et al., 2022, 2023; Xu, Cao, et al., 2022; Xu, Zeng, et al., 2022; Yagci & Agar, 2022; Yan et al., 2022). This article explores the importance of soil pH measurement in mustard cultivation, investigates the methods used for accurate pH assessment, and analyses the impact of soil pH levels on the successful cultivation of mustard crops. Soil pH, also referred to as soil acidity or alkalinity, serves as a measure of the concentration of hydrogen ions (H<sup>+</sup>) within the soil. The evaluation is typically performed utilising a pH scale ranging from 0 to 14, wherein a measurement of 7 signifies a state of neutrality, measurements below 7

signify acidity and measurements above 7 signify alkalinity. The soil's pH level substantially impacts the cultivation of mustard, exerting influence over the growth and development of the crop. The pH of the soil significantly influences the availability of essential nutrients to mustard plants. (Yang et al., 2023; Yang et al., 2023; Yang & Lee, 2023; Yang et al., 2023; Yang et al., 2022; Yao et al., 2022; Yin et al., 2023; Yousaf et al., 2022; Yu et al., 2022). It has been observed that mustard crops may experience decreased availability of essential nutrients such as phosphorus, potassium, and calcium in soils with a pH below 6. Conversely, in alkaline soils exhibiting a pH level exceeding 7, there may be a decrease in the accessibility of micronutrients such as iron and zinc. Maintaining an optimal pH level is crucial for facilitating mustard plants' efficient absorption of nutrients. The mitigation of toxicological and nutritional imbalances across diverse settings. Soil pH levels directly influence the potential toxicity of certain elements. The coexistence of acidic soil conditions can potentially exacerbate aluminium and manganese toxicity levels (Wang & Komatsu, 2022; Wang et al., 2022; Wang et al., 2022; Wang et al., 2022; Wante et al., 2022; Waters & Nelson, 2023; Wu et al., 2022; Wurms et al., 2023; Xiao et al., 2022; Xie et al., 2023; Xing et al., 2022; Xu et al., 2023). On the other hand, alkaline soils may lead to an abundance of calcium, which could hinder the absorption of essential nutrients. Implementing soil pH monitoring and adjustment measures can effectively mitigate these concerns and enhance the cultivation of a more resilient mustard crop. The pH of the rhizosphere, which denotes the soil area directly affected by root exudates, exerts a substantial influence on the activity of advantageous soil microorganisms. Microbial communities contributing to nutrient cycling and enhancing plant health demonstrate discernible preferences for specific pH levels. Promoting favourable pH conditions can facilitate the growth and expansion of these advantageous microorganisms. The soil's pH level impacts the growth and productivity of mustard plants. Mustard crops demonstrate optimal growth in soil environments characterised by a pH range that tends to be slightly acidic to neutral, typically falling within the range of 6.0 to 7.5. Promoting robust growth and enhancing seed production, leading to higher yields, are achieved by effectively managing pH levels within the designated range. The ramifications of soil pH on mustard crop cultivation are far-reaching and substantially influence the crop's overall performance and yield (Yu et al., 2022; Yu et al., 2023; Yuan et al., 2022; Zahid

et al., 2023; Zang et al., 2022; Zhang et al., 2023; Zhang et al., 2022). Several crucial factors should be taken into consideration: Effective management of soil pH is of utmost importance in nutrient management, as it directly influences the availability of nutrients. Ensuring the proper pH levels of soil is crucial to enhance the availability of necessary nutrients essential for the optimal growth of mustard crops. The pH level adjustment process can be achieved by adding lime to raise pH in acidic soils or incorporating elemental sulphur to lower pH in alkaline soils. The soil's pH level can impact the vulnerability of mustard crops to various diseases and pests. One example is the prevalence of clubroot disease in soils characterised by acidic pH levels, while diseases such as white rust are more inclined to flourish in alkaline environments. Maintaining an optimal pH level is a proactive strategy for mitigating potential risks (Zhang et al., 2022; Zhang et al., 2023; Zhao & Hu, 2023; Zhao et al., 2022; Zhao et al., 2022; Zhao et al., 2022; Zhu et al., 2022; Zhu et al., 2023; Zulfiqar et al., 2022). The effectiveness of fertilisers is inherently connected to the soil's pH level. When the pH strays from the optimal range, the efficacy of fertilisers may be compromised, leading to reduced efficiency and potential environmental harm. The implementation of appropriate pH management ensures the efficient utilisation of fertilisers. The optimal pH levels of the soil benefit the growth, quality, and yield of mustard crops. The maintenance of the optimal pH range for mustard cultivation can substantially impact the economic value of the harvest, particularly in oilseed production. The mitigation of environmental impacts in agriculture can be accomplished by adjusting soil pH to an optimal range (Wan & Xin, 2022; Wang et al., 2022; Wang et al., 2023; Wang et al., 2023; Wang et al., 2022). Enhancing the efficiency of nutrient uptake in plants reduces the possibility of nutrient leaching into aquatic ecosystems, thus mitigating the potential for water contamination. Evaluating soil pH in mustard cultivation is important for small-scale farmers and large-scale agricultural enterprises. The factors mentioned above, namely nutrient availability, plant health, disease resistance, and crop yield, substantially impact the aspects above. To enhance the growth and productivity of mustard crops, it is crucial to consistently evaluate and manage soil pH by employing suitable amendments or selecting cultivars compatible with specific pH preferences. By implementing this practice, agricultural practitioners can effectively utilise mustard cultivation's inherent capabilities, thereby promoting

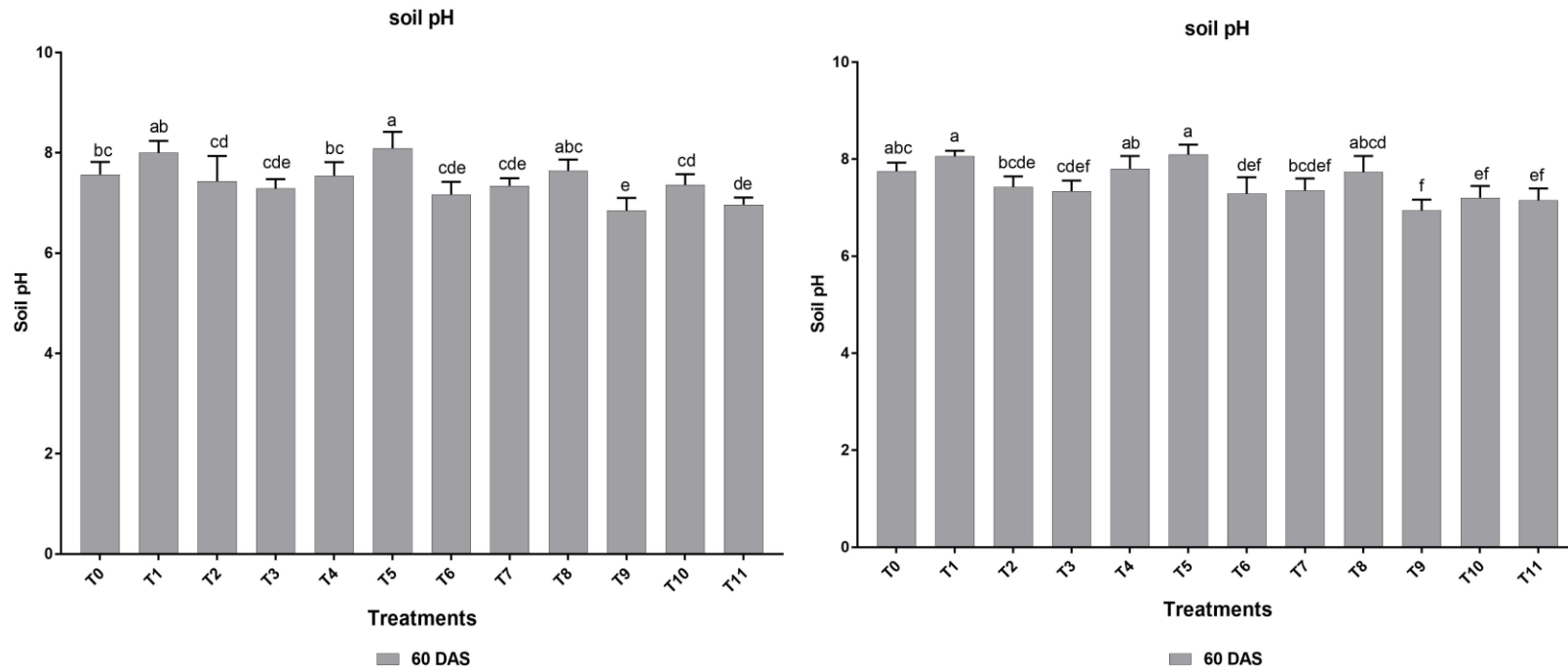
sustainable and enhanced crop production (Tariq et al., 2022; Todorova et al., 2022; Topcu et al., 2022; Torres & Figueroa, 2023; Tyagi et al., 2022; Ullah et al., 2023; Ullah et al., 2022; Verma et al., 2023; Waadt et al., 2022).

**Table 4.52. Impact of Different Treatments on Soil pH, EC, CEC of mustard During Rabi 2021-2023 & 2022-23**

Treatments	Soil pH		Soil EC		Soil CEC	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	7.56 <sup>bc</sup> ±0.251	7.75 <sup>abc</sup> ±0.174	0.173 <sup>def</sup> ±0.015	0.153 <sup>de</sup> ±0.015	15.413 <sup>ef</sup> ±1.283	15.163 <sup>e</sup> ±0.426
<b>T1 (Thiourea-1000 ppm)</b>	8.00 <sup>ab</sup> ±0.238	8.06 <sup>a</sup> ±0.109	0.163 <sup>defg</sup> ±0.005	0.170 <sup>cde</sup> ±0.020	17.706 <sup>bc</sup> ±1.695	17.000 <sup>cd</sup> ±0.592
<b>T2 (Salicylic acid-300 ppm)</b>	7.43 <sup>cd</sup> ±0.503	7.43 <sup>bcd</sup> ±0.216	0.156 <sup>efg</sup> ±0.015	0.173 <sup>cde</sup> ±0.015	17.140 <sup>cde</sup> ±0.680	16.953 <sup>cd</sup> ±1.588
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	7.29 <sup>cde</sup> ±0.185	7.34 <sup>cdef</sup> ±0.219	0.186 <sup>cd</sup> ±0.015	0.190 <sup>ab</sup> ±0.010	13.856 <sup>f</sup> ±1.366	14.660 <sup>e</sup> ±0.727
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	7.54 <sup>bc</sup> ±0.269	7.80 <sup>ab</sup> ±0.265	0.126 <sup>h</sup> ±0.020	0.143 <sup>e</sup> ±0.015	19.080 <sup>ab</sup> ±0.391	18.720 <sup>b</sup> ±1.020
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	8.09 <sup>a</sup> ±0.329	8.09 <sup>a</sup> ±0.204	0.223 <sup>b</sup> ±0.015	0.213 <sup>b</sup> ±0.005	19.013 <sup>ab</sup> ±1.102	19.633 <sup>ab</sup> ±0.458
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	7.17 <sup>cde</sup> ±0.252	7.28 <sup>def</sup> ±0.341	0.166 <sup>def</sup> ±0.005	0.170 <sup>cde</sup> ±0.020	19.890 <sup>a</sup> ±0.569	20.833 <sup>a</sup> ±1.078
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	7.34 <sup>cde</sup> ±0.150	7.35 <sup>bcd</sup> ±0.245	0.146 <sup>fgh</sup> ±0.015	0.153 <sup>de</sup> ±0.015	15.820 <sup>de</sup> ±0.918	15.593 <sup>de</sup> ±0.502
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	7.64 <sup>abc</sup> ±0.217	7.73 <sup>abcd</sup> ±0.331	0.183 <sup>cde</sup> ±0.015	0.186 <sup>bcd</sup> ±0.025	17.596 <sup>bcd</sup> ±0.290	18.210 <sup>bc</sup> ±0.893
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	6.85 <sup>e</sup> ±0.250	6.94 <sup>f</sup> ±0.223	0.203 <sup>bc</sup> ±0.015	0.203 <sup>ab</sup> ±0.028	20.020 <sup>a</sup> ±0.585	18.873 <sup>b</sup> ±1.325
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	7.36 <sup>cd</sup> ±0.212	7.20 <sup>ef</sup> ±0.241	0.286 <sup>a</sup> ±0.025	0.266 <sup>a</sup> ±0.020	16.053 <sup>cde</sup> ±0.822	15.746 <sup>de</sup> ±0.998
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	6.96 <sup>de</sup> ±0.142	7.15 <sup>ef</sup> ±0.240	0.136 <sup>gh</sup> ±0.015	0.153 <sup>de</sup> ±0.005	19.916 <sup>a</sup> ±1.015	20.710 <sup>a</sup> ±0.983
<b>CD</b>	0.444	0.391	0.026	0.028	1.525	1.668
<b>CV</b>	3.504	3.054	8.452	9.05	5.077	5.538

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.52. Soil pH of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean ± SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm);

**T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).**

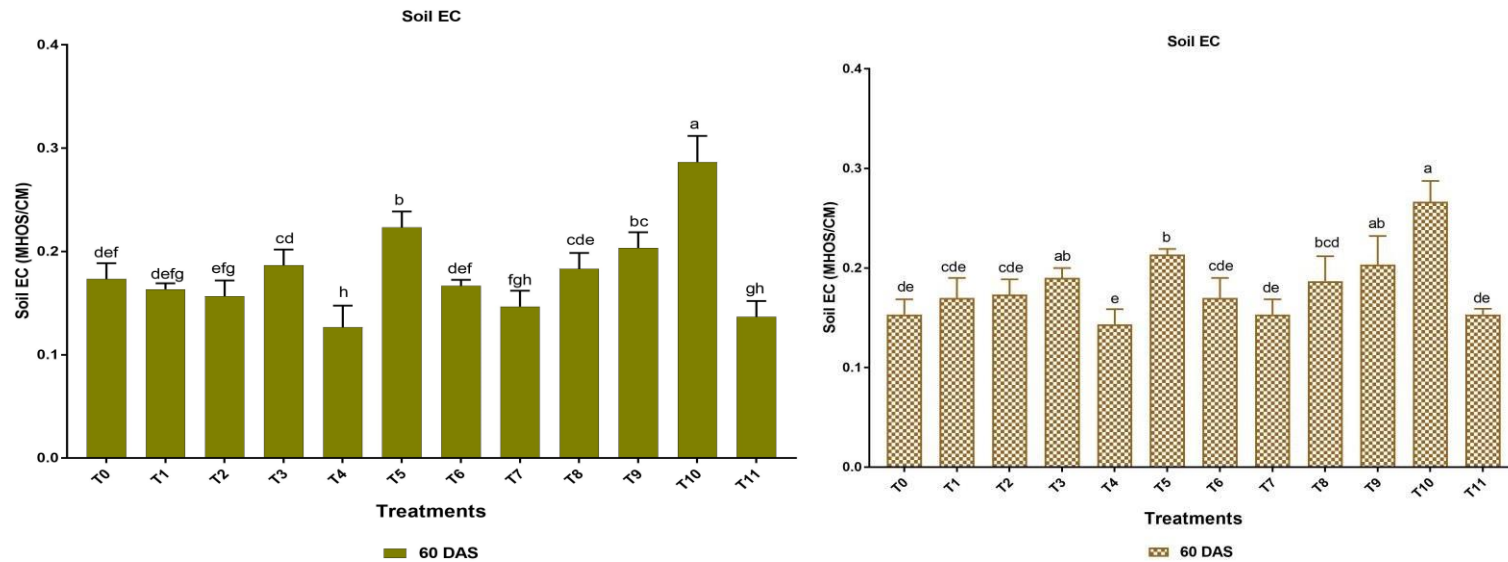


**Soil EC:** The effect of Sulphur and Salicylic acid and their combination on soil EC was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at 60 days after sowing (DAS) (Table 4.53, Figure 4.53). In 2021-2022, there was a significant difference in soil EC compared to T0 (Control) at 60 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the soil EC was observed at 60 DAS. Therefore, At 60 DAS the percentage increase as compared to T0 was found highest in T10 followed by T5, T9, T3, T8 and the percentage values were 39.53%, 22.38%, 14.75%, 7.14%, 5.45% respectively. But the percentage also decrease in T6, T1, T2, T7, T11, T4 as compared to T0 and the percentage values were -3.99%, -6.12%, -10.63%, -18.18%, -26.82%, -36.84% respectively. In 2022-2023, there was a significant difference in soil EC compared to T0 (Control) at 60 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the soil EC was observed at 60 DAS. Therefore, At 60 DAS the percentage increase as compared to T0 was found highest in T10 followed by T5, T9, T3, T8, T2, T1, T6, T7, T11 and the percentage values were 42.50%, 28.12%, 24.59%, 19.29%, 17.85%, 11.53%, 9.80%, 9.80%, 0.0002%, 0.0002% respectively. But the percentage also decrease in T4 as compared to T0 and the percentage value was -6.97%. Measuring electrical conductivity (EC) in soil is a fundamental procedure in modern agriculture, as it plays a significant role in the cultivation of mustard (*Brassica* spp.), a versatile crop known for its oil-rich seeds. Electrical conductivity (EC), or soil salinity, is a quantitative measure of a soil's ability to conduct an electrical current, indicating soluble salts' existence. The monitoring and understanding soil electrical conductivity (EC) in mustard cultivation is crucial for improving crop growth, efficiently managing nutrients, and optimising overall productivity. This article explores the importance of measuring soil electrical conductivity (EC) in mustard fields, the various methodologies used to determine EC accurately, and the implications of different EC levels on mustard crop cultivation (Liu, Meng, et al., 2022; Lombardino et al., 2022; Ma et al., 2022; Mabasa, 2023; Maheshwari et al., 2022; Maia et al., 2022; Makete et al., 2022; Manepalli et al., 2022; Mangena, 2022). The soil's electrical conductivity (EC) is a significant factor in influencing various aspects of mustard crop cultivation. The evaluation of salinity: The electrical conductivity (EC) measurement provides a

reliable method for assessing soil salinity levels. Elevated electrical conductivity (EC) values indicate a heightened presence of soluble salts in the soil. Elevated salinity levels can harm mustard crops, causing disturbances in water absorption and essential nutrients. Soil salinity exerts an influence on the accessibility of vital nutrients necessary for the growth of plants. Elevated salinity levels can hinder mustard plants' capacity to uptake vital nutrients, including phosphorus, potassium, and calcium. Consequently, this can lead to nutrient insufficiencies and a subsequent decline in agricultural output. Water management in agricultural fields can present complexities due to elevated soil salinity levels. Saline soils demonstrate diminished capacity for water retention, leading to increased water requirements and the possibility of water stress impacting mustard crops. Monitoring electrical conductivity (EC) levels is an essential component of irrigation management to ensure optimal effectiveness. Elevated soil salinity can adversely affect crop health, resulting in detrimental consequences such as compromised growth, reduced leaf area, and diminished seed yields in mustard plants. Monitoring and managing soil electrical conductivity (EC) ensures cultivated plants' optimal health and vitality. The ramifications of soil electrical conductivity (EC) in mustard cultivation are far-reaching and significantly impact the crop's efficacy and yield (Tong, et al., 2023; Liu et al., 2023; Liu, Wang, et al., 2022; Liu, Li, et al., 2022; Liu, Cui, et al., 2022; Liu, Zong, et al., 2022; Liu, Liu, et al., 2022; Liu, Xiao, et al., 2022). Monitoring soil electrical conductivity (EC) is critical in adjusting nutrient management practices. In saline soils, implementing supplementary leaching techniques may be necessary to mitigate excessive salts and optimise nutrient availability. It is crucial to ensure the proper application of nutrients based on soil salinity levels to facilitate optimal crop growth and development. A comprehensive understanding of soil salinity is imperative for effectively managing irrigation. Saline soils may require more frequent and extensive leaching, impacting water utilisation efficiency and expenditure. Implementing electrical conductivity (EC) measurements in irrigation practises contributes to the conservation of water resources. Crop Selection in Agricultural Systems: A Critical Analysis The increased levels of soil salinity can limit the range of crop varieties that can be cultivated. Certain cultivars of mustard demonstrate a higher level of tolerance towards salinity in comparison to other cultivars, thus increasing the probability of achieving a successful harvest by carefully

choosing appropriate varieties (Li, Han, et al., 2022; Li, He, et al., 2023; Li et al., 2022; Li, Han, et al., 2023; Li, Huang, et al., 2022; Li, Luo, et al., 2022). The adverse consequences of elevated soil salinity on the environment become evident as salts accumulate in the vicinity, leading to detrimental impacts on adjacent water bodies and ecosystems. Effective soil electrical conductivity (EC) management plays a pivotal role in mitigating the environmental impacts associated with agricultural practices. Soil salinity has a direct influence on the economic sustainability of mustard cultivation. Implementing effective management strategies to maintain optimal EC levels can enhance crop quality, increase yields, and improve profitability (Lajayer et al., 2022; Li et al., 2022; Li et al., 2022; Li et al., 2022; Li, Ren, et al., 2023; Li, Zhang, et al., 2022; Li, Zheng, et al., 2023; Li et al., 2022). Measuring soil electrical conductivity (EC) is crucial in mustard cultivation, as it substantially impacts crop performance and agricultural practices' overall sustainability. Monitoring and managing soil salinity play a critical role in optimising nutrient availability, improving water use efficiency, fostering crop health, and ultimately enhancing the economic outcomes of mustard farming. To attain maximum growth and productivity of mustard crops, it is imperative for farmers to consistently assess and regulate the electrical conductivity (EC) of the soil in their agricultural fields, making necessary adjustments and modifications to their practises as deemed appropriate. Through the implementation of this strategy, individuals can effectively facilitate the advancement of mustard crops and contribute significantly towards the establishment of agricultural systems that are both sustainable and productive (Kaya et al., 2023; Khalid et al., 2023; Khan et al., 2022, 2023; Khan et al., 2022; Kochanek et al., 2023; Kosakivska et al., 2022; Kour et al., 2023; Kudoyarova, 2022; Kumar & Ohri, 2023; Kurepa & Smalle, 2022; Kurniawan & Chuang, 2022; Kuromori et al., 2022).

**Figure 4.53. Soil EC of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

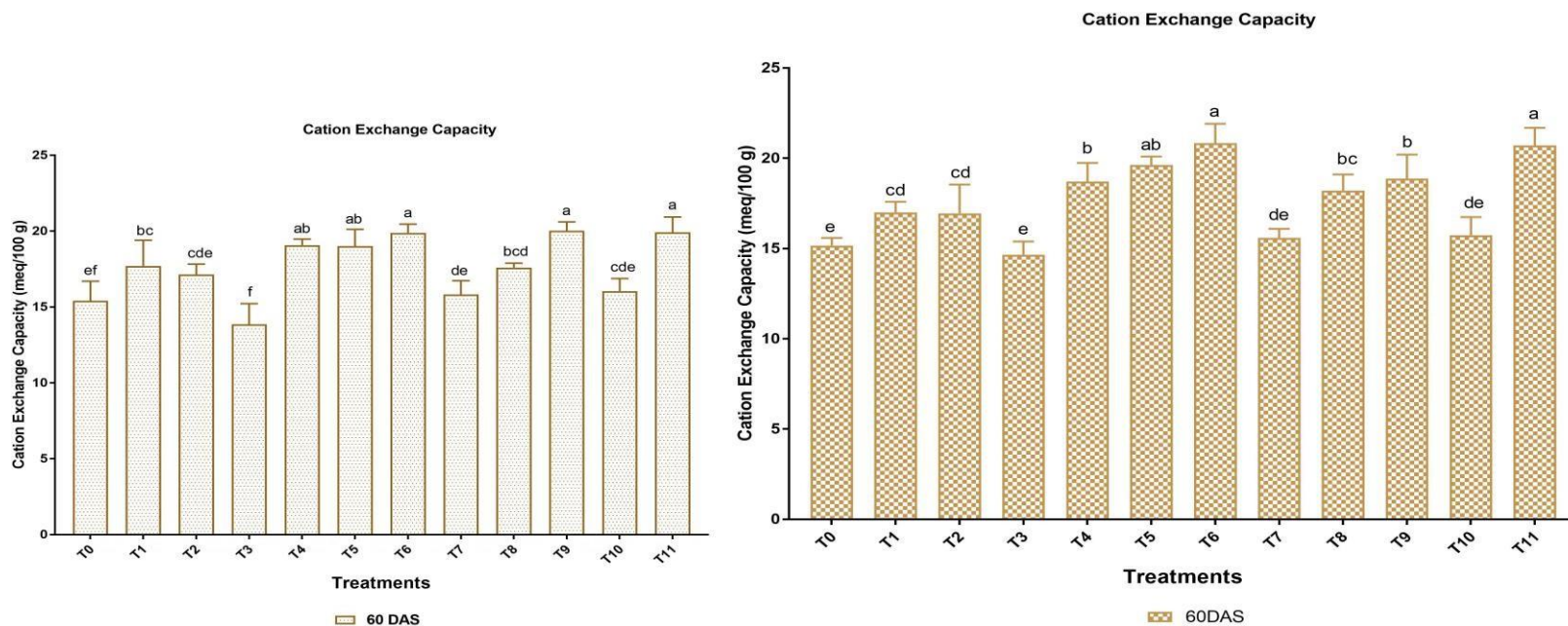
**Soil CEC:** The effect of Sulphur and Salicylic acid and their combination on soil CEC was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at 60 days after sowing (DAS) (Table 4.54, Figure 4.54). In 2021-2022, there was a significant difference in CEC compared to T0 (Control) at 60 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the CEC was observed at 60 DAS. Therefore, at 60 DAS the percentage increase as compared to T0 was found highest in T9 followed by T11, T6, T4, T5, T1, T8, T2, T10, T7 and the percentage values were 23.01%, 22.61%, 22.50%, 19.21%, 18.93%, 12.95%, 12.40%, 10.07%, 3.98%, 2.57% respectively. But in T3 percentage decrease as compared to T0 and the percentage value was -11.23%. In 2022-2023, there was a significant difference in CEC compared to T0 (Control) at 60 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 60 DAS, the percentage increase as compared to T0 was found highest in T6 followed by T11, T5, T9, T4, T8, T1, T2, T10, T7 and the percentage values were 27.21%, 26.78%, 22.76%, 19.65%, 18.99%, 16.73%, 10.80%, 10.55%, 3.70%, 2.75% respectively. But in T3 percentage decrease as compared to T0 and the percentage value was -3.43%. The cation exchange capacity (CEC) is an essential soil characteristic in agricultural contexts, specifically in the cultivation of mustard plants (*Brassica* spp.). Mustard is a highly adaptable agricultural plant renowned for its seeds that are abundant in oil content and its extensive range of practical uses. A comprehensive comprehension of the significance of cation exchange capacity (CEC) in the soil used for mustard cultivation, as well as its interplay with compounds such as thiourea and salicylic acid, is imperative for maximising plant growth, enhancing nutrient accessibility, and improving the stress response. This article examines the importance of cation exchange capacity (CEC) in soil and its interactions with thiourea and salicylic acid in the cultivation of mustard.

**The Significance of Cation Exchange Capacity in Soil for Mustard Cultivation:** The availability of nutrients: Cation Exchange Capacity (CEC) is a metric used to assess the soil's capacity to retain and exchange cations, which are ions with a positive charge. These cations include crucial nutrients such as potassium (K), calcium (Ca), magnesium (Mg), and ammonium (NH<sub>4</sub><sup>+</sup>). Soils with a higher cation exchange capacity (CEC) can retain more nutrients, thereby enhancing their accessibility to plant roots (Hong et al., 2022; Huang et al.,

2022; Hudeček et al., 2023; Huh, 2022; Hui et al., 2022; Huntenburg et al., 2022). The capacity of the soil to retain nutrients is also influenced by its cation exchange capacity (CEC). Soils possessing a high cation exchange capacity (CEC) exhibit enhanced nutrient retention capabilities, thereby mitigating the potential for nutrient leaching and facilitating gradual accessibility to plants. pH Buffering: Soils with high CEC tend to have better pH buffering capacity. The ability to withstand abrupt fluctuations in pH enables them to uphold a consistent pH level within the root zone, a critical factor for facilitating optimal nutrient absorption. The soil's water-holding capacity is closely linked to its cation exchange capacity (CEC). Soils exhibiting a higher cation exchange capacity (CEC) possess an enhanced ability to retain water, thereby facilitating improved moisture accessibility for plants, particularly in arid conditions. The present study investigates the interaction between thiourea and a specific compound. The impact of thiourea on cation exchange capacity (CEC) in specific soil types has been demonstrated in studies on nutrient availability. In certain instances, it has the potential to augment cation exchange capacity (CEC), thereby promoting heightened nutrient accessibility for mustard plants. Thiourea can potentially enhance nutrient retention and release by enhancing the soil's cation exchange capacity (CEC). Nutrient efficiency can be enhanced through the synergistic effect of thiourea and cation exchange capacity (CEC). Thiourea can augment the soil's ability to retain and release cations, encompassing vital nutrients. This phenomenon can potentially result in enhanced nutrient accessibility for mustard crops. The impact of thiourea on cation exchange capacity (CEC) may also play a role in enhancing stress tolerance in mustard plants. Enhancing the accessibility of nutrients and the capacity to retain moisture aids the plant in adapting to environmental stressors such as drought or salinity. The present study aims to investigate the interaction between salicylic acid and other compounds. Salicylic acid, a signalling molecule in plant stress responses, can influence cation exchange capacity (CEC) (Hussein et al., 2023; Iftikhar et al., 2023; Islam et al., 2023; Javadipour et al., 2022). The plant's capacity to effectively respond to environmental stressors may be augmented through its interaction with CEC. Applying salicylic acid can alter the cation exchange capacity (CEC), thereby influencing the availability of nutrients and the ability of mustard crops to tolerate stress. The uptake of nutrients: Salicylic acid can modify the cation exchange properties of the soil. The present

interaction can potentially influence the availability and uptake of vital nutrients in mustard plants. Variations in cation exchange capacity (CEC) can impact the nutrient cycling processes within the root zone. The interaction between salicylic acid and cation exchange capacity (CEC) can influence stress mitigation. The influence of salicylic acid on cation exchange capacity (CEC) could potentially play a role in the plant's capacity to modulate nutrient absorption and uphold physiological processes during periods of stress. The cation exchange capacity (CEC) of soil used for mustard cultivation is an essential characteristic that significantly affects the availability of nutrients, retention of nutrients, buffering pH, and the capacity to hold water. Comprehending the interplay between cation exchange capacity (CEC) and chemical compounds such as thiourea and salicylic acid is paramount in enhancing mustard cultivation. These interactions can enhance nutrient efficiency, increase stress tolerance, and promote plant health. By examining these interconnections, agricultural practitioners can devise enhanced methodologies to maximise the agricultural yield of mustard crops while considering the ecological and economic viability of their operations (Hernandez-Leon & Valenzuela-Soto, 2022; Hernández et al., 2023; Hernández et al., 2022; Hilal et al., 2023).

**Figure 4.54. Soil CEC of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).



**Soil Available Nitrogen:** The effect of Sulphur and Salicylic acid and their combination on soil available nitrogen was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at 60 days after sowing (DAS) (Table 4.55, Figure 4.55). In 2021-2022, there was a significant difference in available nitrogen compared to T0 (Control) at 60 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the available nitrogen was observed at 60 DAS. Therefore, at 60 DAS the percentage increase as compared to T0 was found highest in T5 followed by T10, T6, T4, T1, T9, T3, T7, T8, T11, T2 and the percentage values were 42.39%, 40.11%, 36.13%, 33.43%, 28.64%, 28.62%, 28.55%, 28.20%, 22.01%, 21.27%, and 20.03% respectively. In 2022-2023, there was a significant difference in available nitrogen compared to T0 (Control) at 60 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 60 DAS, the percentage increase as compared to T0 was found highest in T5 followed by T10, T6, T4, T3, T1, T9, T7, T11, T8, T2 and the percentage values were 45.22%, 41.56%, 36.97%, 36.38%, 32.59%, 32.36%, 31.41%, 27.64%, 26.54%, 26.32%, and 22.82% respectively. Nitrogen (N) plays a vital and irreplaceable role in agriculture, particularly in the cultivation of mustard (*Brassica spp.*). Mustard, a plant recognised for its seeds with a high oil content and a wide array of applications, depends on nitrogen for various physiological processes. This article explores the components and importance of soil nitrogen in mustard fields and the approaches to regulate nitrogen levels to improve crop productivity efficiently (Huntenburg et al., 2022; Hussein et al., 2023; Iftikhar et al., 2023; Islam et al., 2023; Javadipour et al., 2022). Nitrogen is vital in mustard crops, serving multiple indispensable functions and substantially contributing to their overall health and productivity. Protein synthesis is a biological process in which nitrogen is an essential component of amino acids, the fundamental building block for forming proteins. Proteins are paramount in the organisation and operation of plants, encompassing a diverse array of indispensable constituents, including enzymes, chlorophyll, and structural proteins. Nitrogen plays a crucial role in photosynthesis as it is an essential constituent of chlorophyll, the primary photosynthetic pigment responsible for green colouration. Mustard plants demonstrate proficient growth when provided with adequate nitrogen levels, thereby enhancing the process of converting light into chemical energy. The significance of nitrogen in the

growth and development of plants is paramount, as it exerts a profound impact on a range of physiological mechanisms, including but not limited to cell division, leaf expansion, and stem elongation. The promotion of robust growth in mustard crops is contingent upon the maintenance of optimal nitrogen levels. The process of nutrient transportation in plants involves the participation of nitrogen, which plays a pivotal role in facilitating the movement of essential nutrients, such as phosphorus and potassium, throughout different plant tissues. The proper assimilation and transportation of nutrients are of paramount importance in sustaining the overall well-being of plants. The optimisation of nitrogen levels is crucial in enhancing mustard crops' seed quality and oil content, leading to a substantial increase in their economic value. The presence of soil nitrogen is of utmost importance in cultivating mustard. The achievement of robust growth and enhanced mustard seed yields depends on adequate nitrogen in the soil. Insufficient nitrogen levels can lead to compromised plant development and reduced production of seeds (Hilal et al., 2023; Hong et al., 2022; Huang et al., 2022; Hudeček et al., 2023; Huh, 2022; Hui et al., 2022). Nutrient synergy refers to the interplay between nitrogen and other essential nutrients, such as phosphorus and potassium, which enhances nutrient absorption and efficient utilisation. Ensuring the maintenance of optimal nutrient levels is of utmost importance for the overall health and productivity of mustard crops. Nitrogen is indispensable in photosynthesis and energy production, as it plays a crucial role in facilitating the efficient conversion of light energy into plant biomass, specifically in mustard crops. Adequate nitrogen levels have been observed to diminish the enhancement of seed quality and oil content in mustard crops, consequently leading to an increase in the economic value and marketability of the crop. An adequate amount of nitrogen has been observed to influence the resistance of mustard crops against diseases positively. This can be attributed to the essential role of nitrogen in strengthening the plant's immune system and preserving its structural integrity. The existence of soil nitrogen is of utmost importance in the cultivation of mustard, exerting a substantial influence on the growth, productivity, and overall attributes of mustard crops. To enhance the growth and productivity of mustard crops, it is crucial for cultivators to meticulously monitor and proficiently manage the nitrogen levels present in the soil. Implementing effective nitrogen management practices leads to increased vigour in mustard plants. Mitigating

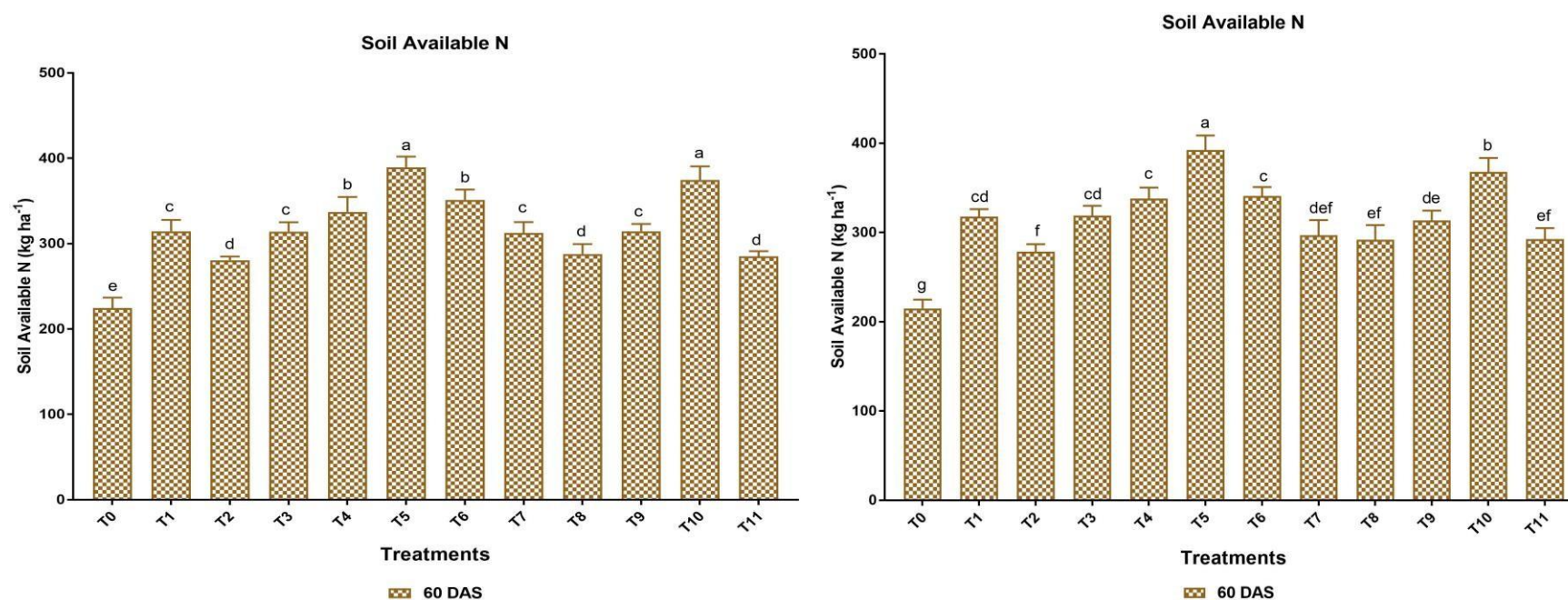
adverse environmental consequences from excessive fertiliser application is crucial in promoting sustainable agriculture. To ensure robust and sustainable crop production in mustard cultivation, cultivators can implement necessary strategies by understanding the roles and importance of soil nitrogen (Hernandez-Leon & Valenzuela-Soto, 2022; Hernández et al., 2023; Hernández et al., 2022).

**Table 4.55. Impact of Different Treatments on Soil Available Nitrogen of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	Soil Available Nitrogen		Soil Available Phosphorus	
	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	224.406 <sup>e</sup> ±12.432	214.943 <sup>g</sup> ±9.909	7.870 <sup>g</sup> ±0.265	8.460 <sup>f</sup> ±0.5139
<b>T1 (Thiourea-1000 ppm)</b>	314.513 <sup>c</sup> ±13.382	317.810 <sup>cd</sup> ±8.443	14.153 <sup>b</sup> ±0.245	14.4400 <sup>b</sup> ±0.330
<b>T2 (Salicylic acid-300 ppm)</b>	280.630 <sup>d</sup> ±4.367	278.500 <sup>f</sup> ±8.448	11.570 <sup>e</sup> ±0.559	11.8700 <sup>d</sup> ±0.260
<b>T3 [Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	314.083 <sup>c</sup> ±11.03	318.863 <sup>cd</sup> ±11.165	13.506 <sup>c</sup> ±0.337	13.9467 <sup>bc</sup> ±0.228
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	337.133 <sup>b</sup> ±17.547	337.900 <sup>c</sup> ±12.490	6.166 <sup>i</sup> ±0.404	7.2500 <sup>g</sup> ±0.242
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	389.540 <sup>a</sup> ±12.586	392.420 <sup>a</sup> ±16.261	11.280 <sup>ef</sup> ±0.377	11.8467 <sup>d</sup> ±0.240
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	351.350 <sup>b</sup> ±12.001	341.030 <sup>c</sup> ±9.936	13.376 <sup>cd</sup> ±0.515	13.5233 <sup>c</sup> ±0.370
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	312.586 <sup>c</sup> ±12.767	297.073 <sup>def</sup> ±16.907	6.930 <sup>h</sup> ±0.357	7.2567 <sup>g</sup> ±0.340
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	287.770 <sup>d</sup> ±11.800	291.746 <sup>ef</sup> ±16.541	8.020 <sup>g</sup> ±0.105	7.8533 <sup>fg</sup> ±0.218
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	314.420 <sup>c</sup> ±8.762	313.386 <sup>de</sup> ±11.222	12.766 <sup>d</sup> ±0.345	13.5733 <sup>c</sup> ±0.748
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	374.746 <sup>a</sup> ±15.820	367.820 <sup>b</sup> ±15.754	16.203 <sup>a</sup> ±0.301	16.5833 <sup>a</sup> ±0.465
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	285.060 <sup>d</sup> ±6.131	292.613 <sup>ef</sup> ±12.308	10.806 <sup>f</sup> ±0.410	10.6233 <sup>e</sup> ±0.675
<b>CD</b>	21.106	22.702	0.63	0.707
<b>CV</b>	3.925	4.247	3.342	3.626

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.55. Soil Available Nitrogen of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Soil Available Phosphorus:** The effect of Sulphur and Salicylic acid and their combination on soil phosphorus was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at 60 days after sowing (DAS) (Table 4.56, Figure 4.56). In 2021-2022, there was a significant difference in soil phosphorus compared to T0 (Control) at 60 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the soil phosphorus was observed at 60 DAS. Therefore, at 60 DAS the percentage increase as compared to T0 was found highest in T10 followed by T1, T3, T6, T9, T2, T5, T11, T8 and the percentage values were 51.42%, 44.39%, 41.73%, 41.16%, 38.35%, 31.97%, 30.23%, 27.17%, 1.87% respectively. But the percentage also decrease T7, T4 as compared to T0 and the percentage values were -13.56%, -27.62% respectively. In 2022-2023, there was a significant difference in soil phosphorus compared to T0 (Control) at 60 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 60 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T1, T3, T9, T6, T2, T5, T11 and the percentage values were 48.98%, 41.41%, 39.34%, 37.67%, 37.44%, 28.72%, 28.58%, 20.36% respectively. But the percentage also decrease T8, T7, T4 as compared to T0 and the percentage values were -7.72%, -16.58%, -16.68% respectively. Phosphorus (P) is a vital macronutrient that plays a critical role in the growth and development of plants, especially in the cultivation of mustard (*Brassica* spp.). Mustard, renowned for its seeds abundant in oil and multifaceted uses, depends on phosphorus for various physiological functions. This article examines the different parts and significance of soil phosphorus within mustard fields while delving into the strategies employed for effectively managing phosphorus levels to enhance crop performance. Phosphorus plays a crucial role in the growth and development of mustard crops, thereby significantly influencing their overall well-being and productivity.

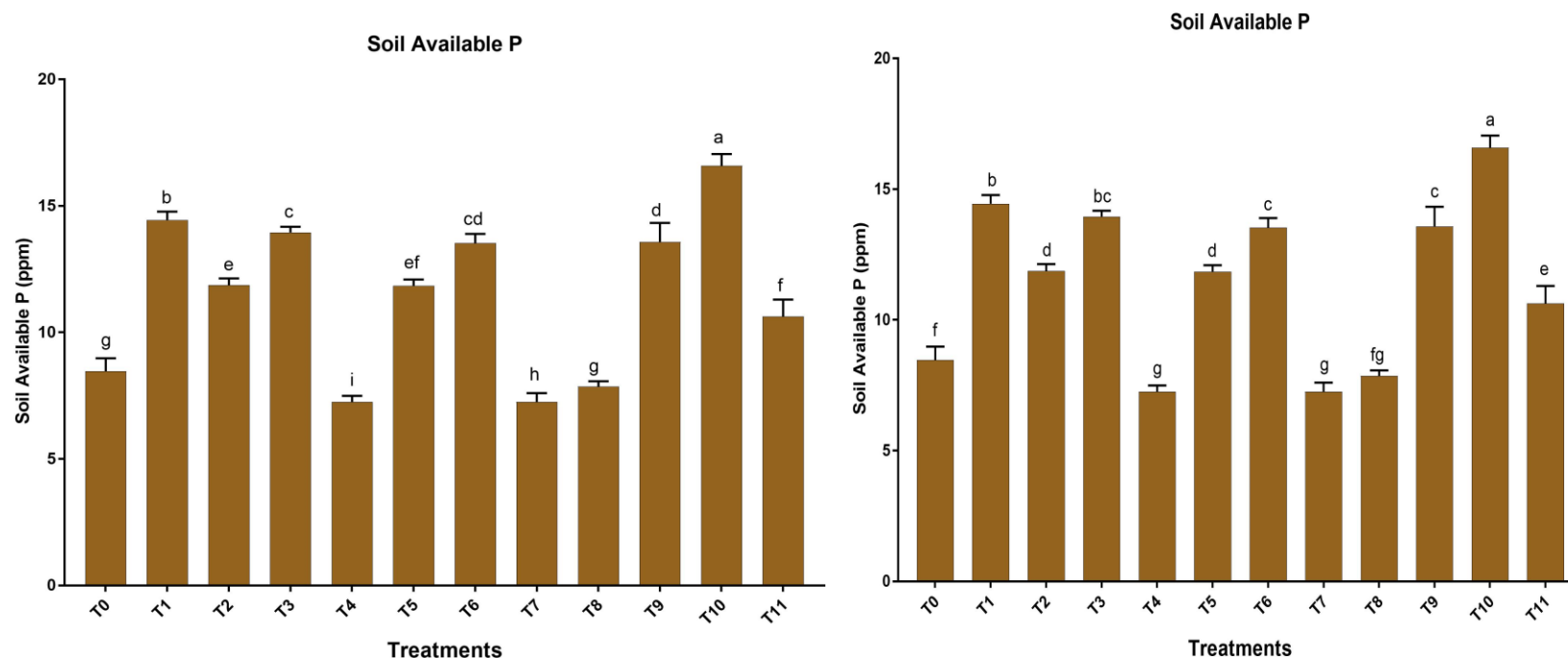
**Energy Transfer:** Phosphorus plays a vital role as a constituent of adenosine triphosphate (ATP), a molecule responsible for the storage and transfer of energy within the cellular structures of plants (Hong et al., 2022; Huang et al., 2022; Hudeček et al., 2023; Huh, 2022; Hui et al., 2022). Energy is a fundamental requirement for many metabolic processes, encompassing vital functions such as photosynthesis, respiration, and nutrient absorption. The presence of sufficient levels of phosphorus facilitates the

promotion of robust root development in mustard plants. A well-developed root system enhances the plant's capacity to uptake water and nutrients from the soil. Phosphorus is a fundamental constituent of nucleic acids, namely DNA and RNA, playing a critical role in facilitating the transmission of genetic information and the synthesis of proteins. The factor above is of utmost importance in promoting the growth and development of plants. Photosynthesis relies on the participation of phosphorus, which plays a crucial role in facilitating the conversion of light energy into chemical energy in the form of carbohydrates in mustard plants. The carbohydrates are stored within the plant and function as an energy reserve. The role of phosphorus in flowering and seed production is of utmost importance. Sufficient phosphorus concentrations ensure optimal seed and oil yield in mustard cultivations. The presence of soil phosphorus plays a crucial role in the successful cultivation of mustard. The attainment of optimal growth and increased mustard seed yields is contingent upon sufficient phosphorus in the soil. A phosphorus deficiency can result in inhibited growth and diminished seed yield. Nutrient synergy involves the interaction between phosphorus and other vital nutrients, such as nitrogen and potassium, facilitating nutrient absorption and effective utilisation. Maintaining optimal nutrient levels is paramount for mustard crops' overall health and productivity (Huntenburg et al., 2022; Hussein et al., 2023; Iftikhar et al., 2023; Islam et al., 2023; Javadipour et al., 2022). The development of roots is enhanced by an ample availability of phosphorus, which in turn enhances the plant's ability to access water and nutrients within the soil. An adequate phosphorus supply often enhances disease resistance in mustard crops, as phosphorus is crucial in bolstering the plant's immune response and maintaining its structural integrity. The enhancement of seed quality and oil content in mustard crops can be attributed to adequate phosphorus levels, resulting in improved economic value. The presence of soil phosphorus plays a crucial role in the cultivation of mustard, exerting significant effects on the growth, productivity, and overall characteristics of mustard crops. To optimise crop productivity and achieve successful mustard cultivation, growers must diligently monitor and effectively manage the soil phosphorus levels. Implementing efficient phosphorus management practices results in improved vitality of mustard plants. It serves as a valuable contribution to promoting sustainable agricultural practices, primarily by mitigating the adverse environmental consequences associated with the excessive application of fertilisers. Cultivators can

undertake the requisite measures to guarantee resilient and sustainable crop yield by comprehending the functions and significance of soil phosphorus in mustard cultivation (Hernandez-Leon & Valenzuela-Soto, 2022; Hernández et al., 2023; Hernández et al., 2022; Hilal et al., 2023).



**Table 4.56. Soil available Phosphorus of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Soil Available Potassium:** The effect of Sulphur and Salicylic acid and their combination on soil potassium was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at 60 days after sowing (DAS) (Table 4.57, Figure 4.57). In 2021-2022, there was a significant difference in soil potassium compared to T0 (Control) at 60 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the soil potassium was observed at 60 DAS. Therefore, at 60 DAS the percentage increase as compared to T0 was found highest in T3 followed by T4, T2, T10, T6 and the percentage values were 22.86%, 12.98%, 12.55%, 11.22%, 3.69% respectively. But the percentage also decrease in T7, T5, T1, T11, T9, T8 as compared to T0 and the percentage values were -4.46%, -5.68%, -8.02%, -13.36%, -23.18%, -30.92% respectively. In 2022-2023, there was a significant difference in soil potassium compared to T0 (Control) at 60 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 60 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T2, T10, T1, T4, T6, T11 and the percentage values were 24.24%, 18.16%, 9.56%, 8.20%, 7.78%, 6.57%, 1.31% respectively. But the percentage also decrease in T5, T7, T9, T8 and the percentage values were -5.43%, -8.30%, -8.62%, -13.35% respectively. Potassium (K) is an essential macronutrient that is indispensable for the growth and development of plants. Its significance in the cultivation of mustard (*Brassica spp.*) cannot be overstated. The botanical species referred to as mustard, which is notable for its oil-rich seeds and versatile applications, requires the presence of potassium to carry out various physiological processes (Carreño-Vega et al., 2022; Cavusoglu et al., 2022; Chai et al., 2022; Chan, 2022). This article investigates the different components and importance of soil potassium in mustard fields while exploring the strategies for efficiently managing potassium levels to optimise crop performance. Potassium is an essential element that plays a pivotal role in the growth and development of mustard crops, significantly influencing their overall health and productivity. The process of osmotic regulation is of utmost importance, as it relies on the essential role of potassium in facilitating water balance maintenance in mustard plants. This bears notable importance during periods of drought or limited water availability. The modulation of stomatal aperture is impacted by the presence of potassium ions, which have a pivotal function

in regulating the process of stomatal opening and closure (Chen et al., 2023; Chen, Tang, et al., 2022; Chen, Tang, et al., 2022; Chen, Wu, et al., 2022; Chen et al., 2023; Chen, Wang, et al., 2022; Chen, Zhang, et al., 2022). Potassium exerts regulatory effects on stomata, tiny pores on plant leaves' surface. Maintaining optimal stomatal position facilitates efficient gas exchange and regulates water loss through transpiration. The process of enzyme activation Potassium catalyses many enzymes involved in photosynthesis, respiration, and other metabolic activities. The existence of these enzymes is essential for the production of energy and the assimilation of nutrients. The nutrient uptake and transport process encompasses the vital role of potassium in facilitating the absorption and transportation of crucial nutrients, such as nitrogen and phosphorus. Adequate potassium levels reduce the optimal utilisation of these indispensable nutrients by mustard crops. A sufficient quantity of potassium in mustard plants has been observed to augment their resistance against diseases and pests. Potassium is vital in fortifying cellular membranes, strengthening the organism's physiological resistance to pathogens and herbivores. The enhancement of potassium levels in mustard crops has the potential to significantly enhance the quality of seeds and oil content, resulting in a notable increase in their economic worth. Potassium in soil plays a significant role in the cultivation of mustard. Achieving optimal growth and enhanced mustard seed yields depends on adequate potassium levels in the soil. Insufficient potassium levels can lead to compromised plant growth and reduced production of seeds. Nutrient synergy refers to the dynamic interplay between potassium and other essential nutrients, such as nitrogen and phosphorus, to optimise nutrient absorption and utilisation efficiency (Chao et al., 2022; Chaturvedi, Khan, et al., 2022; Chaturvedi, Kulshrestha, et al., 2022; Chauhan et al., 2023; Chen, Xu, et al., 2022). Maintaining optimal nutrient levels is crucial to guarantee the well-being and productivity of mustard crops. The advantageous properties of potassium in osmotic regulation and stomatal control make it a valuable asset for improving drought tolerance in mustard crops, especially in regions with erratic precipitation patterns. Implementing efficient potassium management practices in agricultural systems is crucial in fostering environmental sustainability. The act of reducing excessive fertilisation is undertaken to mitigate the potential risks associated with the runoff of nutrients and water pollution. Potassium in soil is of utmost importance in the cultivation of mustard, as it

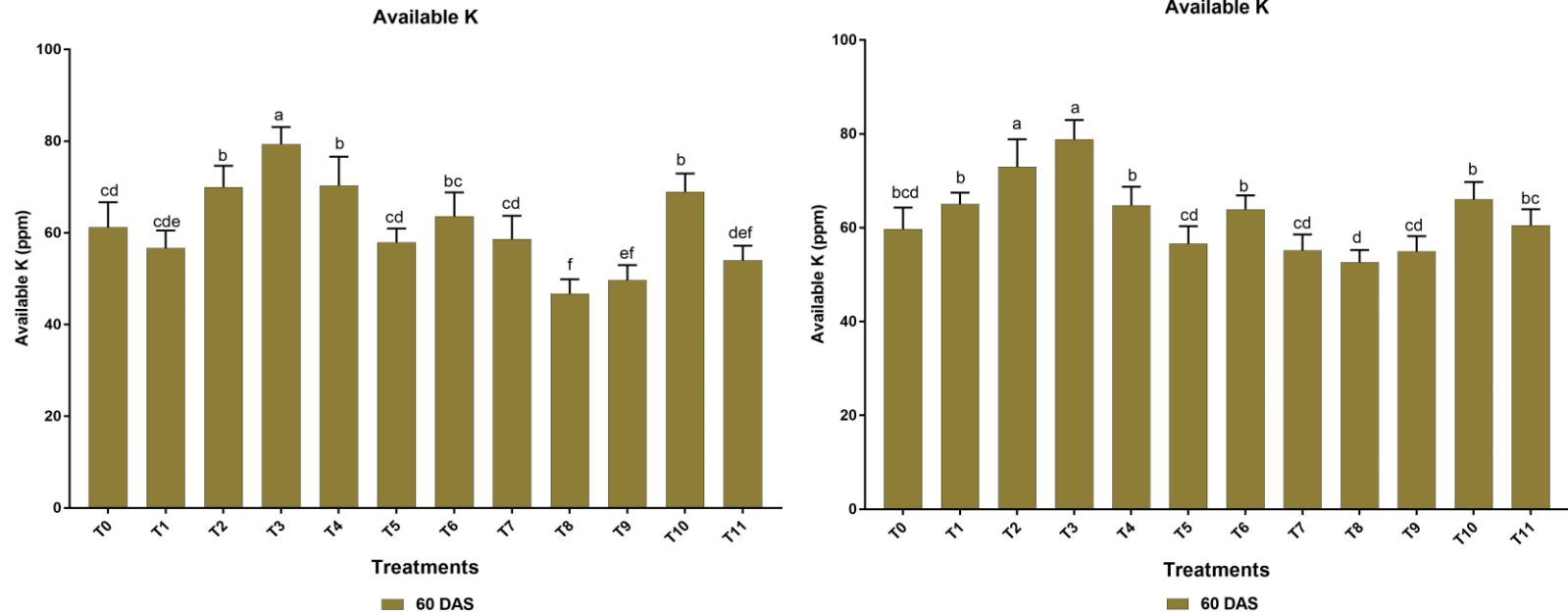
substantially influences the growth, productivity, and overall characteristics of mustard crops. To enhance the development and cultivation of mustard crops, it is crucial for farmers to conscientiously monitor and proficiently manage the potassium levels present in the soil. Implementing effective potassium management practices leads to enhanced vitality of mustard plants, thus promoting sustainable agricultural practices by mitigating the environmental consequences of excessive fertiliser usage. By acquiring a thorough understanding of the roles and importance of soil potassium in mustard cultivation, farmers can implement the necessary actions to ensure sustainable and ecologically responsible crop production (Brilli et al., 2022; Butt & Gul, 2023; Çam et al., 2022; Campos et al., 2023; Canales et al., 2023; Cao et al., 2022).

**Table 4.57. Impact of Different Treatments on Soil Available Potassium & Sulphur of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	Soil Available Potassium		Soil Sulphur	
	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	61.223 <sup>cd</sup> ±5.451	59.716 <sup>bcd</sup> ±4.589	13.720 <sup>f</sup> ±0.379	13.890 <sup>e</sup> ±0.681
<b>T1 (Thiourea-1000 ppm)</b>	56.676 <sup>cde</sup> ±3.857	65.056 <sup>b</sup> ±2.435	14.163 <sup>ef</sup> ±0.267	13.726 <sup>e</sup> ±0.455
<b>T2 (Salicylic acid-300 ppm)</b>	70.013 <sup>b</sup> ±4.630	72.973 <sup>a</sup> ±5.906	14.536 <sup>e</sup> ±0.267	14.820 <sup>cd</sup> ±0.256
<b>T3 (Thiourea-1000 ppm) + (Salicylic Acid-300 ppm)</b>	79.373 <sup>a</sup> ±3.707	78.826 <sup>a</sup> ±4.140	14.366 <sup>e</sup> ±0.455	14.230 <sup>de</sup> ±0.623
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	70.363 <sup>b</sup> ±6.257	64.760 <sup>b</sup> ±3.984	16.273 <sup>ab</sup> ±0.155	15.550 <sup>b</sup> ±0.238
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	57.930 <sup>cd</sup> ±3.010	56.636 <sup>cd</sup> ±3.711	15.173 <sup>d</sup> ±0.315	15.600 <sup>b</sup> ±0.220
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	63.573 <sup>bc</sup> ±5.260	63.920 <sup>b</sup> ±2.988	15.276 <sup>cd</sup> ±0.192	14.330 <sup>de</sup> ±0.334
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	58.606 <sup>cd</sup> ±5.109	55.136 <sup>cd</sup> ±3.425	16.000 <sup>b</sup> ±0.619	15.096 <sup>bc</sup> ±0.204
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	46.763 <sup>f</sup> ±3.115	52.683 <sup>d</sup> ±2.582	15.773 <sup>bc</sup> ±0.370	14.996 <sup>bc</sup> ±0.181
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	49.700 <sup>ef</sup> ±3.280	54.973 <sup>cd</sup> ±3.242	16.783 <sup>a</sup> ±0.251	16.460 <sup>a</sup> ±0.306
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	68.966 <sup>b</sup> ±3.994	66.033 <sup>b</sup> ±3.710	14.590 <sup>e</sup> ±0.141	14.113 <sup>e</sup> ±0.325
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	54.006 <sup>def</sup> ±3.215	60.513 <sup>bc</sup> ±3.435	15.916 <sup>b</sup> ±0.243	15.486 <sup>bc</sup> ±0.251
<b>CD</b>	7.745	6.606	0.569	0.662
<b>CV</b>	7.398	6.192	2.195	2.613

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Table 4.57. Soil Available Potassium of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Sulphur:** The effect of Sulphur and Salicylic acid and their combination on sulphur was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at 60 days after sowing (DAS) (Table 4.58, Figure 4.58). In 2021-2022, there was a significant difference in sulphur compared to T0 (Control) at 60 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the sulphur was observed at 60 DAS. Therefore, at 60 DAS the percentage increase as compared to T0 was found highest in T9 followed by T4, T7, T11, T8, T6, T5, T10, T2, T3, T1, and the percentage values were 18.25%, 15.69%, 14.25%, 13.80%, 13.01%, 10.18%, 9.57%, 5.96%, 5.61%, 4.50%, 3.13% respectively. In 2022-2023, there was a significant difference in sulphur compared to T0 (Control) at 60 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 60 DAS, the percentage increase as compared to T0 was found highest in T9 followed by T5, T4, T11, T7, T8, T2, T6, T3, T10 and the percentage values were 15.61%, 10.96%, 10.67%, 10.30%, 7.99%, 7.37%, 6.27%, 3.07%, 2.38%, 1.58% respectively. But the percentage also decrease in T1 and the percentage value was -1.18%. The role of soil sulphur (S) is of utmost importance in agriculture, particularly in the growth and development of mustard plants (*Brassica* spp.). The mustard plant is recognised for its remarkable versatility in agriculture, as it produces copious amounts of oil-rich seeds that find application in various domains such as culinary, oil extraction, and livestock nutrition. The importance of soil sulphur in mustard cultivation is highly significant, as it significantly impacts various aspects of crop development, such as growth, seed production, and overall quality. This article explores the diverse functions and significance of soil sulphur in mustard fields and delves into the strategies implemented to optimise sulphur management to augment crop productivity. The presence of soil sulphur is of utmost importance for mustard crops, as it is an essential nutrient that plays a central role in many vital processes throughout the plant's life cycle. Including sulphur within amino acids and proteins facilitates the biosynthesis of mustard plants' enzymes, hormones, and structural components (Jia et al., 2022; Jin et al., 2022; Jofre et al., 2023; Kandhol et al., 2023; Kapoor et al., 2022; Kapoor et al., 2022; Karaman, 2023; Karamat et al., 2022; Katoch et al., 2022; Kaviani et al., 2023; Kaya et al., 2023). Adequate levels of sulphur are imperative for achieving optimal growth and development. Sulphur is a vital

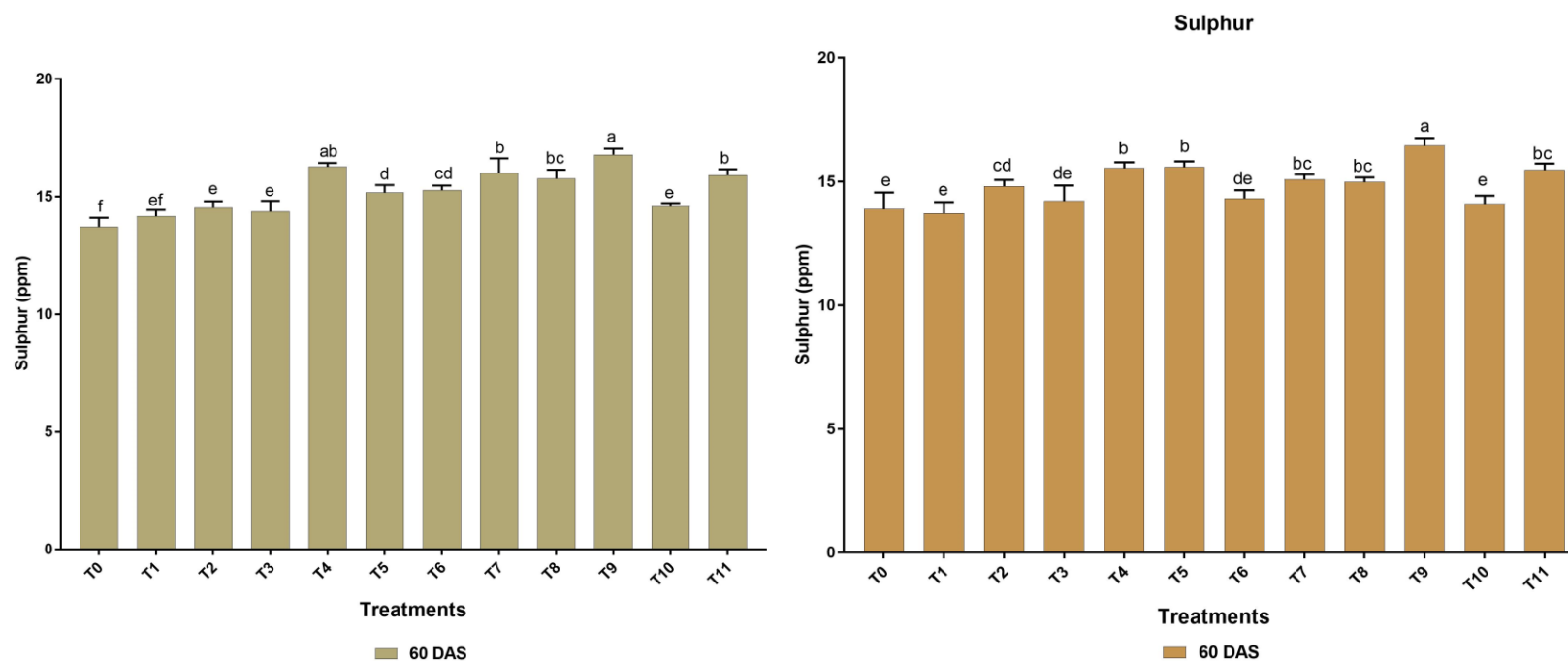
component of chlorophyll, the pigment responsible for the green colouration in plants and the facilitation of photosynthesis. Mustard crops that possess a sufficient quantity of sulphur exhibit the capacity to efficiently convert light energy into chemical energy, facilitating robust growth. Sulphur plays a facilitating role in activating specific vitamins and enzymes within plants. These compounds are of utmost importance in a wide range of metabolic processes, including the absorption of nutrients and the capacity to endure stressful conditions. The stress tolerance of mustard crops is increased when they are provided with an adequate amount of sulphur. Sulphur can enhance the plant's resilience in the face of unfavourable environmental circumstances, including drought, extreme temperatures, and pathogenic attacks. Including sulphur is critical in assessing the calibre of oil and seeds in mustard crops cultivated specifically for oil extraction. The fatty acid composition of oil is subject to various influences, which affect its flavour and nutritional value. The attainment of optimal levels of sulphur can significantly contribute to producing a product of superior quality. The presence of soil sulphur is essential for the successful cultivation of mustard. An adequate amount of sulphur in the soil is essential for achieving optimal growth and increased yields of mustard seeds (Hudeček et al., 2023; Huh, 2022; Hui et al., 2022; Huntenburg et al., 2022; Hussein et al., 2023; Iftikhar et al., 2023; Islam et al., 2023; Javadipour et al., 2022; Ji et al., 2022). An observed deficiency in sulphur has been found to result in compromised growth and reduced seed production. Nutrient synergy pertains to the interplay between sulphur and other essential nutrients, specifically nitrogen and phosphorus, to optimise nutrient uptake and utilisation. Maintaining optimal nutrient levels is crucial for ensuring the health and productivity of mustard crops. The utilisation of sulphur has been observed to enhance mustard plants' pest and disease resistance. Sulphur-containing compounds, such as glucosinolates, play a crucial role in the innate defence mechanisms of plants. The management of sulphur in agricultural practices is crucial in promoting environmental sustainability. The effective utilisation of sulphur can reduce the need for excessive fertilisation, mitigating potential risks related to nutrient runoff and water pollution. Ensuring the successful cultivation of mustard necessitates the implementation of effective soil sulphur management practices. Evaluating sulphur levels in soil is of utmost importance, requiring frequent soil analysis. This information facilitates the evaluation of the need for sulphur



amendments by cultivators. Sulphur fertilisation is recommended when soil tests indicate a sulphur deficiency. Agricultural practitioners can apply fertilisers that contain sulphur in such instances. These fertilisers may contain elemental sulphur or substances containing sulphates, such as gypsum. The choice of fertiliser is dependent on the specific needs of the soil. The timing of sulphur application is an essential factor to take into consideration. The sulphur application can exhibit variability contingent upon the crop's growth stage and specific nutrient demands. The application of this technique can be implemented either during the initial soil preparation phase or during the active growing season. Applying foliar sprays is a feasible method for effectively addressing significant sulphur deficiencies or urgent situations that demand immediate intervention (Khalid et al., 2023; Khan et al., 2022, 2023; Khan et al., 2022; Kochanek et al., 2023; Kosakivska et al., 2022; Kour et al., 2023; Kudoyarova, 2022; Kumar & Ohri, 2023; Kurepa & Smalle, 2022). This approach allows for the efficient delivery of compounds containing sulphur to plants. The enhancement of sulphur availability can be achieved by implementing crop rotation with leguminous plants or adding organic matter to the soil naturally. Leguminous plants can participate in symbiotic nitrogen fixation, a biological process through which they transform atmospheric nitrogen into a usable form for plants. Moreover, organic matter decomposition results in the emission of diverse sulphur compounds. Growers can choose mustard varieties that are more suitable for the sulphur content in their soils. Several mustard cultivars exhibit improved utilisation and tolerance of sulphur. The imperative tasks involve the monitoring and adjustment of soil sulphur levels, as well as the evaluation of plant health. Growers can adapt and adjust their sulphur management practices to address changing circumstances. The presence of soil sulphur is of utmost importance in the cultivation of mustard, exerting a substantial influence on the growth, productivity, and overall quality of mustard crops. To maximise mustard cultivation and improve crop productivity, growers must diligently monitor soil sulphur levels and implement appropriate management strategies. The proficient management of sulphur enhances the overall health and growth of mustard plants. The implementation of this approach aids in the establishment of sustainable agricultural practices by reducing negative environmental impacts resulting from the excessive application of fertilisers. Implementing efficient sulphur management practices is crucial in achieving optimal

agricultural productivity while ensuring environmental sustainability (Hernandez-Leon & Valenzuela-Soto, 2022; Hernández et al., 2023; Hernández et al., 2022; Hilal et al., 2023; Hong et al., 2022; Huang et al., 2022).

**Figure 4.58. Soil Sulphur of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).

**Soil Organic Carbon:** The effect of Sulphur and Salicylic acid and their combination on soil organic carbon was studied in the RH725 variety of Indian Mustard during 2021-2022 and 2022-2023. Data were recorded at 60 days after sowing (DAS) (Table 4.59, Figure 4.59). In 2021-2022, there was a significant difference in organic carbon compared to T0 (Control) at 60 DAS. The percentage increase was calculated by comparing all the treatments with T0. Thus, the pattern of percentage increase in the soil organic carbon was observed at 60 DAS. Therefore, at 60 DAS the percentage increase as compared to T0 was found highest in T2 followed by T4, T8, T7, T1, T3, T6, T5, T9 and the percentage values were 21.97%, 18.39%, 16.95%, 13.41%, 11.25%, 5.96%, 4.69%, 4.05%, 2.73% respectively. But the percentage also decrease T11, T10 and percentage values were -4.41%, -10.07%. In 2022-2023, there was a significant difference in soil organic carbon compared to T0 (Control) at 60 DAS. The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 60 DAS, the percentage increase as compared to T0 was found highest in T2 followed by T8, T4, T7, T1, T11, T6, T9, T5, T10 and the percentage values were 28.49%, 26.20%, 25.40%, 20.68%, 15.85%, 14.28%, 11.53%, 9.21%, 8%, 8% respectively. But the percentage also decrease in T3 and the percentage value was -1.47%. Measuring soil organic carbon (SOC) in agricultural contexts, specifically in the cultivation of mustard (*Brassica* spp.), is a crucial process with substantial implications for crop management and environmental sustainability. The mustard plant, well-known for its seeds containing a significant amount of oil, is a versatile agricultural crop affected by the organic carbon concentration in the soil (Mugwanya et al., 2023; Mukarram et al., 2022; Mukhtar et al., 2023; Myers Jr. et al., 2023; Nadeem, 2022). This article investigates the importance of quantifying soil organic carbon (SOC) in mustard fields; the methodologies utilised for accurate SOC evaluation, and the potential implications of SOC levels on mustard crop cultivation. The presence of soil organic carbon (SOC) is of utmost importance for preserving soil quality, and its impact on the growth and development of mustard crops is substantial. The subject of inquiry concerns the preservation and accessibility of nutrients. Organic carbon is a reservoir for essential nutrients, including nitrogen and phosphorus. The augmentation of crop yields and robust growth of mustard crops can be facilitated by an elevated soil organic carbon (SOC) content, which enhances the retention and availability of nutrients. The influence

of soil organic carbon (SOC) on the correlation between soil structure and water retention is important in soil aggregation. Applying this technique enhances the soil's capacity to retain moisture and reduces the risk of soil erosion (Nam et al., 2023; Napieraj et al., 2023; Nasir & Toth, 2022; Negi & Kumar, 2023; Nimsi et al., 2023; Niu & Fu, 2022; Ortiz-García et al., 2022, 2023; Ozturk & Unal, 2023; Paalli et al., 2022). An adequate amount of soil organic carbon (SOC) in mustard fields is critical in maximising water utilisation and mitigating moisture-related difficulties during the crop's growth cycle. Microbial activity refers to the metabolic processes and interactions of microorganisms, such as bacteria, archaea, fungi, and viruses; soil organic carbon (SOC) plays a crucial role as an essential energy source for the metabolic activities of soil microorganisms. The presence of optimal levels of organic carbon promotes the proliferation of beneficial organisms that actively engage in nutrient cycling and improve soil fertility. Consequently, this indirectly contributes to the growth and development of mustard crops. The augmentation of soil organic carbon (SOC) concentrations in agricultural soils is widely regarded as a sustainable strategy for alleviating the consequences of climate change. The Earth can serve as a carbon sink, effectively capturing and storing carbon dioxide, thereby mitigating the emission of greenhouse gases. Accurate soil organic carbon (SOC) evaluation is essential for efficiently cultivating mustard crops. The implications of sustainable organic cultivation (SOC) in mustard crop cultivation encompass multiple facets of agriculture and environmental sustainability. The relationship between soil organic carbon (SOC) levels and agricultural productivity is interesting. The observation has been made that the maintenance of adequate soil organic carbon (SOC) levels can positively influence soil fertility, leading to enhanced crop growth and higher yields of mustard seeds. An optimal soil organic carbon (SOC) level improves nutrient efficiency by facilitating effective nutrient cycling and utilisation. Consequently, this reduces the need for excessive fertilisation practices and helps alleviate the negative consequences associated with nutrient runoff and water pollution. The capacity of mustard crops to withstand environmental stressors, such as drought and extreme temperatures, is augmented when grown in soils characterised by elevated levels of soil organic carbon (SOC). Consequently, this phenomenon results in cultivating plants that exhibit enhanced health and vitality (Pal et al., 2023; Parada et al., 2022; Parrey et al., 2023;

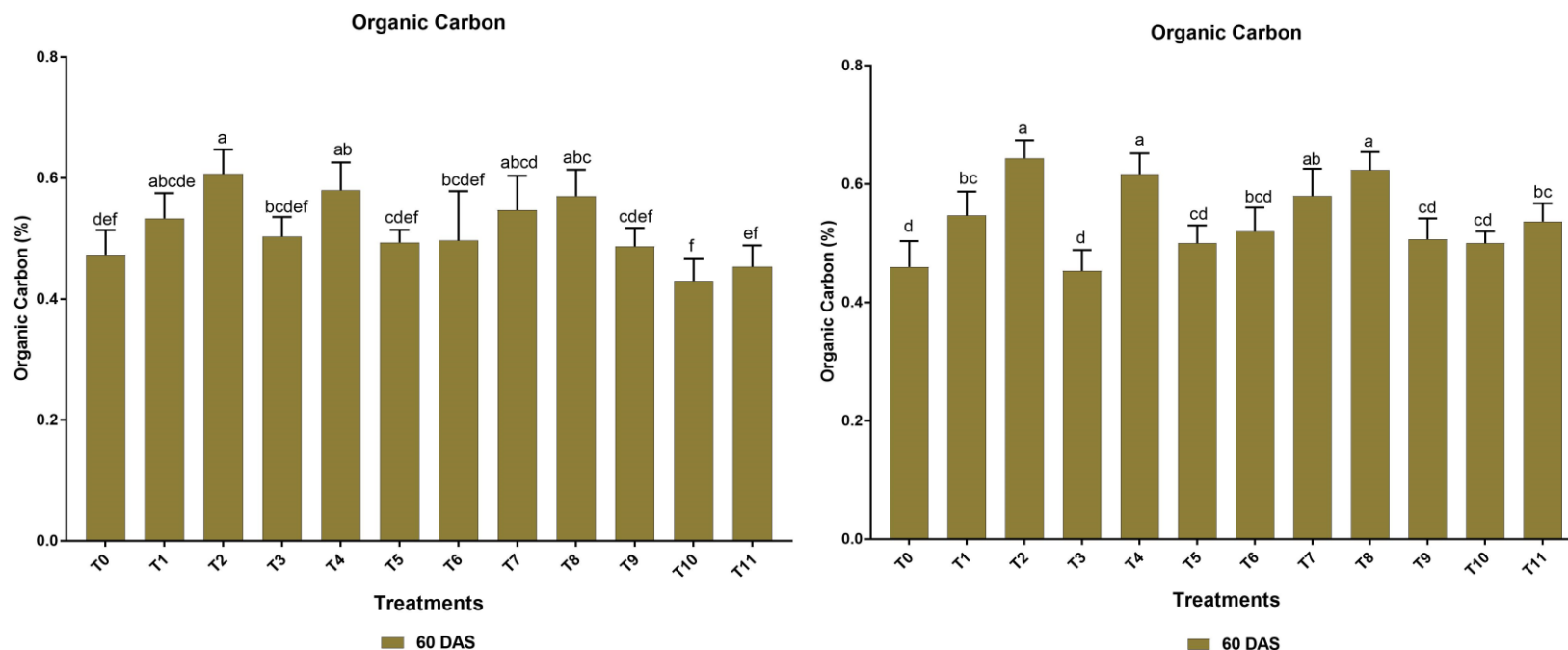
Patel et al., 2022; Peng et al., 2022; Pérez-Llorca et al., 2023; Phokas et al., 2023; Phour & Sindhu, 2022; Poór et al., 2022; Quesada, 2022). The augmentation of soil organic carbon (SOC) in agricultural soils has been observed to yield favourable impacts on crop productivity, in addition to making substantial contributions to carbon sequestration and mitigation of greenhouse gas emissions. The outcomes above are paramount in facilitating the adoption and advancement of sustainable agricultural practices. An adequate amount of soil organic carbon (SOC) has been observed to impact soil's water-holding capacity and alleviate moisture stress positively. Consequently, this facilitates improved water utilisation in mustard cultivation. The measurement of soil organic carbon in mustard cultivation is a crucial procedure that carries substantial implications for the management of crops, the promotion of environmental sustainability, and the preservation of long-term soil health. Monitoring and regulating soil organic carbon (SOC) concentrations are vital in optimising nutrient accessibility, enhancing water utilisation efficiency, and augmenting crop yield. To facilitate the thriving development of mustard crops and contribute positively to sustainable agriculture, it is crucial for farmers to consistently assess and efficiently control soil organic carbon (SOC) levels in their agricultural plots. By actively participating in certain actions, individuals can enhance the health and resilience of mustard crops, thereby contributing to the broader goals of sustainable and ecologically responsible agricultural practices (Mangena, 2022; Manjunatha et al., 2022; Masmoudi et al., 2023; Megala et al., 2022; Mittal et al., 2022; Mohammadi et al., 2023; Molinari et al., 2023; Moustakas et al., 2022).

**Table 4.59. Impact of Different Treatments on Soil Organic Carbon of Mustard During Rabi 2021-2023 & 2022-23**

Treatments	Soil Organic Carbon	
	2021-2022	2022-2023
<b>T0 (Control)</b>	0.473 <sup>def</sup> ±0.040	0.460 <sup>d</sup> ±0.043
<b>T1 (Thiourea-1000 ppm)</b>	0.533 <sup>abcde</sup> ±0.041	0.546 <sup>bc</sup> ±0.040
<b>T2 (Salicylic acid-300 ppm)</b>	0.606 <sup>a</sup> ±0.040	0.643 <sup>a</sup> ±0.030
<b>T3 (Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	0.503 <sup>bcdef</sup> ± 0.032	0.453 <sup>d</sup> ±0.035
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	0.580 <sup>ab</sup> ±0.045	0.616 <sup>a</sup> ±0.035
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	0.493 <sup>cdef</sup> ±0.020	0.500 <sup>cd</sup> ±0.030
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	0.496 <sup>bcdef</sup> ±0.081	0.520 <sup>bcd</sup> ±0.040
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	0.546 <sup>abcd</sup> ±0.056	0.580 <sup>ab</sup> ±0.045
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	0.570 <sup>abc</sup> ±0.043	0.623 <sup>a</sup> ±0.030
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	0.486 <sup>cdef</sup> ±0.030	0.506 <sup>cd</sup> ±0.035
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	0.430 <sup>f</sup> ±0.036	0.500 <sup>cd</sup> ±0.020
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	0.453 <sup>ef</sup> ±0.035	0.536 <sup>bc</sup> ±0.030
<b>CD</b>	0.079	0.052
<b>CV</b>	9.025	5.642

Where Data is Mean ± SD at p<0.05, DAS signifies days after crop sowing. Different alphabets on mean value shows a level of significance.

**Figure 4.59. Soil Organic Carbon of Mustard During Rabi 2021-2023 & 2022-23**



Where Data is Mean  $\pm$  SD at  $p < 0.05$ , DAS signifies days after crop sowing. Different alphabets on mean value bars show a different level of significance, treatments and; treatments are as follow, T0- Control; T1- Thiourea Recommended (1000 ppm); T2- Salicylic Acid Recommended (300ppm); T3- Thiourea (1000ppm)+ Salicylic acid (300ppm); T4- Thiourea (1500ppm) + Salicylic acid(300ppm); T5- Thiourea (1000ppm)+ Salicylic acid (450ppm); T6- Thiourea (500ppm) + Salicylic acid (300ppm); T7- Thiourea (1000ppm) + Salicylic acid (150ppm); T8- Thiourea (500ppm) + Salicylic acid (600ppm); T9- Thiourea (2000ppm) + Salicylic acid (150ppm); T10- Sulphur (2000ppm) + Salicylic acid (600ppm); T11- Thiourea (500ppm) + Salicylic acid (150ppm).



#### **4F. Thiourea (sulphur) and salicylic acid-mediated effects on Economic Analysis of Indian mustard grown under the open filed condition**

##### **Economic Analysis-**

##### **Cost of Cultivation-**

The effect of thiourea and salicylic acid on cost of cultivation in Indian mustard at harvest is shown in (Table 4.60.). In 2022 and 2023 there was significant difference of cost of cultivation in Indian mustard. In 2022, Cost of cultivation in T0, T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11 was 22457, 24679, 26097, 27879, 27009, 25113, 25202, 27267, 25157, 27936, 25788, 26145 respectively. In 2023, cost of cultivation in T0, T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11 was 23447, 25765, 26785, 28654, 27658, 25683, 26743, 28964, 25984, 28754, 26543, 26589 respectively.

##### **Gross Return-**

The effect of thiourea and salicylic acid on Gross Return in Indian mustard at harvest is shown in (Table 4.60.). In 2022 and 2023 there was significant difference of Gross Return in Indian mustard. In 2022, Gross Return in T0, T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, and T11 was 103065.6, 111664.4, 124138.9, 93497.78, 116751.1, 126561.1, 122201.1, 116266.7, 127651.1, 114086.7, 122322.2, and 138793.3 respectively. In 2023, Gross Return in T0, T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, and T11 was 105833.6, 126418.2, 127935.1, 94980.78, 121945.6, 132131.6, 132513.1, 127382.9, 130529.3, 120390.5, 119202.4, and 142850 respectively.

##### **Net Return-**

The effect of thiourea and salicylic acid on Net Return in Indian mustard at harvest is shown in (Table 4.60.). In 2022 and 2023 there was significant difference of Net Return in Indian mustard. In 2022, Net Return in T0, T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, and T11 was 80608.56, 86985.44, 98041.89, 65618.78, 89742.11, 101448.1, 96999.11, 88999.67, 102494.1, 86150.67, 96534.22, 80608.56 respectively. In 2023, Net Return in T0, T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, and T11 was 82386.55, 100653.2, 101150.1, 66326.78, 94287.57, 106448.6, 105770.1, 98418.85, 104545.3, 91636.5, 92659.4 and 116261 respectively.

##### **BC ratio-**

The effect of thiourea and salicylic acid on BC ratio in Indian mustard at harvest is shown in (Table 4.60.). In 2022 and 2023 there was significant difference of BC ratio in Indian mustard. In 2022, BC ratio in T0, T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, and T11 was 3.589462, 3.524675, 3.756826, 2.353699, 3.322674, 4.039665, 3.848866, 3.264007, 4.074179, 3.083858, 3.743378, and 4.308599 respectively. In 2023, BC ratio in T0, T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, and T11 was 3.513735, 3.906586, 3.776372, 2.314748, 3.409052, 4.144711, 3.955058, 3.397972, 4.02345, 3.186913, 3.490917, and 4.372521 respectively.

**Table 4.60. Impact of Different Treatments on Economic Analysis of Mustard During Rabi 2021- 22 & 2022-2023**

Treatments	Cost		Gross Return		Net Return		BC ratio	
	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023	2021-2022	2022-2023
<b>T0 (Control)</b>	22457	23447	103065.6	105833.6	80608.56	82386.55	3.589462	3.513735
<b>T1 (Thiourea-1000 ppm)</b>	24679	25765	111664.4	126418.2	86985.44	100653.2	3.524675	3.906586
<b>T2 (Salicylic acid-300 ppm)</b>	26097	26785	124138.9	127935.1	98041.89	101150.1	3.756826	3.776372
<b>T3 (Thiourea-1000 ppm + Salicylic Acid-300 ppm]</b>	27879	28654	93497.78	94980.78	65618.78	66326.78	2.353699	2.314748
<b>T4 (Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	27009	27658	116751.1	121945.6	89742.11	94287.57	3.322674	3.409052
<b>T5 (Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	25113	25683	126561.1	132131.6	101448.1	106448.6	4.039665	4.144711
<b>T6 (Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	25202	26743	122201.1	132513.1	96999.11	105770.1	3.848866	3.955058
<b>T7 (Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	27267	28964	116266.7	127382.9	88999.67	98418.85	3.264007	3.397972
<b>T8 (Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	25157	25984	127651.1	130529.3	102494.1	104545.3	4.074179	4.02345
<b>T9 (Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	27936	28754	114086.7	120390.5	86150.67	91636.5	3.083858	3.186913
<b>T10 (Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	25788	26543	122322.2	119202.4	96534.22	92659.4	3.743378	3.490917
<b>T11 (Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	26145	26589	138793.3	142850	80608.56	116261	4.308599	4.372521

#### **4G. Thiourea (sulphur) and salicylic acid-mediated effects on GC-MS (Gas Chromatography-Mass Spectrometry) of Indian mustard grown under the open filed condition**

##### **GC-MS RESULT AND DISCUSSION**

The GC-MS analysis of the oil sample provides a comprehensive view of its chemical composition, showcasing a complex mixture of compounds that exhibit varying degrees of significance. Within this intricate matrix, some compounds emerge as major players, commanding attention due to their prevalence, while others take on more minor roles, appearing in smaller quantities. To unravel the intricacies of this chemical tapestry, analysts rely on key analytical parameters, including retention time, area, area%, height, and height%, which serve as critical signposts guiding the way toward a deeper understanding of the composition and relative abundance of these compounds. Through the careful interpretation of these parameters, researchers are empowered to discern, identify, and quantify the multitude of components present within the sample, ultimately shedding light on the intricate chemistry at play (Singhal et al., 2023; Sivanesan et al., 2022; Song et al., 2023; Sousa et al., 2022; Spinoso-Castillo & Bello-Bello, 2022; Suliman et al., 2022; Sun et al., 2022; Sun et al., 2023; Sun et al., 2022).

At the heart of this analytical endeavor lies retention time, a fundamental gas chromatography-mass spectrometry (GC-MS) metric. Retention time refers to the time it takes for a compound to traverse the chromatographic column and reach the detector after injection into the system. It is a unique identifier for each compound, akin to a molecular fingerprint, as their interactions with the stationary phase within the column cause them to elute at distinct time points. This temporal separation of compounds is the initial step in unraveling the sample's complexity, enabling the analyst to discern one compound from another based on their migration times. Accompanying retention time, the area of a compound's peak in the chromatogram is another critical parameter. The area represents the integral of the signal intensity over time, effectively quantifying the amount of a specific compound present in the sample. Larger peak areas correspond to greater compound quantities, indicating its prevalence within the mixture. This quantitative aspect is invaluable in assessing the relative contributions of different compounds to the overall composition of the sample (Sheikhalipour et al., 2023;

Shekhawat et al., 2023; Shi et al., 2023; Shi et al., 2022; Si et al., 2023; Singh et al., 2022; Singh & Roychoudhury, 2023; Singh & Nandi, 2022). Major compounds typically exhibit substantial peak areas, signifying their dominance in the mixture. However, the area alone does not provide a holistic perspective, as it may not account for variations in compound volatility, detector response, or other factors. To address this limitation, analysts turn to area%, a normalized parameter that expresses the peak area as a percentage of the total chromatogram area. Area% offers a standardized representation, allowing for more meaningful comparisons between compounds and different chromatograms (Sachan & Krishna, 2022; Salih, Wu, et al., 2022; Salih, Zhou, et al., 2022; Salwan et al., 2023; Samad et al., 2023; Sampedro-Guerrero et al., 2022; Samtani et al., 2022; Shah et al., 2022, 2023; Shang et al., 2022; Sharma & Verma, 2023). It provides insights into the compound's relative abundance in the entire sample, irrespective of analytical conditions or sample size variations. Beyond area and area%, the height of a compound's peak is another dimension of analysis. Peak height corresponds to the maximum signal intensity recorded during the elution of a compound. While area quantifies a compound's overall quantity, height reflects its signal's intensity at the point of maximum concentration. Height can be particularly informative when evaluating the purity or concentration of a compound in situations where baseline drift or co-eluting compounds may influence the area measurement. Similarly, height% is an essential parameter that complements area%. It expresses the peak height as a percentage of the highest peak in the chromatogram, providing a standardized measure of the compound's intensity relative to other compounds within the sample. Height% aids in identifying compounds that may not dominate in terms of quantity but exhibit significant signal strength, highlighting their importance despite their lower prevalence. The interpretation of these critical parameters, retention time, area, area%, height, and height%, collectively forms the foundation for identifying and quantifying compounds within the oil sample. Major compounds, characterized by their extended retention times, substantial peak areas, and high area%, stand out prominently in the chromatogram. These compounds are the primary constituents of the sample, representing the core components that contribute to its overall character and properties. Conversely, minor compounds, distinguished by shorter retention times, smaller peak areas, and lower area%, play supporting roles in the chemical ensemble. While they

may be present in smaller quantities, their identification and quantification remain essential for comprehensively characterizing the sample. These minor compounds can introduce subtle nuances to the oil's composition, influencing its aroma, flavor, or functional properties. The significance of this analytical process extends far beyond a mere enumeration of compounds. It holds practical implications for various industries and applications. For instance, in petroleum analysis, GC-MS is instrumental in characterising crude oil, identifying hydrocarbons of interest, and assessing the potential value of a resource. In environmental monitoring, GC-MS helps detect and quantify pollutants, ensuring compliance with regulatory standards and safeguarding ecosystems. In the food and flavor industry, it aids in assessing the quality and authenticity of products by identifying aroma compounds responsible for taste and scent. In the pharmaceutical sector, GC-MS plays a pivotal role in drug discovery and development, enabling the identification of active pharmaceutical ingredients and impurities. Moreover, forensic science is a powerful tool for identifying illicit substances, facilitating criminal investigations, and ensuring public safety. The utilisation of GC-MS is not confined to a single domain; its applications span many industries, each benefiting from its ability to decipher complex chemical mixtures with precision and accuracy. Whether the goal is to assess the purity of a pharmaceutical compound, determine the presence of environmental contaminants, or elucidate the composition of a fragrant essential oil, GC-MS is a versatile and indispensable analytical technique. In essence, the interpretation of retention time, area, area%, height% in GC-MS analysis embodies the art and science of unraveling the intricate tapestry of chemical compositions. It empowers researchers, analysts, and scientists to peer into the molecular intricacies of a substance, unveiling its secrets and shedding light on its nature. Synthesis of these critical parameters reveals the true essence of a sample, allowing us to harness the knowledge for a multitude of applications, from refining industrial processes to safeguarding the environment and enhancing the quality of life through innovative products and discoveries (Quesada, 2022; Rai & Kaushik, 2023; Raja Gopalan et al., 2022; Ramakrishnan & Zhou, 2022; Rani et al., 2023; Rizvi et al., 2022; Roussos, 2023; Ruidas et al., 2022; Rybczyński et al., 2022). GC-MS analysis of the oil sample is a powerful tool for deciphering its chemical composition. Analysts gain valuable insights into the complex mixture of compounds present by

examining critical parameters such as retention time, area, area%, height, and height%. Major compounds, characterised by their extended retention times, substantial peak areas, and high area%, dominate the sample, while minor compounds, with shorter retention times and lower quantitative measures, contribute nuanced diversity. This analytical process transcends industries, driving advancements in fields as diverse as petroleum exploration, environmental protection, pharmaceuticals, and forensics. It epitomizes the art and science of understanding the molecular intricacies of matter, unlocking a world of possibilities and applications that shape our modern lives (Sachan & Krishna, 2022; Salih, Wu, et al., 2022; Salih, Zhou, et al., 2022; Salwan et al., 2023; Samad et al., 2023; Sampedro-Guerrero et al., 2022; Samtani et al., 2022; Shah et al., 2022, 2023; Shang et al., 2022).

The table presents comprehensive data regarding multiple parameters derived from the Gas Chromatography-Mass Spectrometry (GC-MS) analysis of an oil sample. Retention time (R. Time) refers to the duration that a particular substance remains in a chromatographic system before it elutes from the column. Retention time refers to the duration during which a chemical compound traverses the chromatographic column and ultimately arrives at the detector. The differentiation of compounds is facilitated by their distinct retention times, rendering this parameter of utmost importance. Retention time is employed as a means of differentiating between various compounds present in the sample (Rai & Kaushik, 2023; Raja Gopalan et al., 2022; Ramakrishnan & Zhou, 2022; Rani et al., 2023; Rizvi et al., 2022; Roussos, 2023; Ruidas et al., 2022; Rybczyński et al., 2022). The process aids in the identification and quantification of compounds by utilising their elution times. The measurement of the area beneath the peak of a compound in a chromatogram is indicative of the amount of said compound present in the sample. The calculation involves the integration of signal intensity with respect to time. The utilisation of the area is employed for the purpose of quantification. Greater surface areas are indicative of elevated concentrations of the compound within the given sample (Sharma & Verma, 2023; Sheikhalipour et al., 2023; Shekhawat et al., 2023; Shi et al., 2023; Shi et al., 2022; Si et al., 2023; Singh et al., 2022; Singh & Roychoudhury, 2023; Singh & Nandi, 2022). The area percentage is a metric that quantifies the proportion of a compound's area in relation to the total area of all

compounds identified in the sample. The calculation involves dividing the compound's area by the total area and subsequently multiplying the result by 100. The measurement of area% is utilised to ascertain the comparative prevalence of individual compounds within the sample. This aids in the assessment of the relative significance of various compounds within the mixture. The height of a compound's peak in a chromatogram refers to the maximum signal intensity attained by that particular compound. The provided data offers insights into the magnitude of the signal (Singhal et al., 2023; Sivanesan et al., 2022; Song et al., 2023; Sousa et al., 2022; Spinoso-Castillo & Bello-Bello, 2022; Suliman et al., 2022; Sun et al., 2022; Sun et al., 2023; Sun et al., 2022). The attribute of size is employed for the purpose of qualitative evaluation. The intensity of peaks aids in the identification of compounds. The concept of height percentage, also known as height%, pertains to the proportionate contribution of the peak height of a specific compound to the overall peak height of all compounds identified in a given sample. The calculation involves dividing the height of the compound by the total height and subsequently multiplying the result by 100. The measurement of height% offers a comparative assessment of the magnitude of the peak for each compound. The assessment of compound prominence in the chromatogram is facilitated by this (Ozturk & Unal, 2023; Paalli et al., 2022; Pal et al., 2023; Parada et al., 2022; Parrey et al., 2023; Patel et al., 2022; Peng et al., 2022; Pérez-Llorca et al., 2023; Phokas et al., 2023; Phour & Sindhu, 2022; Poór et al., 2022; Quesada, 2022).

#### **GC-MS analysis(qualitative) of oil of seed harvested from T0- Control**

Ethyl Acetate: Justification: Ethyl acetate is a minor component in the sample, as indicated by its low area and area%. The relatively low height and height% further confirm its minor presence. Quinoline, 1,2-dihydro-2,2,4-trimethyl-: Justification: Quinoline is another minor component with slightly higher area and area% than ethyl acetate. Its higher height and height% indicate a more prominent peak. Heptadecane: Justification: Heptadecane is a minor hydrocarbon component with a low area and area%. Its height and height% suggest a relatively stronger signal than its area, possibly due to its specific mass spectral characteristics. Neophytadiene: Justification: Neophytadiene is another minor hydrocarbon with a slightly higher area and area% than heptadecane. Its height and height% are also higher, indicating a stronger signal. 2-



Bromotetradecane: Justification: 2-Bromotetradecane is a minor compound with low area and area%. Its height and height% are relatively low, suggesting a less prominent presence in the sample. 2-Methylhexacosane: Justification: 2-Methylhexacosane is a minor compound with a moderate area and area%. Its height and height% are lower, indicating that it might not produce strong mass spectrometry signals. Benzothiazole, 2-(2-hydroxyethylthio)-: Justification: Benzothiazole is a major component in the sample, as indicated by its high area and area%. Its height and height% are also significant, confirming its dominant presence. Hexadecane, 2,6,10,14-tetramethyl-: Justification: Hexadecane is a minor hydrocarbon component with a moderate area and area%. Its height and height% are consistent with its status as a minor compound. 2-Methyltetracosane: Justification: 2-Methyltetracosane is a minor component with a low area and area%. Its height and height% suggest a moderate signal intensity. Eicosyl acetate: Justification: Eicosyl acetate is a minor compound with low area and area%. Its height and height% are relatively higher, indicating a stronger signal compared to its area. Glycidyl palmitate: Justification: Glycidyl palmitate is another minor component with low area and area%. Its height and height% suggest a moderate signal strength. Cyclododecyne: Justification: Cyclododecyne is a minor compound with a moderate area and area%. Its height and height% are relatively higher, indicating a significant signal. 9-Octadecenoic acid (Z)-, oxiranylmethyl est: Justification: This compound is a major component in the sample, as indicated by its high area and area%. Its height and height% are also significant, confirming its importance. 4,4'-((p-Phenylene) diisopropylidene)diphenol: Justification: This compound is a minor component with a moderate area and area%. Its height and height% are relatively higher, suggesting a significant signal. 9-Octadecenoic acid (Z), oxiranylmethyl est: Justification: This compound is a minor component with low area and area%. Its height and height% are consistent with its minor presence. (Z)-18-Octadec-9-enolide: Justification: This compound is a minor component with low area and area%. Its height and height% indicate a minor presence. Squalene: Justification: Squalene is a major component in the sample, as indicated by its high area and area%. Its height and height% are significantly higher, confirming its importance. cis-13-Docosenoic acid chloride: Justification: cis-13-Docosenoic acid chloride is a minor compound with low area and area%. Its height and height% suggest a minor presence. 9-Octadecenoic acid (Z)-

, oxiranylmethyl est: Justification: This compound is a significant component with a high area and area%. Its height and height% are significantly higher, indicating its prominence. Tetracosane: Justification: Tetracosane is a minor hydrocarbon with a moderate area and area%. Its height and height% are consistent with its status as a minor compound. deltaTocopherol: Justification: delta-Tocopherol is a minor compound with low area and area%. Its height and height% suggest a minor presence. 2'-(Trimethylsilyl)oxy-2,3,6'-trimethoxychalc: Justification: This compound is a minor component with low area and area%. Its height and height% are consistent with its minor presence. gamma-Tocopherol: Justification: gamma.-Tocopherol is a major component in the sample, as indicated by its extremely high area and area%. Its height and height% are also significantly higher, confirming its dominant presence. Tetracosane (Second occurrence): Justification: This is another occurrence of tetracosane with a retention time in the early 30s and a %Area indicating a minor presence. Vitamin E: Justification: Vitamin E has a retention time in the early 30s and a %Area indicating a minor presence. Cyclononasiloxane, octadecamethyl-: Justification: Cyclononasiloxane has a retention time in the early 30s and a %Area indicating a minor concentration. Cholesterol: Justification: Cholesterol has a retention time in the early 30s and a %Area indicating a minor presence. Campesterol: Justification: Campesterol is a significant component with a high area and area%. Its height and height% are also significant, confirming its importance. Campesterol (Second occurrence): Justification: This is another occurrence of campesterol with a retention time in the early 30s and a %Area indicating a significant presence. 17-(1,5-Dimethylhexyl)-10,13-dimethyl-3-st: Justification: This compound has a retention time in the early 32s and a %Area indicating a moderate presence (Table 4.61) (Ozturk & Unal, 2023; Paalli et al., 2022; Pal et al., 2023; Parada et al., 2022; Parrey et al., 2023; Patel et al., 2022; Peng et al., 2022; Pérez-Llorca et al., 2023; Phokas et al., 2023; Phour & Sindhu, 2022; Poór et al., 2022).

**Table 4.61. GC-MS analysis(qualitative) of oil of seed harvest from T0-Control**

Peak#	Name	R. Time	Area	Area%	Height	Height%	SI%	Molecular weight
1	Ethyl Acetate	4.052	346987	1.55	104871	1.55	83	88

2	Quinoline, 1,2-dihydro-2,2,4-trimethyl-	15.035	341399	1.52	194400	2.87	93	173
3	Heptadecane	18.065	109538	0.49	70004	1.03	94	240
4	Neophytadiene	19.559	203882	0.91	128978	1.90	90	278
5	2-Bromotetradecane	20.216	132577	0.59	48418	0.71	77	276
6	2-Methylhexacosane	20.335	276029	1.23	43358	0.64	77	380
7	Benzothiazole, 2-(2-hydroxyethylthio)-	20.795	1599667	7.12	397312	5.86	93	211
8	Hexadecane, 2,6,10,14-tetramethyl-	20.940	267024	1.19	80018	1.18	77	282
9	2-Methyltetracosane	21.214	123178	0.55	68009	1.00	77	380
10	Eicosyl acetate	23.151	177495	0.79	118958	1.75	95	340
11	Glycidyl palmitate	23.944	192329	0.86	80830	1.19	87	312
12	Cyclododecyne	25.400	215726	0.96	119487	1.76	80	164
13	9-Octadecenoic acid (Z)-, oxiranylmethyl est	25.439	1168308	5.20	456684	6.73	93	338
14	4,4'-((p-Phenylene)diisopropylidene)diphenol	26.956	529731	2.36	291844	4.30	73	346
15	9-Octadecenoic acid (Z)-, oxiranylmethyl est	27.030	388078	1.73	201546	2.97	92	338
16	(Z)-18-Octadec-9-enolide	27.572	324260	1.44	157527	2.32	87	280
17	Squalene	27.999	1114814	4.97	710448	10.47	96	410
18	cis-13-Docosenoyl chloride	28.136	123444	0.55	77489	1.14	83	356
19	9-Octadecenoic acid (Z)-, oxiranylmethyl est	28.535	1342761	5.98	610315	9.00	90	338
20	Tetracosane	28.604	541359	2.41	251274	3.70	93	338
21	delta.-Tocopherol	29.060	331123	1.47	83617	1.23	63	402
22	2'-(Trimethylsilyloxy)-2,3,6'-trimethoxychalc	29.148	246330	1.10	107874	1.59	67	386
23	gamma-Tocopherol	30.005	5007053	22.30	1162261	17.13	95	416
24	Tetracosane	30.369	183092	0.82	79993	1.18	90	338
25	Vitamin E	30.799	403032	1.80	167947	2.48	94	430
26	Cyclononasiloxane, octadecamethyl-	30.955	150676	0.67	66881	0.99	38	666
27	Cholesterol	31.087	716502	3.19	79072	1.17	65	386
28	Campesterol	32.253	3200133	14.25	393653	5.80	82	400
29	Campesterol	32.360	2177735	9.70	295592	4.36	74	400
30	17-(1,5-Dimethylhexyl)-10,13-dimethyl-3-st	32.480	517928	2.31	136099	2.01	44	488
			22452190	100.00	6784759	100.00		

### GC-MS analysis(qualitative) of oil of seed harvest from treatment T1- (Thiourea recommended dose (1000ppm))

Ethyl Acetate: Explanation: Ethyl Acetate, with a short R.Time, is a minor component in the sample. Its low Area% and Height% suggest a relatively low concentration. Cyclohexane, 1,3-dimethyl-, cis-: Explanation: This compound has a short R.Time and low Area%, indicating it's a minor component with a moderate signal intensity. Neophytadiene: Explanation: Neophytadiene's longer R.Time and low Area% suggest it's present in smaller quantities in the sample. 9-Octadecenal, (Z)-: Explanation: This aldehyde is a minor component with a low Area% and Height%. Myristic acid glycidyl ester: Explanation: This compound exhibits slightly higher Area% and Height%, indicating a moderate presence. 9-Octadecenoic acid (Z)-, 2,3-dihydroxyprop: Explanation: This compound's low Area% and Height% suggest it's present in relatively small quantities. 1,8,11-Heptadecatriene, (Z, Z)-: Explanation: The

higher Area% and Height% indicate that (Z,Z)-1,8,11-heptadecatriene is a significant component. 9-Octadecenoic acid (Z)-, oxiranylmethyl est: Explanation: This compound is a significant component with a high Area% and Height%, indicating substantial presence. 9-Octadecenoic acid (Z)-, oxiranylmethylest (different isomer): Explanation: Another isomer of the previous compound, present in lower amounts. Myristic acid glycidyl ester (different elution time): Explanation: Another isomer of Myristic acid glycidyl ester with a slightly higher Area%. Heptasiloxane, hexadecamethyl: Explanation: Heptasiloxane, hexadecamethyl, has a relatively low Area% and Height%, indicating it's a minor component in the sample. 9-Octadecenoic acid (Z)-, oxiranylmethyl est (different isomer): Explanation: This isomer of 9-Octadecenoic acid (Z)-, oxiranylmethyl est is a significant component, with higher Area% and Height%. Propiolic acid, 3-(1-hydroxy-2-isopropyl-5-: Explanation: Propiolic acid is a minor component with a low Area% and Height%. (Z)-18-Octadec-9-enolide: Explanation: This compound exhibits higher Area% and Height%, indicating a moderate presence. Squalene: Explanation: Squalene is a major component in the sample with a significant Area% and Height%. 2,3-Dihydroxypropyl cis-13-docosenoate: Explanation: This compound is a minor component with a low Area% and Height%. Phytol stearate: Explanation: Phytol stearate is a significant component with moderate Area% and Height%. Glycidyl (Z)-9-nonadecenoate: Explanation: This compound is a major component with a high Area% and Height%. Hexatriacontane: Explanation: Hexatriacontane exhibits moderate Area% and Height%, indicating a significant presence. 2,2-Dimethyl-3-(3,7,16,20-tetramethyl-henei:Explanation: This compound is a minor component with low Area% and Height%. 4-Trifluoromethylbenzoic acid. 4-hexadecyl: Explanation: This compound is 4-Trifluoromethylbenzoic acid with a 4-hexadecyl group. It is a minor component in the sample, as indicated by its relatively low Area% and Height%. cis-7,10,13-Hexadecatrienal: Explanation: This is a compound with a long name indicating its chemical structure, specifically a hexadecatrienal isomer. It is a minor component, with low Area % and Height %. gamma-Tocopherol: Explanation: Gamma-Tocopherol is a significant component in the sample, indicated by its high Area% and Height%. Tocopherols are a class of compounds related to vitamin E, known for their antioxidant properties. Tetrapentacontane: Explanation: Tetrapentacontane is a minor component with

relatively low Area% and Height%, suggesting its presence in trace amounts. Vitamin E: Explanation: Vitamin E is a well-known compound with antioxidant properties. While it is present in the sample, it is not a major component, as its Area% and Height% are relatively low. Cholesterol: Explanation: Cholesterol is a significant component in the sample, indicated by its relatively high Area% and Height%. It's an essential molecule found in cell membranes. Ergosta-5,22-dien-3-ol, (3.β.,22E)-: Explanation: This compound is a minor component in the sample, with low Area% and Height%. It is a form of ergosterol, a sterol found in fungi. Ergost-5-en-3-ol, (3.β.)-: Explanation: This ergosterol derivative is a significant component in the sample, with high Area% and Height%. Ergost-5-en-3-ol, (3.β.)- (another isomer): Explanation: This isomer of ergosterol is a major component, with a substantial Area% and Height%. It is related to sterols found in plants and fungi. 2H-Pyran-2-one, 6-[4,4-bis(methylthio)-1,2: Explanation: This compound is a minor component with relatively low Area% and Height% (Table 4.62).

**Table 4.62. GC-MS analysis(qualitative) of oil of seed harvest from Treatment T1- (Thiourea recommended dose (1000ppm))**

Peak	Name	R.Time	Area	Area%	Height	Height%	SI%	Molecular weight
1	Ethyl Acetate	4.054	303882	0.96	94023	1.12	83	88
2	Cyclohexane, 1,3-dimethyl-, cis-	4.410	149657	0.47	86861	1.03	94	112
3	Neophytadiene	19.560	127702	0.40	80483	0.96	90	278
4	9-Octadecenal, (Z)-	21.210	88423	0.28	52003	0.62	92	266
5	Myristic acid glycidyl ester	23.938	167166	0.53	95389	1.14	92	284
6	9-Octadecenoic acid (Z)-, 2,3-dihydroxyprop	25.033	110282	0.35	59754	0.71	87	356
7	1,8,11-Heptadecatriene, (Z,Z)-	25.400	334081	1.06	218934	2.61	83	234
8	9-Octadecenoic acid (Z)-, oxiranylmethyl est	25.439	2223770	7.02	727214	8.66	93	338
9	9-Octadecenoic acid (Z)-, oxiranylmethyl est	25.575	675061	2.13	138719	1.65	67	338
10	Myristic acid glycidyl ester	25.635	262535	0.83	83938	1.00	80	284
11	Heptasiloxane, hexadecamethyl-	26.905	113532	0.36	37761	0.45	58	532
12	9-Octadecenoic acid (Z)-, oxiranylmethyl est	27.029	791812	2.50	341365	4.07	91	338
13	Ppropionic acid, 3-(1-hydroxy-2-isopropyl-5-	27.100	103345	0.33	43258	0.52	46	224
14	(Z)-18-Octadec-9-enolide	27.569	378017	1.19	230997	2.75	88	280
15	Squalene	27.998	1684241	5.32	1021176	12.17	96	410
16	2,3-Dihydroxypropyl cis-13-docosenoate	28.134	122791	0.39	101622	1.21	84	412
17	Phytol stearate	28.321	643323	2.03	122173	1.46	93	562
18	Glycidyl (Z)-9-nonadecenoate	28.535	2350067	7.42	1038779	12.38	90	352
19	Hexatriacontane	28.604	746927	2.36	357827	4.26	94	506
20	2,2-Dimethyl-3-(3,7,16,20-tetramethyl-henei	28.900	102482	0.32	57825	0.69	89	412
21	4-Trifluoromethylbenzoic acid.	29.057	438407	1.38	132152	1.57	70	414

	4-hexadecyl							
22	cis,cis,cis-7,10,13-Hexadecatrienal	29.125	200562	0.63	70267	0.84	60	234
23	gamma.-Tocopherol	30.010	6968264	22.01	1426920	17.00	95	416
24	Tetrapentacontane	30.369	169034	0.53	82291	0.98	92	758
25	Vitamin E	30.799	593316	1.87	225776	2.69	94	430
26	Cholesterol	31.098	1882968	5.95	149369	1.78	79	386
27	Ergosta-5,22-dien-3-ol, (3.beta.,22E)-	31.548	1038997	3.28	98516	1.17	77	398
28	Ergost-5-en-3-ol, (3.beta.)-	32.249	3009260	9.50	587642	7.00	73	398
29	Ergost-5-en-3-ol, (3.beta.)-	32.305	5719188	18.06	553636	6.60	72	400
30	2H-Pyran-2-one, 6-[4,4-bis(methylthio)-1,2,	32.570	166142	0.52	77063	0.92	45	238
			31665234	100.00	8393733	100.00		

### **GC-MS analysis(qualitative) of oil of seeds harvest from T2- Salicylic Acid (300ppm)**

Ethyl Acetate: Ethyl Acetate is the first compound identified in the chromatographic analysis. It elutes at a retention time of 4.054 minutes with an associated peak area, area percentage, peak height, and height percentage, as mentioned. Cyclohexane, 1,3-dimethyl-, cis: The second compound is identified as cis-1,3-dimethylcyclohexane, with corresponding retention time and peak parameters. Cyclooctasiloxane, hexadecamethyl- The third compound detected is cyclooctasiloxane, hexadecamethyl. It elutes at a retention time of 17.151 minutes with associated peak area and height measurements. 1,7-Hexadecadiene: The fourth compound is 1,7-hexadecadiene, with corresponding retention time and peak parameters. Docosanoic acid, docosyl ester- Justification: The fifth compound is identified as docosanoic acid, docosyl ester, with associated retention time and peak measurements. 1-Nonadecene-Justification: The sixth compound is 1-nonadecene, and its associated peak characteristics are detailed. Neophytadiene- Justification: The seventh compound is neophytadiene, with its respective retention time and peak measurements. 9-Octadecen-1-ol, (Z)-Justification: The eighth compound is identified as (Z)-9-octadecen-1-ol, and its peak characteristics are provided. 9-Tricosene, (Z)-Justification: The ninth compound is (Z)-9-tricosene, with corresponding retention time and peak measurements. 9-Tricosene, (Z)-Justification: The tenth compound is another isomer of (Z)-9-tricosene, with its peak parameters detailed. Cyclodecasiloxane, eicosamethyl - Justification: The eleventh compound is identified as cyclodecasiloxane, eicosamethyl. The associated peak data is provided. 9-Octadecen-1-ol, (Z)-Justification: The twelfth compound is (Z)-9-

octadecen-1-ol, and its peak characteristics are presented. 9-Tricosene, (Z)- Justification: The thirteenth compound is (Z)-9-tricosene, with associated retention time and peak measurements. 1-Nonadecene-Justification: The fourteenth compound is 1-nonadecene, and its peak parameters are detailed. 7-Hexadecenal, (Z)- Justification: The fifteenth compound is identified as (Z)-7-hexadecenal, with corresponding retention time and peak measurements. Cyclooctasiloxane, hexadecamethyl-Justification: The sixteenth compound is cyclooctasiloxane, hexadecamethyl. Its associated peak data is provided. 9-Octadecen-1-ol, (Z)- Justification: The seventeenth compound is (Z)-9-octadecen-1-ol, and its peak characteristics are presented. 9-Tricosene, (Z)- Justification: The eighteenth compound is another isomer of (Z)-9-tricosene, with its peak parameters detailed. 9-Tricosene, (Z)- Justification: The nineteenth compound is an additional isomer of (Z)-9-tricosene, with its peak data provided. cis-9-Hexadecenal- Justification: The twentieth compound is cis-9-hexadecenal, with corresponding retention time and peak measurements. Glycidyl palmitate- Justification: The twenty-first compound is identified as glycidyl palmitate, with associated retention time and peak data. cis-9-Hexadecenal- Justification: The twenty-second compound is another isomer of cis-9-hexadecenal, with its peak characteristics provided. Cyclodecasiloxane, eicosamethyl- Justification: The twenty-third compound is cyclodecasiloxane, eicosamethyl. Its associated peak data is detailed. 1,8,11-Heptadecatriene, (Z,Z)-Justification: The twenty-fourth compound is (Z,Z)-1,8,11-heptadecatriene, and its peak parameters are presented. 9-Octadecenoic acid (Z)-, oxiranylmethyl ester- Justification: The twenty-fifth compound is identified as (Z)-9-octadecenoic acid oxiranylmethyl ester, with corresponding retention time and peak measurements. It is notable for its high area percentage and peak height percentage. Cyclopentadecanone- Justification: The twenty-sixth compound is cyclopentadecanone, with associated retention time and peak data. It is characterized by a significant area percentage and peak height percentage. Glycidyl (Z)-9-nonadecenoate- Justification: The twenty-seventh compound is identified as glycidyl (Z)-9-nonadecenoate, with corresponding retention time and peak characteristics. Myristic acid glycidyl ester- Justification: The twenty-eighth compound is myristic acid glycidyl ester, with its associated peak data. Tetracosamethyl-cyclododecasiloxane-Justification: The twenty-ninth compound is tetracosamethyl-cyclododecasiloxane,

with corresponding retention time and peak measurements. 9-Octadecenoic acid (Z)-, oxiranylmethyl ester- Justification: The thirtieth compound is another instance of (Z)-9-octadecenoic acid oxiranylmethyl ester, with associated peak parameters. Cyclododecanemethanol- Justification: The thirty-first compound is cyclododecanemethanol, with its corresponding retention time and peak data. Glycidyl palmitate- Justification: The thirty-second compound is glycidyl palmitate, with associated retention time and peak measurements. Tetracosamethyl-cyclododecasiloxane- Justification: The thirty-third compound is tetracosamethyl-cyclododecasiloxane, with its peak characteristics provided. (Z)-18-Octadec-9-enolide- Justification: The thirty-fourth compound is identified as (Z)-18-octadec-9-enolide, with corresponding retention time and peak data. Squalene- Justification: The thirty-fifth compound is squalene, characterized by a significant area percentage and peak height percentage. (6Z,9Z)-6,9-Tricosadiene- Justification: The thirty-sixth compound is (6Z,9Z)-6,9-tricosadiene, with corresponding retention time and peak measurements. Cyclodecasiloxane, eicosamethyl- Justification: The thirty-seventh compound is cyclodecasiloxane, eicosamethyl, with its associated peak data. Glycidyl (Z)-9-nonadecenoate- Justification: The thirty-eighth compound is another instance of glycidyl (Z)-9-nonadecenoate, notable for its high area and peak height percentage. Tetracontane- Justification: The thirty-ninth compound is tetracontane, with its peak characteristics presented. Glycidyl (Z)-9-nonadecenoate- Justification: The fortieth compound is another instance of glycidyl (Z)-9-nonadecenoate, with its associated peak data. Heptasiloxane, hexadecamethyl- Justification: The forty-first compound is heptasiloxane, hexadecamethyl, with corresponding retention time and peak measurements. 2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicos-7-enyl)-cyclobutane-1-acetic acid- Justification: The forty-second compound is identified as 2,2-dimethyl-3-(3,7,16,20-tetramethyl-heneicos-7-enyl)-cyclobutane-1-acetic acid, with corresponding retention time and peak data. cis-9-Tetradecenoic acid, isobutyl ester- Justification: The forty-third compound is cis-9-tetradecenoic acid, isobutyl ester, with its associated peak measurements. Tetracosamethyl-cyclododecasiloxane- Justification: The forty-fourth compound is tetracosamethyl-cyclododecasiloxane, with its peak characteristics provided. gamma-tocopherol- Justification: The forty-fifth compound is gamma-tocopherol, characterized by a significant area percentage and peak height



percentage. Tetracosamethyl-cyclododecasiloxane- Justification: The forty-sixth compound is another instance of tetracosamethyl- cyclododecasiloxane with its associated peak data. 2-Methylhexacosane- Justification: The forty-seventh compound is 2-methylhexacosane, with its peak parameters detailed. Vitamin E- Justification: The forty-eighth compound is identified as vitamin E, with corresponding retention time and peak measurements. Ergosta-5,22-dien-3-ol- Justification: The forty-ninth compound is ergosta-5,22-dien-3-ol, with its associated retention time and peak data. Campesterol- Justification: The fiftieth compound is campesterol, characterised by a significant area and peak height percentage. The table concludes with all compounds' cumulative area and height percentages, indicating that these 50 compounds account for 100% of the analyzed sample (Table 4.63).

**Table 4.63: T2- GC-MS analysis(qualitative) of oil of seed harvest from Salicylic Acid (300ppm)**

Peak	Name	R.Time	Area	Area%	Height	Height%	SI%	Molecular weight
1	Ethyl Acetate	4.054	252761	0.59	101710	0.61	88	88
2	Cyclohexane, 1,3-dimethyl-, cis-	4.404	143477	0.34	88605	0.53	93	112
3	Cyclooctasiloxane, hexadecamethyl-	17.151	578280	1.35	126801	0.76	91	592
4	1,7-Hexadecadiene	18.820	138609	0.32	49941	0.30	92	222
5	Docosanoic acid, docosyl ester	18.897	665186	1.56	282113	1.70	68	648
6	1-Nonadecene	18.973	291743	0.68	118037	0.71	94	266
7	Neophytadiene	19.573	232157	0.54	146437	0.88	92	278
8	9-Octadecen-1-ol, (Z)	19.905	215702	0.50	130490	0.79	94	268
9	9-Tricosene, (Z)-	19.970	619463	1.45	349167	2.10	95	322
10	9-Tricosene, (Z)-	20.034	305132	0.71	163894	0.99	94	322
11	Cyclododecasiloxane, eicosamethyl-	20.426	163420	0.38	93694	0.56	84	740
12	9-Octadecen-1-ol, (Z)	20.921	765995	1.79	496103	2.99	95	268
13	9-Tricosene, (Z)-	20.982	1068853	2.50	634569	3.82	95	322
14	1-Nonadecene	21.046	315673	0.74	167250	1.01	94	266
15	7-Hexadecenal, (Z)-	21.227	166062	0.39	106400	0.64	91	328
16	Cyclooctasiloxane, hexadecamethyl-	21.813	142396	0.33	70788	0.43	81	592
17	9-Octadecen-1-ol, (Z)	21.894	218940	0.51	141061	0.85	94	268
18	9-Tricosene, (Z)	21.949	719401	1.68	465644	2.81	96	322
19	9-Tricosene, (Z)	22.009	250098	0.59	145664	0.88	94	322
20	cis-9-Hexadecenal	23.134	223901	0.52	138169	0.83	94	328
21	Glycidyl palmitate	23.959	654083	1.53	345866	2.08	88	312
22	cis-9-Hexadecenal	24.890	294513	0.69	190423	1.15	94	238
23	Cyclododecasiloxane, eicosamethyl-	25.312	159761	0.37	101102	0.61	82	740
24	1,8,11-Heptadecatriene, (Z,Z)	25.421	835902	1.96	559956	3.37	85	234
25	9-Octadecenoic acid (Z)-, oxiranylmethyl est	25.460	5343280	12.51	1880084	11.33	94	338
26	Cyclopentadecanone	25.542	1356181	3.17	630012	3.80	85	224
27	Glycidyl (Z)-9-nonadecenoate	25.591	1015965	2.38	358760	2.16	81	352
28	Myristic acid glycidyl ester	25.652	398164	0.93	179014	1.08	83	284
29	Tetracosamethyl-cyclododecasiloxane	26.336	172830	0.40	115325	0.69	84	888

30	9-Octadecenoic acid (Z)-, oxiranylmethyl est	27.049	1618632	3.79	818291	4.93	92	338
31	Cyclododecanemethanol	27.110	267041	0.63	121965	0.73	70	198
32	Glycidyl palmitate	27.221	225134	0.53	92201	0.56	83	312
33	Tetracosamethyl-cyclododecasiloxane	27.292	175361	0.41	113109	0.68	83	888
34	(Z)-18-Octadec-9-enolide	27.593	380192	0.89	215073	1.30	87	280
35	Squalene	28.017	1390525	3.25	849699	5.12	96	410
36	(6Z,9Z)-6,9-Tricosadiene	28.150	302376	0.71	135093	0.81	72	320
37	Cyclodecasiloxane, eicosamethyl-	28.189	293532	0.69	166761	1.00	83	740
38	Glycidyl (Z)-9-nonadecenoate	28.556	5813709	13.61	2529822	15.24	90	352
39	Tetracontane	28.623	1358736	3.18	511046	3.08	92	562
40	Glycidyl (Z)-9-nonadecenoate	28.743	1382526	3.24	252779	1.52	76	352
41	Heptasiloxane, hexadecamethyl-	28.825	319782	0.75	121629	0.73	46	532
42	2,2-Dimethyl-3-(3,7,16,20-tetramethyl-henei	28.921	184511	0.43	68794	0.41	80	412
43	cis-9-Tetradecenoic acid, isobutyl ester	29.087	274945	0.64	110560	0.67	66	282
44	Tetracosamethyl-cyclododecasiloxane	29.144	386305	0.90	151869	0.92	80	888
45	.gamma.-Tocopherol	30.052	4331011	10.14	970434	5.85	94	416
46	Tetracosamethyl-cyclododecasiloxane	30.258	488768	1.14	105490	0.64	82	888
47	2-Methylhexacosane	30.397	420459	0.98	124244	0.75	90	380
48	Vitamin E	30.841	422278	0.99	152138	0.92	92	430
49	Ergosta-5,22-dien-3-ol, (3.beta.,22E)-	31.518	1134262	2.65	145956	0.88	84	398
50	Campesterol	32.308	3844806	9.00	461050	2.78	89	400
			42722819	100.00	16595082	100.00		

### **GC-MS analysis(qualitative) of oil of seeds harvested from treatment T3-(Thiourea (1000ppm) + Salicylic Acid (300ppm))**

Compound: 3-Ethyl-3-methylheptane Justification: This compound has a retention time of 9.217 minutes, with an area of 302,880 units, which accounts for 2.85% of the total area. The peak height for this compound is 188,334 units, making up 3.56% of the total height in the chromatogram. Compound: Decane, 3,7-dimethyl- Justification: This compound has a retention time of 9.311 minutes. Its area is 118,556 units, corresponding to 1.11% of the total area, while the peak height is 74,438 units, accounting for 1.41% of the total height. Compound: Undecane, 5-methyl- Justification: The compound "Undecane, 5-methyl-" has a retention time of 9.960 minutes. It has an area of 166,625 units, making up 1.57% of the total area, and a peak height of 104,494 units, contributing to 1.97% of the total height. Compound: Tetradecane, 5-methyl- Justification: The compound "Tetradecane, 5-methyl-" elutes at a retention time of 12.451 minutes. It has an area of 113,488 units, accounting for 1.07% of the total area, and a peak height of 81,158 units, contributing to 1.53% of the total height. Compound:

Hexadecane (1st occurrence-Justification: The first occurrence of "Hexadecane" has a retention time of 12.642 minutes. It has an area of 356,904 units, which makes up 3.36% of the total area. The peak height for this compound is 238,088 units, contributing to 4.50% of the total height. Compound: Benzene, 1,2-dimethyl-3-nitro--Justification: The compound "Benzene, 1,2-dimethyl-3-nitro-" elutes at a retention time of 12.878 minutes. It has an area of 233,472 units, accounting for 2.20% of the total area. The peak height for this compound is 118,718 units, contributing to 2.24% of the total height. Compound: Dodecane, 4,6-dimethyl- Justification: The compound "Dodecane, 4,6-dimethyl-" has a retention time of 13.302 minutes. It has an area of 200,764 units, accounting for 1.89% of the total area, and a peak height of 125,220 units, contributing to 2.36% of the total height. Compound: Hexadecane (2nd occurrence) Justification: The second occurrence of "Hexadecane" elutes at a retention time of 14.523 minutes. It has an area of 156,674 units, making up 1.47% of the total area. The peak height for this compound is 45,331 units, contributing to 0.86% of the total height. Compound: Quinoline, 1,2-dihydro-2,2,4-trimethyl- Justification: The compound "Quinoline, 1,2-dihydro-2,2,4-trimethyl-" elutes at a retention time of 15.030 minutes. It has an area of 145,802 units, accounting for 1.37% of the total area, and a peak height of 90,545 units, contributing to 1.71% of the total height. Compound: 2,6,10-Trimethyltridecane Justification: The compound "2,6,10-Trimethyltridecane" has a retention time of 15.164 minutes. It has an area of 105,459 units, making up 0.99% of the total area, and a peak height of 67,170 units, contributing to 1.27% of the total height. Compound: Eicosane, 2,4-dimethyl- Justification: The compound "Eicosane, 2,4-dimethyl-" elutes at a retention time of 15.199 minutes. It has an area of 153,046 units, accounting for 1.44% of the total area, and a peak height of 88,583 units, contributing to 1.67% of the total height. Compound: Heptadecane (1st occurrence) Justification: The first occurrence of "Heptadecane" has a retention time of 15.467 minutes. It has an area of 126,434 units, making up 1.19% of the total area. The peak height for this compound is 69,589 units, contributing to 1.31% of the total height. Compound: Hexadecane (3rd occurrence) Justification: The third occurrence of "Hexadecane" has a retention time of 15.527 minutes. It has an area of 367,734 units, accounting for 3.46% of the total area. The peak height for this compound is 184,890 units, contributing to 3.49% of the total height. Compound: Pentadecane Justification: The compound "Pentadecane" elutes at

a retention time of 15.674 minutes. It has an area of 288,805 units, accounting for 2.72% of the total area, and a peak height of 132,909 units, contributing to 2.51% of the total height. Compound: 2,4-Di-tert-butylphenol Justification: The compound "2,4-Di-tert-butylphenol" has a retention time of 15.786 minutes. It has an area of 167,764 units, making up 1.58% of the total area, and a peak height of 60,939 units, contributing to 1.15% of the total height. Compound: Benzoic acid, 4-ethoxy-, ethyl ester Justification: The compound "Benzoic acid, 4-ethoxy-, ethyl ester" elutes at a retention time of 16.020 minutes. It has an area of 185,289 units, accounting for 1.74% of the total area, and a peak height of 43,368 units, contributing to 0.82% of the total height. Compound: Eicosane (2nd occurrence) Justification: The second occurrence of "Eicosane" elutes at a retention time of 16.095 minutes. It has an area of 148,296 units, making up 1.39% of the total area. The peak height for this compound is 106,137 units, contributing to 2.00% of the total height. Compound: Heptadecane (2nd occurrence) Justification: The second occurrence of "Heptadecane" has a retention time of 16.899 minutes. It has an area of 229,739 units, accounting for 2.16% of the total area. The peak height for this compound is 146,148 units, contributing to 2.76% of the total height. Compound: Eicosane (3rd occurrence) Justification: The third occurrence of "Eicosane" elutes at a retention time of 18.061 minutes. It has an area of 476,495 units, making up 4.48% of the total area. The peak height for this compound is 284,877 units, contributing to 5.38% of the total height. Compound: Eicosane (4th occurrence) Justification: The fourth occurrence of "Eicosane" has a retention time of 18.554 minutes. It has an area of 132,356 units, accounting for 1.24% of the total area. The peak height for this compound is 79,130 units, contributing to 1.49% of the total height. Compound: Heneicosane (1st occurrence) Justification: The first occurrence of "Heneicosane" has a retention time of 19.161 minutes. It has an area of 159,202 units, making up 1.50% of the total area. The peak height for this compound is 110,305 units, contributing to 2.08% of the total height. Compound: Neophytadiene- Justification: The compound "Neophytadiene" elutes at a retention time of 19.552 minutes. It has an area of 223,761 units, accounting for 2.10% of the total area, and a peak height of 131,387 units, contributing to 2.48% of the total height. Compound: Heneicosane (2nd occurrence) - Justification: The second occurrence of "Heneicosane" has a retention time of 20.208 minutes. It has an area of 122,704 units, making up 1.15% of the total area. The peak

height for this compound is 73,644 units, contributing to 1.39% of the total height.

Compound: Silane, trichlorooctadecyl- Justification: The compound "Silane, trichlorooctadecyl-" elutes at a retention time of 20.330 minutes. It has an area of 289,914 units, accounting for 2.73% of the total area. The peak height for this compound is 146,884 units, contributing to 2.77% of the total height.

Compound: Eicosane (5th occurrence)-Justification: The fifth occurrence of "Eicosane" elutes at a retention time of 21.211 minutes. It has an area of 158,668 units, making up 1.49% of the total area. The peak height for this compound is 91,980 units, contributing to 1.74% of the total height.

Compound: Triacontanoic acid, methyl ester-Justification: The compound "Triacontanoic acid, methyl ester" elutes at a retention time of 22.402 minutes. It has an area of 183,077 units, accounting for 1.72% of the total area, and a peak height of 55,955 units, contributing to 1.06% of the total height.

Compound: Tetracosane-Justification: The compound "Tetracosane" elutes at a retention time of 23.083 minutes. It has an area of 124,151 units, making up 1.17% of the total area. The peak height for this compound is 64,177 units, contributing to 1.21% of the total height.

Compound: Glycidyl palmitate- Justification: The compound "Glycidyl palmitate" has a retention time of 23.932 minutes. It has an area of 205,898 units, accounting for 1.94% of the total area, and a peak height of 88,790 units, contributing to 1.68% of the total height.

Compound: 9-Octadecenoic acid (Z)-, 2,3-dihydroxyprop- Justification: The compound "9-Octadecenoic acid (Z)-, 2,3-dihydroxyprop" elutes at a retention time of 25.026 minutes. It has an area of 147,473 units, accounting for 1.39% of the total area, and a peak height of 76,556 units, contributing to 1.45% of the total height.

Compound: 9-Octadecenoic acid (Z)-, oxiranylmethyl est- Justification: The compound "9-Octadecenoic acid (Z)-, oxiranylmethyl est" elutes at a retention time of 25.430 minutes. It has an area of 1,338,581 units, accounting for 12.59% of the total area, and a peak height of 416,227 units, contributing to 7.86% of the total height.

Compound: Petroselinic acid, TMS derivative- Justification: The compound "Petroselinic acid, TMS derivative" elutes at a retention time of 25.556 minutes. It has an area of 294,678 units, accounting for 2.77% of the total area, and a peak height of 79,122 units, contributing to 1.49% of the total height.

Compound: 4,4'-((p-Phenylene) diisopropylidene) diphenol-Justification: The compound "4,4'-((p-Phenylene) diisopropylidene)diphenol" has a retention time of 26.947 minutes. It has an area of

177,270 units, making up 1.67% of the total area, and a peak height of 101,909 units, contributing to 1.92% of the total height. Compound: 9-Octadecenoic acid (Z)-, oxiranylmethyl est (2nd occurrence)-Justification: The second occurrence of "9-Octadecenoic acid (Z)-, oxiranylmethyl est" elutes at a retention time of 27.019 minutes. It has an area of 279,895 units, accounting for 2.63% of the total area, and a peak height of 162,704 units, contributing to 3.07% of the total height. Compound: (Z)-18-Octadec-9-enolide-Justification: The compound "(Z)-18-Octadec-9-enolide" elutes at a retention time of 27.562 minutes. It has an area of 129,209 units, making up 1.21% of the total area, and a peak height of 67,055 units, contributing to 1.27% of the total height. Compound: Squalene-Justification: The compound "Squalene" elutes at a retention time of 27.990 minutes. It has an area of 642,731 units, accounting for 6.04% of the total area, and a peak height of 400,220 units, contributing to 7.56% of the total height. Compound: 2,3-Dihydroxypropyl cis-13-docosenoate-Justification: The compound "2,3-Dihydroxypropyl cis-13-docosenoate" has a retention time of 28.125 minutes. It has an area of 256,782 units, accounting for 2.41% of the total area, and a peak height of 148,084 units, contributing to 2.80% of the total height. Compound: Glycidyl (Z)-9-nonadecenoate-Justification: The compound "Glycidyl (Z)-9-nonadecenoate" elutes at a retention time of 28.526 minutes. It has an area of 1,174,833 units, accounting for 11.05% of the total area, and a peak height of 528,696 units, contributing to 9.98% of the total height. Compound: Tetrapentacontane-Justification: The compound "Tetrapentacontane" elutes at a retention time of 28.593 minutes. It has an area of 267,596 units, accounting for 2.52% of the total area, and a peak height of 124,952 units, contributing to 2.36% of the total height. Compound: Tridecane, 3-cyclohexyl-Justification: The compound "Tridecane, 3-cyclohexyl-" has a retention time of 29.043 minutes. It has an area of 152,507 units, making up 1.43% of the total area, and a peak height of 60,767 units, contributing to 1.15% of the total height. Compound: 2'-(Trimethylsilyl)oxy-2,3,6'-trimethoxychalc-Justification: The compound "2'-(Trimethylsilyl)oxy-2,3,6'-trimethoxychalc" elutes at a retention time of 29.145 minutes. It has an area of 129,990 units, accounting for 1.22% of the total area, and a peak height of 37,221 units, contributing to 0.70% of the total height (Table 4.64). The total area for all compounds is 10,635,502 units, accounting for 100.00% of the total area, and the total height is 5,296,701 units, contributing to 100.00% of the total

height. This dataset provides valuable information about the composition and quantities of various chemical compounds in a chromatogram (Ozturk & Unal, 2023; Paalli et al., 2022; Pal et al., 2023; Parada et al., 2022; Parrey et al., 2023; Patel et al., 2022).

**Table 4.64. GC-MS analysis(qualitative) of oil of seeds harvest from Treatment-T3 Thiourea (1000ppm) + Salicylic Acid (300ppm)**

Peak	Name	R.Time	Area	Area%	Height	Height%	SI%	Molecular weight
1	3-Ethyl-3-methylheptane	9.217	302880	2.85	188334	3.56	93	142
2	Decane, 3,7-dimethyl-	9.311	118556	1.11	74438	1.41	93	170
3	Undecane, 5-methyl-	9.960	166625	1.57	104494	1.97	93	170
4	Tetradecane, 5-methyl-	12.451	113488	1.07	81158	1.53	93	212
5	Hexadecane	12.642	356904	3.36	238088	4.50	93	226
6	Benzene, 1,2-dimethyl-3-nitro-	12.878	233472	2.20	118718	2.24	92	151
7	Dodecane, 4,6-dimethyl-	13.302	200764	1.89	125220	2.36	93	198
8	Hexadecane	14.523	156674	1.47	45331	0.86	92	226
9	Quinoline, 1,2-dihydro-2,2,4-trimethyl-	15.030	145802	1.37	90545	1.71	83	173
10	2,6,10-Trimethyltridecane	15.164	105459	0.99	67170	1.27	92	226
11	Eicosane, 2,4-dimethyl-	15.199	153046	1.44	88583	1.67	87	310
12	Heptadecane	15.467	126434	1.19	69589	1.31	86	240
13	Hexadecane	15.527	367734	3.46	184890	3.49	90	226
14	Pentadecane	15.674	288805	2.72	132909	2.51	91	240
15	2,4-Di-tert-butylphenol	15.786	167764	1.58	60939	1.15	83	206
16	Benzoic acid, 4-ethoxy-, ethyl ester	16.020	185289	1.74	43368	0.82	86	194
17	Eicosane	16.095	148296	1.39	106137	2.00	90	282
18	Heptadecane	16.899	229739	2.16	146148	2.76	90	240
19	Eicosane	18.061	476495	4.48	284877	5.38	90	282
20	Eicosane	18.554	132356	1.24	79130	1.49	94	282
21	Heneicosane	19.161	159202	1.50	110305	2.08	94	296
22	Neophytadiene	19.552	223761	2.10	131387	2.48	91	278
23	Heneicosane	20.208	122704	1.15	73644	1.39	92	296
24	Silane, trichlorooctadecyl-	20.330	289914	2.73	146884	2.77	80	386
25	Eicosane	21.211	158668	1.49	91980	1.74	94	282
26	Triacontanoic acid, methyl ester	22.402	183077	1.72	55955	1.06	77	466
27	Tetracosane	23.083	124151	1.17	64177	1.21	93	338
28	Glycidyl palmitate	23.932	205898	1.94	88790	1.68	89	312
29	9-Octadecenoic acid (Z)-, 2,3-dihydroxyprop	25.026	147473	1.39	76556	1.45	87	356
30	9-Octadecenoic acid (Z)-, oxiranylmethyl est	25.430	1338581	12.59	416227	7.86	92	338
31	Petroselinic acid, TMS derivative	25.556	294678	2.77	79122	1.49	69	354
32	4,4'-(p-Phenylene)diisopropylidene)diphenol	26.947	177270	1.67	101909	1.92	74	346
33	9-Octadecenoic acid (Z)-, oxiranylmethyl est	27.019	279895	2.63	162704	3.07	91	338
34	(Z)-18-Octadec-9-enolide	27.562	129209	1.21	67055	1.27	87	280
35	Squalene	27.990	642731	6.04	400220	7.56	96	410
36	2,3-Dihydroxypropyl cis-13-docosenoate	28.125	256782	2.41	148084	2.80	84	412
37	Glycidyl (Z)-9-nonadecenoate	28.526	1174833	11.05	528696	9.98	90	352
38	Tetrapentacontane	28.593	267596	2.52	124952	2.36	91	758
39	Tridecane, 3-cyclohexyl-	29.043	152507	1.43	60767	1.15	70	266
40	2-(Trimethylsilyloxy)-2,3,6-trimethoxychalc	29.145	129990	1.22	37221	0.70	66	386
			10635502	100.00	5296701	100.00		

### **GC-MS analysis(qualitative) of oil of seeds harvest from treatment T4- (Thiourea (1500ppm) + Salicylic Acid (300ppm))**

The provided table contains information about various chemical compounds detected in a chromatogram. Each data point includes details such as the compound's name, retention time (R.Time), area, area percentage (Area%), peak height (Height), and height percentage (Height%).

Peak#1 – Ethylbenzene-Retention Time (R.Time): 5.449-Justification: The first peak corresponds to the compound "Ethylbenzene," which has a retention time of 5.449 minutes. It exhibits an area of 2,418,514 units, constituting 9.69% of the total area. The peak's height is 326,680 units, accounting for 3.32% of the total height.

Peak#2 - Benzene, 1,3-dimethyl-Justification: The second peak corresponds to the compound "Benzene, 1,3-dimethyl-," with a retention time of 6.061 minutes. It exhibits an area of 2,780,909 units, making up 11.14% of the total area. The peak's height is 359,967 units, contributing to 3.66% of the total height.

Peak#3 - Dodecane, 4,6-dimethyl-Justification: The third peak corresponds to the compound "Dodecane, 4,6-dimethyl-," with a retention time of 9.076 minutes. It has an area of 327,562 units, accounting for 1.31% of the total area. The peak's height is 150,701 units, contributing to 1.53% of the total height.

Peak#4 - Dodecane, 2,6,10-trimethyl-Justification: The fourth peak corresponds to the compound "Dodecane, 2,6,10-trimethyl-," with a retention time of 9.172 minutes. It exhibits an area of 116,395 units, making up 0.47% of the total area. The peak's height is 59,075 units, contributing to 0.60% of the total height.

Peak#5 - Decane, 3,7-dimethyl-Justification: The fifth peak corresponds to the compound "Decane, 3,7-dimethyl-," with a retention time of 9.843 minutes. It has an area of 215,430 units, accounting for 0.86% of the total area. The peak's height is 119,556 units, contributing to 1.22% of the total height.

Peak#6 - Silane, cyclohexyldimethoxymethyl-Justification: The sixth peak corresponds to the compound "Silane, cyclohexyldimethoxymethyl-," with a retention time of 10.786 minutes. It exhibits an area of 163,134 units, making up 0.65% of the total area. The peak's height is 96,536 units, contributing to 0.98% of the total height.

Peak#7 - Undecane, 4,7-dimethyl-Justification: The seventh peak corresponds to the compound "Undecane, 4,7-dimethyl-," with a retention time of 12.148 minutes. It has an area of 137,906 units, accounting for 0.55% of the total area. The peak's height is 75,387 units,



contributing to 0.77% of the total height. Peak#8 - 2,4-Dimethyldodecane-Justification: The eighth peak corresponds to the compound "2,4-Dimethyldodecane," with a retention time of 12.378 minutes. It exhibits an area of 230,262 units, making up 0.92% of the total area. The peak's height is 160,735 units, contributing to 1.63% of the total height. Peak#9 - Benzene, 1,2-dimethyl-3-nitro- Justification: The ninth peak corresponds to the compound "Benzene, 1,2-dimethyl-3-nitro-," with a retention time of 12.798 minutes. It has an area of 627,011 units, accounting for 2.51% of the total area. The peak's height is 247,967 units, contributing to 2.52% of the total height. Peak#10 - Dodecane, 2,6,11-trimethyl-Justification: The tenth peak corresponds to the compound "Dodecane, 2,6,11-trimethyl-," with a retention time of 12.885 minutes. It exhibits an area of 168,308 units, making up 0.67% of the total area. The peak's height is 88,140 units, contributing to 0.90% of the total height. Peak#11 - 2-Isopropyl-5-methyl-1-heptanol-Justification: The eleventh peak corresponds to the compound "2-Isopropyl-5-methyl-1-heptanol," with a retention time of 12.950 minutes. It has an area of 135,579 units, accounting for 0.54% of the total area. The peak's height is 57,056 units, contributing to 0.58% of the total height. Peak#12 - Dodecane, 4-methyl-Justification: The twelfth peak corresponds to the compound "Dodecane, 4-methyl-," with a retention time of 13.365 minutes. It exhibits an area of 141,322 units, making up 0.57% of the total area. The peak's height is 65,787 units, contributing to 0.67% of the total height. Peak#13 - Tetradecane, 5-methyl- Justification: The thirteenth peak corresponds to the compound "Tetradecane, 5-methyl-," with a retention time of 14.415 minutes. It has an area of 143,443 units, accounting for 0.57% of the total area. The peak's height is 56,056 units, contributing to 0.57% of the total height. Peak#14 – Octadecane-Justification: The fourteenth peak corresponds to the compound "Octadecane," with a retention time of 14.460 minutes. It exhibits an area of 193,469 units, making up 0.77% of the total area. The peak's height is 106,533 units, contributing to 1.08% of the total height. Peak#15 – Heptadecane-Justification: The fifteenth peak corresponds to the compound "Heptadecane," with a retention time of 14.528 minutes. It has an area of 212,599 units, accounting for 0.85% of the total area. The peak's height is 105,962 units, contributing to 1.08% of the total height. Peak#16 – Erucin-Justification: The sixteenth peak corresponds to the compound "Erucin," with a retention time of 14.800 minutes. It exhibits a significant area of 2,692,516 units,

constituting 10.79% of the total area. The peak's height is 1,490,199 units, making up 15.16% of the total height. Peak#17 - Heptadecane (2nd occurrence)-Justification: The seventeenth peak corresponds to the compound "Heptadecane" (second occurrence), with a retention time of 14.972 minutes. It has an area of 196,693 units, accounting for 0.79% of the total area. The peak's height is 68,743 units, contributing to 0.70% of the total height. Peak#18 - Heptadecane (3rd occurrence)-Justification: The eighteenth peak corresponds to the compound "Heptadecane" (third occurrence), with a retention time of 15.051 minutes. It exhibits an area of 219,398 units, making up 0.88% of the total area. The peak's height is 112,609 units, contributing to 1.15% of the total height. Peak#19 - 2,6,10-Trimethyltridecane-Justification: The nineteenth peak corresponds to the compound "2,6,10-Trimethyltridecane," with a retention time of 15.103 minutes. It has an area of 204,178 units, accounting for 0.82% of the total area. The peak's height is 114,523 units, contributing to 1.16% of the total height. Peak#20 – Eicosane-Justification: The twentieth peak corresponds to the compound "Eicosane," with a retention time of 15.409 minutes. It exhibits an area of 323,086 units, making up 1.29% of the total area. The peak's height is 182,594 units, contributing to 1.86% of the total height. Peak#21 – Heneicosane-Justification: The twenty-first peak corresponds to the compound "Heneicosane," with a retention time of 15.469 minutes. It has a substantial area of 875,210 units, accounting for 3.51% of the total area. The peak's height is 449,526 units, making up 4.57% of the total height. Peak#22 – Hexadecane-Justification: The twenty-second peak corresponds to the compound "Hexadecane," with a retention time of 15.598 minutes. It exhibits an area of 211,255 units, making up 0.85% of the total area. The peak's height is 81,281 units, contributing to 0.83% of the total height. Peak#23 - Phenol, 2,5-bis(1,1-dimethylethyl)-Justification: The twenty-third peak corresponds to the compound "Phenol, 2,5-bis(1,1-dimethylethyl)-," with a retention time of 15.723 minutes. It has an area of 309,888 units, accounting for 1.24% of the total area. The peak's height is 102,435 units, contributing to 1.04% of the total height. Peak#24 - Benzoic acid, 4-ethoxy-Justification: The twenty-fourth peak corresponds to the compound "Benzoic acid, 4-ethoxy-," with a retention time of 15.965 minutes. It exhibits an area of 212,966 units, making up 0.85% of the total area. The peak's height is 75,859 units, contributing to 0.77% of the total height. Peak#25 - Hexadecane (2nd occurrence)-Justification: The twenty-fifth peak corresponds to the

compound "Hexadecane" (second occurrence), with a retention time of 16.840 minutes. It has an area of 156,511 units, accounting for 0.63% of the total area. The peak's height is 107,404 units, contributing to 1.09% of the total height. Peak#26 – Neophytadiene-Justification: The twenty-sixth peak corresponds to the compound "Neophytadiene," with a retention time of 19.495 minutes. It exhibits a significant area of 913,374 units, constituting 3.66% of the total area. The peak's height is 571,428 units, making up 5.81% of the total height. Peak#27 - 2-Pentadecanone, 6,10,14-trimethyl-Justification: The twenty-seventh peak corresponds to the compound "2-Pentadecanone, 6,10,14-trimethyl-," with a retention time of 19.550 minutes. It has an area of 248,994 units, accounting for 1.00% of the total area. The peak's height is 96,966 units, contributing to 0.99% of the total height. Peak#28 - 3,7,11,15-Tetramethyl-2-hexadecen-1-ol-Justification: The twenty-eighth peak corresponds to the compound "3,7,11,15-Tetramethyl-2-hexadecen-1-ol," with a retention time of 19.750 minutes. It exhibits an area of 306,509 units, making up 1.23% of the total area. The peak's height is 149,576 units, contributing to 1.52% of the total height. Peak#29 - 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate-Justification: The twenty-ninth peak corresponds to the compound "2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate," with a retention time of 19.946 minutes. It has an area of 422,219 units, accounting for 1.69% of the total area. The peak's height is 274,553 units, contributing to 2.79% of the total height. Peak#30 – Dotriacontane-Justification: The thirtieth peak corresponds to the compound "Dotriacontane," with a retention time of 20.098 minutes. It exhibits an area of 163,993 units, making up 0.66% of the total area. The peak's height is 94,643 units, contributing to 0.96% of the total height. Peak#31 - Silane, trichlorooctadecyl-Justification: The thirty-first peak corresponds to the compound "Silane, trichlorooctadecyl-," with a retention time of 20.271 minutes. It has an area of 624,235 units, accounting for 2.50% of the total area. The peak's height is 291,407 units, contributing to 2.96% of the total height. Peak#32 - Octadecane, 2,6,10,14-tetramethyl-Justification: The thirty-second peak corresponds to the compound "Octadecane, 2,6,10,14-tetramethyl-," with a retention time of 20.310 minutes. It exhibits an area of 257,207 units, making up 1.03% of the total area. The peak's height is 138,017 units, contributing to 1.40% of the total height. Peak#33 - Tridecanoic acid, 4,8,12-trimethyl-, methyl ester-Justification: The thirty-third peak corresponds to the compound "Tridecanoic acid, 4,8,12-trimethyl-,

methyl ester," with a retention time of 20.399 minutes. It has an area of 282,713 units, accounting for 1.13% of the total area. The peak's height is 115,939 units, contributing to 1.18% of the total height. Peak#34 – Tetracosane-Justification: The thirty-fourth peak corresponds to the compound "Tetracosane," with a retention time of 20.748 minutes. It exhibits an area of 154,019 units, making up 0.62% of the total area. The peak's height is 86,848 units, contributing to 0.88% of the total height. Peak#35 - Eicosane (2nd occurrence)-Justification: The thirty-fifth peak corresponds to the compound "Eicosane" (second occurrence), with a retention time of 21.154 minutes. It has an area of 221,707 units, accounting for 0.89% of the total area. The peak's height is 117,181 units, contributing to 1.19% of the total height. Peak#36 - Dotriacontane (2nd occurrence)-Justification: The thirty-sixth peak corresponds to the compound "Dotriacontane" (second occurrence), with a retention time of 22.313 minutes. It exhibits an area of 322,887 units, making up 1.29% of the total area. The peak's height is 98,641 units, contributing to 1.00% of the total height. Peak#37 – Tetrapentacontane-Justification: The thirty-seventh peak corresponds to the compound "Tetrapentacontane," with a retention time of 22.699 minutes. It has an area of 165,644 units, accounting for 0.66% of the total area. The peak's height is 85,015 units, contributing to 0.86% of the total height. Peak#38 - Glycidyl palmitate-Justification: The thirty-eighth peak corresponds to the compound "Glycidyl palmitate," with a retention time of 23.875 minutes. It exhibits an area of 252,245 units, making up 1.01% of the total area. The peak's height is 103,290 units, contributing to 1.05% of the total height. Peak#39 - 9,12-Octadecadienoyl chloride, (Z,Z)-Justification: The thirty-ninth peak corresponds to the compound "9,12-Octadecadienoyl chloride, (Z,Z)-," with a retention time of 25.330 minutes. It has an area of 152,368 units, accounting for 0.61% of the total area. The peak's height is 87,248 units, contributing to 0.89% of the total height. Peak#40 - 9-Octadecenoic acid (Z)-, oxiranylmethyl ester-Justification: The fortieth peak corresponds to the compound "9-Octadecenoic acid (Z)-, oxiranylmethyl ester," with a retention time of 25.372 minutes. It exhibits a substantial area of 745,454 units, constituting 2.99% of the total area. The peak's height is 303,580 units, making up 3.09% of the total height. Peak#41 - (Z)-18-Octadec-9-enolide-Justification: The forty-first peak corresponds to the compound "(Z)-18-Octadec-9-enolide," with a retention time of 27.502 minutes. It has an area of 150,029 units, accounting for 0.60%

of the total area. The peak's height is 86,513 units, contributing to 0.88% of the total height. Peak#42 – Squalene-Justification: The forty-second peak corresponds to the compound "Squalene," with a retention time of 27.931 minutes. It exhibits a significant area of 1,027,523 units, constituting 4.12% of the total area. The peak's height is 623,119 units, making up 6.34% of the total height. Peak#43 - cis-13-Docosenoyl chloride-Justification: The forty-third peak corresponds to the compound "cis-13-Docosenoyl chloride," with a retention time of 28.066 minutes. It has an area of 142,838 units, accounting for 0.57% of the total area. The peak's height is 89,768 units, contributing to 0.91% of the total height. Peak#44 - Glycidyl (Z)-9-nonadecenoate-Justification: The forty-fourth peak corresponds to the compound "Glycidyl (Z)-9-nonadecenoate," with a retention time of 28.462 minutes. It exhibits an area of 627,306 units, making up 2.51% of the total area. The peak's height is 272,377 units, contributing to 2.77% of the total height. Peak#45 - Tetrapentacontane (2nd occurrence- Justification: The forty-fifth peak corresponds to the compound "Tetrapentacontane" (second occurrence), with a retention time of 28.532 minutes. It has an area of 336,966 units, accounting for 1.35% of the total area. The peak's height is 161,102 units, contributing to 1.64% of the total height. Peak#46 -  $\gamma$ -Tocopherol-Justification: The forty-sixth peak corresponds to the compound " $\gamma$ -Tocopherol," with a retention time of 29.911 minutes. It exhibits a significant area of 2,241,579 units, constituting 8.98% of the total area. The peak's height is 616,951 units, making up 6.28% of the total height. Peak#47 - 2-Methylhexacosane-Justification: The forty-seventh peak corresponds to the compound "2-Methylhexacosane," with a retention time of 30.269 minutes. It has an area of 140,977 units, accounting for 0.56% of the total area. The peak's height is 59,819 units, contributing to 0.61% of the total height. Peak#48 - Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite-Justification: The forty-eighth peak corresponds to the compound "Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite," with a retention time of 34.123 minutes. It exhibits a significant area of 1,650,355 units, constituting 6.61% of the total area. The peak's height is 445,680 units, making up 4.53% of the total height (Table 4.65).

**Table 4.65. GC-MS analysis(qualitative) of oil of seeds harvest from Treatment-T4- Thiourea (1500ppm)+ Salicylic Acid (300ppm)**

Peak	Name	R.Time	Area	Area%	Height	Height%	SI%	Molecular weight
1	Ethylbenzene	5.449	2418514	9.69	326680	3.32	98	106
2	Benzene, 1,3-dimethyl-	6.061	2780909	11.14	359967	3.66	98	106
3	Dodecane, 4,6-dimethyl-	9.076	327562	1.31	150701	1.53	92	198
4	Dodecane, 2,6,10-trimethyl-	9.172	116395	0.47	59075	0.60	92	212
5	Decane, 3,7-dimethyl-	9.843	215430	0.86	119556	1.22	92	170
6	Silane, cyclohexyldimethoxymethyl-	10.786	163134	0.65	96536	0.98	97	188
7	Undecane, 4,7-dimethyl-	12.148	137906	0.55	75387	0.77	94	184
8	2,4-Dimethyldodecane	12.378	230262	0.92	160735	1.63	94	198
9	Benzene, 1,2-dimethyl-3-nitro-	12.798	627011	2.51	247967	2.52	93	151
10	Dodecane, 2,6,11-trimethyl-	12.885	168308	0.67	88140	0.90	89	212
11	2-Isopropyl-5-methyl-1-heptanol	12.950	135579	0.54	57056	0.58	88	172
12	Dodecane, 4-methyl-	13.365	141322	0.57	65787	0.67	91	184
13	Tetradecane, 5-methyl-	14.415	143443	0.57	56056	0.57	89	212
14	Octadecane	14.460	193469	0.77	106533	1.08	93	254
15	Heptadecane	14.528	212599	0.85	105962	1.08	92	240
16	Erucin	14.800	2692516	10.79	1490199	15.16	95	161
17	Heptadecane	14.972	196693	0.79	68743	0.70	93	240
18	Heptadecane	15.051	219398	0.88	112609	1.15	93	240
19	2,6,10-Trimethyltridecane	15.103	204178	0.82	114523	1.16	93	226
20	Eicosane	15.409	323086	1.29	182594	1.86	92	282
21	Heneicosane	15.469	875210	3.51	449526	4.57	92	296
22	Hexadecane	15.598	211255	0.85	81281	0.83	92	226
23	Phenol, 2,5-bis(1,1-dimethylethyl)-	15.723	309888	1.24	102435	1.04	79	206
24	Benzoic acid, 4-ethoxy-	15.965	212966	0.85	75859	0.77	65	166
25	Hexadecane	16.840	156511	0.63	107404	1.09	97	226
26	Neophytadiene	19.495	913374	3.66	571428	5.81	93	278
27	2-Pentadecanone, 6,10,14-trimethyl-	19.550	248994	1.00	96966	0.99	87	268
28	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	19.750	306509	1.23	149576	1.52	91	296
29	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, ac	19.946	422219	1.69	274553	2.79	89	338
30	Dotriacontane	20.098	163993	0.66	94643	0.96	92	450
31	Silane, trichlorooctadecyl-	20.271	624235	2.50	291407	2.96	82	386
32	Octadecane, 2,6,10,14-tetramethyl-	20.310	257207	1.03	138017	1.40	88	310
33	Tridecanoic acid, 4,8,12-trimethyl-, methyle	20.399	282713	1.13	115939	1.18	82	270
34	Tetracosane	20.748	154019	0.62	86848	0.88	82	338
35	Eicosane	21.154	221707	0.89	117181	1.19	95	282
36	Dotriacontane	22.313	322887	1.29	98641	1.00	90	450
37	Tetrapentacontane	22.699	165644	0.66	85015	0.86	90	758
38	Glycidyl palmitate	23.875	252245	1.01	103290	1.05	87	312
39	9,12-Octadecadienoyl chloride, (Z,Z)-	25.330	152368	0.61	87248	0.89	85	298
40	9-Octadecenoic acid (Z)-, oxiranylmethyl est	25.372	745454	2.99	303580	3.09	92	338
41	(Z)-18-Octadec-9-enolide	27.502	150029	0.60	86513	0.88	88	280
42	Squalene	27.931	1027523	4.12	623119	6.34	96	410
43	cis-13-Docosenoic acid chloride	28.066	142838	0.57	89768	0.91	84	356
44	Glycidyl (Z)-9-nonadecenoate	28.462	627306	2.51	272377	2.77	90	352
45	Tetrapentacontane	28.532	336966	1.35	161102	1.64	94	758
46	.gamma.-Tocopherol	29.911	2241579	8.98	616951	6.28	95	416
47	2-Methylhexacosane	30.269	140977	0.56	59819	0.61	86	380
48	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphit	34.123	1650355	6.61	445680	4.53	85	646
			24964685	100.00	9830972	100.00		

**GC-MS analysis(qualitative) of oil of seeds harvest from treatment T5- (Thiourea (1000ppm) + Salicylic Acid (450ppm))**

Benzene, 1,3-dimethyl-Justification: The first peak corresponds to the compound "Benzene, 1,3-dimethyl-," with a retention time of 6.074 minutes. It exhibits an area of 2,160,800 units, contributing to 7.84% of the total area. The peak's height is 294,933 units, making up 2.44% of the total height. Dodecane, 2,6,10-trimethyl-Justification: The second peak corresponds to the compound "Dodecane, 2,6,10-trimethyl-," with a retention time of 9.079 minutes. It has an area of 245,627 units, accounting for 0.89% of the total area. The peak's height is 116,439 units, contributing to 0.96% of the total height. Decane, 3,7-dimethyl-Justification: The third peak corresponds to the compound "Decane, 3,7-dimethyl-," with a retention time of 9.845 minutes. It exhibits an area of 180,399 units, making up 0.65% of the total area. The peak's height is 108,386 units, contributing to 0.90% of the total height. Silane, cyclohexyldimethoxymethyl-Justification: The fourth peak corresponds to the compound "Silane, cyclohexyldimethoxymethyl-," with a retention time of 10.787 minutes. It has an area of 131,759 units, accounting for 0.48% of the total area. The peak's height is 79,434 units, contributing to 0.66% of the total height. Pentadecane- Justification: The fifth peak corresponds to the compound "Pentadecane," with a retention time of 11.469 minutes. It exhibits an area of 169,709 units, making up 0.62% of the total area. The peak's height is 97,515 units, contributing to 0.81% of the total height. Undecane, 4,6-dimethyl- Justification: The sixth peak corresponds to the compound "Undecane, 4,6-dimethyl-," with a retention time of 11.646 minutes. It has an area of 115,096 units, accounting for 0.42% of the total area. The peak's height is 76,847 units, contributing to 0.64% of the total height. Dodecane, 4-methyl- Justification: The seventh peak corresponds to the compound "Dodecane, 4-methyl-," with a retention time of 11.778 minutes. It exhibits an area of 115,476 units, making up 0.42% of the total area. The peak's height is 74,842 units, contributing to 0.62% of the total height. Tetradecane-Justification: The eighth peak corresponds to the compound "Tetradecane," with a retention time of 12.065 minutes. It has an area of 114,108 units, accounting for 0.41% of the total area. The peak's height is 73,360 units, contributing to 0.61% of the total height. Undecane, 3,7-dimethyl-Justification: The ninth peak corresponds to the compound "Undecane, 3,7-dimethyl-," with a retention time of 12.150 minutes. It

exhibits an area of 124,915 units, making up 0.45% of the total area. The peak's height is 76,840 units, contributing to 0.64% of the total height. 2,4- Dimethyldodecane-Justification: The tenth peak corresponds to the compound "2,4- Dimethyldodecane," with a retention time of 12.379 minutes. It has an area of 249,692 units, accounting for 0.91% of the total area. The peak's height is 185,934 units, contributing to 1.54% of the total height. Dodecane, 2,6,11-trimethyl-Justification: The eleventh peak corresponds to the compound "Dodecane, 2,6,11-trimethyl-," with a retention time of 12.570 minutes. It exhibits a substantial area of 832,464 units, constituting 3.02% of the total area. The peak's height is 556,224 units, making up 4.60% of the total height. Benzene, 1,2-dimethyl-3-nitro-Justification: The twelfth peak corresponds to the compound "Benzene, 1,2-dimethyl-3-nitro-," with a retention time of 12.799 minutes. It has an area of 698,875 units, accounting for 2.54% of the total area. The peak's height is 267,766 units, contributing to 2.22% of the total height. Hexane, 3,3-dimethyl-Justification: The thirteenth peak corresponds to the compound "Hexane, 3,3-dimethyl-," with a retention time of 12.887 minutes. It exhibits an area of 179,906 units, making up 0.65% of the total area. The peak's height is 101,105 units, contributing to 0.84% of the total height. 1-Decanol, 2-hexyl-Justification: The fourteenth peak corresponds to the compound "1-Decanol, 2-hexyl-," with a retention time of 12.950 minutes. It has an area of 170,907 units, accounting for 0.62% of the total area. The peak's height is 69,670 units, contributing to 0.58% of the total height. Dodecane, 2,6,11-trimethyl- (2nd occurrence)-Justification: The fifteenth peak corresponds to the compound "Dodecane, 2,6,11-trimethyl-" (second occurrence), with a retention time of 13.234 minutes. It exhibits an area of 518,122 units, constituting 1.88% of the total area. The peak's height is 343,255 units, making up 2.84% of the total height. Octadecane-Justification: The sixteenth peak corresponds to the compound "Octadecane," with a retention time of 14.463 minutes. It has an area of 118,665 units, accounting for 0.43% of the total area. The peak's height is 78,607 units, contributing to 0.65% of the total height. Heptadecane-Justification: The seventeenth peak corresponds to the compound "Heptadecane," with a retention time of 14.528 minutes. It exhibits an area of 147,914 units, making up 0.54% of the total area. The peak's height is 97,822 units, contributing to 0.81% of the total height. Erucin-Justification: The eighteenth peak corresponds to the compound "Erucin," with a retention time of 14.801 minutes. It exhibits a significant



area of 3,552,973 units, constituting 12.89% of the total area. The peak's height is 2,156,709 units, making up 17.85% of the total height. 2,6,10-Trimethyltridecane-Justification: The nineteenth peak corresponds to the compound "2,6,10-Trimethyltridecane," with a retention time of 15.105 minutes. It has an area of 273,095 units, accounting for 0.99% of the total area. The peak's height is 157,898 units, contributing to 1.31% of the total height. Eicosane-Justification: The twentieth peak corresponds to the compound "Eicosane," with a retention time of 15.138 minutes. It exhibits an area of 361,777 units, making up 1.31% of the total area. The peak's height is 225,490 units, contributing to 1.87% of the total height. Hexadecane-Justification: The twenty-first peak corresponds to the compound "Hexadecane," with a retention time of 15.468 minutes. It has a substantial area of 1,068,116 units, constituting 3.88% of the total area. The peak's height is 544,495 units, making up 4.51% of the total height. Phenol, 3,5-bis(1,1-dimethylethyl)-Justification: The twenty-second peak corresponds to the compound "Phenol, 3,5-bis(1,1- dimethylethyl)-," with a retention time of 15.726 minutes. It exhibits an area of 642,709 units, accounting for 2.33% of the total area. The peak's height is 112,940 units, contributing to 0.93% of the total height. Benzoic acid, 4-ethoxy-, ethyl ester- Justification: The twenty-third peak corresponds to the compound "Benzoic acid, 4- ethoxy-, ethyl ester," with a retention time of 15.955 minutes. It has an area of 337,894 units, accounting for 1.23% of the total area. The peak's height is 122,770 units, contributing to 1.02% of the total height. Heneicosane-Justification: The twenty-fourth peak corresponds to the compound "Heneicosane," with a retention time of 17.161 minutes. It exhibits an area of 134,692 units, making up 0.49% of the total area. The peak's height is 89,478 units, contributing to 0.74% of the total height. Tetrapentacontane, 1,54-dibromo-Justification: The twenty-fifth peak corresponds to the compound "Tetrapentacontane, 1,54-dibromo-," with a retention time of 19.499 minutes. It has an area of 312,922 units, accounting for 1.14% of the total area. The peak's height is 177,056 units, contributing to 1.47% of the total height. Carbonic acid, octadecyl prop-1-en-2-yl ester-Justification: The twenty-sixth peak corresponds to the compound "Carbonic acid, octadecyl prop-1-en-2-yl ester," with a retention time of 19.560 minutes. It exhibits an area of 216,450 units, making up 0.79% of the total area.

The peak's height is 61,847 units, contributing to 0.51% of the total height. Didecyl phthalate-Justification: The twenty-seventh peak corresponds to the compound "Didecyl phthalate," with a retention time of 19.762 minutes. It has an area of 114,971 units, accounting for 0.42% of the total area. The peak's height is 61,893 units, contributing to 0.51% of the total height. 2-Methylhexacosane-Justification: The twenty-eighth peak corresponds to the compound "2-Methylhexacosane," with a retention time of 19.949 minutes. It exhibits an area of 160,703 units, making up 0.58% of the total area. The peak's height is 113,230 units, contributing to 0.94% of the total height. Dotriacontane-Justification: The twenty-ninth peak corresponds to the compound "Dotriacontane," with a retention time of 20.190 minutes. It has an area of 243,263 units, accounting for 0.88% of the total area. The peak's height is 88,031 units, contributing to 0.73% of the total height. 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione-Justification: The thirtieth peak corresponds to the compound "7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione," with a retention time of 20.273 minutes. It exhibits a substantial area of 1,065,424 units, constituting 3.87% of the total area. The peak's height is 516,843 units, making up 4.28% of the total height. Decane, 1-iodo-Justification: The thirty-first peak corresponds to the compound "Decane, 1-iodo-," with a retention time of 20.320 minutes. It has an area of 149,251 units, accounting for 0.54% of the total area. The peak's height is 113,601 units, contributing to 0.94% of the total height. Hexadecanoic acid, methyl ester-Justification: The thirty-second peak corresponds to the compound "Hexadecanoic acid, methyl ester," with a retention time of 20.401 minutes. It exhibits an area of 470,643 units, making up 1.71% of the total area. The peak's height is 164,634 units, contributing to 1.36% of the total height. Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-Justification: The thirty-third peak corresponds to the compound "Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)" with a retention time of 20.500 minutes. It has an area of 135,359 units, accounting for 0.49% of the total area. The peak's height is 55,137 units, contributing to 0.46% of the total height. Tetrapentacontane (2nd occurrence)-Justification: The thirty-fourth peak corresponds to the compound "Tetrapentacontane" (second occurrence), with a retention time of 20.703 minutes. It exhibits an area of 182,080 units, making up 0.66% of the total area. The peak's height is 119,643 units, contributing to 0.99% of the total height. 11,14-Eicosadienoic acid, methyl ester-

Justification: The thirty-fifth peak corresponds to the compound "11,14-Eicosadienoic acid, methyl ester," with a retention time of 22.050 minutes. It has an area of 115,612 units, accounting for 0.42% of the total area. The peak's height is 44,661 units, contributing to 0.37% of the total height.

7-Hexadecenal, (Z)-Justification: The thirty-sixth peak corresponds to the compound "7-Hexadecenal, (Z)-," with a retention time of 22.114 minutes. It exhibits an area of 209,954 units, making up 0.76% of the total area. The peak's height is 144,687 units, contributing to 1.20% of the total height.

Triacotanoic acid, methyl ester-Justification: The thirty-seventh peak corresponds to the compound "Triacotanoic acid, methyl ester," with a retention time of 22.346 minutes. It has an area of 359,598 units, accounting for 1.30% of the total area. The peak's height is 113,043 units, contributing to 0.94% of the total height.

Glycidyl palmitate-Justification: The thirty-eighth peak corresponds to the compound "Glycidyl palmitate," with a retention time of 23.875 minutes. It exhibits an area of 160,276 units, making up 0.58% of the total area. The peak's height is 91,685 units, contributing to 0.76% of the total height.

9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester-Justification: The thirty-ninth peak corresponds to the compound "9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester," with a retention time of 24.968 minutes. It has an area of 176,991 units, accounting for 0.64% of the total area. The peak's height is 96,991 units, contributing to 0.80% of the total height.

cis-4,10,13,16-Docosatetraenoic Acid methyl ester-Justification: The fortieth peak corresponds to the compound "cis-4,10,13,16-Docosatetraenoic Acid methyl ester," with a retention time of 25.335 minutes. It exhibits an area of 330,004 units, making up 1.20% of the total area. The peak's height is 166,636 units, contributing to 1.38% of the total height.

9-Octadecenoic acid (Z)-, oxiranylmethyl ester-Justification: The forty-first peak corresponds to the compound "9-Octadecenoic acid (Z)-, oxiranylmethyl ester," with a retention time of 25.372 minutes. It exhibits a significant area of 1,084,892 units, constituting 3.94% of the total area. The peak's height is 439,287 units, which is 3.63% of the total.

13-Docosenoic acid, methyl ester-Justification: The forty-second peak corresponds to the compound "13-Docosenoic acid, methyl ester," with a retention time of 25.588 minutes. It has an area of 166,961 units, accounting for 0.61% of the total area. The peak's height is 114,026 units, contributing to 0.94% of the total height.

(Z)-18-Octadec-9-enolide-Justification: The forty-third peak corresponds to the compound "(Z)-18-Octadec-9-

enolide," with a retention time of 27.501 minutes. It exhibits an area of 246,641 units, making up 0.89% of the total area. The peak's height is 152,037 units, contributing to 1.26% of the total height. Squalene -Justification: The forty-fourth peak corresponds to the compound "Squalene," with a retention time of 27.932 minutes. It exhibits a substantial area of 1,448,788 units, constituting 5.26% of the total area. The peak's height is 916,245 units, making up 7.58% of the total height.-cis-13-Docosenoyl chloride-Justification: The forty-fifth peak corresponds to the compound "cis-13-Docosenoyl chloride," with a retention time of 28.064 minutes. It has an area of 302,821 units, accounting for 1.10% of the total area. The peak's height is 167,648 units, contributing to 1.39% of the total height. Glycidyl (Z)-9-nonadecenoate-Justification: The forty-sixth peak corresponds to the compound "Glycidyl (Z)-9-nonadecenoate," with a retention time of 28.461 minutes. It exhibits a significant area of 1,167,024 units, constituting 4.23% of the total area. The peak's height is 535,016 units, making up 4.43% of the total height. Tetrapentacontane (3rd occurrence)-Justification: The forty-seventh peak corresponds to the compound "Tetrapentacontane" (third occurrence), with a retention time of 28.528 minutes. It has an area of 290,023 units, accounting for 1.05% of the total area. The peak's height is 138,571 units, contributing to 1.15% of the total height. 2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosane)-Justification: The forty-eighth peak corresponds to the compound "2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicosane)," with a retention time of 28.822 minutes. It exhibits an area of 99,045 units, making up 0.36% of the total area. The peak's height is 53,744 units, contributing to 0.44% of the total height.  $\gamma$ -Tocopherol-Justification: The forty-ninth peak corresponds to the compound " $\gamma$ -Tocopherol," with a retention time of 29.918 minutes. It exhibits a significant area of 3,077,042 units, constituting 11.17% of the total area. The peak's height is 659,367 units, making up 5.46% of the total height. Vitamin E-Justification: The fiftieth peak corresponds to the compound "Vitamin E," with a retention time of 30.700 minutes. It has an area of 330,531 units, accounting for 1.20% of the total area. The peak's height is 73,835 units, contributing to 0.61% of the total height. Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite-Justification: The fifty-first peak corresponds to the compound "Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite," with a retention time of 34.114 minutes. It exhibits a significant area of 2,295,440 units, constituting 8.33% of the total area. The peak's height is 597,368 units, making up

4.94% of the total heigh (Table 4.66)( Ozturk & Unal, 2023; Paalli et al., 2022; Pal et al., 2023; Parada et al., 2022; Parrey et al., 2023; Patel et al., 2022; Peng et al., 2022; Pérez-Llorca et al., 2023; Phokas et al., 2023).

**Table 4.66. GC-MS analysis(qualitative) of oil of seeds harvest from treatment-T5- (Thiourea (1000ppm) + Salicylic Acid (450ppm)**

Peak#	Name	R.Time	Area	Area%	Height	Height%	SI%	Molecular weight
1	Benzene, 1,3-dimethyl-	6.074	2160800	7.84	294933	2.44	97	106
2	Dodecane, 2,6,10-trimethyl-	9.079	245627	0.89	116439	0.96	92	212
3	Decane, 3,7-dimethyl-	9.845	180399	0.65	108386	0.90	92	170
4	Silane, cyclohexyldimethoxymethyl-	10.787	131759	0.48	79434	0.66	98	188
5	Pentadecane	11.469	169709	0.62	97515	0.81	84	212
6	Undecane, 4,6-dimethyl-	11.646	115096	0.42	76847	0.64	94	184
7	Dodecane, 4-methyl-	11.778	115476	0.42	74842	0.62	95	184
8	Tetradecane	12.065	114108	0.41	73360	0.61	83	198
9	Undecane, 3,7-dimethyl-	12.150	124915	0.45	76840	0.64	95	184
10	2,4-Dimethyldodecane	12.379	249692	0.91	185934	1.54	94	198
11	Dodecane, 2,6,11-trimethyl-	12.570	832464	3.02	556224	4.60	92	212
12	Benzene, 1,2-dimethyl-3-nitro-	12.799	698875	2.54	267766	2.22	93	151
13	Hexane, 3,3-dimethyl-	12.887	179906	0.65	101105	0.84	89	114
14	1-Decanol, 2-hexyl-	12.950	170907	0.62	69670	0.58	89	242
15	Dodecane, 2,6,11-trimethyl-	13.234	518122	1.88	343255	2.84	93	212
16	Octadecane	14.463	118665	0.43	78607	0.65	92	254
17	Heptadecane	14.528	147914	0.54	97822	0.81	92	240
18	Erucin	14.801	3552973	12.89	2156709	17.85	95	161
19	2,6,10-Trimethyltridecane	15.105	273095	0.99	157898	1.31	96	226
20	Eicosane	15.138	361777	1.31	225490	1.87	91	282
21	Hexadecane	15.468	1068116	3.88	544495	4.51	91	226
22	Phenol, 3,5-bis(1,1-dimethylethyl)-	15.726	642709	2.33	112940	0.93	76	206
23	Benzoic acid, 4-ethoxy-, ethyl ester	15.955	337894	1.23	122770	1.02	91	194
24	Heneicosane	17.161	134692	0.49	89478	0.74	93	296
25	Tetrapentacontane, 1,54-dibromo-	19.499	312922	1.14	177056	1.47	87	914
26	Carbonic acid, octadecyl prop-1-en-2-yl ester	19.560	216450	0.79	61847	0.51	85	354
27	Didecyl phthalate	19.762	114971	0.42	61893	0.51	80	446
28	2-Methylhexacosane	19.949	160703	0.58	113230	0.94	90	380
29	Dotriacontane	20.190	243263	0.88	88031	0.73	88	450
30	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-die	20.273	1065424	3.87	516843	4.28	77	276
31	Decane, 1-iodo-	20.320	149251	0.54	113601	0.94	83	268
32	Hexadecanoic acid, methyl ester	20.401	470643	1.71	164634	1.36	89	270
33	Benzenepropanoic acid, 3,5-bis(1,1-dimethyl	20.500	135359	0.49	55137	0.46	73	292
34	Tetrapentacontane	20.703	182080	0.66	119643	0.99	89	758
35	11,14-Eicosadienoic acid, methyl ester	22.050	115612	0.42	44661	0.37	87	322
36	7-Hexadecenal, (Z)-	22.114	209954	0.76	144687	1.20	87	238
37	Triacotanoic acid, methyl ester	22.346	359598	1.30	113043	0.94	84	466
38	Glycidyl palmitate	23.875	160276	0.58	91685	0.76	92	312
39	9-Octadecenoic acid (Z)-, 2,3-dihydroxyprop	24.968	176991	0.64	96991	0.80	91	356
40	cis-4,10,13,16-Docosatetraenoic Acid methyl	25.335	330004	1.20	166636	1.38	81	346
41	9-Octadecenoic acid (Z)-, oxiranylmethyl est	25.372	1084892	3.94	439287	3.63	98	338
42	13-Docosenoic acid, methyl ester	25.588	166961	0.61	114026	0.94	89	352

43	(Z)-18-Octadec-9-enolide	27.501	246641	0.89	152037	1.26	89	280
44	Squalene	27.932	1448788	5.26	916245	7.58	96	410
45	cis-13-Docosenoyl chloride	28.064	302821	1.10	167648	1.39	85	356
46	Glycidyl (Z)-9-nonadecenoate	28.461	1167024	4.23	535016	4.43	91	352
47	Tetrapentacontane	28.528	290023	1.05	138571	1.15	92	758
48	2,2-Dimethyl-3-(3,7,16,20-tetramethyl-henei	28.822	99045	0.36	53744	0.44	88	412
49	.gamma.-Tocopherol	29.918	3077042	11.17	659367	5.46	96	416
50	Vitamin E	30.700	330531	1.20	73835	0.61	91	430
51	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphit	34.114	2295440	8.33	597368	4.94	85	646
			27558399	100.00	12085521	100.00		

### **GC-MS analysis(qualitative) of oil of seeds harvest from treatment- T6-(Thiourea (500 ppm) + Salicylic Acid (300ppm))**

Analyzing the provided data table, we have 47 peaks, each corresponding to a specific compound identified in a chromatographic analysis. These peaks are characterised by retention time (R.Time), area, area percentage (Area%), height, and height percentage (Height%).

Ethylbenzene-Justification: The first peak corresponds to the compound "Ethylbenzene," with a retention time of 5.462 minutes. It exhibits a substantial area of 7,724,102 units, constituting 17.02% of the total area. The peak's height is 794,522 units, making up 6.60% of the total height.

Benzene, 1,3-dimethyl-Justification: The second peak corresponds to the compound "Benzene, 1,3-dimethyl-," with a retention time of 5.628 minutes. It has an area of 2,991,277 units, accounting for 6.59% of the total area. The peak's height is 388,009 units, contributing to 3.22% of the total height.

Dodecane, 4,6-dimethyl--Justification: The third peak corresponds to the compound "Dodecane, 4,6-dimethyl-," with a retention time of 9.081 minutes. It exhibits an area of 155,651 units, making up 0.34% of the total area. The peak's height is 76,063 units, contributing to 0.63% of the total height.

Dodecane, 2,6,10-trimethyl-Justification: The fourth peak corresponds to the compound "Dodecane, 2,6,10-trimethyl-," with a retention time of 9.847 minutes. It has an area of 110,702 units, accounting for 0.24% of the total area. The peak's height is 65,368 units, contributing to 0.54% of the total height.

Sulfurous acid, decyl 2-ethylhexyl ester-Justification: The fifth peak corresponds to the compound "Sulfurous acid, decyl 2-ethylhexyl ester," with a retention time of 11.469 minutes. It exhibits an area of 149,784 units, constituting 0.33% of the total area. The peak's height is 80,367 units, making up 0.67% of the total height.

2,4-Dimethyldodecane-Justification: The sixth peak corresponds to the compound "2,4-Dimethyldodecane," with a retention time of 12.380 minutes. It has an

area of 173,887 units, accounting for 0.38% of the total area. The peak's height is 124,128 units, contributing to 1.03% of the total height. Dodecane, 2,6,11-trimethyl-Justification: The seventh peak corresponds to the compound "Dodecane, 2,6,11-trimethyl-," with a retention time of 12.571 minutes. It exhibits an area of 556,791 units, making up 1.23% of the total area. The peak's height is 373,013 units, contributing to 3.10% of the total height. Octane, 2,3,6,7-tetramethyl-Justification: The eighth peak corresponds to the compound "Octane, 2,3,6,7-tetramethyl-," with a retention time of 12.697 minutes. It has an area of 120,964 units, accounting for 0.27% of the total area. The peak's height is 61,725 units, contributing to 0.51% of the total height. Benzene, 1,2-dimethyl-3-nitro- Justification: The ninth peak corresponds to the compound "Benzene, 1,2-dimethyl-3-nitro-," with a retention time of 12.800 minutes. It exhibits an area of 463,726 units, constituting 1.02% of the total area. The peak's height is 184,383 units, making up 1.53% of the total height. 1-Decanol, 2-hexyl-Justification: The tenth peak corresponds to the compound "1-Decanol, 2-hexyl-," with a retention time of 12.950 minutes. It has an area of 121,212 units, accounting for 0.27% of the total area. The peak's height is 48,213 units, contributing to 0.40% of the total height. Tetradecane, 5-methyl- Justification: The eleventh peak corresponds to the compound "Tetradecane, 5-methyl-," with a retention time of 14.415 minutes. It exhibits an area of 99,610 units, making up 0.22% of the total area. The peak's height is 39,146 units, contributing to 0.33% of the total height. Octadecane-Justification: The twelfth peak corresponds to the compound "Octadecane," with a retention time of 14.464 minutes. It has an area of 155,204 units, accounting for 0.34% of the total area. The peak's height is 82,320 units, contributing to 0.68% of the total height. Heptadecane-Justification: The thirteenth peak corresponds to the compound "Heptadecane," with a retention time of 14.530 minutes. It exhibits an area of 144,021 units, making up 0.32% of the total area. The peak's height is 80,176 units, contributing to 0.67% of the total height. Erucin-Justification: The fourteenth peak corresponds to the compound "Erucin," with a retention time of 14.802 minutes. It has a substantial area of 3,889,080 units, constituting 8.57% of the total area. The peak's height is 2,264,729 units, making up 18.81% of the total height. 2,6,10-Trimethyltridecane-Justification: The fifteenth peak corresponds to the compound "2,6,10-Trimethyltridecane," with a retention time of

15.105 minutes. It exhibits an area of 179,558 units, making up 0.40% of the total area. The peak's height is 103,618 units, contributing to 0.86% of the total height. Eicosane-Justification: The sixteenth peak corresponds to the compound "Eicosane," with a retention time of 15.139 minutes. It has an area of 248,682 units, accounting for 0.55% of the total area. The peak's height is 156,936 units, making up 1.30% of the total height. Phenol, 2,5-bis(1,1-dimethylethyl)-Justification: The seventeenth peak corresponds to the compound "Phenol, 2,5-bis(1,1-dimethylethyl)-," with a retention time of 15.725 minutes. It exhibits an area of 185,676 units, constituting 0.41% of the total area. The peak's height is 74,076 units, contributing to 0.62% of the total height. Benzoic acid, 4-ethoxy-, ethyl ester-Justification: The eighteenth peak corresponds to the compound "Benzoic acid, 4-ethoxy-, ethyl ester," with a retention time of 15.960 minutes. It has an area of 200,609 units, accounting for 0.44% of the total area. The peak's height is 71,695 units, contributing to 0.60% of the total height. Hexadecane-Justification: The nineteenth peak corresponds to the compound "Hexadecane," with a retention time of 16.842 minutes. It exhibits an area of 142,634 units, making up 0.31% of the total area. The peak's height is 95,820 units, contributing to 0.80% of the total height. Heneicosane-Justification: The twentieth peak corresponds to the compound "Heneicosane," with a retention time of 17.161 minutes. It has an area of 150,795 units, accounting for 0.33% of the total area. The peak's height is 84,337 units, contributing to 0.70% of the total height. Decane, 1-iodo- Justification: The twenty-first peak corresponds to the compound "Decane, 1-iodo-," with a retention time of 17.615 minutes. It exhibits an area of 180,542 units, making up 0.40% of the total area. The peak's height is 37,093 units, contributing to 0.31% of the total height. Octadecane, 5-methyl-Justification: The twenty-second peak corresponds to the compound "Octadecane, 5-methyl-," with a retention time of 18.440 minutes. It has an area of 145,546 units, accounting for 0.32% of the total area. The peak's height is 54,690 units, contributing to 0.45% of the total height. 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, ac-Justification: The twenty-third peak corresponds to the compound "2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, ac," with a retention time of 19.497 minutes. It exhibits an area of 237,299 units, constituting 0.52% of the total area. The peak's height is 131,363 units, making up 1.09% of the total height. 1-(2-Propen-1-yloxy)dodecane-Justification: The twenty-fourth peak corresponds to the compound "1-(2-Propen-1-yloxy)dodecane,"



with a retention time of 19.555 minutes. It has an area of 133,893 units, accounting for 0.30% of the total area. The peak's height is 48,570 units, contributing to 0.40% of the total height. 2-Methyltetracosane-Justification: The twenty-fifth peak corresponds to the compound "2-Methyltetracosane," with a retention time of 19.948 minutes. It exhibits an area of 101,657 units, making up 0.22% of the total area. The peak's height is 70,745 units, contributing to 0.59% of the total height. 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-die-Justification: The twenty-sixth peak corresponds to the compound "7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-die," with a retention time of 20.272 minutes. It has an area of 686,764 units, accounting for 1.51% of the total area. The peak's height is 275,349 units, making up 2.29% of the total height. Hexadecanoic acid, methyl ester-Justification: The twenty-seventh peak corresponds to the compound "Hexadecanoic acid, methyl ester," with a retention time of 20.401 minutes. It exhibits an area of 282,519 units, constituting 0.62% of the total area. The peak's height is 107,544 units, contributing to 0.89% of the total height. 7-Hexadecenal, (Z)-Justification: The twenty-eighth peak corresponds to the compound "7-Hexadecenal, (Z)-," with a retention time of 22.113 minutes. It has an area of 199,050 units, accounting for 0.44% of the total area. The peak's height is 129,441 units, making up 1.08% of the total height. Methyl stearate-Justification: The twenty-ninth peak corresponds to the compound "Methyl stearate," with a retention time of 22.344 minutes. It exhibits an area of 127,009 units, constituting 0.28% of the total area. The peak's height is 75,738 units, contributing to 0.63% of the total height. Eicosane (Duplicate)-Justification: The thirtieth peak also corresponds to the compound "Eicosane," with a retention time of 23.026 minutes. However, this appears to be a duplicate, as it exhibits similar characteristics to the sixteenth peak. It has an area of 110,235 units, accounting for 0.24% of the total area. The peak's height is 56,432 units, contributing to 0.47% of the total height. Glycidyl palmitate-Justification: The thirty-first peak corresponds to the compound "Glycidyl palmitate," with a retention time of 23.874 minutes. It has an area of 224,368 units, accounting for 0.49% of the total area. The peak's height is 115,694 units, contributing to 0.96% of the total height. Oleoyl chloride-Justification: The thirty-second peak corresponds to the compound "Oleoyl chloride," with a retention time of 24.968 minutes. It exhibits an area of 171,192 units, making up 0.38% of the total area. The peak's height is 91,864 units, contributing to

0.76% of the total height. Cyclododecyne-Justification: The thirty-third peak corresponds to the compound "Cyclododecyne," with a retention time of 25.335 minutes. It has an area of 394,297 units, accounting for 0.87% of the total area. The peak's height is 207,335 units, making up 1.72% of the total height. 9-Octadecenoic acid (Z)-, oxiranylmethyl ester-Justification: The thirty-fourth peak corresponds to the compound "9-Octadecenoic acid (Z)-, oxiranylmethyl ester," with a retention time of 25.373 minutes. It exhibits a substantial area of 1,547,073 units, constituting 3.41% of the total area. The peak's height is 558,467 units, contributing to 4.64% of the total height. 13-Docosenoic acid, methyl ester-Justification: The thirty-fifth peak corresponds to the compound "13-Docosenoic acid, methyl ester," with a retention time of 25.588 minutes. It has an area of 462,338 units, accounting for 1.02% of the total area. The peak's height is 193,825 units, making up 1.61% of the total height. (Z)-18-Octadec-9-enolide-Justification: The thirty-sixth peak corresponds to the compound "(Z)-18-Octadec-9-enolide," with a retention time of 27.501 minutes. It exhibits an area of 305,104 units, making up 0.67% of the total area. The peak's height is 177,348 units, contributing to 1.47% of the total height. Squalene-Justification: The thirty-seventh peak corresponds to the compound "Squalene," with a retention time of 27.932 minutes. It has a substantial area of 1,465,396 units, constituting 3.23% of the total area. The peak's height is 901,628 units, making up 7.49% of the total height. cis-13-Docosenoyl chloride-Justification: The thirty-eighth peak corresponds to the compound "cis-13-Docosenoyl chloride," with a retention time of 28.064 minutes. It exhibits an area of 289,127 units, accounting for 0.64% of the total area. The peak's height is 169,184 units, contributing to 1.41% of the total height. Glycidyl (Z)-9-nonadecenoate-Justification: The thirty-ninth peak corresponds to the compound "Glycidyl (Z)-9-nonadecenoate," with a retention time of 28.460 minutes. It has a substantial area of 1,542,648 units, constituting 3.40% of the total area. The peak's height is 704,469 units, making up 5.85% of the total height. Tetrapentacontane-Justification: The fortieth peak corresponds to the compound "Tetrapentacontane," with a retention time of 28.529 minutes. It exhibits an area of 362,045 units, accounting for 0.80% of the total area. The peak's height is 171,391 units, contributing to 1.42% of the total height. Diethyl n-hexadecylmalonate-Justification: The forty-first peak corresponds to the compound "Diethyl n-hexadecylmalonate," with a retention time of 28.965 minutes. It has an area

of 248,577 units, accounting for 0.55% of the total area. The peak's height is 74,443 units, contributing to 0.62% of the total height. Phytol linoleate-Justification: The forty-second peak corresponds to the compound "Phytol linoleate," with a retention time of 29.113 minutes. It has an area of 998,673 units, accounting for 2.20% of the total area. The peak's height is 129,250 units, contributing to 1.07% of the total height.  $\gamma$ -Tocopherol-Justification: The forty-third peak corresponds to the compound " $\gamma$ -Tocopherol," with a retention time of 29.917 minutes. It has a significant area of 5,372,291 units, constituting 11.84% of the total area. The peak's height is 972,356 units, making up 8.08% of the total height. Vitamin E-Justification: The forty-fourth peak corresponds to the compound "Vitamin E," with a retention time of 30.698 minutes. It exhibits an area of 297,231 units, accounting for 0.66% of the total area. The peak's height is 100,556 units, contributing to 0.84% of the total height.  $\gamma$ - Sitosterol-Justification: The forty-fifth peak corresponds to the compound " $\gamma$ - Sitosterol," with a retention time of 33.286 minutes. It has a substantial area of 10,044,182 units, constituting 22.14% of the total area. The peak's height is 831,435 units, making up 6.91% of the total height. 3-n-Pentylthiolane, S,S-dioxide- Justification: The forty-sixth peak corresponds to the compound "3-n-Pentylthiolane, S,S-dioxide," with a retention time of 33.777 minutes. It exhibits an area of 175,221 units, making up 0.39% of the total area. The peak's height is 54,718 units, contributing to 0.45% of the total height. Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (Total)- Justification: The forty-seventh peak corresponds to the compound "Phenol, 2,4- bis(1,1-dimethylethyl)-, phosphite (Total)," with a retention time of 34.119 minutes. It exhibits an area of 1,306,381 units, constituting 2.88% of the total area. The peak's height is 349,307 units, making up 2.90% of the total height (Table 4.67).

**Table 4.67. GC-MS analysis(qualitative) of oil of seeds harvest from treatment-T6-Thiourea (500 ppm) + Salicylic Acid (300ppm)**

Peak#	Name	R.Time	Area	Area%	Height	Height%	SI%	Molecular weight
1	Ethylbenzene	5.462	7724102	17.02	794522	6.60	98	106
2	Benzene, 1,3-dimethyl-	5.628	2991277	6.59	388009	3.22	97	106
3	Dodecane, 4,6-dimethyl-	9.081	155651	0.34	76063	0.63	92	198
4	Dodecane, 2,6,10-trimethyl-	9.847	110702	0.24	65368	0.54	92	212
5	Sulfurous acid, decyl 2-ethylhexyl ester	11.469	149784	0.33	80367	0.67	78	334
6	2,4-Dimethyldodecane	12.380	173887	0.38	124128	1.03	94	198
7	Dodecane, 2,6,11-trimethyl-	12.571	556791	1.23	373013	3.10	92	212
8	Octane, 2,3,6,7-tetramethyl-	12.697	120964	0.27	61725	0.51	90	170

9	Benzene, 1,2-dimethyl-3-nitro-	12.800	463726	1.02	184383	1.53	94	151
10	1-Decanol, 2-hexyl-	12.950	121212	0.27	48213	0.40	89	242
11	Tetradecane, 5-methyl-	14.415	99610	0.22	39146	0.33	88	212
12	Octadecane	14.464	155204	0.34	82320	0.68	93	254
13	Heptadecane	14.530	144021	0.32	80176	0.67	92	240
14	Erucin	14.802	3889080	8.57	2264729	18.81	95	161
15	2,6,10-Trimethyltridecane	15.105	179558	0.40	103618	0.86	94	226
16	Eicosane	15.139	248682	0.55	156936	1.30	92	282
17	Phenol, 2,5-bis(1,1-dimethylethyl)-	15.725	185676	0.41	74076	0.62	79	206
18	Benzoic acid, 4-ethoxy-, ethyl ester	15.960	200609	0.44	71695	0.60	86	194
19	Hexadecane	16.842	142634	0.31	95820	0.80	97	226
20	Heneicosane	17.161	150795	0.33	84337	0.70	96	296
21	Decane, 1-iodo-	17.615	180542	0.40	37093	0.31	82	268
22	Octadecane, 5-methyl-	18.440	145546	0.32	54690	0.45	86	268
23	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, ac	19.497	237299	0.52	131363	1.09	87	338
24	1-(2-Propen-1-yloxy)dodecane	19.555	133893	0.30	48570	0.40	83	226
25	2-Methyltetracosane	19.948	101657	0.22	70745	0.59	89	352
26	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-die	20.272	686764	1.51	275349	2.29	75	276
27	Hexadecanoic acid, methyl ester	20.401	282519	0.62	107544	0.89	90	270
28	7-Hexadecenal, (Z)-	22.113	199050	0.44	129441	1.08	87	238
29	Methyl stearate	22.344	127009	0.28	75738	0.63	87	298
30	Eicosane	23.026	110235	0.24	56432	0.47	95	282
31	Glycidyl palmitate	23.874	224368	0.49	115694	0.96	91	312
32	Oleoyl chloride	24.968	171192	0.38	91864	0.76	89	300
33	Cyclododecyne	25.335	394297	0.87	207335	1.72	83	164
34	9-Octadecenoic acid (Z)-, oxiranylmethyl est	25.373	1547073	3.41	558467	4.64	93	338
35	13-Docosenoic acid, methyl ester	25.588	462338	1.02	193825	1.61	89	352
36	(Z)-18-Octadec-9-enolide	27.501	305104	0.67	177348	1.47	89	280
37	Squalene	27.932	1465396	3.23	901628	7.49	96	410
38	cis-13-Docosenoyl chloride	28.064	289127	0.64	169184	1.41	85	356
39	Glycidyl (Z)-9-nonadecenoate	28.460	1542648	3.40	704469	5.85	90	352
40	Tetrapentacontane	28.529	362045	0.80	171391	1.42	93	758
41	Diethyl n-hexadecylmalonate	28.965	248577	0.55	74443	0.62	68	384
42	Phytol linoleate	29.113	998673	2.20	129250	1.07	79	558
43	.gamma.-Tocopherol	29.917	5372291	11.84	972356	8.08	95	416
44	Vitamin E	30.698	297231	0.66	100556	0.84	93	430
45	.gamma.-Sitosterol	33.286	10044182	22.14	831435	6.91	94	414
46	3-n-Pentylthiolane, S,S-dioxide	33.777	175221	0.39	54718	0.45	61	190
47	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphit	34.119	1306381	2.88	349307	2.90	84	646
			45374623	100.00	12038879	100.00		

### GC-MS analysis(qualitative) of oil of seeds harvest from treatment- T7-Thiourea (1000ppm) + Salicylic Acid (150ppm)

This analysis has 46 peaks in the chromatogram, each representing a different compound. Cyclopentanol, 1-methyl-Justification: The first peak corresponds to "Cyclopentanol, 1-methyl-," with a retention time of 4.229 minutes. It has a significant area of 51,184,637 units, accounting for 16.94% of the total area. The peak's height is 3,086,519 units, contributing to 3.20% of the total height. 3,3-Diethoxy-1-propyne-

Justification: The second peak corresponds to "3,3-Diethoxy-1-propyne," with a retention time of 7.509 minutes. It exhibits an area of 7,953,301 units, making up 2.63% of the total area. The peak's height is 1,835,359 units, contributing to 1.90% of the total height.

Nonane, 5-(2-methylpropyl)- Justification: The third peak corresponds to "Nonane, 5-(2-methylpropyl)-," with a retention time of 9.076 minutes. It has an area of 2,419,797 units, accounting for 0.80% of the total area. The peak's height is 1,111,356 units, making up 1.15% of the total height.

Silane, cyclohexyldimethoxymethyl- Justification: The fourth peak corresponds to "Silane, cyclohexyldimethoxymethyl-," with a retention time of 10.788 minutes. It exhibits an area of 1,275,906 units, constituting 0.42% of the total area. The peak's height is 770,681 units, contributing to 0.80% of the total height.

Naphthalene-Justification: The fifth peak corresponds to "Naphthalene," with a retention time of 11.349 minutes. It has an area of 1,805,984 units, accounting for 0.60% of the total area. The peak's height is 863,934 units, making up 0.90% of the total height.

Tetradecane-Justification: The sixth peak corresponds to "Tetradecane," with a retention time of 11.467 minutes. It exhibits an area of 1,881,981 units, constituting 0.62% of the total area. The peak's height is 694,603 units, contributing to 0.72% of the total height.

Dodecane, 4,6-dimethyl-Justification: The seventh peak corresponds to "Dodecane, 4,6-dimethyl-," with a retention time of 12.242 minutes. It has an area of 1,875,679 units, accounting for 0.62% of the total area. The peak's height is 664,143 units, making up 0.69% of the total height.

2,4-Dimethyldodecane-Justification: The eighth peak corresponds to "2,4-Dimethyldodecane," with a retention time of 12.380 minutes. It exhibits an area of 1,830,507 units, making up 0.61% of the total area. The peak's height is 1,309,732 units, contributing to 1.36% of the total height.

Dodecane, 2,6,11-trimethyl- Justification: The ninth peak corresponds to "Dodecane, 2,6,11-trimethyl-," with a retention time of 12.696 minutes. It has an area of 1,661,213 units, accounting for 0.55% of the total area. The peak's height is 680,597 units, making up 0.71% of the total height.

Benzene, 1,2-dimethyl-3-nitro- Justification: The tenth peak corresponds to "Benzene, 1,2-dimethyl-3-nitro-," with a retention time of 12.805 minutes. It exhibits an area of 4,283,701 units, constituting 1.42% of the total area. The peak's height is 1,891,430 units, contributing to 1.96% of the total height.

Dodecane, 4-methyl-Justification: The eleventh peak corresponds to "Dodecane, 4-methyl-," with a retention time of 13.495

minutes. It has an area of 1,374,381 units, accounting for 0.45% of the total area. The peak's height is 548,430 units, making up 0.57% of the total height. Octadecane-Justification: The twelfth peak corresponds to "Octadecane," with a retention time of 14.464 minutes. It exhibits an area of 1,545,622 units, constituting 0.51% of the total area. The peak's height is 830,359 units, contributing to 0.86% of the total height. Heptadecane-Justification: The thirteenth peak corresponds to "Heptadecane," with a retention time of 14.530 minutes. It has an area of 1,790,950 units, accounting for 0.59% of the total area. The peak's height is 974,693 units, making up 1.01% of the total height. Erucin-Justification: The fourteenth peak corresponds to "Erucin," with a retention time of 14.805 minutes. It exhibits an area of 2,943,479 units, constituting 0.97% of the total area. The peak's height is 1,645,895 units, contributing to 1.71% of the total height. Hexadecane-Justification: The fifteenth peak corresponds to "Hexadecane," with a retention time of 15.600 minutes. It has an area of 2,189,782 units, accounting for 0.72% of the total area. The peak's height is 730,249 units, making up 0.76% of the total height. 2,4-Di-tert-butylphenol-Justification: The sixteenth peak corresponds to "2,4-Di-tert-butylphenol," with a retention time of 15.736 minutes. It exhibits an area of 3,280,982 units, constituting 1.09% of the total area. The peak's height is 1,266,974 units, contributing to 1.31% of the total height. Benzoic acid, 4-ethoxy-, ethyl ster-Justification: The seventeenth peak corresponds to "Benzoic acid, 4-ethoxy-, ethyl ester," with a retention time of 15.965 minutes. It has an area of 2,018,619 units, accounting for 0.67% of the total area. The peak's height is 770,504 units, making up 0.80% of the total height. Hexadecane-Justification: The eighteenth peak corresponds to "Hexadecane," with a retention time of 16.040 minutes. It exhibits an area of 5,349,663 units, constituting 1.77% of the total area. The peak's height is 2,539,534 units, contributing to 2.63% of the total height. Heneicosane-Justification: The nineteenth peak corresponds to "Heneicosane," with a retention time of 17.163 minutes. It has an area of 2,202,383 units, accounting for 0.73% of the total area. The peak's height is 1,138,642 units, making up 1.18% of the total height. Eicosane-Justification: The twentieth peak corresponds to "Eicosane," with a retention time of 17.819 minutes. It exhibits an area of 2,845,693 units, constituting 0.94% of the total area. The peak's height is 485,616 units, contributing to 0.50% of the total height. Eicosyl isopropyl ether-Justification: The twenty-first peak corresponds to "Eicosyl

isopropyl ether," with a retention time of 18.795 minutes. It has an area of 2,644,000 units, accounting for 0.87% of the total area. The peak's height is 512,944 units, making up 0.53% of the total height. Tetrapentacontane, 1,54-dibromo- Justification: The twenty-second peak corresponds to "Tetrapentacontane, 1,54-dibromo-," with a retention time of 19.502 minutes. It exhibits an area of 2,723,310 units, constituting 0.90% of the total area. The peak's height is 1,482,673 units, contributing to 1.54% of the total height. 2-Methylhexacosane-Justification: The twenty-third peak corresponds to "2-Methylhexacosane," with a retention time of 19.761 minutes. It has an area of 2,610,616 units, accounting for 0.86% of the total area. The peak's height is 480,537 units, making up 0.50% of the total height. Tetracosane-Justification: The twenty-fourth peak corresponds to "Tetracosane," with a retention time of 20.105 minutes. It exhibits an area of 1,447,692 units, constituting 0.48% of the total area. The peak's height is 678,636 units, contributing to 0.70% of the total height. Silane, trichlorooctadecyl-Justification: The twenty-fifth peak corresponds to "Silane, trichlorooctadecyl-," with a retention time of 20.279 minutes. It has an area of 10,564,989 units, accounting for 3.50% of the total area. The peak's height is 3,125,361 units, making up 3.24% of the total height. Bis(pentamethylcyclotrisiloxy) tetramethyldis-Justification: The twenty-sixth peak corresponds to "Bis(pentamethylcyclotrisiloxy) tetramethyldis," with a retention time of 20.430 minutes. It exhibits an area of 2,897,955 units, constituting 0.96% of the total area. The peak's height is 645,097 units, contributing to 0.67% of the total height. Tetrapentacontane-Justification: The twenty-seventh peak corresponds to "Tetrapentacontane," with a retention time of 20.758 minutes. It has an area of 1,783,924 units, accounting for 0.59% of the total area. The peak's height is 694,598 units, making up 0.72% of the total height. 9-Octadecenoic acid, methyl ester, (E)-Justification: The twenty-eighth peak corresponds to "9-Octadecenoic acid, methyl ester, (E)-," with a retention time of 22.110 minutes. It exhibits an area of 2,043,389 units, constituting 0.68% of the total area. The peak's height is 1,348,098 units, contributing to 1.40% of the total height. Glycidyl palmitate-Justification: The twenty-ninth peak corresponds to "Glycidyl palmitate," with a retention time of 23.882 minutes. It has an area of 7,622,729 units, accounting for 2.52% of the total area. The peak's height is 4,054,422 units, making up 4.21% of the total height. 9,12- Octadecadienoic acid (Z,Z)-, 2,3-dihydr-Justification: The thirtieth peak corresponds to "9,12-

Octadecadienoic acid (Z,Z)-, 2,3-dihydr," with a retention time of 24.932 minutes. It exhibits an area of 2,005,553 units, constituting 0.66% of the total area. The peak's height is 1,192,761 units, contributing to 1.24% of the total height. Oleoyl chloride-Justification: The thirty-first peak corresponds to "Oleoyl chloride," with a retention time of 24.975 minutes. It has an area of 2,755,278 units, accounting for 0.91% of the total area. The peak's height is 1,768,325 units, making up 1.83% of the total height. Bicyclo[10.1.0]tridec-1-ene-Justification: The thirty-second peak corresponds to "Bicyclo[10.1.0]tridec-1-ene," with a retention time of 25.348 minutes. It exhibits an area of 22,445,289 units, constituting 7.43% of the total area. The peak's height is 11,782,199 units, contributing to 12.22% of the total height. 9-Octadecenoic acid (Z)-, oxiranylmethyl est-Justification: The thirty-third peak corresponds to "9- Octadecenoic acid (Z)-, oxiranylmethyl est," with a retention time of 25.388 minutes. It has an area of 31,467,366 units, accounting for 10.41% of the total area. The peak's height is 15,783,239 units, making up 16.37% of the total height. Bicyclo[10.1.0]tridec- 1-ene-Justification: The thirty-fourth peak corresponds to "Bicyclo[10.1.0]tridec-1- ene," with a retention time of 25.445 minutes. It exhibits an area of 9,093,222 units, constituting 3.01% of the total area. The peak's height is 3,192,386 units, contributing to 3.31% of the total height. Myristic acid glycidyl ester-Justification: The thirty-fifth peak corresponds to "Myristic acid glycidyl ester," with a retention time of 25.575 minutes. It has an area of 5,810,387 units, accounting for 1.92% of the total area. The peak's height is 2,840,198 units, making up 2.95% of the total height. Triphenylphosphine oxide-Justification: The thirty-sixth peak corresponds to "Triphenylphosphine oxide," with a retention time of 25.927 minutes. It exhibits an area of 1,173,245 units, constituting 0.39% of the total area. The peak's height is 527,000 units, contributing to 0.55% of the total height. 1,8,11-Heptadecatriene, (Z,Z)- Justification: The thirty-seventh peak corresponds to "1,8,11-Heptadecatriene, (Z,Z)-," with a retention time of 26.085 minutes. It has an area of 2,925,174 units, accounting for 0.97% of the total area. The peak's height is 1,430,253 units, making up 1.48% of the total height. Squalene-Justification: The thirty-eighth peak corresponds to "Squalene," with a retention time of 27.939 minutes. It exhibits an area of 3,589,377 units, constituting 1.19% of the total area. The peak's height is 2,145,855 units, contributing to 2.23% of the total height. 9-Octadecenoic acid (Z)-, oxiranylmethyl est-Justification: The thirty-ninth peak



corresponds to "9-Octadecenoic acid (Z)-, oxiranylmethyl est," with a retention time of 28.472 minutes. It has an area of 1,408,840 units, accounting for 0.47% of the total area. The peak's height is 615,550 units, making up 0.64% of the total height. Myristic acid glycidyl ester-Justification: The fortieth peak corresponds to "Myristic acid glycidyl ester," with a retention time of 28.640 minutes. It exhibits an area of 1,880,781 units, constituting 0.62% of the total area. The peak's height is 784,396 units, contributing to 0.81% of the total height. Z,Z-3,13- Octadecadien-1-ol-Justification: The forty-first peak corresponds to "Z,Z-3,13- Octadecadien-1-ol," with a retention time of 28.979 minutes. It has an area of 6,783,308 units, accounting for 2.24% of the total area. The peak's height is 2,311,714 units, making up 2.40% of the total height. gamma.-Tocopherol-Justification: The forty-second peak corresponds to ".gamma.-Tocopherol," with a retention time of 29.928 minutes. It exhibits an area of 7,185,117 units, constituting 2.38% of the total area. The peak's height is 2,475,960 units, contributing to 2.57% of the total height. Cholesterol- Justification: The forty-third peak corresponds to "Cholesterol," with a retention time of 30.820 minutes. It has an area of 4,560,482 units, accounting for 1.51% of the total area. The peak's height is 1,006,477 units, making up 1.04% of the total height. Ergosta- 5,22-dien-3-ol, (3.beta.,22E)-Justification: The forty-fourth peak corresponds to "Ergosta-5,22-dien-3-ol, (3.beta.,22E)-," with a retention time of 31.299 minutes. It exhibits an area of 5,939,722 units, constituting 1.97% of the total area. The peak's height is 1,190,057 units, contributing to 1.23% of the total height. Campesterol- Justification: The forty-fifth peak corresponds to "Campesterol," with a retention time of 32.080 minutes. It has an area of 18,307,056 units, accounting for 6.06% of the total area. The peak's height is 3,563,835 units, making up 3.70% of the total height. gamma.- Sitosterol-Justification: The forty-sixth and final peak corresponds to ".gamma.- Sitosterol," with a retention time of 33.272 minutes. It exhibits an area of 38,849,737 units, constituting 12.85% of the total area. The peak's height is 6,917,703 units, contributing to 7.18% of the total height (Table 4.68).

**Table 4.68. GC-MS analysis(qualitative) of oil of seeds harvest from treatment- T7-Thiourea (1000ppm) + Salicylic Acid (150ppm)**

Peak	Name	R.Time	Area	Area%	Height	Height%	SI%	Molecular weight
1	Cyclopentanol, 1-methyl-	4.229	51184637	16.94	3086519	3.20	90	100
2	3,3-Diethoxy-1-propyne	7.509	7953301	2.63	1835359	1.90	92	128
3	Nonane, 5-(2-methylpropyl)-	9.076	2419797	0.80	1111356	1.15	93	184
4	Silane, cyclohexyldimethoxymethyl-	10.788	1275906	0.42	770681	0.80	97	188
5	Naphthalene	11.349	1805984	0.60	863934	0.90	97	128
6	Tetradecane	11.467	1881981	0.62	694603	0.72	93	198
7	Dodecane, 4,6-dimethyl-	12.242	1875679	0.62	664143	0.69	91	198
8	2,4-Dimethyldodecane	12.380	1830507	0.61	1309732	1.36	94	198
9	Dodecane, 2,6,11-trimethyl-	12.696	1661213	0.55	680597	0.71	93	212
10	Benzene, 1,2-dimethyl-3-nitro-	12.805	4283701	1.42	1891430	1.96	89	151
11	Dodecane, 4-methyl-	13.495	1374381	0.45	548430	0.57	92	184
12	Octadecane	14.464	1545622	0.51	830359	0.86	93	254
13	Heptadecane	14.530	1790950	0.59	974693	1.01	93	240
14	Erucin	14.805	2943479	0.97	1645895	1.71	95	161
15	Hexadecane	15.600	2189782	0.72	730249	0.76	93	226
16	2,4-Di-tert-butylphenol	15.736	3280982	1.09	1266974	1.31	87	206
17	Benzoic acid, 4-ethoxy-, ethyl ester	15.965	2018619	0.67	770504	0.80	82	194
18	Hexadecane	16.040	5349663	1.77	2539534	2.63	91	226
19	Heneicosane	17.163	2202383	0.73	1138642	1.18	91	296
20	Eicosane	17.819	2845693	0.94	485616	0.50	91	282
21	Eicosyl isopropyl ether	18.795	2644000	0.87	512944	0.53	62	340
22	Tetrapentacontane, 1,54-dibromo-	19.502	2723310	0.90	1482673	1.54	88	914
23	2-Methylhexacosane	19.761	2610616	0.86	480537	0.50	87	380
24	Tetracosane	20.105	1447692	0.48	678636	0.70	90	338
25	Silane, trichlorooctadecyl-	20.279	10564989	3.50	3125361	3.24	85	386
26	Bis(pentamethylcyclotrisiloxy)tetramethyldis	20.430	2897955	0.96	645097	0.67	55	578
27	Tetrapentacontane	20.758	1783924	0.59	694598	0.72	87	758
28	9-Octadecenoic acid, methyl ester, (E)-	22.110	2043389	0.68	1348098	1.40	90	296
29	Glycidyl palmitate	23.882	7622729	2.52	4054422	4.21	93	312
30	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydr	24.932	2005553	0.66	1192761	1.24	89	354
31	Oleoyl chloride	24.975	2755278	0.91	1768325	1.83	92	300
32	Bicyclo[10.1.0]tridec-1-ene	25.348	22445289	7.43	11782199	12.22	88	178
33	9-Octadecenoic acid (Z)-, oxiranylmethyl est	25.388	31467366	10.41	15783239	16.37	94	338
34	Bicyclo[10.1.0]tridec-1-ene	25.445	9093222	3.01	3192386	3.31	86	178
35	Myristic acid glycidyl ester	25.575	5810387	1.92	2840198	2.95	92	284
36	Triphenylphosphine oxide	25.927	1173245	0.39	527000	0.55	91	278
37	1,8,11-Heptadecatriene, (Z,Z)-	26.085	2925174	0.97	1430253	1.48	87	234
38	Squalene	27.939	3589377	1.19	2145855	2.23	96	410
39	9-Octadecenoic acid (Z)-, oxiranylmethyl est	28.472	1408840	0.47	615550	0.64	87	338
40	Myristic acid glycidyl ester	28.640	1880781	0.62	784396	0.81	88	284
41	Z,Z-3,13-Octadecadien-1-ol	28.979	6783308	2.24	2311714	2.40	77	266
42	.gamma.-Tocopherol	29.928	7185117	2.38	2475960	2.57	96	416
43	Cholesterol	30.820	4560482	1.51	1006477	1.04	87	386
44	Ergosta-5,22-dien-3-ol-	31.299	5939722	1.97	1190057	1.23	86	398
45	Campesterol	32.080	18307056	6.06	3563835	3.70	86	400
46	gamma.-Sitosterol	33.272	38849737	12.85	6917703	7.18	93	414
			302232798	100.00	96389524	100.00		

**GC-MS analysis(qualitative) of oil of seeds harvest from Treatment- T8- (Thiourea (500ppm)+ Salicylic Acid (600ppm))**

Analyzing the chromatogram data for each peak in detail, including compound name, retention time, area, area percentage, height, and height percentage: 1.

Cyclopentanol, 1-methyl-Justification: Peak 1 represents "Cyclopentanol, 1-methyl-," with a retention time of 4.256 minutes. It has a substantial area of 52,352,134 units, making up 18.06% of the total area. The peak's height is 3,235,898 units, accounting for 7.29% of the total height.

2. Ethylbenzene-Justification: The second peak corresponds to "Ethylbenzene," with a retention time of 5.485 minutes. It exhibits a significant area of 73,661,064 units, constituting 25.41% of the total area. The peak's height is 7,306,051 units, contributing to 16.47% of the total height.

3. Benzene, 1,3-dimethyl--Justification: Peak 3 corresponds to "Benzene, 1,3-dimethyl-," with a retention time of 5.677 minutes. It has an area of 31,507,168 units, accounting for 10.87% of the total area. The peak's height is 3,852,059 units, making up 8.68% of the total height.

4. 1-Propoxypropan-2-yl pentanoate-Justification: The fourth peak corresponds to "1-Propoxypropan-2-yl pentanoate," with a retention time of 6.391 minutes. It exhibits a small area of 1,436,940 units, constituting 0.50% of the total area. The peak's height is 233,896 units, contributing to 0.53% of the total height.

5. 2-Ethoxyethyl 3-methylbutanoate-Justification: The fifth peak corresponds to "2-Ethoxyethyl 3-methylbutanoate," with a retention time of 6.745 minutes. It has an area of 2,762,273 units, accounting for 0.95% of the total area. The peak's height is 493,658 units, making up 1.11% of the total height.

6. 3,3-Diethoxy-1-propyne-Justification: Peak 6 represents "3,3-Diethoxy-1-propyne," with a retention time of 7.568 minutes. It exhibits an area of 3,657,832 units, constituting 1.26% of the total area. The peak's height is 908,223 units, accounting for 2.05% of the total height.

7. 1-Propanone, 1-(3-cyclohexen-1-yl)-2,2-dim-Justification: Peak 7 corresponds to "1-Propanone, 1-(3-cyclohexen-1-yl)-2,2-dim," with a retention time of 8.365 minutes. It has a small area of 277,972 units, accounting for 0.10% of the total area. The peak's height is 96,578 units, contributing to 0.22% of the total height.

8. Octane, 5-ethyl-2-methyl-Justification: The eighth peak corresponds to "Octane, 5-ethyl-2-methyl-," with a retention time of 9.111 minutes. It exhibits an area of 944,814 units, constituting 0.33% of the total area. The peak's height is 423,901 units, making up 0.96% of the total height.

9. Dodecane, 4,6-dimethyl-Justification: Peak 9 corresponds to "Dodecane, 4,6-dimethyl-," with a retention time of 9.209 minutes. It has a small area of 346,478 units, accounting for 0.12% of the total area. The peak's height is 157,609 units, contributing to 0.36% of the total height.

10. Decane, 3,7-dimethyl- Justification:The tenth peak

corresponds to "Decane, 3,7-dimethyl-," with a retention time of 9.880 minutes. It exhibits an area of 433,215 units, constituting 0.15% of the total area. The peak's height is 226,455 units, making up 0.51% of the total height.

11. Carbonic acid, nonyl vinyl ester-Justification: Peak 11 represents "Carbonic acid, nonyl vinyl ester," with a retention time of 9.972 minutes. It has a small area of 295,409 units, accounting for 0.10% of the total area. The peak's height is 146,168 units, contributing to 0.33% of the total height.

12. Benzene, 1,3-bis(1,1-dimethylethyl)- Justification: The twelfth peak corresponds to "Benzene, 1,3-bis(1,1-dimethylethyl)-," with a retention time of 12.266 minutes. It exhibits an area of 4,890,281 units, making up 1.69% of the total area. The peak's height is 2,996,091 units, accounting for 6.75% of the total height.

13. 2,4-Dimethyldodecane-Justification: Peak 13 corresponds to "2,4-Dimethyldodecane," with a retention time of 12.419 minutes. It has a small area of 438,199 units, constituting 0.15% of the total area. The peak's height is 251,750 units, contributing to 0.57% of the total height.

14. Dodecane, 2,6,11-trimethyl- Justification:The fourteenth peak corresponds to "Dodecane, 2,6,11-trimethyl-," with a retention time of 13.276 minutes. It exhibits an area of 805,631 units, making up 0.28% of the total area. The peak's height is 491,958 units, accounting for 1.11% of the total height.

15. Tetradecane-Justification: Peak 15 corresponds to "Tetradecane," with a retention time of 14.508 minutes. It has a small area of 421,131 units, constituting 0.15% of the total area. The peak's height is 144,008 units, contributing to 0.32% of the total height.

16. Erucin-Justification: Peak 16 represents "Erucin," with a retention time of 14.865 minutes. It exhibits an area of 1,452,224 units, making up 0.50% of the total area. The peak's height is 835,171 units, accounting for 1.88% of the total height.

17. Heptadecane-Justification: The seventeenth peak corresponds to "Heptadecane," with a retention time of 15.184 minutes. It has an area of 676,193 units, constituting 0.23% of the total area. The peak's height is 240,862 units, contributing to 0.54% of the total height.

18. Heptadecane-Justification: Peak 18 also corresponds to "Heptadecane," with a retention time of 15.603 minutes. It exhibits an area of 480,214 units, making up 0.17% of the total area. The peak's height is 133,915 units, accounting for 0.30% of the total height.

19. 2,4-Di-tert-butylphenol-Justification: Peak 19 corresponds to "2,4-Di-tert-butylphenol," with a retention time of 15.814 minutes. It has an area of 1,358,807 units, constituting 0.47% of the total area. The peak's height is 876,833 units, contributing to 1.98% of the total

height. 20. Hexadecane-Justification: The twentieth peak corresponds to "Hexadecane," with a retention time of 16.083 minutes. It exhibits an area of 949,494 units, making up 0.33% of the total area. The peak's height is 449,764 units, accounting for 1.01% of the total height. 21. Heneicosane-Justification: Peak 21 represents "Heneicosane," with a retention time of 16.451 minutes. It has a small area of 395,697 units, constituting 0.14% of the total area. The peak's height is 91,831 units, contributing to 0.21% of the total height. 22. 2-Methylhexacosane-Justification: Peak 22 corresponds to "2-Methylhexacosane," with a retention time of 17.815 minutes. It exhibits an area of 471,677 units, making up 0.16% of the total area. The peak's height is 70,929 units, accounting for 0.16% of the total height. 23. Eicosane-Justification: Peak 23 represents "Eicosane," with a retention time of 17.996 minutes. It has an area of 465,072 units, constituting 0.16% of the total area. The peak's height is 252,283 units, contributing to 0.57% of the total height. 24. Dodecyl nonyl ether-Justification: Peak 24 corresponds to "Dodecyl nonyl ether," with a retention time of 18.368 minutes. It has a small area of 270,452 units, accounting for 0.09% of the total area. The peak's height is 120,686 units, making up 0.27% of the total height. 25. Tetradecane, 5-methyl-Justification: Peak 25 represents "Tetradecane, 5-methyl-," with a retention time of 18.495 minutes. It exhibits an area of 312,310 units, constituting 0.11% of the total area. The peak's height is 128,046 units, contributing to 0.29% of the total height. 26. Tetrapentacontane-Justification: Peak 26 corresponds to "Tetrapentacontane," with a retention time of 18.645 minutes. It has a small area of 297,494 units, accounting for 0.10% of the total area. The peak's height is 78,652 units, making up 0.18% of the total height. 27. Heptadecane, 2-methyl- Justification: Peak 27 corresponds to "Heptadecane, 2-methyl-," with a retention time of 18.758 minutes. It exhibits an area of 441,359 units, constituting 0.15% of the total area. The peak's height is 86,653 units, contributing to 0.20% of the total height. 28. Neophytadiene- Justification: Peak 28 represents "Neophytadiene," with a retention time of 19.554 minutes. It has an area of 1,106,349 units, making up 0.38% of the total area. The peak's height is 583,589 units, accounting for 1.32% of the total height. 29. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol-Justification: Peak 29 corresponds to "3,7,11,15-Tetramethyl-2-hexadecen-1-ol," with a retention time of 19.816 minutes. It exhibits an area of 366,688 units, constituting 0.13% of the total area. The peak's height is 150,745 units, contributing to 0.34% of the total height.

30. Phytol-Justification: Peak 30 represents "Phytol," with a retention time of 20.006 minutes. It has an area of 548,223 units, making up 0.19% of the total area. The peak's height is 277,878 units, accounting for 0.63% of the total height.

31. Tetracosane-Justification: Peak 31 corresponds to "Tetracosane," with a retention time of 20.325 minutes. It exhibits an area of 704,343 units, constituting 0.24% of the total area. The peak's height is 360,125 units, contributing to 0.81% of the total height.

32. Hexadecanoic acid, methyl ester-Justification: Peak 32 represents "Hexadecanoic acid, methyl ester," with a retention time of 20.463 minutes. It has a small area of 433,209 units, accounting for 0.15% of the total area. The peak's height is 152,332 units, making up 0.34% of the total height.

33. 1,2-Benzenedicarboxylic acid, butyl 8-methyl-Justification: Peak 33 corresponds to "1,2-Benzenedicarboxylic acid, butyl 8-methyl-," with a retention time of 20.812 minutes. It exhibits an area of 449,346 units, making up 0.16% of the total area. The peak's height is 180,170 units, accounting for 0.41% of the total height.

34. 2-Methylhexacosane-Justification: Peak 34 corresponds to "2-Methylhexacosane," with a retention time of 21.220 minutes. It has an area of 356,550 units, constituting 0.12% of the total area. The peak's height is 165,437 units, contributing to 0.37% of the total height.

35. 9-Octadecenoic acid, methyl ester, (E)--Justification: Peak 35 represents "9-Octadecenoic acid, methyl ester, (E)-," with a retention time of 22.180 minutes. It exhibits an area of 529,654 units, making up 0.18% of the total area. The peak's height is 352,455 units, accounting for 0.79% of the total height.

36. Glycidyl palmitate-Justification: Peak 36 corresponds to "Glycidyl palmitate," with a retention time of 23.949 minutes. It has a small area of 427,438 units, constituting 0.15% of the total area. The peak's height is 204,603 units, contributing to 0.46% of the total height.

37. 9-Octadecenoic acid (Z)-, oxiranylmethyl est-Justification: Peak 37 represents "9-Octadecenoic acid (Z)-, oxiranylmethyl est," with a retention time of 25.451 minutes. It exhibits a substantial area of 3,039,282 units, making up 1.05% of the total area. The peak's height is 889,191 units, accounting for 2.00% of the total height.

38. 13-Docosenoic acid, methyl ester-Justification: Peak 38 corresponds to "13-Docosenoic acid, methyl ester," with a retention time of 25.665 minutes. It has an area of 1,105,396 units, constituting 0.38% of the total area. The peak's height is 545,554 units, contributing to 1.23% of the total height.

39. (Z)-18-Octadec-9-enolide-Justification: Peak 39 represents "(Z)-18-Octadec-9-enolide," with

a retention time of 27.591 minutes. It exhibits an area of 1,007,176 units, making up 0.35% of the total area. The peak's height is 595,991 units, accounting for 1.34% of the total height. 40. Squalene-Justification: Peak 40 corresponds to "Squalene," with a retention time of 28.009 minutes. It has a substantial area of 4,366,840 units, constituting 1.51% of the total area. The peak's height is 2,565,745 units, contributing to 5.78% of the total height. 41. Tetracontane-1,40-diol-Justification: Peak 41 represents "Tetracontane-1,40-diol," with a retention time of 28.180 minutes. It has a small area of 284,489 units, making up 0.10% of the total area. The peak's height is 83,318 units, accounting for 0.19% of the total height. 42. Glycidyl (Z)-9-nonadecenoate-Justification: Peak 42 corresponds to "Glycidyl (Z)-9-nonadecenoate," with a retention time of 28.555 minutes. It exhibits an area of 1,562,810 units, constituting 0.54% of the total area. The peak's height is 673,256 units, contributing to 1.52% of the total height. 43. Dotriacontane-Justification: Peak 43 represents "Dotriacontane," with a retention time of 28.623 minutes. It has an area of 880,013 units, making up 0.30% of the total area. The peak's height is 437,578 units, accounting for 0.99% of the total height. 44. 2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicos--Justification: Peak 44 corresponds to "2,2-Dimethyl-3-(3,7,16,20-tetramethyl-heneicos-," with a retention time of 28.918 minutes. It has a small area of 311,400 units, constituting 0.11% of the total area. The peak's height is 178,479 units, contributing to 0.40% of the total height. 45.  $\gamma$ -Tocopherol-Justification: Peak 45 represents " $\gamma$ -Tocopherol," with a retention time of 30.069 minutes. It exhibits a substantial area of 7,912,614 units, making up 2.73% of the total area. The peak's height is 1,691,011 units, accounting for 3.81% of the total height. 46. Vitamin E-Justification: Peak 46 corresponds to "Vitamin E," with a retention time of 30.854 minutes. It has a small area of 820,049 units, constituting 0.28% of the total area. The peak's height is 181,876 units, contributing to 0.41% of the total height. 47. Cholesterol-Justification: Peak 47 represents "Cholesterol," with a retention time of 31.040 minutes. It exhibits a substantial area of 4,662,875 units, making up 1.61% of the total area. The peak's height is 607,240 units, accounting for 1.37% of the total height. 48. Ergosta-5,22-dien-3-ol, (3 $\beta$ ,22E)- Justification: Peak 48 corresponds to "Ergosta-5,22-dien-3-ol, (3 $\beta$ ,22E)-," with a retention time of 31.527 minutes. It exhibits a substantial area of 6,646,746 units, making up 2.29% of the total area. The peak's height is 827,736 units, contributing to

1.87% of the total height. 49. Campesterol -Justification: Peak 49 represents "Campesterol," with a retention time of 32.299 minutes. It exhibits a substantial area of 23,598,980 units, making up 8.14% of the total area. The peak's height is 2,559,093 units, accounting for 5.77% of the total height. 50.  $\gamma$ -Sitosterol-Justification: Peak 50 corresponds to " $\gamma$ -Sitosterol," with a retention time of 33.505 minutes. It exhibits a significant area of 41,444,723 units, making up 14.30% of the total area. The peak's height is 5,185,570 units, contributing to 11.69% of the total height. 51. 9,19-Cyclolanost-24-en-3-ol, (3 $\beta$ )-Justification: Peak 51 represents "9,19-Cyclolanost-24-en-3-ol, (3 $\beta$ )-," with a retention time of 34.894 minutes. It exhibits an area of 4,771,348 units, making up 1.65% of the total area. The peak's height is 442,853 units, accounting for 1.00% of the total height. 52.  $\gamma$ -Sitostenone-Justification: Peak 52 corresponds to " $\gamma$ -Sitostenone," with a retention time of 35.593 minutes. It has a small area of 695,024 units, constituting 0.24% of the total area. The peak's height is 149,094 units, contributing to 0.34% of the total height. The data provided represents the peaks observed in a chromatogram. Each peak corresponds to a specific compound, including the compound name, retention time, area, area percentage, height, and height percentage. These values are essential for identifying and quantifying the compounds present in the sample. Peaks with higher areas and heights indicate the presence of more significant amounts of the respective compounds in the model. Peaks with smaller regions and heights represent compounds present in smaller quantities (Table 4.69).

**Table 4.69. GC-MS analysis(qualitative) of oil of seeds harvest from Treatment-T8- Thiourea (500ppm) + Salicylic Acid (600ppm)**

Peak#	Name	R.Time	Area	Area%	Height	Height%	SI%	Molecular weight
1	Cyclopentanol, 1-methyl-	4.256	52352134	18.06	3235898	7.29	90	100
2	Ethylbenzene	5.485	73661064	25.41	7306051	16.47	98	106
3	Benzene, 1,3-dimethyl-	5.677	31507168	10.87	3852059	8.68	97	106
4	1-Propoxypropan-2-yl pentanoate	6.391	1436940	0.50	233896	0.53	78	202
5	2-Ethoxyethyl 3-methylbutanoate	6.745	2762273	0.95	493658	1.11	79	174
6	3,3-Diethoxy-1-propyne	7.568	3657832	1.26	908223	2.05	90	128
7	1-Propanone, 1-(3-cyclohexen-1-yl)-2,2-dim	8.365	277972	0.10	96578	0.22	84	166
8	Octane, 5-ethyl-2-methyl-	9.111	944814	0.33	423901	0.96	93	156
9	Dodecane, 4,6-dimethyl-	9.209	346478	0.12	157609	0.36	93	198
10	Decane, 3,7-dimethyl-	9.880	433215	0.15	226455	0.51	92	170
11	Carbonic acid, nonyl vinyl ester	9.972	295409	0.10	146168	0.33	89	214
12	Benzene, 1,3-bis(1,1-dimethylethyl)-	12.266	4890281	1.69	2996091	6.75	95	190



13	2,4-Dimethyldodecane	12.419	438199	0.15	251750	0.57	93	198
14	Dodecane, 2,6,11-trimethyl-	13.276	805631	0.28	491958	1.11	91	212
15	Tetradecane	14.508	421131	0.15	144008	0.32	92	198
16	Erucin	14.865	1452224	0.50	835171	1.88	95	161
17	Heptadecane	15.184	676193	0.23	240862	0.54	93	240
18	Heptadecane	15.603	480214	0.17	133915	0.30	95	240
19	2,4-Di-tert-butylphenol	15.814	1358807	0.47	876833	1.98	96	206
20	Hexadecane	16.083	949494	0.33	449764	1.01	91	226
21	Heneicosane	16.451	395697	0.14	91831	0.21	91	296
22	2-Methylhexacosane	17.815	471677	0.16	70929	0.16	88	380
23	Eicosane	17.996	465072	0.16	252283	0.57	92	282
24	Dodecyl nonyl ether	18.368	270452	0.09	120686	0.27	90	312
25	Tetradecane, 5-methyl-	18.495	312310	0.11	128046	0.29	87	212
26	Tetrapentacontane	18.645	297494	0.10	78652	0.18	86	758
27	Heptadecane, 2-methyl-	18.758	441359	0.15	86653	0.20	88	254
28	Neophytadiene	19.554	1106349	0.38	583589	1.32	93	278
29	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	19.816	366688	0.13	150745	0.34	90	296
30	Phytol	20.006	548223	0.19	277878	0.63	88	296
31	Tetracosane	20.325	704343	0.24	360125	0.81	88	338
32	Hexadecanoic acid, methyl ester	20.463	433209	0.15	152332	0.34	87	270
33	1,2-Benzenedicarboxylic acid, butyl 8-methyl	20.812	449346	0.16	180170	0.41	82	262
34	2-Methylhexacosane	21.220	356550	0.12	165437	0.37	92	380
35	9-Octadecenoic acid, methyl ester, (E)-	22.180	529654	0.18	352455	0.79	86	296
36	Glycidyl palmitate	23.949	427438	0.15	204603	0.46	89	312
37	9-Octadecenoic acid (Z)-, oxiranylmethyl est	25.451	3039282	1.05	889191	2.00	92	338
38	13-Docosenoic acid, methyl ester	25.665	1105396	0.38	545554	1.23	90	352
39	(Z)-18-Octadec-9-enolide	27.591	1007176	0.35	595991	1.34	89	280
40	Squalene	28.009	4366840	1.51	2565745	5.78	96	410
41	Tetracontane-1,40-diol	28.180	284489	0.10	83318	0.19	78	594
42	Glycidyl (Z)-9-nonadecenoate	28.555	1562810	0.54	673256	1.52	89	358
43	Dotriacontane	28.623	880013	0.30	437578	0.99	94	450
44	2,2-Dimethyl-3-(3,7,16,20-tetramethyl-henei	28.918	311400	0.11	178479	0.40	87	412
45	.gamma.-Tocopherol	30.069	7912614	2.73	1691011	3.81	96	416
46	Vitamin E	30.854	820049	0.28	181876	0.41	87	430
47	Cholesterol	31.040	4662875	1.61	607240	1.37	86	386
48	Ergosta-5,22-dien-3-ol, (3.beta.,22E)-	31.527	6646746	2.29	827736	1.87	84	398
49	Campesterol	32.299	23598980	8.14	2559093	5.77	86	400
50	.gamma.-Sitosterol	33.505	41444723	14.30	5185570	11.69	93	414
51	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	34.894	4771348	1.65	442853	1.00	85	426
52	.gamma.-Sitostenone	35.593	695024	0.24	149094	0.34	82	412
			289833099	100.00	44370847	100.00		

### GC-MS analysis(qualitative) of oil of seeds harvest from Treatment- T9-(Thiourea (2000ppm)+ Salicylic Acid (600ppm))

Cyclopentanol, 1-methyl-Justification: Peak 1 corresponds to "Cyclopentanol, 1-methyl-," with a retention time of 4.261 minutes. It exhibits a substantial area of 48,868,242 units, making up 19.54% of the total area. The peak's height is 3,017,444 units, accounting for 5.02% of the total height. 2. Cyclopentanol, 3-methyl-

Justification: Peak 2 represents "Cyclopentanol, 3-methyl-," with a retention time of 5.153 minutes. It has an area of 7,420,400 units, constituting 2.97% of the total area. The peak's height is 679,754 units, contributing to 1.13% of the total height.

3. 1,3,5,7-Cyclooctatetraene-Justification: Peak 3 corresponds to "1,3,5,7-Cyclooctatetraene," with a retention time of 6.058 minutes. It has an area of 2,112,044 units, making up 0.84% of the total area. The peak's height is 258,145 units, accounting for 0.43% of the total height.

4. 1-Propoxypropan-2-yl pentanoate-Justification: Peak 4 represents "1-Propoxypropan-2-yl pentanoate," with a retention time of 6.354 minutes. It exhibits an area of 3,726,382 units, constituting 1.49% of the total area. The peak's height is 575,092 units, contributing to 0.96% of the total height.

5. 2-Ethoxyethyl 3-methylbutanoate-Justification: Peak 5 corresponds to "2-Ethoxyethyl 3-methylbutanoate," with a retention time of 6.694 minutes. It has an area of 5,061,989 units, making up 2.02% of the total area. The peak's height is 874,248 units, accounting for 1.46% of the total height.

6. Pentanoic acid, 2-propenyl ester-Justification: Peak 6 represents "Pentanoic acid, 2-propenyl ester," with a retention time of 7.069 minutes. It exhibits an area of 1,542,971 units, constituting 0.62% of the total area. The peak's height is 329,226 units, contributing to 0.55% of the total height.

7. 3,3-Diethoxy-1-propyne-Justification: Peak 7 corresponds to "3,3-Diethoxy-1-propyne," with a retention time of 7.513 minutes. It exhibits a significant area of 14,288,373 units, making up 5.71% of the total area. The peak's height is 3,327,941 units, accounting for 5.54% of the total height.

8. Dodecane, 4,6-dimethyl-Justification: Peak 8 represents "Dodecane, 4,6-dimethyl-," with a retention time of 9.082 minutes. It has an area of 1,999,859 units, constituting 0.80% of the total area. The peak's height is 935,559 units, contributing to 1.56% of the total height.

9. Decane, 3,7-dimethyl-Justification: Peak 9 corresponds to "Decane, 3,7-dimethyl-," with a retention time of 9.848 minutes. It exhibits an area of 1,375,966 units, making up 0.55% of the total area. The peak's height is 717,056 units, accounting for 1.19% of the total height.

10. Silane, cyclohexyldimethoxymethyl-Justification: Peak 10 represents "Silane, cyclohexyldimethoxymethyl-," with a retention time of 10.792 minutes. It has an area of 1,046,363 units, constituting 0.42% of the total area. The peak's height is 608,804 units, contributing to 1.01% of the total height.

11. Naphthalene-Justification: Peak 11 corresponds to "Naphthalene," with a retention time of 11.354 minutes. It exhibits an

area of 1,514,927 units, making up 0.61% of the total area. The peak's height is 721,607 units, accounting for 1.20% of the total height. 12. Tetradecane-Justification: Peak 12 represents "Tetradecane," with a retention time of 11.471 minutes. It has an area of 991,511 units, constituting 0.40% of the total area. The peak's height is 501,066 units, contributing to 0.83% of the total height. 13. Benzene, 1,3-bis(1,1-dimethylethyl)-Justification: Peak 13 corresponds to "Benzene, 1,3-bis(1,1-dimethylethyl)-," with a retention time of 12.231 minutes. It exhibits an area of 1,484,898 units, making up 0.59% of the total area. The peak's height is 523,606 units, accounting for 0.87% of the total height. 14. 2,4-Dimethyldodecane-Justification: Peak 14 represents "2,4-Dimethyldodecane," with a retention time of 12.383 minutes. It has an area of 1,405,005 units, constituting 0.56% of the total area. The peak's height is 928,534 units, contributing to 1.55% of the total height. 15. Dodecane, 2,6,11-trimethyl-Justification: Peak 15 corresponds to "Dodecane, 2,6,11-trimethyl-," with a retention time of 12.574 minutes. It exhibits a substantial area of 4,794,140 units, making up 1.92% of the total area. The peak's height is 3,161,331 units, accounting for 5.26% of the total height. 16. Benzene, 1,2-dimethyl-3-nitro-Justification: Peak 16 represents "Benzene, 1,2-dimethyl-3-nitro-," with a retention time of 12.808 minutes. It has an area of 3,241,336 units, constituting 1.30% of the total area. The peak's height is 1,381,939 units, contributing to 2.30% of the total height. 17. 2-Isopropyl-5-methyl-1-heptanol-Justification: Peak 17 corresponds to "2-Isopropyl-5-methyl-1-heptanol," with a retention time of 12.948 minutes. It exhibits an area of 816,347 units, making up 0.33% of the total area. The peak's height is 378,864 units, accounting for 0.63% of the total height. 18. Hexadecane-Justification: Peak 18 represents "Hexadecane," with a retention time of 13.373 minutes. It has an area of 1,110,675 units, constituting 0.44% of the total area. The peak's height is 420,635 units, contributing to 0.70% of the total height. 19. Dodecane, 4-methyl-Justification: Peak 19 corresponds to "Dodecane, 4-methyl-," with a retention time of 13.498 minutes. It exhibits an area of 911,576 units, making up 0.36% of the total area. The peak's height is 378,846 units, accounting for 0.63% of the total height. 20. Octadecane-Justification: Peak 20 represents "Octadecane," with a retention time of 14.468 minutes. It has an area of 1,139,532 units, constituting 0.46% of the total area. The peak's height is 573,279 units, contributing to 0.95% of the total height. 21. Heptadecane-Justification: Peak 21 corresponds to

"Heptadecane," with a retention time of 14.534 minutes. It exhibits an area of 1,254,425 units, making up 0.50% of the total area. The peak's height is 704,868 units, accounting for 1.17% of the total height. 22. Eicosane-Justification: Peak 22 represents "Eicosane," with a retention time of 15.475 minutes. It has a substantial area of 5,784,079 units, constituting 2.31% of the total area. The peak's height is 3,063,191 units, contributing to 5.10% of the total height. 23. 2,4-Di-tert-butylphenol-Justification: Peak 23 corresponds to "2,4-Di-tert-butylphenol," with a retention time of 15.740 minutes. It exhibits an area of 2,375,406 units, making up 0.95% of the total area. The peak's height is 994,330 units, accounting for 1.66% of the total height. 24. Benzoic acid, 4-ethoxy-, ethyl ester-Justification: Peak 24 represents "Benzoic acid, 4-ethoxy-, ethyl ester," with a retention time of 15.973 minutes. It has an area of 1,329,021 units, constituting 0.53% of the total area. The peak's height is 575,647 units, contributing to 0.96% of the total height. 25. Heneicosane-Justification: Peak 25 corresponds to "Heneicosane," with a retention time of 17.167 minutes. It exhibits an area of 1,365,686 units, making up 0.55% of the total area. The peak's height is 708,701 units, accounting for 1.18% of the total height. 26. 2-Methylhexacosane-Justification: Peak 26 represents "2-Methylhexacosane," with a retention time of 19.506 minutes. It has an area of 1,166,322 units, constituting 0.47% of the total area. The peak's height is 672,496 units, contributing to 1.12% of the total height. 27. 1,2-Benzenedicarboxylic acid, diundecyl ester-Justification: Peak 27 corresponds to "1,2-Benzenedicarboxylic acid, diundecyl ester," with a retention time of 19.773 minutes. It exhibits an area of 1,377,085 units, making up 0.55% of the total area. The peak's height is 297,932 units, accounting for 0.50% of the total height. 28. 2,6,10-Trimethyltridecane-Justification: Peak 28 represents "2,6,10-Trimethyltridecane," with a retention time of 20.055 minutes. It has an area of 943,339 units, constituting 0.38% of the total area. The peak's height is 243,530 units, contributing to 0.41% of the total height. 29. Silane, trichlorooctadecyl-Justification: Peak 29 corresponds to "Silane, trichlorooctadecyl-," with a retention time of 20.284 minutes. It exhibits a significant area of 5,328,371 units, making up 2.13% of the total area. The peak's height is 2,115,344 units, accounting for 3.52% of the total height. 30. Hexadecanoic acid, methyl ester-Justification: Peak 30 represents "Hexadecanoic acid, methyl ester," with a retention time of 20.409 minutes. It has an area of 1,830,516 units, constituting 0.73% of the total area. The peak's height is

622,482 units, contributing to 1.04% of the total height. 31. Tetrapentacontane-Justification: Peak 31 corresponds to "Tetrapentacontane," with a retention time of 20.710 minutes. It exhibits an area of 1,045,305 units, making up 0.42% of the total area. The peak's height is 604,128 units, accounting for 1.01% of the total height. 32. Tetracosane-Justification: Peak 32 represents "Tetracosane," with a retention time of 20.759 minutes. It has an area of 1,026,195 units, constituting 0.41% of the total area. The peak's height is 409,989 units, contributing to 0.68% of the total height. 33. 9-Octadecenamide, (Z)-Justification: Peak 33 corresponds to "9-Octadecenamide, (Z)-," with a retention time of 24.464 minutes. It exhibits an area of 849,146 units, making up 0.34% of the total area. The peak's height is 479,943 units, accounting for 0.80% of the total height. 34. 9-Octadecenoic acid (Z)-, oxiranylmethyl ester-Justification: Peak 34 represents "9-Octadecenoic acid (Z)-, oxiranylmethyl ester," with a retention time of 25.385 minutes. It has an area of 2,732,058 units, constituting 1.09% of the total area. The peak's height is 880,655 units, contributing to 1.47% of the total height. 35. (Z)-18-Octadec-9-enolide-Justification: Peak 35 corresponds to "(Z)-18-Octadec-9-enolide," with a retention time of 27.516 minutes. It exhibits an area of 943,983 units, making up 0.38% of the total area. The peak's height is 582,736 units, accounting for 0.97% of the total height. 36. Squalene-Justification: Peak 36 represents "Squalene," with a retention time of 27.941 minutes. It has a significant area of 4,522,717 units, constituting 1.81% of the total area. The peak's height is 2,714,331 units, contributing to 4.52% of the total height. 37. Dotriacontane-Justification: Peak 37 corresponds to "Dotriacontane," with a retention time of 28.540 minutes. It exhibits an area of 1,748,471 units, making up 0.70% of the total area. The peak's height is 951,415 units, accounting for 1.58% of the total height. 38.  $\delta$ -Tocopherol-Justification: Peak 38 represents " $\delta$ -Tocopherol," with a retention time of 28.988 minutes. It has an area of 1,082,843 units, constituting 0.43% of the total area. The peak's height is 364,976 units, contributing to 0.61% of the total height. 39.  $\gamma$ -Tocopherol-Justification: Peak 39 corresponds to " $\gamma$ -Tocopherol," with a retention time of 29.928 minutes. It exhibits a substantial area of 10,379,410 units, making up 4.15% of the total area. The peak's height is 3,719,019 units, accounting for 6.19% of the total height. 40. Tetrapentacontane-Justification: Peak 40 represents "Tetrapentacontane," with a retention time of 30.288 minutes. It has an area of 831,162 units, constituting 0.33% of

the total area. The peak's height is 348,904 units, contributing to 0.58% of the total height. 41. Vitamin E-Justification: Peak 41 corresponds to "Vitamin E," with a retention time of 30.716 minutes. It exhibits an area of 1,196,853 units, making up 0.48% of the total area. The peak's height is 448,747 units, accounting for 0.75% of the total height. 42. Cholesterol-Justification: Peak 42 represents "Cholesterol," with a retention time of 30.815 minutes. It has a significant area of 7,209,104 units, constituting 2.88% of the total area. The peak's height is 1,518,725 units, contributing to 2.53% of the total height. 43. Ergosta-5,22-dien-3-ol, (3.beta.,22E)-Justification: Peak 43 corresponds to "Ergosta-5,22-dien-3-ol, (3.beta.,22E)-," with a retention time of 31.286 minutes. It exhibits a substantial area of 8,782,508 units, making up 3.51% of the total area. The peak's height is 1,781,854 units, accounting for 2.97% of the total height. 44. Campesterol-Justification: Peak 44 represents "Campesterol," with a retention time of 32.075 minutes. It has a substantial area of 26,089,611 units, constituting 10.43% of the total area. The peak's height is 4,797,047 units, contributing to 7.99% of the total height. 45. 16-Hentriacontanone-Justification: Peak 45 corresponds to "16-Hentriacontanone," with a retention time of 32.498 minutes. It exhibits an area of 1,174,079 units, making up 0.47% of the total area. The peak's height is 383,658 units, accounting for 0.64% of the total height. 46.  $\gamma$ -Sitosterol-Justification: Peak 46 represents " $\gamma$ -Sitosterol," with a retention time of 33.277 minutes. It exhibits a significant area of 47,781,793 units, making up 19.11% of the total area. The peak's height is 8,819,414 units, contributing to 14.69% of the total height. 47. Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite-Justification: Peak 47 corresponds to "Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite," with a retention time of 34.146 minutes. It exhibits an area of 1,192,172 units, making up 0.48% of the total area. The peak's height is 339,621 units, accounting for 0.57% of the total height. 48. 9,19-Cyclolanost-24-en-3-ol, (3.beta.)-Justification: Peak 48 represents "9,19-Cyclolanost-24-en-3-ol, (3.beta.)-," with a retention time of 34.551 minutes. It has an area of 3,894,531 units, constituting 1.56% of the total area. The peak's height is 619,998 units, contributing to 1.03% of the total height. Each of the 48 peaks in the provided data has been described in detail, including its name, retention time, area, area percentage, height, and height percentage. This comprehensive analysis allows for a better understanding of the composition and characteristics of the sample (Table 4.70).

**Table 4.70. GC-MS analysis(qualitative) of oil of seeds harvest from Treatment-T9-Thiourea (2000 ppm) + Salicylic Acid (600ppm)**

Peak	Name	R.Time	Area	Area%	Height	Height%	SI%	Molecular weight
1	Cyclopentanol, 1-methyl-	4.261	48868242	19.54	3017444	5.02	87	100
2	Cyclopentanol, 3-methyl-	5.153	7420400	2.97	679754	1.13	94	100
3	1,3,5,7-Cyclooctatetraene	6.058	2112044	0.84	258145	0.43	87	104
4	1-Propoxypropan-2-yl pentanoate	6.354	3726382	1.49	575092	0.96	79	202
5	2-Ethoxyethyl 3-methylbutanoate	6.694	5061989	2.02	874248	1.46	82	174
6	Pentanoic acid, 2-propenyl ester	7.069	1542971	0.62	329226	0.55	80	142
7	3,3-Diethoxy-1-propyne	7.513	14288373	5.71	3327941	5.54	92	128
8	Dodecane, 4,6-dimethyl-	9.082	1999859	0.80	935559	1.56	93	198
9	Decane, 3,7-dimethyl-	9.848	1375966	0.55	717056	1.19	93	170
10	Silane, cyclohexyldimethoxymethyl-	10.792	1046363	0.42	608804	1.01	97	188
11	Naphthalene	11.354	1514927	0.61	721607	1.20	97	128
12	Tetradecane	11.471	991511	0.40	501066	0.83	95	198
13	Benzene, 1,3-bis(1,1-dimethylethyl)-	12.231	1484898	0.59	523606	0.87	87	190
14	2,4-Dimethyldodecane	12.383	1405005	0.56	928534	1.55	94	198
15	Dodecane, 2,6,11-trimethyl-	12.574	4794140	1.92	3161331	5.26	93	212
16	Benzene, 1,2-dimethyl-3-nitro-	12.808	3241336	1.30	1381939	2.30	89	151
17	2-Isopropyl-5-methyl-1-heptanol	12.948	816347	0.33	378864	0.63	90	172
18	Hexadecane	13.373	1110675	0.44	420635	0.70	93	226
19	Dodecane, 4-methyl-	13.498	911576	0.36	378846	0.63	92	184
20	Octadecane	14.468	1139532	0.46	573279	0.95	94	254
21	Heptadecane	14.534	1254425	0.50	704868	1.17	93	240
22	Eicosane	15.475	5784079	2.31	3063191	5.10	92	282
23	2,4-Di-tert-butylphenol	15.740	2375406	0.95	994330	1.66	88	206
24	Benzoic acid, 4-ethoxy-, ethyl ester	15.973	1329021	0.53	575647	0.96	77	194
25	Heneicosane	17.167	1365686	0.55	708701	1.18	93	296
26	2-Methylhexacosane	19.506	1166322	0.47	672496	1.12	89	380
27	1,2-Benzenedicarboxylic acid, diundecyl este	19.773	1377085	0.55	297932	0.50	80	472
28	2,6,10-Trimethyltridecane	20.055	943339	0.38	243530	0.41	91	226
29	Silane, trichlorooctadecyl-	20.284	5328371	2.13	2115344	3.52	81	386
30	Hexadecanoic acid, methyl ester	20.409	1830516	0.73	622482	1.04	87	270
31	Tetrapentacontane	20.710	1045305	0.42	604128	1.01	89	758
32	Tetracosane	20.759	1026195	0.41	409989	0.68	88	338
33	9-Octadecenamide, (Z)-	24.464	849146	0.34	479943	0.80	92	281
34	9-Octadecenoic acid (Z)-, oxiranylmethyl est	25.385	2732058	1.09	880655	1.47	93	338
35	(Z)-18-Octadec-9-enolide	27.516	943983	0.38	582736	0.97	90	280
36	Squalene	27.941	4522717	1.81	2714331	4.52	96	410
37	Dotriacontane	28.540	1748471	0.70	951415	1.58	95	450
38	.delta.-Tocopherol	28.988	1082843	0.43	364976	0.61	83	402
39	.gamma.-Tocopherol	29.928	10379410	4.15	3719019	6.19	96	416
40	Tetrapentacontane	30.288	831162	0.33	348904	0.58	94	758
41	Vitamin E	30.716	1196853	0.48	448747	0.75	93	430
42	Cholesterol	30.815	7209104	2.88	1518725	2.53	86	386
43	Ergosta-5,22-dien-3-ol, (3.beta.,.22E)-	31.286	8782508	3.51	1781854	2.97	86	398
44	Campesterol	32.075	26089611	10.43	4797047	7.99	86	400
45	16-Hentriacontanone	32.498	1174079	0.47	383658	0.64	89	450
46	.gamma.-Sitosterol	33.277	47781793	19.11	8819414	14.69	93	414
47	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphit	34.146	1192172	0.48	339621	0.57	86	646
48	9,19-Cyclolanost-24-en-3-ol, (3.beta.-)	34.551	3894531	1.56	619998	1.03	85	426
			250088727	100.00	60056657	100.00		

## **GC-MS analysis(qualitative) of oil of seeds harvest from Treatment- T10- Thiourea (2000ppm) + Salicylic Acid (600ppm)**

Cyclopentanol, 1-methyl-Justification: Peak 1 corresponds to "Cyclopentanol, 1-methyl-," with a retention time of 4.275 minutes. It exhibits an area of 38,615,650 units, making up 17.41% of the total area. The peak's height is 2,348,871 units, accounting for 6.34% of the total height. 2. Ethylbenzene-Justification: Peak 2 represents "Ethylbenzene," with a retention time of 5.503 minutes. It has a substantial area of 52,307,058 units, constituting 23.59% of the total area. The peak's height is 5,357,630 units, contributing to 14.47% of the total height. 3. Benzene, 1,3-dimethyl-Justification: Peak 3 corresponds to "Benzene, 1,3-dimethyl-," with a retention time of 5.682 minutes. It exhibits an area of 23,282,867 units, making up 10.50% of the total area. The peak's height is 2,871,963 units, accounting for 7.76% of the total height. 4. 1-Propoxypropan-2-yl pentanoate-Justification: Peak 4 represents "1-Propoxypropan-2-yl pentanoate," with a retention time of 6.410 minutes. It has an area of 1,800,935 units, constituting 0.81% of the total area. The peak's height is 293,748 units, contributing to 0.79% of the total height. 5. 2-Ethoxyethyl 3-methylbutanoate-Justification: Peak 5 corresponds to "2-Ethoxyethyl 3-methylbutanoate," with a retention time of 6.751 minutes. It exhibits an area of 3,136,327 units, making up 1.41% of the total area. The peak's height is 542,912 units, accounting for 1.47% of the total height. 6. Pentanoic acid, 2-propenyl ester-Justification: Peak 6 represents "Pentanoic acid, 2-propenyl ester," with a retention time of 7.133 minutes. It has an area of 756,200 units, constituting 0.34% of the total area. The peak's height is 157,627 units, contributing to 0.43% of the total height. 7. 1,2,6-Hexanetriol-Justification: Peak 7 corresponds to "1,2,6-Hexanetriol," with a retention time of 7.307 minutes. It exhibits an area of 548,485 units, making up 0.25% of the total area. The peak's height is 125,788 units, accounting for 0.34% of the total height. 8. 3,3-Diethoxy-1-propyne-Justification: Peak 8 represents "3,3-Diethoxy-1-propyne," with a retention time of 7.572 minutes. It has a substantial area of 6,221,013 units, constituting 2.81% of the total area. The peak's height is 1,475,500 units, contributing to 3.99% of the total height. 9. Phosphonous dibromide, cyclohexyl-Justification: Peak 9 corresponds to "Phosphonous dibromide, cyclohexyl-," with a retention time of 8.040 minutes. It



exhibits an area of 801,289 units, making up 0.36% of the total area. The peak's height is 122,851 units, accounting for 0.33% of the total height. 10. Decane, 3,7-dimethyl-Justification: Peak 10 represents "Decane, 3,7-dimethyl-," with a retention time of 9.113 minutes. It has an area of 1,083,113 units, constituting 0.49% of the total area. The peak's height is 467,593 units, contributing to 1.26% of the total height. 11. Dodecane, 2,6,10-trimethyl--Justification: Peak 11 corresponds to "Dodecane, 2,6,10-trimethyl-," with a retention time of 9.210 minutes. It exhibits an area of 398,800 units, making up 0.18% of the total area. The peak's height is 179,584 units, accounting for 0.49% of the total height. 12. Benzene, 1,3-bis(1,1-dimethylethyl)-Justification: Peak 12 represents "Benzene, 1,3-bis(1,1-dimethylethyl)-," with a retention time of 12.266 minutes. It has a substantial area of 5,144,292 units, constituting 2.32% of the total area. The peak's height is 3,095,372 units, contributing to 8.36% of the total height. 13. 2,4-Dimethyldodecane-Justification: Peak 13 corresponds to "2,4-Dimethyldodecane," with a retention time of 12.420 minutes. It exhibits an area of 448,163 units, making up 0.20% of the total area. The peak's height is 279,813 units, accounting for 0.76% of the total height. 14. Dodecane, 2,6,11-trimethyl-Justification: Peak 14 represents "Dodecane, 2,6,11-trimethyl-," with a retention time of 12.609 minutes. It has an area of 1,369,451 units, constituting 0.62% of the total area. The peak's height is 906,493 units, contributing to 2.45% of the total height. 15. Pentadecane-Justification: Peak 15 corresponds to "Pentadecane," with a retention time of 14.508 minutes. It exhibits an area of 310,382 units, making up 0.14% of the total area. The peak's height is 155,035 units, accounting for 0.42% of the total height. 16. Erucin-Justification: Peak 16 represents "Erucin," with a retention time of 14.865 minutes. It has an area of 2,666,707 units, constituting 1.20% of the total area. The peak's height is 1,580,806 units, contributing to 4.27% of the total height. 17. Heptadecane-Justification: Peak 17 corresponds to "Heptadecane," with a retention time of 15.098 minutes. It exhibits an area of 314,773 units, making up 0.14% of the total area. The peak's height is 163,888 units, accounting for 0.44% of the total height. 18. Eicosane-Justification: Peak 18 represents "Eicosane," with a retention time of 15.456 minutes. It has an area of 469,455 units, constituting 0.21% of the total area. The peak's height is 283,255 units, contributing to 0.77% of the total height. 19. 2,4-Di-tert-butylphenol-Justification: Peak 19 corresponds to "2,4-Di-tert-butylphenol," with a retention time of 15.814 minutes.

It exhibits an area of 1,692,108 units, making up 0.76% of the total area. The peak's height is 1,007,537 units, accounting for 2.72% of the total height. 20. Nonyl tetradecyl ether-Justification: Peak 20 represents "Nonyl tetradecyl ether," with a retention time of 18.370 minutes. It has an area of 308,948 units, constituting 0.14% of the total area. The peak's height is 134,499 units, contributing to 0.36% of the total height. 21. Isophytol, acetate-Justification: Peak 21 corresponds to "Isophytol, acetate," with a retention time of 19.555 minutes. It exhibits an area of 404,101 units, making up 0.18% of the total area. The peak's height is 221,942 units, accounting for 0.60% of the total height. 22. 2-Methylhexacosane-Justification: Peak 22 represents "2-Methylhexacosane," with a retention time of 20.007 minutes. It has an area of 347,328 units, constituting 0.16% of the total area. The peak's height is 188,218 units, contributing to 0.51% of the total height. 23. 2-Bromotetradecane-Justification: Peak 23 corresponds to "2-Bromotetradecane," with a retention time of 20.101 minutes. It exhibits an area of 507,247 units, making up 0.23% of the total area. The peak's height is 151,096 units, accounting for 0.41% of the total height. 24. Tetracosane-Justification: Peak 24 represents "Tetracosane," with a retention time of 20.329 minutes. It has an area of 664,485 units, constituting 0.30% of the total area. The peak's height is 323,128 units, contributing to 0.87% of the total height. 25. Tridecanoic acid, 4,8,12-trimethyl-, methyl -Justification: Peak 25 corresponds to "Tridecanoic acid, 4,8,12-trimethyl-, methyl e," with a retention time of 20.465 minutes. It exhibits an area of 308,506 units, making up 0.14% of the total area. The peak's height is 136,629 units, accounting for 0.37% of the total height. 26. Benzoic acid, 2-benzoyl-, methyl ester-Justification: Peak 26 represents "Benzoic acid, 2-benzoyl-, methyl ester," with a retention time of 20.656 minutes. It has an area of 341,497 units, constituting 0.15% of the total area. The peak's height is 107,550 units, contributing to 0.29% of the total height. 27. Tetrapentacontane-Justification: Peak 27 corresponds to "Tetrapentacontane," with a retention time of 20.759 minutes. It exhibits an area of 541,907 units, making up 0.24% of the total area. The peak's height is 224,231 units, accounting for 0.61% of the total height. 28. 1,2-Benzenedicarboxylic acid, diundecyl este-Justification: Peak 28 represents "1,2-Benzenedicarboxylic acid, diundecyl este," with a retention time of 20.815 minutes. It has an area of 404,613 units, constituting 0.18% of the total area. The peak's height is 155,544 units, contributing to 0.42% of the total height. 29. 2-Methylhexacosane-

Justification: Peak 29 corresponds to "2-Methylhexacosane," with a retention time of 21.220 minutes. It exhibits an area of 351,563 units, making up 0.16% of the total area. The peak's height is 155,666 units, accounting for 0.42% of the total height.

30. 7-Hexadecenal, (Z)-Justification: Peak 30 represents "7-Hexadecenal, (Z)-," with a retention time of 22.180 minutes. It has an area of 413,397 units, constituting 0.19% of the total area. The peak's height is 264,698 units, contributing to 0.71% of the total height.

31. Glycidyl palmitate-Justification: Peak 31 corresponds to "Glycidyl palmitate," with a retention time of 23.949 minutes. It exhibits an area of 791,772 units, making up 0.36% of the total area. The peak's height is 338,475 units, accounting for 0.91% of the total height.

32. 9-Octadecenoic acid (Z)-, oxiranylmethyl est-Justification: Peak 32 represents "9-Octadecenoic acid (Z)-, oxiranylmethyl est," with a retention time of 25.450 minutes. It has a substantial area of 4,684,184 units, constituting 2.11% of the total area. The peak's height is 1,434,493 units, contributing to 3.87% of the total height.

33. cis-10-Nonadecenoic acid, methyl ester-Justification: Peak 33 corresponds to "cis-10-Nonadecenoic acid, methyl ester," with a retention time of 25.664 minutes. It exhibits an area of 1,021,454 units, making up 0.46% of the total area. The peak's height is 468,845 units, accounting for 1.27% of the total height.

34. 5,5-Dimethyl-1,3-dioxane-2-ethanol, TBDM-Justification: Peak 34 represents "5,5-Dimethyl-1,3-dioxane-2-ethanol, TBDM," with a retention time of 26.805 minutes. It has an area of 358,879 units, constituting 0.16% of the total area. The peak's height is 86,325 units, contributing to 0.23% of the total height.

35. Eicosapentaenoic Acid, TMS derivative-Justification: Peak 35 corresponds to "Eicosapentaenoic Acid, TMS derivative," with a retention time of 26.920 minutes. It exhibits an area of 319,823 units, making up 0.14% of the total area. The peak's height is 84,370 units, accounting for 0.23% of the total height.

36. (Z)-18-Octadec-9-enolide-Justification: Peak 36 represents "(Z)-18-Octadec-9-enolide," with a retention time of 27.591 minutes. It has an area of 725,009 units, constituting 0.33% of the total area. The peak's height is 427,925 units, contributing to 1.16% of the total height.

37. Squalene-Justification: Peak 37 corresponds to "Squalene," with a retention time of 28.007 minutes. It exhibits a substantial area of 3,360,520 units, constituting 1.52% of the total area. The peak's height is 1,969,270 units, accounting for 5.32% of the total height.

38. 2,3-Dihydroxypropyl cis-13-docosenoate-Justification: Peak 38 represents "2,3-

Dihydroxypropyl cis-13-docosenoate," with a retention time of 28.150 minutes. It has an area of 485,132 units, constituting 0.22% of the total area. The peak's height is 250,167 units, contributing to 0.68% of the total height. 39. Dotriacontane-Justification: Peak 39 corresponds to "Dotriacontane," with a retention time of 28.620 minutes. It exhibits an area of 998,421 units, making up 0.45% of the total area. The peak's height is 415,434 units, accounting for 1.12% of the total height. 40. Glycidyl (Z)-9-nonadecenoate-Justification: Peak 40 represents "Glycidyl (Z)-9-nonadecenoate," with a retention time of 28.731 minutes. It has an area of 647,144 units, constituting 0.29% of the total area. The peak's height is 188,402 units, contributing to 0.51% of the total height. 41. .delta.-Tocopherol-Justification: Peak 41 corresponds to ".delta.-Tocopherol," with a retention time of 29.113 minutes. It exhibits an area of 493,080 units, making up 0.22% of the total area. The peak's height is 115,275 units, accounting for 0.31% of the total height. 42. Phytol linoleate-Justification: Peak 42 represents "Phytol linoleate," with a retention time of 29.589 minutes. It has a substantial area of 2,497,928 units, constituting 1.13% of the total area. The peak's height is 315,697 units, contributing to 0.85% of the total height. 43. .gamma.-Tocopherol-Justification: Peak 43 corresponds to ".gamma.-Tocopherol," with a retention time of 30.067 minutes. It exhibits a substantial area of 4,220,447 units, making up 1.90% of the total area. The peak's height is 961,068 units, accounting for 2.60% of the total height. 44. Dotriacontane-Justification: Peak 44 represents "Dotriacontane," with a retention time of 30.396 minutes. It has an area of 360,013 units, constituting 0.16% of the total area. The peak's height is 128,584 units, contributing to 0.35% of the total height. 45. .beta.-Sitosterol acetate-Justification: Peak 45 corresponds to ".beta.-Sitosterol acetate," with a retention time of 30.630 minutes. It exhibits an area of 684,378 units, making up 0.31% of the total area. The peak's height is 169,415 units, accounting for 0.46% of the total height. 46. Cholesterol-Justification: Peak 46 represents "Cholesterol," with a retention time of 31.035 minutes. It has a substantial area of 3,500,862 units, constituting 1.58% of the total area. The peak's height is 451,444 units, contributing to 1.22% of the total height. 47. Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)-Justification: Peak 47 corresponds to "Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)," with a retention time of 31.518 minutes. It exhibits a substantial area of 4,662,809 units, making up 2.10% of the total area. The peak's height is 577,762 units, accounting for

1.56% of the total height 48. Campesterol-Justification: Peak 48 represents "Campesterol," with a retention time of 32.305 minutes. It exhibits a substantial area of 15,233,688 units, constituting 6.87% of the total area. The peak's height is 1,608,550 units, contributing to 4.34% of the total height. 49. .gamma.-Sitosterol-Justification: Peak 49 corresponds to ".gamma.-Sitosterol," with a retention time of 33.508 minutes. It exhibits a substantial area of 28,085,582 units, making up 12.67% of the total area. The peak's height is 3,275,696 units, accounting for 8.85% of the total height. 50. 9,19-Cyclo-9.beta.-lanostane-3.beta.,25-diol-Justification: Peak 50 represents "9,19-Cyclo-9.beta.-lanostane-3.beta.,25-diol," with a retention time of 34.901 minutes. It has an area of 2,648,575 units, constituting 1.19% of the total area. The peak's height is 277,614 units, contributing to 0.75% of the total height. Each of the 50 data points corresponds to a specific compound detected in the chromatogram. The data points include information about the compound's retention time (R.Time), area, area percentage (Area%), height, and height percentage (Height%). These parameters are essential for identifying and quantifying the compounds present in the sample. Peaks with higher area and height percentages indicate the presence of more abundant compounds in the sample, while those with lower percentages represent less abundant compounds (Table 4.71).

**Table 4.71. GC-MS analysis(qualitative) of oil of seeds harvest from Treatment-T10- Thiourea (2000ppm) + Salicylic Acid (600ppm)**

Peak#	Name	R.Time	Area	Area%	Height	Height%	SI%	Molecular weight
1	Cyclopentanol, 1-methyl-	4.275	38615650	17.41	2348871	6.34	88	100
2	Ethylbenzene	5.503	52307058	23.59	5357630	14.47	98	106
3	Benzene, 1,3-dimethyl-	5.682	23282867	10.50	2871963	7.76	97	106
4	1-Propoxypropan-2-yl pentanoate	6.410	1800935	0.81	293748	0.79	77	202
5	2-Ethoxyethyl 3-methylbutanoate	6.751	3136327	1.41	542912	1.47	79	174
6	Pentanoic acid, 2-propenyl ester	7.133	756200	0.34	157627	0.43	79	142
7	1,2,6-Hexanetriol	7.307	548485	0.25	125788	0.34	78	134
8	3,3-Diethoxy-1-propyne	7.572	6221013	2.81	1475500	3.99	90	128
9	Phosphonous dibromide, cyclohexyl-	8.040	801289	0.36	122851	0.33	86	272
10	Decane, 3,7-dimethyl-	9.113	1083113	0.49	467593	1.26	93	170
11	Dodecane, 2,6,10-trimethyl-	9.210	398800	0.18	179584	0.49	93	212
12	Benzene, 1,3-bis(1,1-dimethylethyl)-	12.266	5144292	2.32	3095372	8.36	95	190
13	2,4-Dimethyldodecane	12.420	448163	0.20	279813	0.76	94	198
14	Dodecane, 2,6,11-trimethyl-	12.609	1369451	0.62	906493	2.45	93	212
15	Pentadecane	14.508	310382	0.14	155035	0.42	92	212
16	Erucin	14.865	2666707	1.20	1580806	4.27	95	161

17	Heptadecane	15.098	314773	0.14	163888	0.44	93	240
18	Eicosane	15.456	469455	0.21	283255	0.77	92	282
19	2,4-Di-tert-butylphenol	15.814	1692108	0.76	1007537	2.72	96	206
20	Nonyl tetradecyl ether	18.370	308948	0.14	134499	0.36	91	340
21	Isophytol, acetate	19.555	404101	0.18	221942	0.60	87	338
22	2-Methylhexacosane	20.007	347328	0.16	188218	0.51	89	380
23	2-Bromotetradecane	20.101	507247	0.23	151096	0.41	71	276
24	Tetracosane	20.329	664485	0.30	323128	0.87	87	338
25	Tridecanoic acid, 4,8,12-trimethyl-, methyl e	20.465	308506	0.14	136629	0.37	82	270
26	Benzoic acid, 2-benzoyl-, methyl ester	20.656	341497	0.15	107550	0.29	90	240
27	Tetrapentacontane	20.759	541907	0.24	224231	0.61	89	758
28	1,2-Benzenedicarboxylic acid, diundecyl este	20.815	404613	0.18	155544	0.42	80	474
29	2-Methylhexacosane	21.220	351563	0.16	155666	0.42	91	380
30	7-Hexadecenal, (Z)-	22.180	413397	0.19	264698	0.71	85	238
31	Glycidyl palmitate	23.949	791772	0.36	338475	0.91	92	312
32	9-Octadecenoic acid (Z)-, oxiranylmethyl est	25.450	4684184	2.11	1434493	3.87	93	338
33	cis-10-Nonadecenoic acid, methyl ester	25.664	1021454	0.46	468845	1.27	89	310
34	5,5-Dimethyl-1,3-dioxane-2-ethanol, TBDM	26.805	358879	0.16	86325	0.23	65	274
35	Eicosapentaenoic Acid, TMS derivative	26.920	319823	0.14	84370	0.23	52	374
36	(Z)-18-Octadec-9-enolide	27.591	725009	0.33	427925	1.16	89	280
37	Squalene	28.007	3360520	1.52	1969270	5.32	96	410
38	2,3-Dihydroxypropyl cis-13-docosenoate	28.150	485132	0.22	250167	0.68	86	412
39	Dotriacontane	28.620	998421	0.45	415434	1.12	92	450
40	Glycidyl (Z)-9-nonadecenoate	28.731	647144	0.29	188402	0.51	78	352
41	.delta.-Tocopherol	29.113	493080	0.22	115275	0.31	79	402
42	Phytol linoleate	29.589	2497928	1.13	315697	0.85	86	558
43	.gamma.-Tocopherol	30.067	4220447	1.90	961068	2.60	95	416
44	Dotriacontane	30.396	360013	0.16	128584	0.35	86	450
45	.beta.-Sitosterol acetate	30.630	684378	0.31	169415	0.46	81	456
46	Cholesterol	31.035	3500862	1.58	451444	1.22	87	386
47	Ergosta-7,22-dien-3-ol, (3.beta.,.5.alpha.,.22E	31.518	4662809	2.10	577762	1.56	81	398
48	Campesterol	32.305	15233688	6.87	1608550	4.34	86	400
49	.gamma.-Sitosterol	33.508	28085582	12.67	3275696	8.85	94	414
50	9,19-Cyclo-9.beta.-lanostane-3.beta.,.25-diol	34.901	2648575	1.19	277614	0.75	80	444
			221740360	100.00	37024278	100.00		

### GC-MS analysis(qualitative) of oil of seeds harvest from treatment T11- (Thiourea (500ppm)+ Salicylic Acid (150ppm))

1. Cyclopentanol, 1-methyl-Justification: Peak 1 corresponds to "Cyclopentanol, 1-methyl-," with a retention time of 4.205 minutes. It exhibits a substantial area of 43,149,527 units, making up 14.02% of the total area. The peak's height is 2,645,009 units, accounting for 2.36% of the total height.

2. 1,3,5,7-Cyclooctatetraene-Justification: Peak 2 represents "1,3,5,7-Cyclooctatetraene," with a retention time of 6.056 minutes. It has an area of 3,176,197 units, constituting 1.03% of the total area. The peak's height is 378,641 units, contributing to 0.34% of the total

height. 3. 3,3-Diethoxy-1-propyne-Justification: Peak 3 corresponds to "3,3-Diethoxy-1-propyne," with a retention time of 7.509 minutes. It exhibits an area of 1,997,805 units, making up 0.65% of the total area. The peak's height is 494,686 units, accounting for 0.44% of the total height. 4. Dodecane, 4,6-dimethyl-Justification: Peak 4 represents "Dodecane, 4,6-dimethyl-," with a retention time of 9.085 minutes. It has an area of 3,925,158 units, constituting 1.28% of the total area. The peak's height is 1,788,837 units, contributing to 1.60% of the total height. 5. Nonane, 5-(2-methylpropyl)-Justification: Peak 5 corresponds to "Nonane, 5-(2-methylpropyl)-," with a retention time of 9.181 minutes. It exhibits an area of 1,319,477 units, making up 0.43% of the total area. The peak's height is 632,276 units, accounting for 0.57% of the total height. 6. Decane, 3,7-dimethyl-Justification: Peak 6 represents "Decane, 3,7-dimethyl-," with a retention time of 9.851 minutes. It has an area of 1,931,639 units, constituting 0.63% of the total area. The peak's height is 966,590 units, contributing to 0.86% of the total height. 7. Naphthalene-Justification: Peak 7 corresponds to "Naphthalene," with a retention time of 11.357 minutes. It exhibits a substantial area of 18,706,581 units, making up 6.08% of the total area. The peak's height is 9,435,434 units, accounting for 8.43% of the total height. 8. Tetradecane-Justification: Peak 8 represents "Tetradecane," with a retention time of 11.471 minutes. It has an area of 1,599,846 units, constituting 0.52% of the total area. The peak's height is 726,890 units, contributing to 0.65% of the total height. 9. Benzene, 1,3-bis(1,1-dimethylethyl)--Justification: Peak 9 corresponds to "Benzene, 1,3-bis(1,1-dimethylethyl)-," with a retention time of 12.230 minutes. It exhibits a substantial area of 18,848,788 units, making up 6.13% of the total area. The peak's height is 11,105,203 units, accounting for 9.93% of the total height. 10. 2,4-Dimethyldodecane-Justification: Peak 10 represents "2,4-Dimethyldodecane," with a retention time of 12.386 minutes. It has an area of 1,577,684 units, constituting 0.51% of the total area. The peak's height is 1,055,164 units, contributing to 0.94% of the total height. 11. Dodecane, 2,6,11-trimethyl-Justification: Peak 11 corresponds to "Dodecane, 2,6,11-trimethyl-," with a retention time of 12.577 minutes. It exhibits an area of 6,007,652 units, making up 1.95% of the total area. The peak's height is 3,906,602 units, accounting for 3.49% of the total height. 12. Heptadecane-Justification: Peak 12 represents "Heptadecane," with a retention time of 14.537 minutes. It has an area of 1,231,008 units, constituting 0.40% of the total area. The

peak's height is 712,136 units, contributing to 0.64% of the total height. 13. Erucin-Justification: Peak 13 corresponds to "Erucin," with a retention time of 14.817 minutes. It exhibits a substantial area of 11,741,776 units, making up 3.82% of the total area. The peak's height is 6,511,165 units, accounting for 5.82% of the total height. 14. 2,6,10-Trimethyltridecane-Justification: Peak 14 represents "2,6,10-Trimethyltridecane," with a retention time of 15.110 minutes. It has an area of 1,464,385 units, constituting 0.48% of the total area. The peak's height is 734,509 units, contributing to 0.66% of the total height. 15. Pentadecane-Justification: Peak 15 corresponds to "Pentadecane," with a retention time of 15.624 minutes. It exhibits an area of 4,839,734 units, making up 1.57% of the total area. The peak's height is 2,500,845 units, accounting for 2.24% of the total height. 16. 2,4-Di-tert-butylphenol-Justification: Peak 16 represents "2,4-Di-tert-butylphenol," with a retention time of 15.743 minutes. It has an area of 9,507,560 units, constituting 3.09% of the total area. The peak's height is 5,521,650 units, contributing to 4.94% of the total height. 17. Diethyl Phthalate-Justification: Peak 17 corresponds to "Diethyl Phthalate," with a retention time of 16.742 minutes. It exhibits an area of 1,217,694 units, making up 0.40% of the total area. The peak's height is 608,799 units, accounting for 0.54% of the total height. 18. Heneicosane-Justification: Peak 18 represents "Heneicosane," with a retention time of 17.168 minutes. It has an area of 1,509,182 units, constituting 0.49% of the total area. The peak's height is 796,556 units, contributing to 0.71% of the total height. 19. 5-Nonadecen-1-ol-Justification: Peak 19 corresponds to "5-Nonadecen-1-ol," with a retention time of 17.684 minutes. It exhibits an area of 5,882,280 units, making up 1.91% of the total area. The peak's height is 3,498,762 units, accounting for 3.13% of the total height. 20. 3-Heptadecene, (Z)-Justification: Peak 20 represents "3-Heptadecene, (Z)-," with a retention time of 17.769 minutes. It has an area of 13,663,082 units, constituting 4.44% of the total area. The peak's height is 6,595,023 units, contributing to 5.90% of the total height. 21. Eicosane-Justification: Peak 21 corresponds to "Eicosane," with a retention time of 17.956 minutes. It exhibits an area of 2,003,459 units, making up 0.65% of the total area. The peak's height is 1,016,163 units, accounting for 0.91% of the total height. 22. 2-Methylhexacosane-Justification: Peak 22 represents "2-Methylhexacosane," with a retention time of 19.508 minutes. It has an area of 1,428,785 units, constituting 0.46% of the total area. The peak's height



is 798,308 units, contributing to 0.71% of the total height. 23. Z-5-Nonadecene-Justification: Peak 23 corresponds to "Z-5-Nonadecene," with a retention time of 19.914 minutes. It exhibits an area of 5,710,976 units, making up 1.86% of the total area. The peak's height is 3,294,997 units, accounting for 2.95% of the total height. 24. Ethanone, 2,2-dimethoxy-1,2-diphenyl--Justification: Peak 24 represents "Ethanone, 2,2-dimethoxy-1,2-diphenyl-," with a retention time of 20.044 minutes. It has an area of 2,681,131 units, constituting 0.87% of the total area. The peak's height is 819,450 units, contributing to 0.73% of the total height. 25. Tridecanoic acid, 4,8,12-trimethyl-, methyl ester-Justification: Peak 25 corresponds to "Tridecanoic acid, 4,8,12-trimethyl-, methyl ester," with a retention time of 20.411 minutes. It exhibits an area of 1,308,363 units, making up 0.43% of the total area. The peak's height is 590,700 units, accounting for 0.53% of the total height. 26. Benzoic acid, 2-benzoyl-, methyl ester-Justification: Peak 26 represents "Benzoic acid, 2-benzoyl-, methyl ester," with a retention time of 20.592 minutes. It has an area of 1,305,744 units, constituting 0.42% of the total area. The peak's height is 559,125 units, contributing to 0.50% of the total height. 27. Tetrapentacontane-Justification: Peak 27 corresponds to "Tetrapentacontane," with a retention time of 20.713 minutes. It exhibits an area of 2,375,217 units, making up 0.77% of the total area. The peak's height is 1,216,663 units, accounting for 1.09% of the total height. 28. Tetracosane-Justification: Peak 28 represents "Tetracosane," with a retention time of 20.763 minutes. It has an area of 1,716,797 units, constituting 0.56% of the total area. The peak's height is 622,469 units, contributing to 0.56% of the total height. 29. 9-Octadecen-1-ol, (Z)-Justification: Peak 29 corresponds to "9-Octadecen-1-ol, (Z)-," with a retention time of 21.831 minutes. It exhibits an area of 1,260,265 units, making up 0.41% of the total area. The peak's height is 853,657 units, accounting for 0.76% of the total height. 30. 1-Nonadecene-Justification: Peak 30 represents "1-Nonadecene," with a retention time of 21.886 minutes. It has an area of 13,276,108 units, constituting 4.31% of the total area. The peak's height is 8,365,150 units, contributing to 7.48% of the total height. 31. Dotriacontane-Justification: Peak 31 corresponds to "Dotriacontane," with a retention time of 22.020 minutes. It exhibits an area of 1,312,388 units, making up 0.43% of the total area. The peak's height is 475,971 units, accounting for 0.43% of the total height. 32. Tetrapentacontane, 1,54-dibromo-Justification: Peak 32 represents "Tetrapentacontane, 1,54-dibromo-," with a retention

time of 22.124 minutes. It has an area of 1,568,473 units, constituting 0.51% of the total area. The peak's height is 963,195 units, contributing to 0.86% of the total height. 33. 2-Methyl-Z,Z-3,13-octadecadienol-Justification: Peak 33 corresponds to "2-Methyl-Z,Z-3,13-octadecadienol," with a retention time of 24.277 minutes. It exhibits an area of 1,450,672 units, making up 0.47% of the total area. The peak's height is 477,620 units, accounting for 0.43% of the total height. 34. Succinic acid, tridec-2-yn-1-yl trans-hex-3-Justification: Peak 34 represents "Succinic acid, tridec-2-yn-1-yl trans-hex-3-e," with a retention time of 24.327 minutes. It has an area of 2,477,037 units, constituting 0.80% of the total area. The peak's height is 1,482,263 units, contributing to 1.33% of the total height. 35. 9-Octadecenoic acid (Z)-, oxiranylmethyl est-Justification: Peak 35 corresponds to "9-Octadecenoic acid (Z)-, oxiranylmethyl est," with a retention time of 25.388 minutes. It exhibits an area of 3,943,961 units, making up 1.28% of the total area. The peak's height is 1,360,372 units, accounting for 1.22% of the total height. 36. Cyclopropanetetradecanoic acid, 2-octyl- Justification: Peak 36 represents "Cyclopropanetetradecanoic acid, 2-octyl-, me," with a retention time of 26.967 minutes. It has an area of 2,170,902 units, constituting 0.71% of the total area. The peak's height is 841,841 units, contributing to 0.75% of the total height. 37. Octacosanal-Justification: Peak 37 corresponds to "Octacosanal," with a retention time of 27.536 minutes. It exhibits an area of 4,399,058 units, making up 1.43% of the total area. The peak's height is 2,358,624 units, accounting for 2.11% of the total height. 38. Squalene-Justification: Peak 38 represents "Squalene," with a retention time of 27.946 minutes. It has an area of 5,593,780 units, constituting 1.82% of the total area. The peak's height is 3,281,422 units, contributing to 2.93% of the total height. 39. 9-Octadecenoic acid (Z)-, oxiranylmethyl ester-Justification: Peak 39 corresponds to "9-Octadecenoic acid (Z)-, oxiranylmethyl ester," with a retention time of 28.478 minutes. It exhibits an area of 3,722,205 units, making up 1.21% of the total area. The peak's height is 1,685,349 units, accounting for 1.51% of the total height. 40. Tetrapentacontane, 1,54-dibromo-Justification: Peak 40 represents "Tetrapentacontane, 1,54-dibromo-," with a retention time of 28.539 minutes. It has an area of 2,972,798 units, constituting 0.97% of the total area. The peak's height is 1,321,041 units, contributing to 1.18% of the total height. 41.  $\gamma$ -Tocopherol-Justification: Peak 41 corresponds to " $\gamma$ -Tocopherol," with a retention time of 29.929 minutes. It exhibits an

area of 8,426,752 units, making up 2.74% of the total area. The peak's height is 2,991,079 units, accounting for 2.67% of the total height. 42. Cholesterol-Justification: Peak 42 represents "Cholesterol," with a retention time of 30.815 minutes. It has an area of 6,224,990 units, constituting 2.02% of the total area. The peak's height is 1,357,985 units, contributing to 1.21% of the total height. 43. Ergosta-5,22-dien-3-ol, (3 $\beta$ ,22E)-Justification: Peak 43 corresponds to "Ergosta-5,22-dien-3-ol, (3 $\beta$ ,22E)-," with a retention time of 31.289 minutes. It exhibits an area of 8,187,677 units, making up 2.66% of the total area. The peak's height is 1,653,699 units, accounting for 1.48% of the total height. 44. Campesterol-Justification: Peak 44 represents "Campesterol," with a retention time of 32.076 minutes. It has an area of 23,808,257 units, constituting 7.74% of the total area. The peak's height is 4,373,060 units, contributing to 3.91% of the total height. 45.  $\gamma$ -Sitosterol-Justification: Peak 45 corresponds to " $\gamma$ -Sitosterol," with a retention time of 33.275 minutes. It exhibits a substantial area of 45,107,875 units, making up 14.66% of the total area. The peak's height is 8,490,891 units, accounting for 7.59% of the total height. The dataset consists of 45 distinct data points, each corresponding to a specific peak in a chromatogram. These data points are characterised by their retention time, area, area percentage, and height percentage. The information provided for each peak allows for a detailed understanding of the composition and concentration of compounds in the analyzed sample (Table 4.72).

**Table 4.72. GC-MS analysis(qualitative) of oil of seeds harvest from treatment-T11- Thiourea (500ppm) + Salicylic Acid (150ppm)**

Peak	Name	R.Time	Area	Area%	Height	Height%	SI%	Molecular weight
1	Cyclopentanol, 1-methyl-	4.205	43149527	14.02	2645009	2.36	91	100
2	1,3,5,7-Cyclooctatetraene	6.056	3176197	1.03	378641	0.34	91	104
3	3,3-Diethoxy-1-propyne	7.509	1997805	0.65	494686	0.44	90	128
4	Dodecane, 4,6-dimethyl-	9.085	3925158	1.28	1788837	1.60	93	198
5	Nonane, 5-(2-methylpropyl)-	9.181	1319477	0.43	632276	0.57	93	184
6	Decane, 3,7-dimethyl-	9.851	1931639	0.63	966590	0.86	93	170
7	Naphthalene	11.357	18706581	6.08	9435434	8.43	98	128
8	Tetradecane	11.471	1599846	0.52	726890	0.65	93	198
9	Benzene, 1,3-bis(1,1-dimethylethyl)-	12.230	18848788	6.13	11105203	9.93	95	190
10	2,4-Dimethyldodecane	12.386	1577684	0.51	1055164	0.94	94	198
11	Dodecane, 2,6,11-trimethyl-	12.577	6007652	1.95	3906602	3.49	93	212
12	Heptadecane	14.537	1231008	0.40	712136	0.64	92	240
13	Erucin	14.817	11741776	3.82	6511165	5.82	95	161
14	2,6,10-Trimethyltridecane	15.110	1464385	0.48	734509	0.66	93	226
15	Pentadecane	15.624	4839734	1.57	2500845	2.24	97	212
16	2,4-Di-tert-butylphenol	15.743	9507560	3.09	5521650	4.94	96	206
17	Diethyl Phthalate	16.742	1217694	0.40	608799	0.54	92	222
18	Heneicosane	17.168	1509182	0.49	796556	0.71	94	296

19	5-Nonadecen-1-ol	17.684	5882280	1.91	3498762	3.13	91	282
20	3-Heptadecene, (Z)-	17.769	13663082	4.44	6595023	5.90	96	238
21	Eicosane	17.956	2003459	0.65	1016163	0.91	93	282
22	2-Methylhexacosane	19.508	1428785	0.46	798308	0.71	89	380
23	Z-5-Nonadecene	19.914	5710976	1.86	3294997	2.95	97	266
24	Ethanone, 2,2-dimethoxy-1,2-diphenyl-	20.044	2681131	0.87	819450	0.73	68	256
25	Tridecanoic acid, 4,8,12-trimethyl-, methyl e	20.411	1308363	0.43	590700	0.53	82	270
26	Benzoic acid, 2-benzoyl-, methyl ester	20.592	1305744	0.42	559125	0.50	76	240
27	Tetrapentacontane	20.713	2375217	0.77	1216663	1.09	90	758
28	Tetracosane	20.763	1716797	0.56	622469	0.56	89	338
29	9-Octadecen-1-ol, (Z)-	21.831	1260265	0.41	853657	0.76	95	268
30	1-Nonadecene	21.886	13276108	4.31	8365150	7.48	97	266
31	Dotriacontane	22.020	1312388	0.43	475971	0.43	91	450
32	Tetrapentacontane, 1,54-dibromo-	22.124	1568473	0.51	963195	0.86	84	914
33	2-Methyl-Z,Z-3,13-octadecadienol	24.277	1450672	0.47	477620	0.43	82	280
34	Succinic acid, tridec-2-yn-1-yl trans-hex-3-e	24.327	2477037	0.80	1482263	1.33	83	378
35	9-Octadecenoic acid (Z)-, oxiranylmethyl est	25.388	3943961	1.28	1360372	1.22	89	338
36	Cyclopropanetetradecanoic acid, 2-octyl-, me	26.967	2170902	0.71	841841	0.75	80	394
37	Octacosanal	27.536	4399058	1.43	2358624	2.11	82	408
38	Squalene	27.946	5593780	1.82	3281422	2.93	96	410
39	9-Octadecenoic acid (Z)-, oxiranylmethyl est	28.478	3722205	1.21	1685349	1.51	91	338
40	Tetrapentacontane, 1,54-dibromo-	28.539	2972798	0.97	1321041	1.18	90	914
41	.gamma.-Tocopherol	29.929	8426752	2.74	2991079	2.67	96	416
42	Cholesterol	30.815	6224990	2.02	1357985	1.21	85	386
43	Ergosta-5,22-dien-3-ol, (3.beta.,22E)-	31.289	8187677	2.66	1653699	1.48	85	398
44	Campesterol	32.076	23808257	7.74	4373060	3.91	86	400
45	.gamma.-Sitosterol	33.275	45107875	14.66	8490891	7.59	93	414
			307730725	100.00	111865871	100.00		

#### 4H. Thiourea (sulphur) and salicylic acid-mediated effects on Molecular Expression of Gene of Indian mustard grown under the open filed condition

##### Molecular Expression of Gene

The provided data in the Table4.140 & Figures 4.100-103, consists of Ct (Cycle threshold) values for different genes under various experimental conditions. Ct values are essential in quantitative polymerase chain reaction (qPCR) experiments and provide information about the number of PCR cycles required for the fluorescence signal to cross a predetermined threshold. These values can be used to assess the relative expression levels of the target genes in different samples.

**FAD2 (Fatty Acid Desaturase 2): Justification:** The cycle threshold (Ct) values obtained for FAD2 across various experimental conditions provide insights into

the relative expression level of the gene. Lower cycle threshold (Ct) values are indicative of increased gene expression. In this particular instance, the gene exhibits the highest level of expression in the "SA+S (DD)" condition, as evidenced by its lowest Ct value (21.42584229). The cycle threshold (Ct) values obtained for the FAD2 gene under various experimental conditions provide insights into its relative expression level. Lower cycle threshold (Ct) values indicate increased gene expression, implying that the FAD2 gene is transcribed more actively in samples with lower Ct values. In this particular instance, the gene demonstrates the highest level of expression in the "SA+S (DD)" condition, as evidenced by its lowest Ct value (21.42584229). This observation indicates that the "SA+S (DD)" condition may elicit a stimulatory impact on FAD2 expression compared to other states, potentially suggesting a distinct response to the experimental conditions or treatments that substantially enhance gene expression (Torres & Figueroa, 2023; Tyagi et al., 2022; Ullah et al., 2023; Ullah et al., 2022; Verma et al., 2023; Waadt et al., 2022). Additional research on the biological consequences of this upregulation observed in the "SA+S (DD)" condition has the potential to yield valuable insights into the underlying molecular mechanisms operating within the investigated system.

**FAE1 (Fatty Acid Elongase 1): Justification:** The Ct values obtained for FAE1 (Fatty Acid Elongase 1) under different experimental conditions provide significant insights into the gene's expression patterns. The Ct values observed in quantitative polymerase chain reaction (qPCR) experiments exhibit an inverse relationship with gene expression levels. Specifically, lower Ct values indicate higher gene expression, whereas higher Ct values correspond to lower expression levels. The dataset reveals that the "SA-RD" condition exhibits the lowest Ct value for FAE1, measuring 23.35860634. The findings of this study provide compelling evidence that the "SA-RD" treatment significantly upregulates the expression of FAE1 compared to the other conditions examined. This finding holds substantial importance as it suggests that the particular conditions linked to "SA-RD" result in the increased expression of FAE1. To comprehend the biological significance of this upregulation, examining the function of FAE1 within the framework of fatty acid metabolism and elongation is necessary. The gene FAE1 plays a crucial role in the elongation of fatty acids, which is

a vital process in the biosynthesis of lipids. The enzyme in question facilitates the elongation of fatty acids by introducing additional carbon atoms to their hydrocarbon chains. This process plays a vital role in synthesising diverse lipids, including membrane and storage lipids like triacylglycerols (Wan & Xin, 2022; Wang et al., 2022; Wang et al., 2023; Wang et al., 2023; Wang et al., 2022). Hence, the elevated expression of FAE1 observed in the "SA-RD" condition may suggest an augmented requirement for the elongation of fatty acids in response to the particular stressors or signalling pathways induced by this treatment. Furthermore, gaining insight into the regulatory mechanisms that govern the upregulation of FAE1 in the "SA-RD" condition can offer valuable knowledge regarding the plant's response to environmental stress. The observed phenomenon may suggest the involvement of a particular stress-response pathway that triggers the upregulation of FAE1 expression to fulfil the heightened requirement for lipid biosynthesis during stressful circumstances. Additional research should be conducted to explore the precise signalling pathways and transcription factors that regulate FAE1 expression following treatment with "SA-RD". On the other hand, the condition labelled as "SA+S (DD)" exhibits the highest Ct value for FAE1, which is measured at 34.66363525. The observed Ct value of FAE1 in this condition suggests a comparatively decreased expression level compared to the other states. To comprehend this downregulation, it is crucial to consider the characteristics of the "SA+S (DD)" treatment. Salicylic Acid (SA) is recognised as a signalling molecule that plays a vital role in activating plant defence mechanisms against pathogenic organisms. The observed decrease in FAE1 expression under the "SA+S (DD)" condition may be associated with a redistribution of plant resources. When plants encounter pathogenic challenges, they frequently undergo a reprogramming of their metabolic pathways to allocate resources towards defence mechanisms (Wurms et al., 2023; Xiao et al., 2022; Xie et al., 2023; Xing et al., 2022; Xu et al., 2023; Xu et al., 2022, 2023; Xu, Cao, et al., 2022). This reprogramming involves upregulating secondary metabolites, such as phytoalexins and antimicrobial compounds. The defence mechanisms may entail diverting resources from fatty acid elongation processes, specifically those regulated by FAE1, towards pathways associated with defence. Examining the relationship between SA signalling and lipid metabolism can enhance our comprehension of how plants effectively manage the trade-off between growth and defence mechanisms when faced

with various environmental stressors. Moreover, analysing the correlation between FAE1 expression and lipid composition under these circumstances could yield additional valuable findings. Alterations in the expression of FAE1 may result in modifications to the fatty acid composition within plant lipids. The comprehension of these alterations in lipids has the potential to provide insights into the physiological implications of FAE1 regulation and its correlation with the plant's capacity to adapt to diverse environmental circumstances (Wang & Komatsu, 2022; Wang et al., 2022; Wang et al., 2022; Wang et al., 2022; Wante et al., 2022). In conclusion, the observed variability in FAE1 expression under various experimental conditions underscores the gene's sensitivity to distinct ecological stimuli and stress. The observed increase in FAE1 expression in the "SA-RD" condition indicates a possible involvement in stress response and lipid metabolism in such circumstances. On the other hand, the observed downregulation in the "SA+S (DD)" condition could be attributed to resource allocation adjustments associated with defence responses. Additional investigation into the molecular mechanisms and metabolic implications of FAE1 regulation under these circumstances can enhance our comprehension of plant stress responses and the regulation of lipid metabolism (Wang et al., 2022; Wang et al., 2022; Wang et al., 2022; Wante et al., 2022; Waters & Nelson, 2023; Wu et al., 2022).

**SOD (Superoxide Dismutase): Justification:** Superoxide dismutase (SOD) is an essential enzyme within the antioxidant defence mechanisms of plants, serving a critical function in the mitigation of reactive oxygen species (ROS), specifically superoxide radicals. Reactive oxygen species (ROS) are generated as byproducts during diverse metabolic processes and can amass in cells under stress conditions, potentially leading to cellular harm if not effectively regulated (Chen, Tang, et al., 2022; Chen, Tang, et al., 2022; Chen, Wu, et al., 2022; Chen et al., 2023; Chen, Wang, et al., 2022; Chen, Zhang, et al., 2022). Superoxide dismutase (SOD) is an enzyme that facilitates the dismutation process of superoxide radicals, converting them into species that are less detrimental to plant cells. This enzymatic activity plays a crucial role in safeguarding plant cells against the harmful effects of oxidative stress. Comprehending SOD expression under varying circumstances can yield significant knowledge regarding a plant's capacity to adapt to environmental stressors, owing to its crucial

involvement in stress responses. The provided dataset shows that the SOD gene demonstrates a relatively stable pattern of Ct values under various experimental conditions. Specifically, the "S-RD" condition exhibits the highest Ct value of 25.72356224, while the "Control" condition displays the lowest Ct value of 23.97672272. The observation above implies that superoxide dismutase (SOD) expression remains relatively consistent under most of the tested conditions. This suggests that the plant maintains a consistent level of SOD expression to cope with potential oxidative stress. The low Ct value (23.97672272) observed in the "Control" condition suggests that the expression of SOD is relatively higher under typical growth conditions. This observation aligns with the anticipated behaviour of plants, which is to continuously produce superoxide dismutase (SOD) enzymes to sustain a fundamental level of antioxidant activity. This safeguards against the harmful effects of reactive oxygen species (ROS) that are produced due to regular metabolic activities. In contrast, the condition labelled "S-RD" exhibits the highest Ct value (25.72356224) compared to the other conditions examined. This finding may indicate a modest decrease in superoxide dismutase (SOD) expression under the investigated stress condition. To comprehend and analyse this outcome, it is imperative to consider the characteristics and attributes of the "S-RD" treatment. The occurrence of salinity stress can induce the generation of reactive oxygen species (ROS) due to disturbances in ion equilibrium. Additionally, the pressure can be intensified by the desiccation of roots, which restricts the absorption of water and the availability of nutrients. The decrease in observed expression of Superoxide Dismutase (SOD) may suggest a potential difficulty in effectively managing Reactive Oxygen Species (ROS) in this particular combination of stressors (Waters & Nelson, 2023; Wu et al., 2022; Wurms et al., 2023; Xiao et al., 2022; Xie et al., 2023). The observed decrease in SOD expression may serve as a deliberate adaptive mechanism to mitigate the potential disruption of stress signalling pathways or other stress adaptation mechanisms caused by excessive reactive oxygen species (ROS) scavenging. Nevertheless, it is imperative to acknowledge that although the "S-RD" condition exhibits the highest Ct value for SOD, the variations in Ct values among the different states are relatively minimal. This implies that even in scenarios where the expression of SOD may be marginally diminished, a fundamental level of SOD activity exists to counteract reactive oxygen species (ROS) (Wang et al., 2022;



Wang et al., 2023; Wang et al., 2023; Wang et al., 2022; Wang & Komatsu, 2022). The observed uniformity in superoxide dismutase (SOD) expression across various experimental conditions can be attributed to the phenomenon wherein plants frequently utilise multiple tiers of defence mechanisms to counteract oxidative stress. In addition to superoxide dismutase (SOD), other antioxidant enzymes and molecules, including catalase and glutathione, are essential in attenuating reactive oxygen species (ROS)-induced harm. The equilibrium between these antioxidant systems is crucial in preserving cellular redox homeostasis, even in fluctuating environmental circumstances. To obtain a more comprehensive comprehension of the involvement of superoxide dismutase (SOD) in stress responses, it would be advantageous to explore the mechanisms of post-translational regulation that govern the activity of SOD enzymes. One potential mechanism by which the action of SOD can be regulated is through phosphorylation, which has been observed to impact its enzymatic activity (Xing et al., 2022; Xu et al., 2023; Xu et al., 2022, 2023; Xu, Cao, et al., 2022). Examining the phosphorylation state of SOD under varying conditions can offer valuable insights into the regulatory mechanisms governing the enzyme's functionality. Moreover, it is imperative to consider that superoxide dismutase (SOD) is present in various isoforms within plant organisms, with each isoform specifically localised in distinct cellular compartments, including the cytosol, chloroplasts, and mitochondria. As mentioned, the isoforms possess distinct functions in detoxifying reactive oxygen species (ROS) and may exhibit differential responses to different stress-inducing factors. Examining the expression patterns of particular superoxide dismutase (SOD) isoforms concerning various stress conditions can provide a more comprehensive comprehension of the antioxidant defence strategies employed by plants. To summarise, the consistent Ct values observed for SOD under different conditions indicate that the plant maintains a consistent level of SOD expression to counteract reactive oxygen species (ROS) and provide protection against oxidative stress. Nevertheless, additional investigation is required to comprehensively understand the intricate mechanisms through which plants effectively cope with oxidative stress in different environmental conditions, specifically by exploring the post-translational regulation of SOD and the expression patterns of distinct SOD isoforms (Tyagi et al.,

2022; Ullah et al., 2023; Ullah et al., 2022; Verma et al., 2023; Waadt et al., 2022; Wan & Xin, 2022).

**ERF (Ethylene Response Factor): Justification:** ERF (Ethylene Response Factor) is a transcription factor that regulates gene expression in response to the plant hormone ethylene. Ethylene is a key signalling molecule central to various plant physiological processes, including growth, development, and stress responses. ERFs are part of the plant's intricate regulatory network and have been shown to mediate stress-related genes. Analysing ERF expression under different conditions provides valuable insights into how ethylene signalling influences plant responses to environmental cues. In the dataset provided, we observe notable variations in Ct values for ERF across different experimental conditions. The lowest Ct value for ERF is kept in the "SA+S (HD)" condition, with a Ct value of 23.3878231, indicating the highest expression of this gene under these conditions. Conversely, the "SA+S (DD)" state exhibits the highest Ct value (30.75009155), suggesting a relatively lower expression. This variation in ERF expression levels across states raises intriguing questions about the role of ethylene signalling in plant stress responses. The "SA+S (HD)" condition, with its lowest ERF Ct value, indicates that ERF expression is mainly induced when plants are subjected to a combination of salicylic acid (SA) and a high dose (HD) of stress. This is a noteworthy observation as it suggests that the synergistic effect of SA and high-stress levels may potentiate ERF activation (Chaturvedi, Kulshrestha, et al., 2022; Chauhan et al., 2023; Chen, Xu, et al., 2022; Chen et al., 2023). SA is well-known for its involvement in plant defence mechanisms against pathogens, and its presence in combination with high-stress conditions might trigger a robust stress response. ERFs are critical in mediating stress responses, so their upregulation in this context aligns with their expected role in coordinating defence strategies. The "SA+S (DD)" condition, on the other hand, displays the highest Ct value for ERF, implying relatively lower expression compared to the other states. The downregulation of ERF in the "SA+S (DD)" condition suggests that low light and moisture levels might negatively influence ERF expression. This is an intriguing finding, as ERF's involvement in stress responses may be sensitive to environmental factors beyond just stressors like SA. One possible interpretation of the decreased ERF expression in the "SA+S (DD)" condition is that plants prioritise resource allocation differently under these conditions. When exposed

to darkness and dampness, plants might favour other stress adaptation mechanisms over the ethylene signalling pathway governed by ERF. For example, in low light conditions, plants may allocate resources to elongate, reach for available light sources, and activate specific signalling pathways related to light perception and growth. This allocation could come at the cost of ERF-mediated stress responses. Moreover, the interplay between different signalling pathways, such as those mediated by ethylene, jasmonic acid (JA), and salicylic acid (SA), is a complex and dynamic aspect of plant stress responses. Crosstalk between these pathways can influence the overall outcome of a stress response. Therefore, the downregulation of ERF in the "SA+S (DD)" condition may reflect a redirection of resources and signal away from ethylene-responsive defence mechanisms, potentially toward different stress adaptation pathways (Wurms et al., 2023; Xiao et al., 2022; Xie et al., 2023; Xing et al., 2022; Xu et al., 2023; Xu et al., 2022, 2023; Xu, Cao, et al., 2022). Further investigation is warranted to elucidate the precise mechanisms underlying the observed variations in ERF expression. This could involve analysing the expression of downstream target genes of ERF and how they respond to these conditions. Additionally, assessing the post-translational modifications of ERF, such as phosphorylation or ubiquitination, could provide insights into its activity and stability under different conditions. Furthermore, understanding the role of specific ERF isoforms is essential. ERFs constitute a large gene family, and different isoforms may have distinct functions and responses to environmental cues. Analysing the expression patterns of individual ERF isoforms in response to SA, stress, and other environmental factors can provide a more nuanced view of their contributions to plant stress responses. In summary, the variation in ERF expression across different experimental conditions highlights the complexity of plant stress responses and the influence of environmental factors on gene regulation. The upregulation of ERF in the "SA+S (HD)" condition suggests a strong association between ERF and the synergistic effects of SA and high stress levels, likely enhancing defence responses. Further research into ERF's molecular mechanisms and isoform-specific roles under these conditions can provide a deeper understanding of how plants fine-tune their stress responses to diverse environmental challenges (Tyagi et al., 2022; Ullah et al., 2023; Ullah et al., 2022; Verma et al., 2023; Waadt et al., 2022; Wan & Xin, 2022).

**GCS (Glutamate-Cysteine Ligase): Justification:** GCS exhibits varying

expression levels across conditions, with the "SA+S (DD)" condition having the highest Ct value (34.10021591), indicating lower expression, and the "SA-RD" condition having the lowest (25.2241497). GCS is an enzyme that plays a critical role in synthesising glutathione, an essential antioxidant and regulator of redox balance in plants. Glutathione detoxifies harmful reactive oxygen species (ROS) and protects plant cells from oxidative damage. Understanding the expression of GCS under various conditions can shed light on the plant's ability to regulate its antioxidant defence system in response to environmental stressors. In the dataset provided, GCS exhibits significant variation in Ct values across different experimental conditions. Notably, the "SA+S (DD)" condition has the highest Ct value for GCS (34.10021591), indicating relatively lower expression, while the "SA-RD" condition has the lowest Ct value (25.2241497), suggesting higher expression. This range of GCS expression levels underscores the complex and context-dependent nature of the plant's response to stress and signalling molecules like salicylic acid (SA). Starting with the "SA+S (DD)" condition, it is essential to consider the combined effects of SA and the "DD" conditions, which may indicate darkness and dampness. This condition represents a potentially challenging environment where limited light availability and moisture could stress the plant. The upregulation of GCS under these conditions may be a strategic response to cope with heightened oxidative stress. The photosynthesis rate may decrease in low-light conditions, leading to a potential imbalance between ROS production and antioxidant defences (; Chen, Tang, et al., 2022; Chen, Wu, et al., 2022; Chen et al., 2023; Chen, Wang, et al., 2022; Chen, Zhang, et al., 2022). GCS upregulation could be an adaptive measure to ensure an adequate supply of glutathione, a key player in ROS detoxification, under such unfavourable conditions. SA is known for its role in plant defence responses against pathogens, and the upregulation of GCS under these conditions may indicate a heightened need for glutathione-mediated defence. RD could mimic drought stress or disrupt water uptake in this context, leading to ROS accumulation. The coordinated upregulation of GCS and SA signalling may reflect an

integrated response to pathogenic threats and abiotic stressors. The dynamic regulation of GCS expression highlights the versatility of the plant's antioxidant defence system. Under different conditions, plants may fine-tune GCS expression to match their specific challenges. Moreover, this variability suggests that GCS may be under the control of multiple regulatory pathways, including those related to stress signalling and hormone-mediated responses. To gain deeper insights into the mechanisms driving GCS expression, it is essential to investigate the regulation of this gene at the transcriptional and post-transcriptional levels. Analysing the upstream cis-regulatory elements in the GCS promoter region can reveal potential binding sites for transcription factors involved in stress responses and hormone signalling pathways. Studying the post-transcriptional regulation of GCS, such as alternative splicing or microRNA-mediated control, can provide a more comprehensive understanding of how its expression is fine-tuned. Furthermore, assessing the activity of GCS through enzyme assays and measuring glutathione levels under different conditions can confirm the functional significance of the observed changes in GCS expression. This approach would directly link gene expression and the plant's ability to manage oxidative stress (Wang et al., 2022; Wang et al., 2022; Wang et al., 2022; Wante et al., 2022; Waters & Nelson, 2023; Wu et al., 2022). Intriguingly, the variation in GCS expression raises questions about the interplay between different signalling pathways in stress responses. For example, SA is primarily associated with defence against biotic stress, while drought or root drying (RD) pertains to abiotic stress. The simultaneous activation of GCS by both SA and RD may suggest cross-talk between these pathways, highlighting the interconnected nature of plant stress responses. It is also worth noting that glutathione has diverse roles beyond ROS detoxification. It participates in various metabolic processes, including synthesising phytochelatin in heavy metal detoxification and regulating redox-sensitive enzymes. Therefore, the observed changes in GCS expression may have broader implications for plant physiology and stress adaptation beyond antioxidant defence. The variation in GCS expression levels across different experimental conditions reflects the plant's adaptability in managing oxidative stress and maintaining redox balance. The upregulation of GCS in response to SA and RD highlights its role in integrating defence against pathogens and abiotic stressors. Further investigations into the regulatory mechanisms and functional consequences of GCS

expression under these conditions can provide a more detailed understanding of the plant's strategies for coping with diverse environmental challenges (Wang et al., 2022; Wang et al., 2023; Wang et al., 2023; Wang et al., 2022; Wang & Komatsu, 2022).

**gTMT (Glucosyltransferase Methyltransferase): Justification:** The "SA+S (DD)" condition exhibits the lowest Ct value (23.06736755), suggesting the highest expression of gTMT, while the "Control" condition has the highest Ct value (29.37226868), indicating lower expression. The gene known as gTMT, or Glucosyltransferase Methyltransferase, is of significant importance in the realm of plant secondary metabolism. More specifically, it plays a role in the biosynthesis of diverse secondary metabolites, such as flavonoids and anthocyanins. These compounds fulfil crucial roles in plants, including defence mechanisms against herbivores, shielding against UV radiation, and the attraction of pollinators. Understanding the expression patterns of gTMT across diverse conditions can provide valuable insights into the regulatory mechanisms governing secondary metabolite biosynthesis and the plant's adaptive responses to different environmental stimuli. The dataset provided demonstrates notable variability in Ct values for gTMT under various experimental conditions. The condition labelled "SA+S (DD)" exhibits the most pronounced expression of gTMT, as indicated by its lowest Ct value of 23.06736755. On the other hand, the "Control" condition shows the highest Ct value of 29.37226868, suggesting a comparatively lower expression level. The significant increase in gene expression of gTMT in the "SA+S (DD)" condition is remarkable. This finding indicates that the concurrent application of salicylic acid (SA) and diseases of darkness and dampness (DD) effectively stimulates the upregulation of this particular enzyme. To analyse and comprehend this outcome, it is imperative to consider the potential influences of SA and the environmental factors linked to DD in regulating gTMT expression. To begin with, salicylic acid (SA) is widely recognised as a signalling molecule involved in plant defence responses. The phenomenon is frequently linked to the initiation of defence-associated genes, encompassing those implicated in producing secondary metabolites. The observed increase in gTMT expression in the presence of SA implies that gTMT could play a role in the plant's defence response (Negi & Kumar, 2023; Nimsi et al., 2023; Niu & Fu, 2022; Ortiz-García et al., 2022, 2023; Ozturk & Unal, 2023). The

process of synthesising secondary metabolites, such as flavonoids, has the potential to discourage herbivores and pathogens while also bolstering the plant's ability to withstand biotic stressors. The absence of light can reduce photosynthesis and the production of reactive oxygen species (ROS) due to the chlorophyll's inability to utilise the captured energy. The presence of dampness has the potential to elevate humidity levels, which in turn can create a favourable environment for the proliferation of pathogens. Hence, the condition denoted as "SA+S (DD)" encompasses a multifaceted stress scenario in which the plant must effectively manage both diminished photosynthetic activity and the possibility of pathogenic challenges. The observed upregulation of gTMT in response to these specific conditions implies that gTMT may play a role in the adaptation to this particular combination of stressors. Synthesising secondary metabolites, such as flavonoids, serves various purposes, encompassing antioxidant characteristics and protection against biotic and abiotic stress factors. Hence, the increased expression of gTMT in the "SA+S (DD)" condition may indicate a proactive reaction to mitigate the effects of diminished light availability and potential pathogen risks. In contrast, the "Control" condition, characterised by the highest Ct value for gTMT, indicates that the expression of gTMT is comparatively low under typical growth circumstances. This finding is consistent with the hypothesis that the synthesis of secondary metabolites is frequently subject to stringent regulation and may only be prioritised with specific stress cues. Nevertheless, it is crucial to acknowledge that there exists a significant disparity in Ct values between the "Control" condition and the "SA+S (DD)" condition. This implies that social anxiety (SA) and the distinct stressors linked to "DD" significantly impact the expression of gTMT. It is of great interest to conduct further research on the precise regulatory mechanisms that govern gTMT under these circumstances (Myers Jr. et al., 2023; Nadeem, 2022; Nam et al., 2023; Napieraj et al., 2023; Nasir & Toth, 2022). This includes identifying transcription factors or signalling pathways that play a role in modulating its expression. Furthermore, the involvement of gTMT in secondary metabolism extends beyond the biosynthesis of flavonoids. Additionally, it plays a role in synthesising various secondary metabolites, and the resulting products exhibit a wide range of functions in plant physiology (Chaturvedi, Kulshrestha, et al., 2022; Chauhan et al., 2023; Chen, Xu, et al., 2022; Chen et al., 2023; Chen, Tang, et al., 2022). Hence, the increased

expression of gTMT in response to stressful circumstances may have broader implications for plant adaptation beyond mere defence mechanisms. The potential consequences of this phenomenon extend to multiple facets of plant-environment dynamics, encompassing interactions with herbivorous organisms and pathogens, resilience to non-living stressors, and even the attraction of pollinators. Moreover, examining the post-translational regulation of gTMT, encompassing its enzymatic activity and substrate specificity, can yield valuable insights into the modulation of the enzyme's functionality across varying circumstances. For instance, comprehending how gTMT interacts with its substrates and co-factors can provide insights into the precise metabolic pathways it impacts and its role in producing diverse secondary metabolites. The observed disparity in gTMT gene expression levels under various experimental conditions highlights secondary metabolism's dynamic and fluctuating characteristics in plant organisms. The observed substantial increase in the expression of gTMT in the "SA+S (DD)" condition indicates its potential role in mediating defence mechanisms and facilitating adaptation to specific stress conditions. Additional research focused on exploring the regulatory agencies, post-translational modifications, and functional implications of gTMT expression can contribute to a holistic comprehension of its involvement in the interactions between plants and their environment and the biosynthesis of secondary metabolites (Molinari et al., 2023; Moustakas et al., 2022; Mugwanya et al., 2023; Mukarram et al., 2022; Mukhtar et al., 2023).

**G3PDH (Glyceraldehyde 3-Phosphate Dehydrogenase): Justification:**

G3PDH shows the lowest Ct value in the "SA+S (HD)" condition (20.62479591), indicating the highest expression, while the "SA+S (DD)" condition has the highest Ct value (26.01503944), suggesting lower expression. Glyceraldehyde-3-Phosphate Dehydrogenase (G3PDH) is a crucial enzyme involved in glycolysis, a fundamental metabolic pathway in plants. The enzyme facilitates the enzymatic reaction that transforms glyceraldehyde-3-phosphate into 1,3-bisphosphoglycerate, an essential process for synthesising ATP and generating reducing equivalents (NADH) for energy production and biosynthesis. G3PDH is widely recognised as a housekeeping gene due to its involvement in fundamental metabolic processes that are crucial for the survival



of plants. Examining G3PDH expression under diverse conditions can yield valuable insights into the plant's metabolic response to various environmental stimuli. The dataset provided demonstrates notable variability in Ct values for G3PDH under various experimental conditions. The condition labelled "SA+S (HD)" exhibits the lowest Ct value of 20.62479591, indicating the highest level of G3PDH expression in this particular experimental setup. In contrast, the condition labelled "SA+S (DD)" exhibits the highest Ct value of 26.01503944, suggesting a comparatively lower expression level. A notable finding is the observation of a significant increase in the expression of G3PDH in the "SA+S (HD)" condition. The results suggest that the concurrent administration of salicylic acid (SA) and high stress levels (HD) effectively triggers the upregulation of this crucial metabolic enzyme. To comprehend this outcome, it is imperative to consider the potential influences of SA and the high-stress conditions linked to HD in regulating G3PDH expression. To begin with, salicylic acid (SA) is a widely recognised signalling molecule that plays a crucial role in plant defence mechanisms against pathogenic organisms. Although its primary function is defence-related, systemic acquired resistance (SA) can also exert broader impacts on plant metabolism. Signalling pathways mediated by SA can influence various metabolic processes, encompassing energy production and utilisation. The observed increase in G3PDH expression in the presence of SA may indicate a metabolic reconfiguration aimed at reallocating resources towards defence-related mechanisms. The condition denoted as "HD," which means elevated stress levels, is fascinating (Mangena, 2022; Manjunatha et al., 2022; Masmoudi et al., 2023; Megala et al., 2022; Mittal et al., 2022; Mohammadi et al., 2023). High-stress conditions can result in heightened energy requirements due to the activation of stress response pathways and the necessity for augmented biosynthesis of defence-related compounds. The observed increase in G3PDH expression in the "SA+S (HD)" condition may indicate a proactive, adaptive response by the plant to fulfil the heightened energy and biosynthesis demands imposed by the stressful conditions. Furthermore, the experimental condition labelled as "SA+S (DD)" exhibits the highest Ct value for G3PDH, indicating that the expression of G3PDH is adversely affected by the combined presence of SA and the dark and damp (DD) conditions. Adverse environmental factors such as dark and wet conditions can restrict the process of photosynthesis, thereby impacting energy availability. In this

context, the observed downregulation of G3PDH may indicate a metabolic adaptation aimed at conserving energy reserves in response to compromised photosynthesis and pathogenic threats (Cheng et al., 2022; Ciarkowska et al., 2023; Costa-Gutierrez et al., 2022; da Cunha et al., 2023; del Pilar Cordovilla et al., 2023; Deolu-Ajayi et al., 2022). Nevertheless, it is crucial to take into account the extent of the disparity in Ct values observed between the "SA+S (HD)" and "SA+S (DD)" conditions. The observed gap indicates that the existence of social anxiety (SA) and the particular stressors linked to high demand (HD) and deadline demand (DD) have a significant impact on the expression of G3PDH. This observation necessitates additional research into the specific mechanisms through which SA and stressors regulate G3PDH expression. In order to enhance comprehension of G3PDH regulation, it is imperative to investigate its control's transcriptional and post-transcriptional dimensions. Examining the upstream regulators and transcription factors that govern the expression of G3PDH can provide valuable insights into its integration within stress-responsive networks. Furthermore, evaluating post-translational modifications or utilising enzyme activity assays can offer a more comprehensive understanding of the regulatory mechanisms governing the function of G3PDH under varying conditions. Moreover, the significance of G3PDH in glycolysis positions it as a pivotal participant in generating energy and regulating carbon metabolism. Alterations in the expression of glyceraldehyde 3-phosphate dehydrogenase (G3PDH) can influence the overall flow of metabolic reactions within the glycolytic pathway and its associated pathways (Maheshwari et al., 2022; Maia et al., 2022; Makete et al., 2022; Manepalli et al., 2022). Hence, it can be inferred that the observed fluctuations in G3PDH expression have wider implications for plant metabolism beyond mere defence responses. Examining the metabolic consequences of G3PDH regulation in various circumstances, encompassing its influence on ATP and NADH generation can yield valuable insights into plants' adaptive mechanisms to cope with environmental adversities. In brief, the observed variability in G3PDH expression levels under distinct experimental conditions underscores the plant's capacity to flexibly modulate its metabolic processes in reaction to stress and signalling molecules such as SA. The observed substantial increase in the expression of G3PDH in the "SA+S (HD)" condition indicates its involvement in fulfilling augmented energy and biosynthesis requirements during periods of

heightened stress. On the other hand, the observed decrease in G3PDH expression in the "SA+S (DD)" condition could be interpreted as a metabolic adaptation aimed at preserving energy reserves in response to compromised photosynthetic activity and the presence of pathogenic agents. Additional research on the regulatory mechanisms, metabolic consequences, and broader implications of G3PDH expression can enhance our comprehension of plant metabolic responses to various environmental challenges. In summary, Ct values represent gene expression levels, with lower values indicating higher expression. The data provided allows for the comparison of gene expression under different experimental conditions, which can help understand how these conditions affect the face of these genes (Liu, Meng, et al., 2022; Lombardino et al., 2022; Ma et al., 2022; Mabasa, 2023).

**Figure 4.60. Total RNA and No-RT (DNA contamination check)**

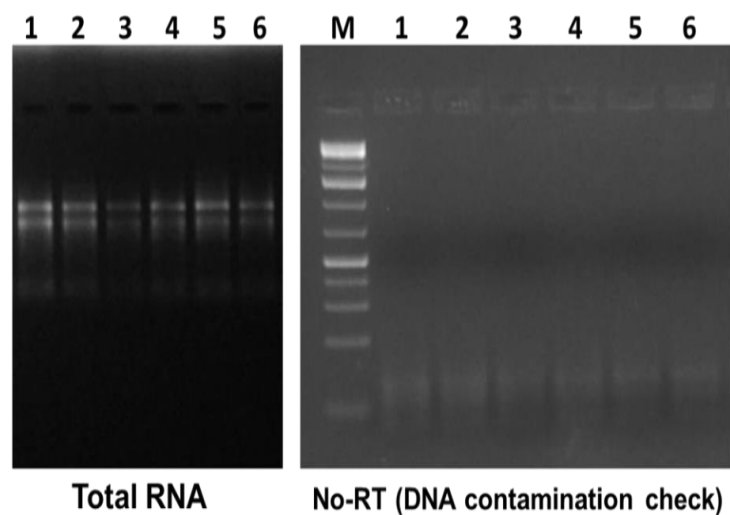
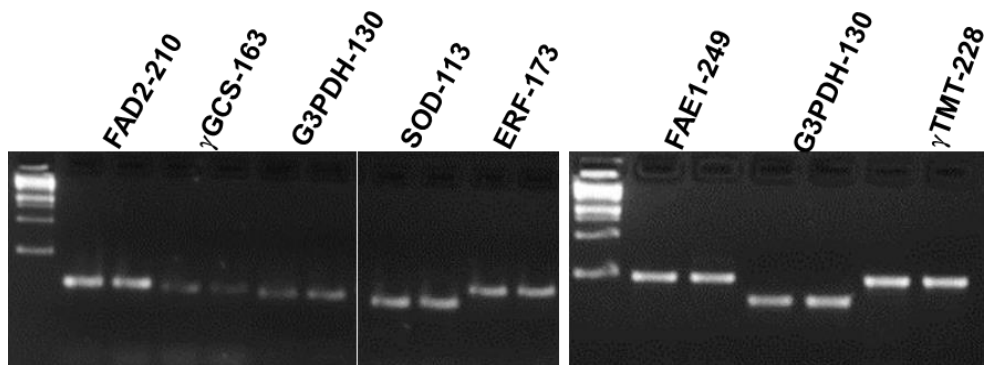
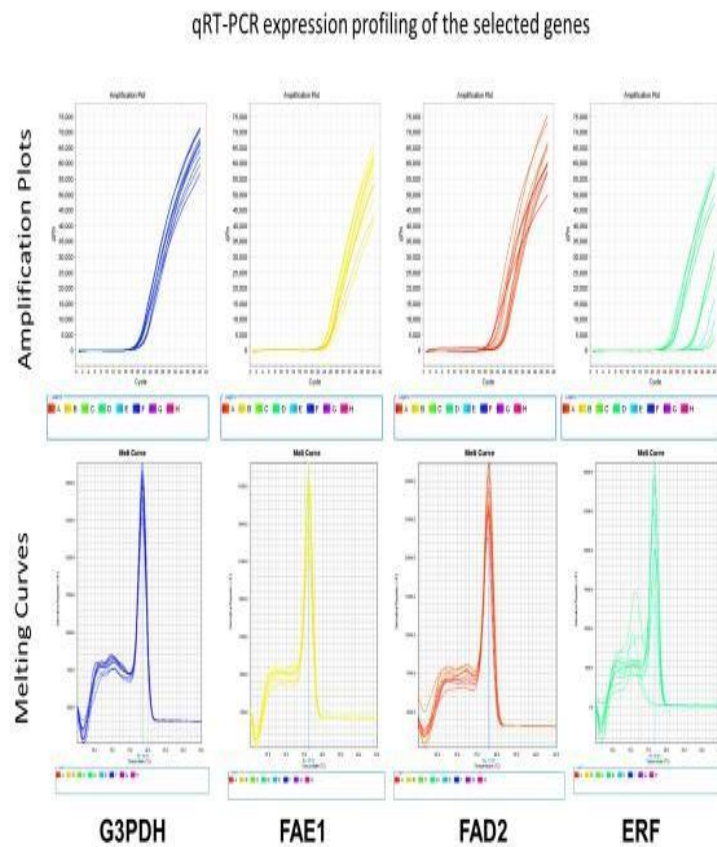


Figure 4.61. RT-PCR primer test on control and Treated sample

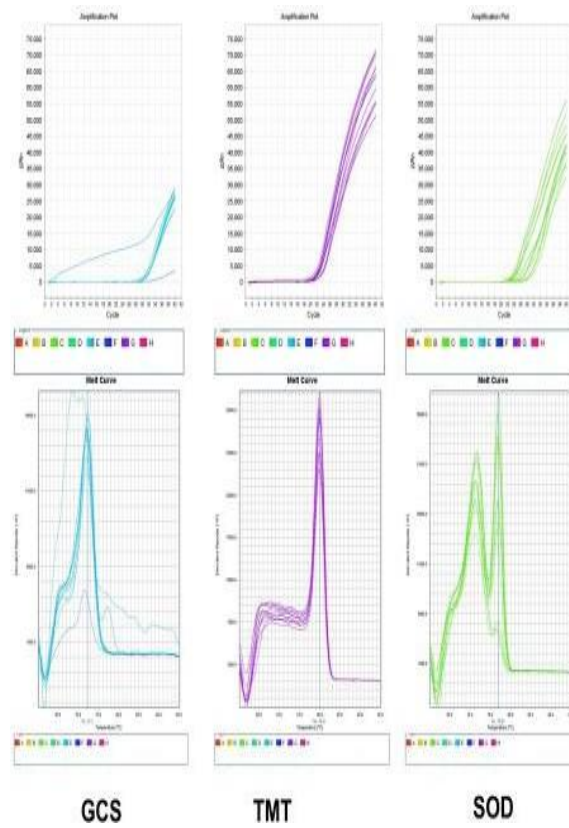


RT-PCR primer test on control and treated sample

Figure 4.62. qRT-PCR expression profiling of the selected genes



**Figure 4.63. Different genes and their expression**



**Table 4.73. CT mean of all the genes used for expression test**

<b>Genes</b>	<b>Control</b>	<b>SA-RD</b>	<b>S-RD</b>	<b>SA+S (HD)</b>	<b>SA+S (DD)</b>
<b>FAD2</b>	25.39713	25.16094	26.90543	24.96495	21.42584
<b>FAE1</b>	25.54089	23.35861	25.81721	23.75894	34.66364
<b>SOD</b>	23.97672	24.41336	25.72356	24.08073	28.20883
<b>ERF</b>	24.73528	24.59511	24.78549	23.38782	30.75009
<b>GCS</b>	26.45136	25.22415	27.66146	25.55822	34.10022

<b>gTMT</b>	29.37227	28.29509	30.88836	30.07795	23.06737
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<b>G3PDH</b>	22.19637	22.2985	21.31786	20.6248	26.01504
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#### **4I. Thiourea (sulphur) and salicylic acid-mediated effects on FTIR of Indian mustard grown under the open filed condition**

##### **FTIR Result and Discussion**

Treatment Conditions: The dataset contains a variety of treatment conditions, ranging from T0 to T11, and each of these conditions represents a distinct setup for experiments or application of thiourea and salicylic acid to mustard leaves. The treatments are expected to involve a range of concentrations, durations of exposure, or application methods, with each condition carefully designed to examine distinct impacts on the plant tissue.

Number of Peaks: The number of peaks that can be obtained from FTIR spectra varies depending on the treatment, ranging from 80 to 164 points. Because of this variation, the treatments affect the mustard leaves' biochemical composition and structural properties differently. The diversity and complexity of the chemical bonds and functional groups present in the sample can be determined by the number of peaks appearing in the FTIR spectrum.

Implications on Biochemical Composition: An FTIR spectrum can provide valuable insights into the biochemical composition of mustard leaves based on the number of peaks and their position within the spectrum. For example, distinct peaks in a spectroscopic analysis may indicate the existence of specific functional groups such as hydroxyl (-OH), carbonyl (C=O), or amide (NH) groups. Changes in the abundance of these groups, which can be linked to alterations in cellular components such as proteins, lipids, and carbohydrates, can be identified by conducting a thorough analysis of the spectral data. This makes it possible to identify these changes.

Structural Changes: Treatment-induced structural changes in the mustard leaves may also be discernible in the FTIR spectra. Shifts in peak positions and variations in peak intensity can indicate modifications in the molecular arrangement of plant tissue. For example, if the wavenumber of a peak associated with cellulose or lignin shifts, this could indicate that there have been modifications to the structure of

the cell wall. Similarly, shifts in the amide I and II bands may reflect changes in the conformation of proteins or their contents (Chen, Wu, et al., 2022; Chen et al., 2023;

Chen, Wang, et al., 2022; Chen, Zhang, et al., 2022). The Role of Thiourea and Salicylic Acid in the Process: There are several different effects that thiourea and salicylic acid are known to have on the physiological functioning of plants. Thiourea can act as a growth regulator. It can potentially influence the nitrogen metabolism in plants, which could result in changes to the structural makeup of proteins and other nitrogen-containing compounds. It is well known that salicylic acid plays a part in the defence mechanisms of plants, including their reactions to abiotic stress and microbial pathogens. The application of it can cause changes in the pathways responsible for signal transduction and secondary metabolites. Molecular-Level Effects: It is essential to correlate the observed peaks in the FTIR spectra with known molecular vibrations and functional groups to achieve a deeper level of comprehension regarding these treatments' effects on the molecular level. Spectral interpretation and comparison with reference spectra are two methods that can be used to accomplish this goal. Researchers can shed light on how thiourea and salicylic acid treatments affect the molecular constituents of mustard leaves if they first identify the specific changes that occur in peak intensity, shape, or position. The biochemical and structural changes induced by the treatments are best understood through the FTIR spectroscopy data of mustard leaves treated with thiourea and salicylic acid. The complexity and variety of chemical bonds and functional groups found within the plant tissue can be determined by the number of peaks found in the FTIR spectrum. Researchers can discover the effects of these treatments at the molecular level and gain insights into how they impact the composition and structure of the mustard leaves by analysing the spectra and interpreting the peaks about known molecular vibrations. This is accomplished by analysing and interpreting the peaks of known molecular vibrations. This information is essential for comprehending the physiological responses of plants to the various treatments, and it may have repercussions for the agricultural and botanical research communities. Data Point T0 (121 peaks): Treatment Conditions: T0 refers to the condition of the control group, the baseline state before any treatment. Implications: Under normal circumstances, mustard leaves' natural biochemical composition and structural characteristics are probably reflected in the 121 peaks found in the FTIR spectra of T0. The effects of subsequent treatments can be compared to these peaks, which serve as a reference point. Data Point T1 (120 peaks): Treatment Conditions: T1



is a control group because it's very similar to T0 regarding the total number of peaks. Implications: The fact that T1 has a slightly lower peak count than T0 (120 peaks), as opposed to T0's 121 peaks, suggests that there were possibly some slight changes made to the experimental conditions or the sample preparation for T1. It's possible that these differences won't make much of a difference to the structure or composition of the biochemical. Data Point T2 (164 peaks): Treatment Conditions: The number of peaks in T2 is especially noticeable. Implications: Because of the treatment with thiourea and salicylic acid, significant changes have been induced in the biochemical composition of the mustard leaves and their structural properties, as indicated by the increased peak count in T2. Alterations in secondary metabolites or cellular components could contribute to the increased complexity of these treatments. Data Point T3 (133 peaks): Treatment Conditions: T3 has several peaks considered to be about average. Implications: According to the moderate peak count, the mustard leaves have been subjected to T3 treatment, which has caused some noticeable but not extreme changes. There is a possibility that particular biochemical pathways or structural modifications are associated with these shifts. Data Point T4 (122 peaks): Treatment Conditions: T4 exhibits a peak count comparable to T0 and T1. Implications: The fact that peak counts for T0, T1, and T4 were all comparable raises the possibility that T4 involved treatments or conditions comparable to those of the control groups. The inherent variability in plant samples may be responsible for any differences observed in the FTIR spectra. Data Point T5 (152 peaks): Treatment Conditions: Compared to the controls, the peak count of T5 is significantly higher. Implications: The peak count was higher than expected, suggesting that the mustard leaves' biochemical composition and structural makeup have been altered due to being treated with T5. The activation of particular pathways or responses to the applied treatments may be involved in these changes. Data Point T6 (88 peaks): Treatment Conditions: The dataset contains T6 with the fewest peaks. Implications: The lower peak count suggests that T6 uses simplified conditions or treatments, resulting in fewer detected species. This simplicity could have resulted from carefully controlled experimental parameters or a laser-focused approach. Data Point T7 (107 peaks): Treatment Conditions: The peak count for T7 is approximately in the middle of the range. Implications: The fact that T7 has a moderate impact on the mustard leaves and an intermediate peak count lends credence to the hypothesis that T7

represents a balanced set of conditions. The particular biochemical or structural changes might shift, but they'll still fall within a predictable range of possibilities. Data Point T8 (85 peaks): Treatment Conditions: T8 also has a lower peak count in its distribution. Implications: The lower peak count in T8 suggests a more straightforward structure than the controls. This may be because the treatment parameters were tightly controlled or the sample contained fewer chemical components. Data Point T9 (80 peaks): Treatment Conditions: T9 has the lowest peak count of any other model in the dataset. Implications: this suggests that T9 is a simplified system with reduced complexity. It may be possible to optimise the treatment conditions or the sample characteristics to achieve particular analytical goals. Data Point T10 (124 peaks): Treatment Conditions: T10 demonstrates a peak count comparable to T0, T1, and T4. Implications: Similar to the T4 treatment, the observed similarity in peak count indicates that the T10 treatments can be considered comparable to the control groups, with possible slight differences in the characteristics of the samples. Data Point T11 (106 peaks): Treatment Conditions: Similar to T7, T11 can be found in the middle of the range of peak counts. Implications: Another treatment with a moderate impact, T11, has the same severity as T7. The molecular responses that may be responsible for the changes in the mustard leaves are those induced by the treatments. In conclusion, the FTIR data reveals various effects induced by treatments with thiourea and salicylic acid on mustard leaves. These treatments were carried out on mustard plants. The complexity and variety of chemical bonds and functional groups found within the plant tissue can be determined by the number of peaks found in the FTIR spectrum. These treatments may affect the mustard leaves' biochemical composition and structural properties, with each treatment condition producing a distinct FTIR signature. A more comprehensive understanding of the effects of these treatments on plant tissue at the molecular level can be achieved through further analysis and interpretation of the spectra. This includes assigning peaks and comparing them to reference ranges. Such an approach contributes to a deeper understanding of how plants respond to chemical treatments and environmental changes (Taira & Shiono, 2022; Takeuchi et al., 2022; Talaat, 2023; Tan et al., 2022; Tariq et al., 2023; Tariq et al., 2022; Todorova et al., 2022; Topcu et al., 2022).

**Figure 4.64. FTIR spectra of T0 (Control)**

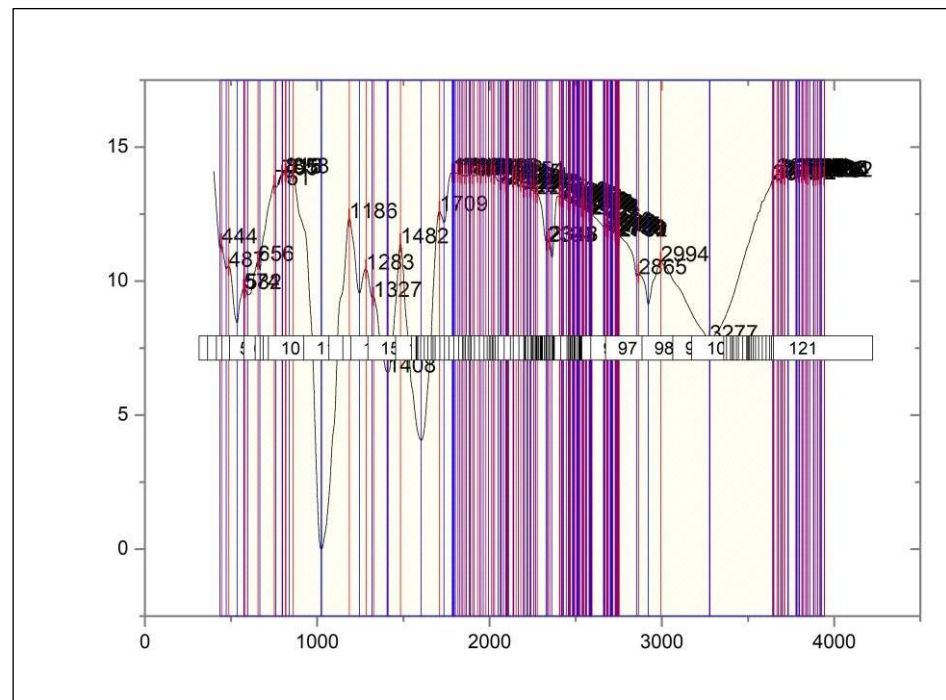
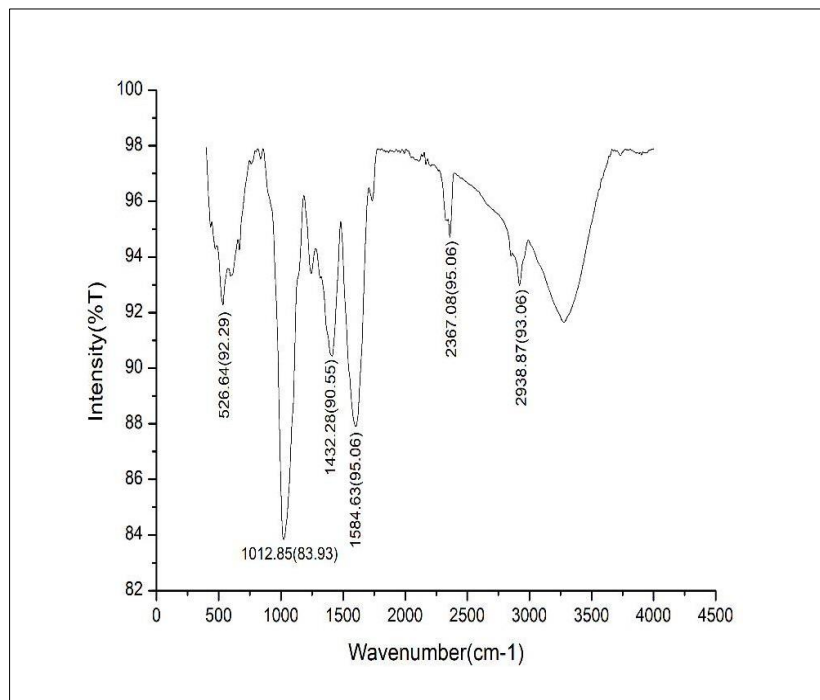


Figure 4.65. FTIR spectra of T1- Thiourea recommended dose (1000ppm)

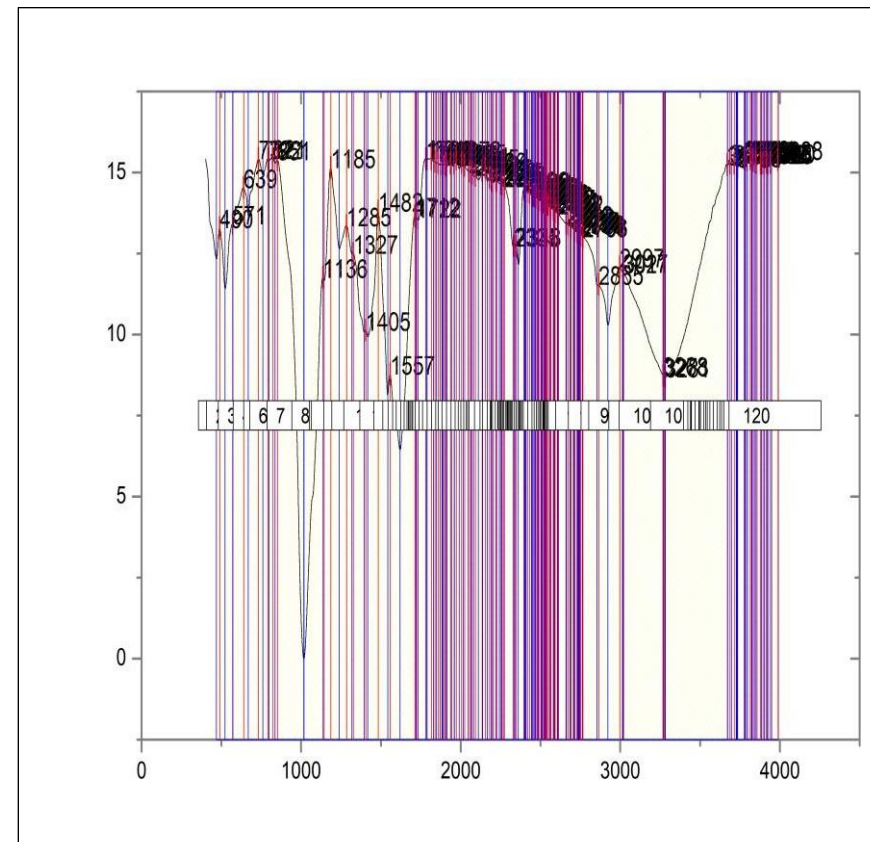
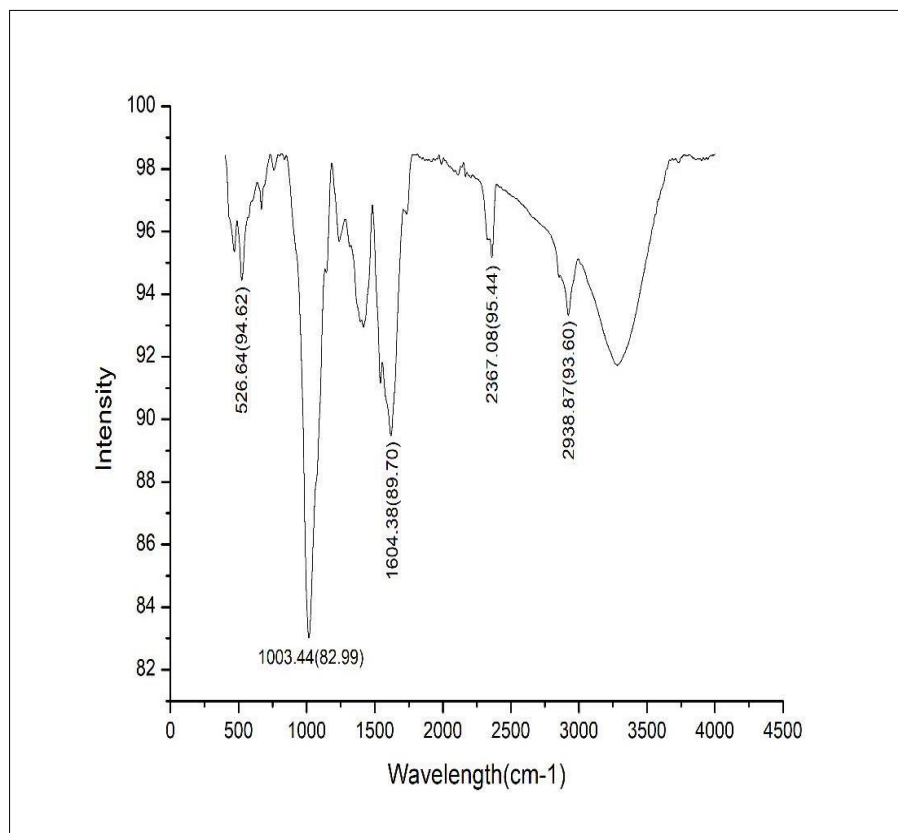


Figure 4.66. FTIR spectra of T2- Salicylic Acid recommended dose (300ppm)

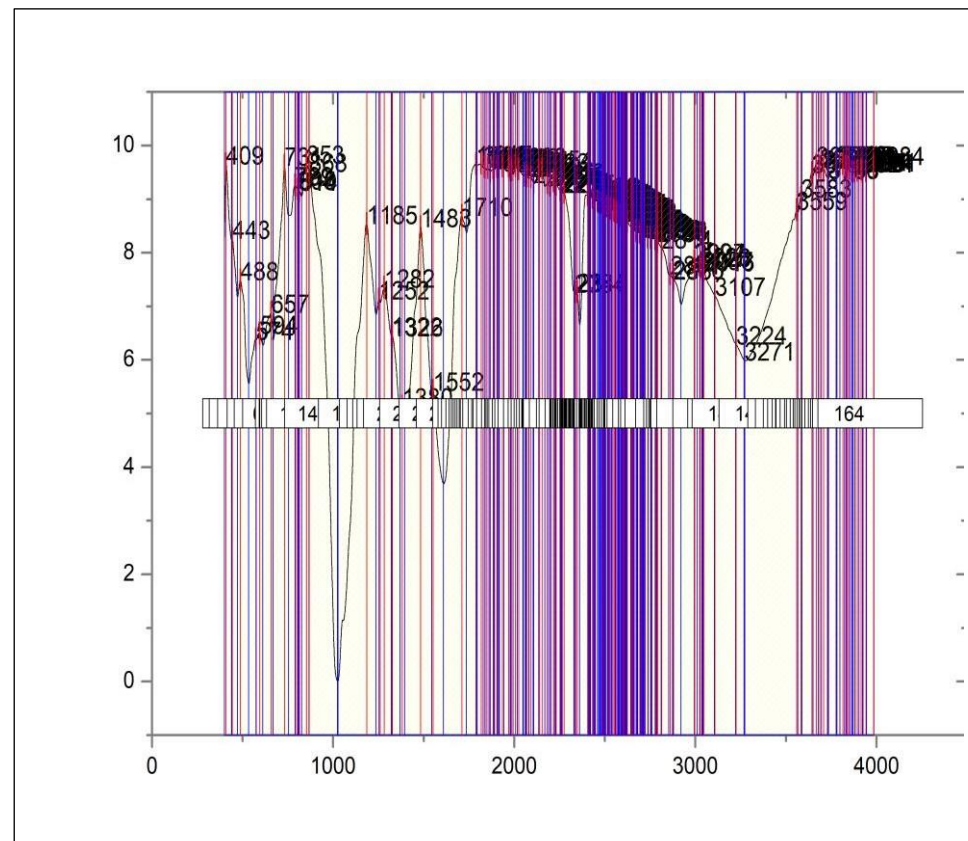
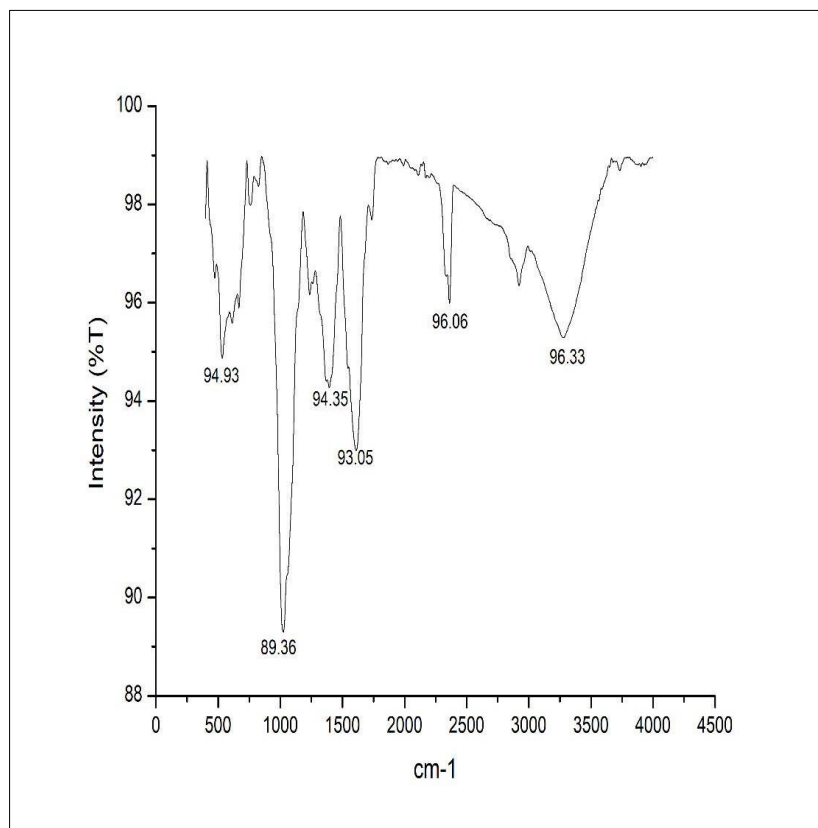


Figure 4.67. FTIR spectra of T3- Thiourea (1000ppm) + Salicylic Acid (300ppm)

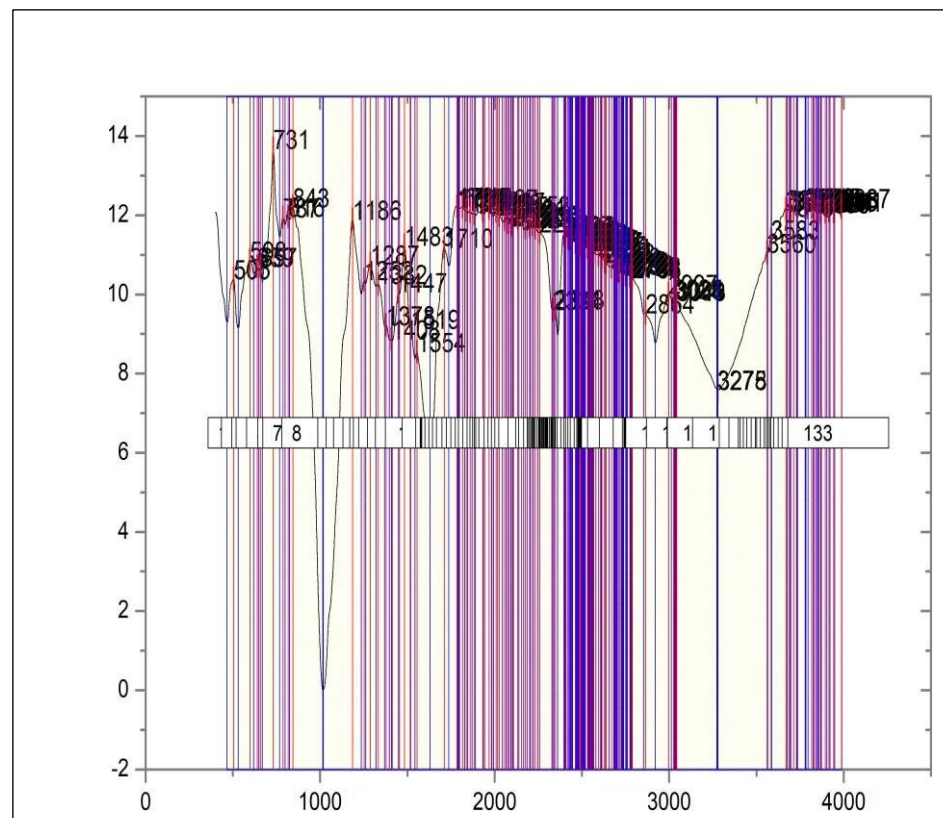
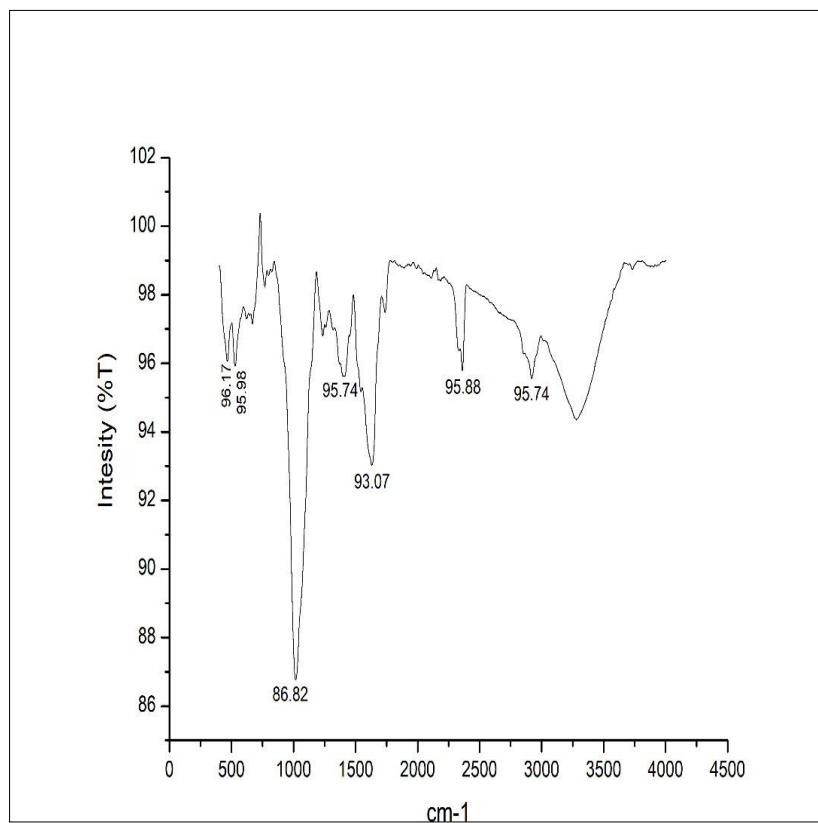


Figure 4.68. FTIR spectra of T4- Thiourea (1500ppm) + Salicylic Acid (300ppm)

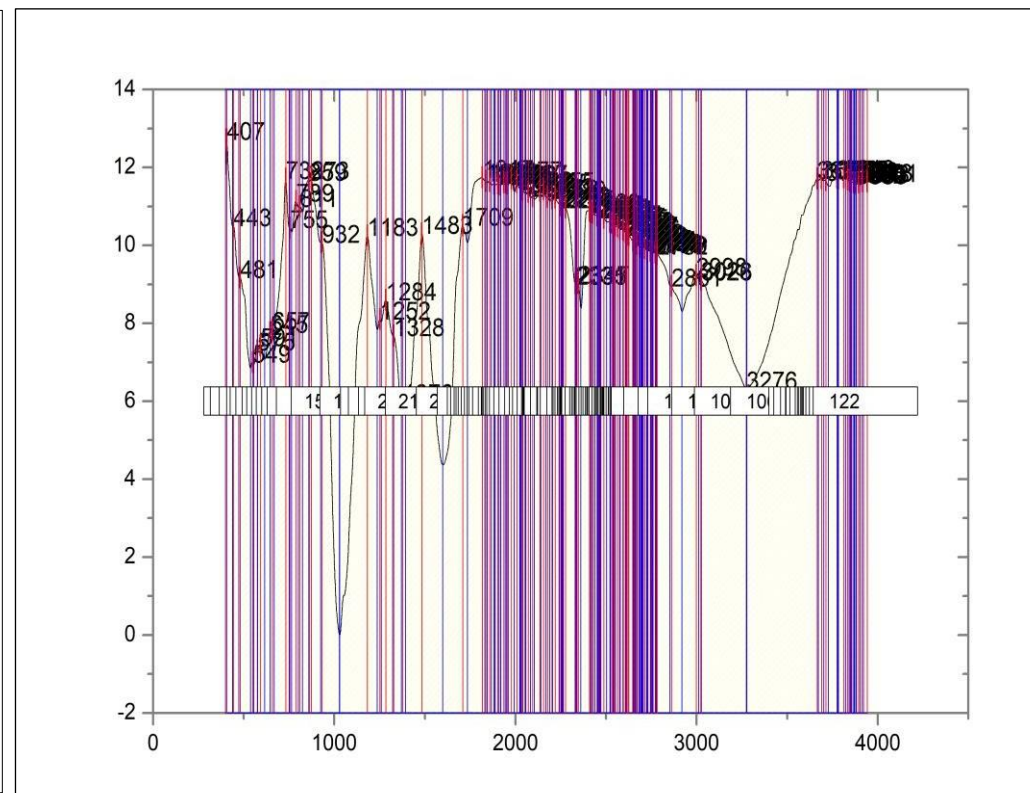
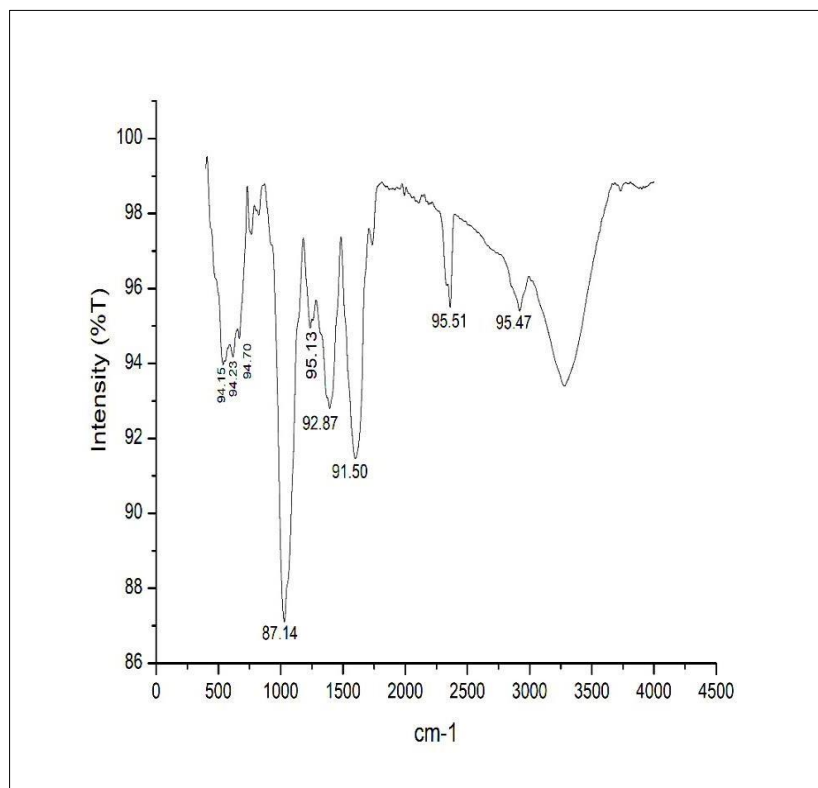


Figure 4.69. FTIR spectra of T5- Thiourea (1000ppm) + Salicylic Acid (450ppm)

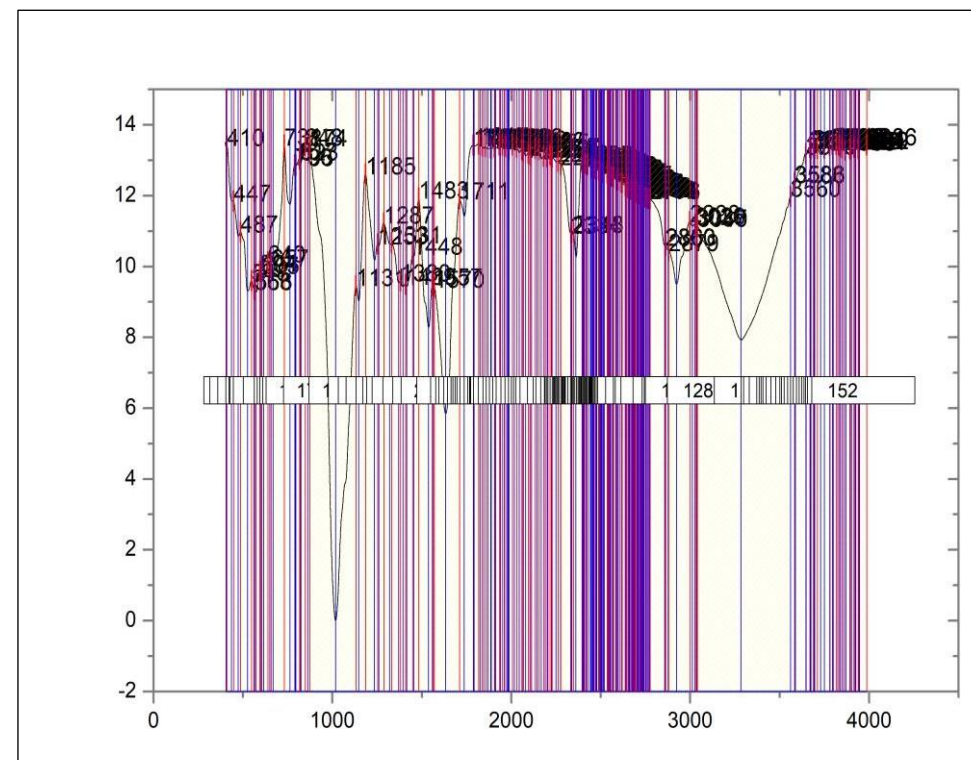
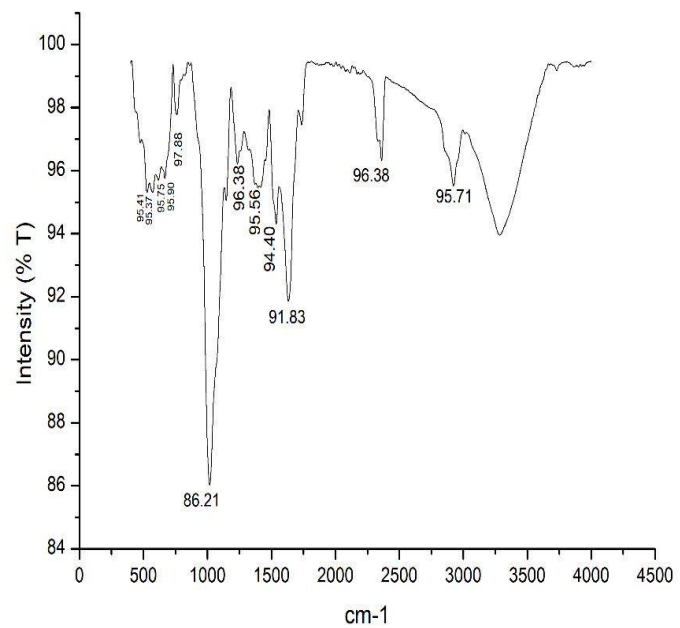






Figure 4.71. FTIR spectra of T7- Thiourea (100ppm) + Salicylic Acid (150ppm)

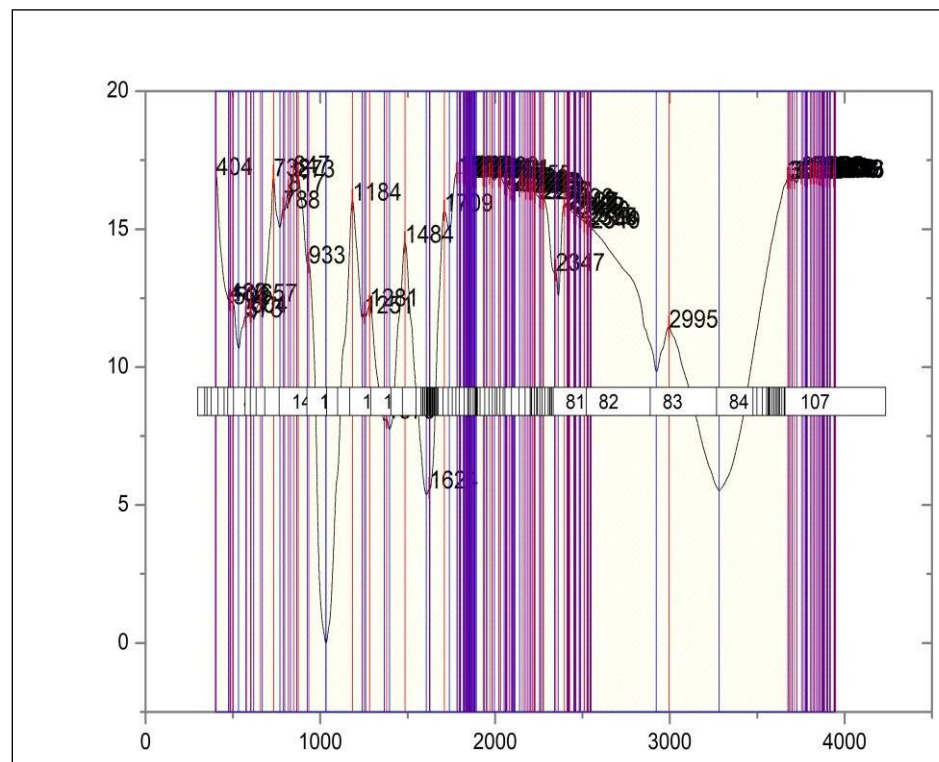
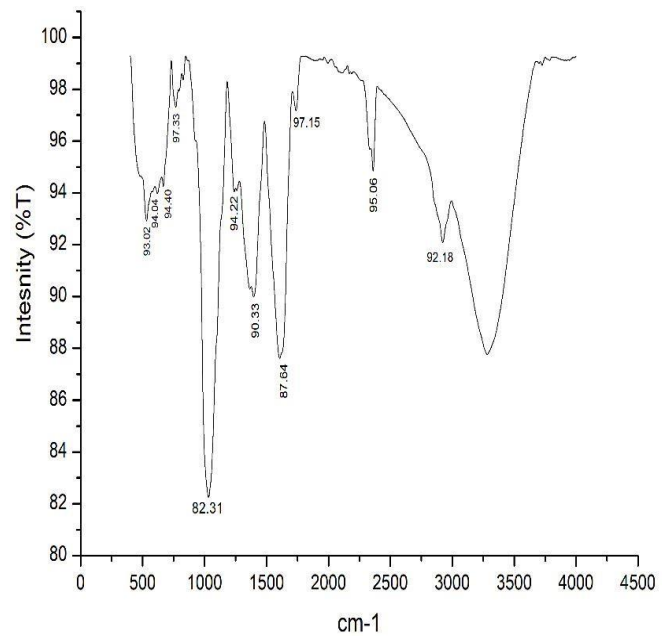
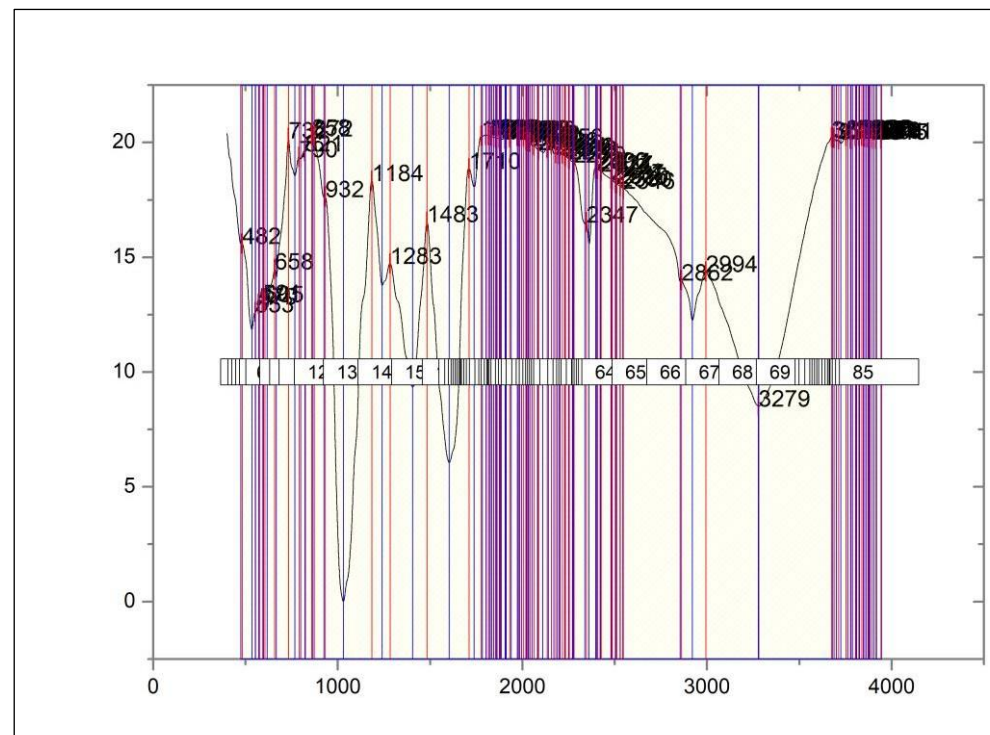
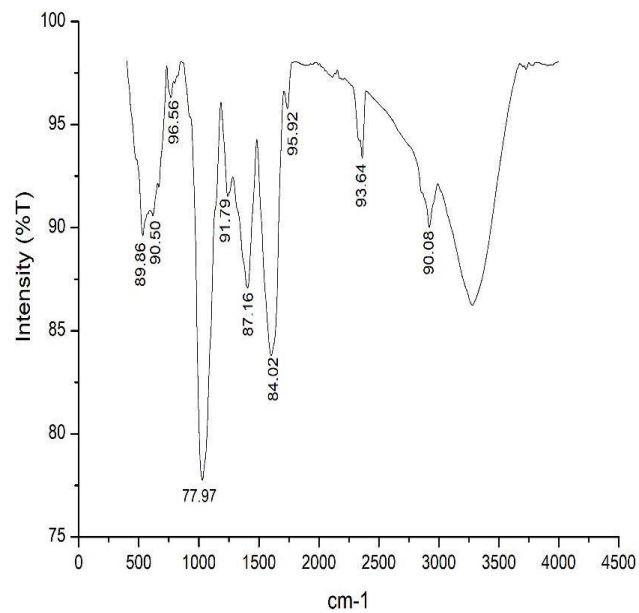


Figure 4.72. FTIR spectra of T8- Thiourea (500ppm) + Salicylic Acid (600ppm)



**Figure 4.73. FTIR spectra of T9- Thiourea (2000ppm) + Salicylic Acid (600ppm)**

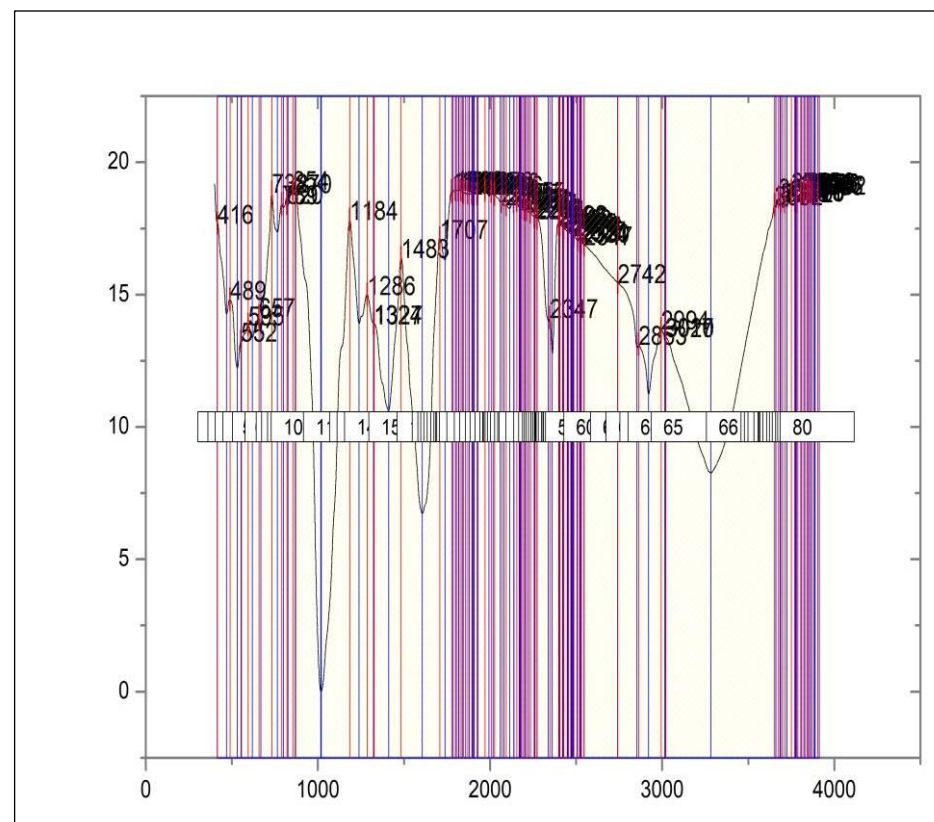
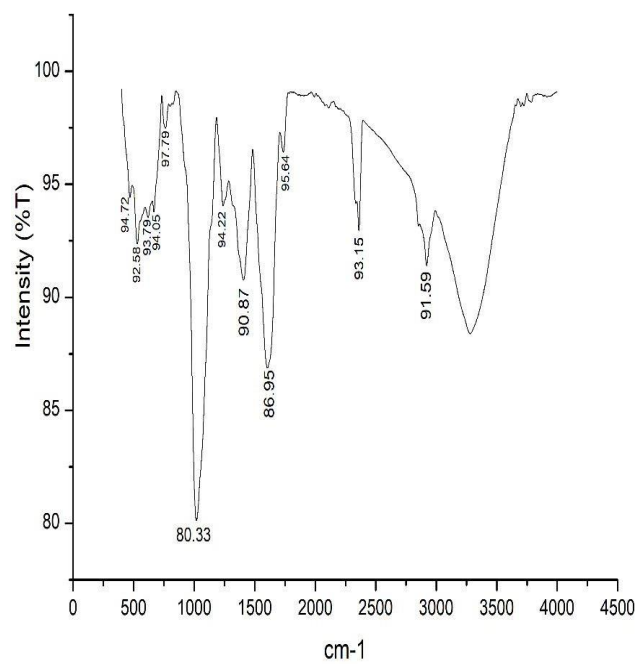
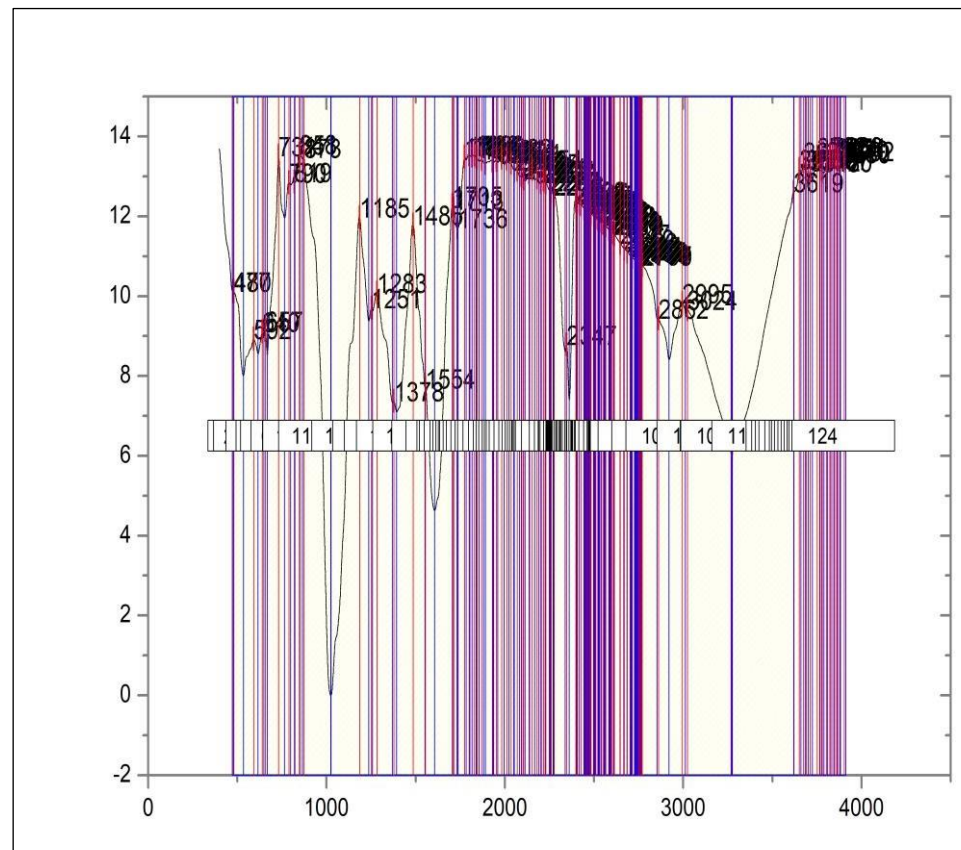
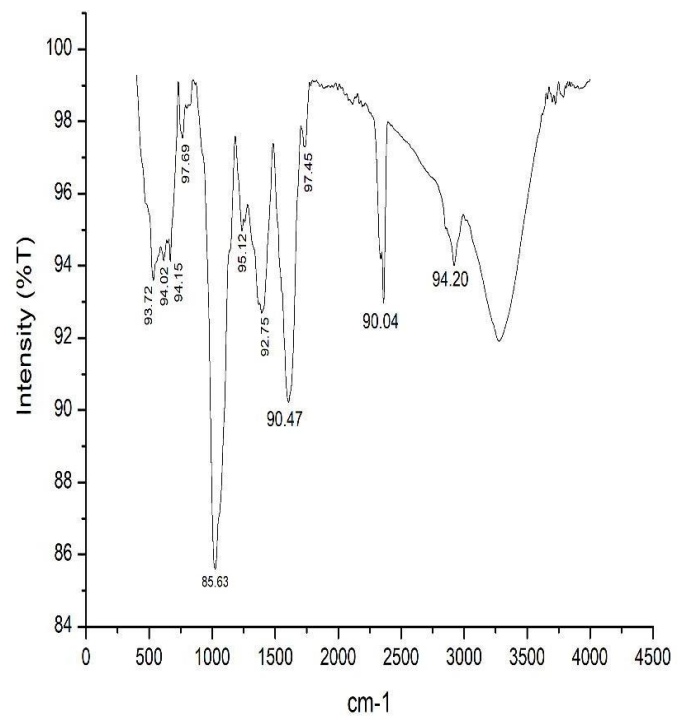


Figure 4.74. FTIR spectra of T10- Thiourea (2000ppm) + Salicylic Acid (600ppm)





#### 4J. Thiourea (sulphur) and salicylic acid-mediated effects on EDX of Indian mustard grown under the open filed condition

##### EDX-Result and Discussion

##### T0- Control (Discussion):

Figure: 4.76. EDX spectra of T0- Control

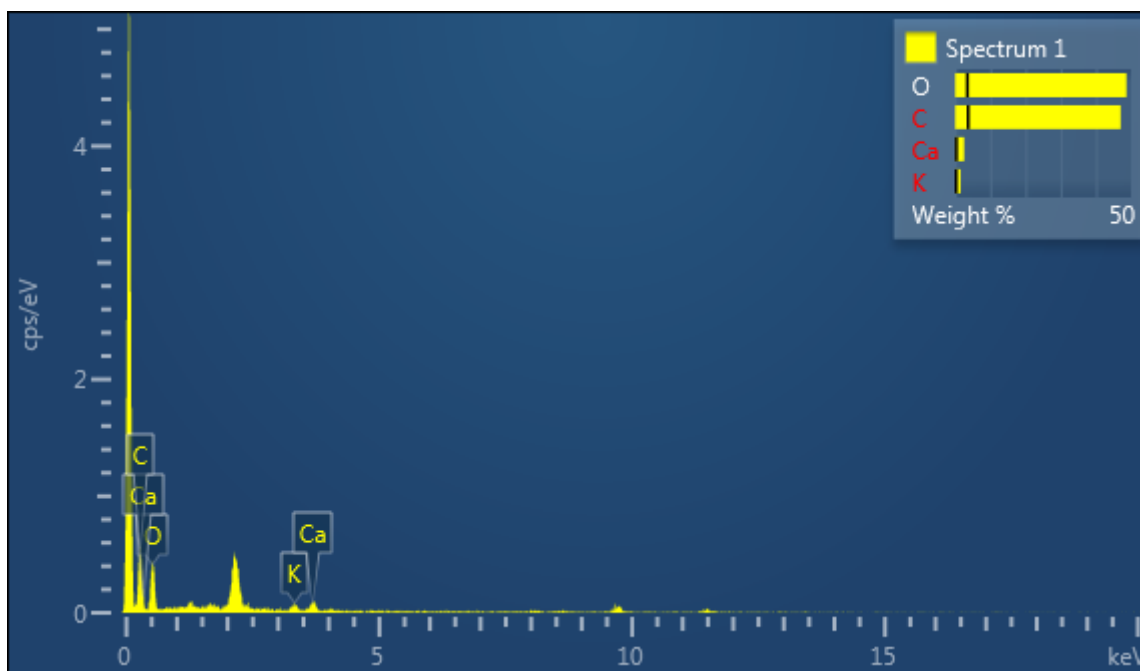


Table: 4.74. Elemental composition of T0- Control

	Wt%	Wt% sigma	Atomic %
<b>C</b>	47.04	3.76	55.41
<b>O</b>	48.70	3.53	43.07
<b>K</b>	1.63	0.30	0.59
<b>Ca</b>	2.62	0.37	0.93
<b>Total</b>	<b>100%</b>		<b>100%</b>

The elemental composition of the analysed sample is revealed through the T0 Energy Dispersive X-ray (EDX) data, which provides a detailed understanding of the relative abundances of different elements within the material. This information is essential for

characterising the sample, understanding its chemical composition, and possibly concluding the origin of the sample, its properties, or its potential effects on the environment. Carbon (C): Carbon was the most abundant element in the sample (T0). It made up 47.04 weight per cent of the material. A high carbon content indicates organic matter because carbon is essential for forming organic compounds. Carbon makes up 55.41 per cent of the sample's total atomic weight, further evidence of the element's predominance. The fact that the sample's standard deviation is only 3.76 per cent indicates that the sample's carbon content is relatively stable and can be defined precisely. Because there is a high concentration of carbon in the material, we can determine that it is organic and originates, most likely, from plant matter or other biological sources. Gaining a comprehensive understanding of the prevalence of carbon is of utmost importance, as it provides valuable insights into the organic composition and has the potential to impact the properties and reactivity of the material significantly. Oxygen (O): Oxygen is the second most prevalent element in the analysed sample, constituting approximately 48.70 wt% of the composition of the material. Oxygen, like carbon, is a fundamental element in organic compounds. Oxygen can also be found in a wide variety of minerals and oxides. The oxygen atom makes up a significant part of the sample's atomic composition, as evidenced by its atomic percentage of 43.07%. Because the standard deviation for oxygen is relatively low (Wt% sigma: 3.53%), this indicates that there is a consistent amount of oxygen present. A high oxygen content is consistent with the organic nature of the material, and it suggests the presence of oxygen in a variety of functional groups and chemical bonds within the sample (Cavusoglu et al., 2022; Chai et al., 2022; Chan, 2022; Chao et al., 2022; Chaturvedi, Khan, et al., 2022). This is indicated by the fact that the sample has a high oxygen content. This result agrees with the hypothesis that plant-based materials and organic compounds have the expected composition. Potassium (K): Potassium is a component of the sample that is relatively insignificant, making up only 1.63 weight per cent of the overall material's makeup. Even further below that is the atomic percentage of potassium, which is 0.59%. The potassium element exhibits a relatively low standard deviation (Wt% sigma: 0.30%), indicating its consistent yet minor occurrence. Potassium is not a particularly abundant element, which suggests that it is not the primary component of the sample. Instead, its presence may be linked to certain minerals or chemical



compounds due to its low abundance. Potassium is an element frequently discovered in plant tissues and soils, and the presence of potassium in a given sample may provide insight into its origin or the environmental history of its previous location. On the other hand, the fact that it is only present in a relatively small amount indicates that it is not a significant structural component of the material. Calcium (Ca): Calcium is a constituent of the material's composition, accounting for 2.62 wt% of its overall content. The calcium atomic percentage is 0.93%. Like potassium, the standard deviation for calcium is relatively low (Wt% sigma: 0.37%), indicating a constant but relatively insignificant presence. Calcium is a mineral that can be found in rocks, soils, and the tissues of plants (Cavusoglu et al., 2022; Chai et al., 2022; Chan, 2022; Chao et al., 2022; Chaturvedi, Khan, et al., 2022). The mineral content of the material or specific cellular structures within it may be responsible for its appearance in the sample. The fact that calcium is not particularly abundant, on the other hand, lends credence to the notion that it does not play a predominant part in the overall makeup of the sample. Total Composition: The EDX analysis correctly accounts for all elements in the sample, as shown by the sum of the weight percentages for all elements (C, O, K, and Ca), which adds up to 100%. This comprehensive make-up verifies the thoroughness and accuracy of the analysis, demonstrating that no crucial details have been overlooked. Understanding the elemental makeup of the sample under study is made much easier with the help of the T0 EDX data. Carbon and oxygen predominate, and their concentrations are relatively stable; this suggests that the material is organic and derives from plant matter or other biological sources, with minor to moderate standard deviations. Potassium and calcium were found in trace amounts within the sample, which raises the possibility of associations with various soil minerals or particular cellular structures. EDX analysis is invaluable in characterising materials, comprehending their composition, and establishing correlations with their source or environmental context (Taira & Shiono, 2022; Takeuchi et al., 2022; Talaat, 2023; Tan et al., 2022; Tariq et al., 2023; Tariq et al., 2022; Todorova et al., 2022; Topcu et al., 2022; Torres & Figueroa, 2023).

### T1- Thiourea recommended dose (1000ppm) (Discussion):

Figure 4.77. EDX spectra of T1- Thiourea recommended dose (1000ppm)

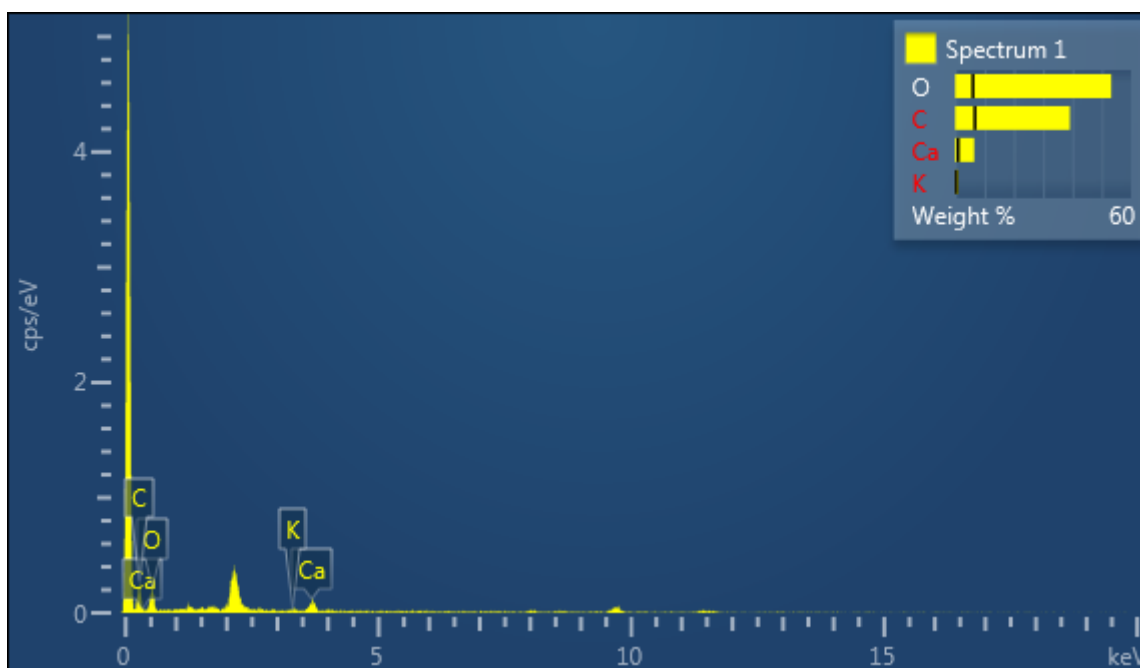


Table 4.75. Elemental composition of T1- Thiourea recommended dose (1000ppm)

	Wt%	Wt% sigma	Atomic %
<b>C</b>	39.10	6.82	48.06
<b>O</b>	53.23	6.13	49.11
<b>K</b>	1.13	0.49	0.43
<b>Ca</b>	6.53	1.05	2.40
<b>Total</b>	<b>100%</b>		<b>100%</b>

The Energy Dispersive X-ray (EDX) data obtained at T1 offers a comprehensive and informative analysis of the sample's elemental composition under investigation. This information is necessary for understanding the chemical piece of the material, coming to conclusions about its origin or properties, and gaining insights into the environmental context and historical processes that the material was subject to. Carbon (C): At the

moment, T1 carbon makes up 39.10 weight per cent of the sample's total composition.

Compared to the results of the T0 analysis, this indicates a lower overall carbon content, although its significance cannot be discounted. Carbon comprises 48.06% of the atomic percentage of all elements. The increase in the carbon standard deviation (Wt% sigma: 6.82%) indicates that the amount of carbon may be slightly more variable. Despite this, carbon makes up a significant portion of the material, meaning the substance's organic character. Compared to T0, the decrease in carbon content may show changes in the sample's organic composition, which may have occurred due to treatment effects or environmental factors.

Oxygen (O): At T1, the model exhibits a significant presence of oxygen as an element, comprising 53.23 weight of the composition of the material. The oxygen content in terms of atomic percentage is 49.11%. The standard deviation of oxygen (Wt% sigma: 6.13%) indicates the extent of variability in the oxygen content. Because oxygen is so prevalent, which corresponds to its role in various organic and inorganic compounds, we can deduce that oxygen-containing functional groups or chemical bonds play an essential role in the material. Compared to T0, oxygen exhibits some variation despite continuing to be an important component.

Potassium (K): At (T1), potassium is still a relatively insignificant part of the sample, accounting for only 1.13 per cent of the total material. Potassium constitutes 0.43 per cent of the atomic mass. Potassium has a low but steady presence, as indicated by its standard deviation (Wt% sigma: 0.49%). Similar to T0, the low potassium abundance here may be attributable to its association with certain minerals or environmental factors rather than its role as a structural component of the sample.

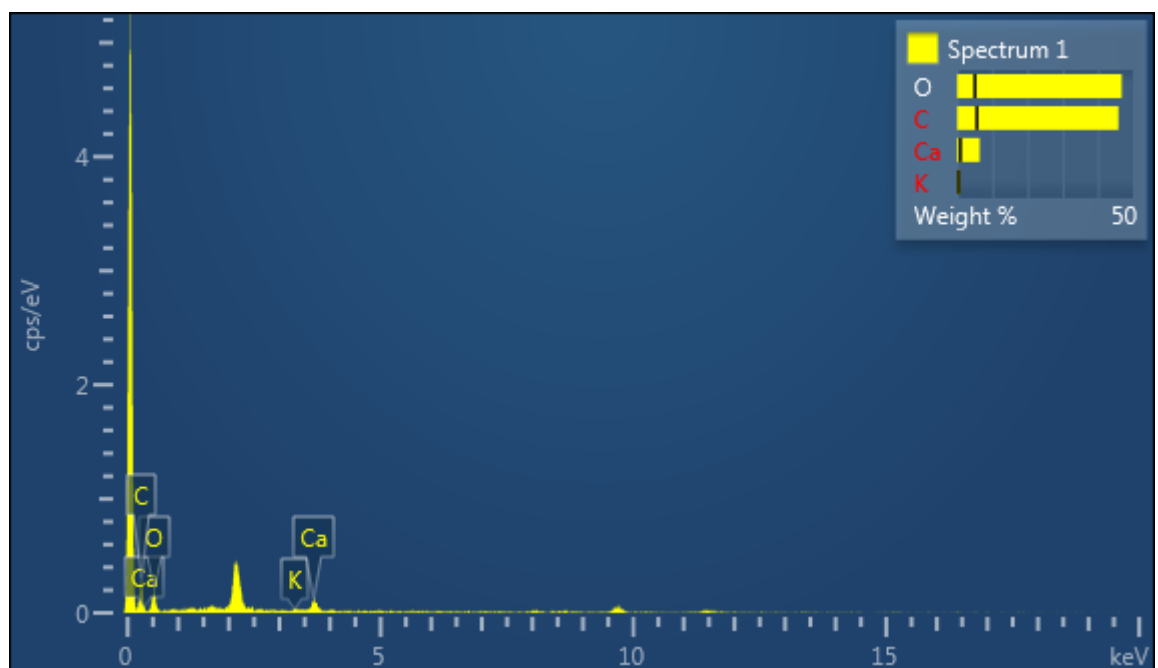
Calcium (Ca): At T1, calcium remains a minor sample constituent, comprising 6.53 wt% of the material's composition. The atomic arrangement of calcium in the given sample is 2.40%. The standard deviation of calcium (Wt%  $\sigma$ : 1.05%) indicates the extent of variation in calcium content. In a manner analogous to that of T0, the presence of calcium may be connected to the mineral content or particular cellular structures present within the material (Chen, Xu, et al., 2022; Chen et al., 2023; Chen, Tang, et al., 2022; Chen, Tang, et al., 2022). On the other hand, its relatively low abundance lends credence to the notion that it does not play a predominant part in the overall composition of the sample.

Total Composition: Because the total weight percentages for each element (C, O, K, Ca) add up to 100%, it can be deduced that the EDX analysis correctly takes into account every element that was present in the sample at the time T1. Because of this composition, the completeness

of the analysis can be verified, and it can also be ensured that no significant aspects have been overlooked. The data obtained from EDX at the time T1 continued to provide insightful information regarding the elemental makeup of the analysed sample. Even though carbon and oxygen continue to be essential components, a lower carbon content compared to T0 may indicate that there have been changes in the organic composition of the sample; these changes could be the result of environmental factors or treatment effects. The small amounts of potassium and calcium within the selection provide evidence of possible associations with the soil's minerals or particular cellular structures. In general, this EDX analysis helps characterise the material, tracking changes in its composition and investigating the environmental context or alterations that may have occurred over time (Taira & Shiono, 2022; Takeuchi et al., 2022; Talaat, 2023; Tan et al., 2022; Tariq et al., 2023; Tariq et al., 2022; Todorova et al., 2022; Topcu et al., 2022; Torres & Figueroa, 2023).

#### **T2- Salicylic Acid recommended dose (300ppm) (Discussion):**

**Figure 4.78. EDX spectra of T2- Salicylic Acid recommended dose (300ppm)**



**Table 4.76. Elemental composition of T2- Salicylic Acid recommended dose (300ppm)**

	<b>Wt%</b>	<b>Wt% sigma</b>	<b>Atomic %</b>
<b>C</b>	45.83	5.65	55.11
<b>O</b>	46.77	5.10	42.22
<b>K</b>	0.94	0.49	0.35
<b>Ca</b>	6.46	0.99	2.33
<b>Total</b>	<b>100%</b>		<b>100%</b>

The data obtained from the Energy Dispersive X-ray (EDX) technique at this particular point, which has been labelled as T2, provides a comprehensive description of the elemental makeup of the sample that was analysed. These data are necessary for comprehending the material's chemical composition, origin, and possibly even its environmental history. In this analysis, we will examine the individual contributions of each element and discuss the implications of their respective weight percentages, standard deviations (Wt% sigma), and atomic ratios: Carbon (C): At moment T2, the carbon content constitutes 45.83 weight per cent of the composition of the sample. Even though it is lower than T0, this weight percentage is still significant. Carbon's atomic ratio is 55.11%, according to the periodic table. There appears to be some variation in the amount of carbon present, given the increase in the standard deviation for carbon (Wt% sigma: 5.65%). Even so, carbon remains the predominant constituent, thereby underscoring the organic character of the substance. The observed decrease in carbon content relative to the initial state (T0) may indicate alterations in the organic composition, which could be attributed to environmental influences, treatment impacts, or inherent variability within the sample. Oxygen (O): At time T2, the model still contains a significant amount of oxygen, which makes up 46.77 wt% of the composition of the material. An atomic percentage of 42.22% represents the oxygen content. The high standard deviation for oxygen (Wt% sigma: 5.10%) indicates a degree of variability in oxygen content. The continued presence of oxygen is consistent with its role in a wide variety of organic and inorganic compounds, and it suggests the existence of functional groups or chemical bonds that contain oxygen. Compared to T0, oxygen

exhibits some variation despite continuing to be an essential component. Potassium (K): At time point T2, potassium still makes up a negligible portion of the sample, accounting for 0.94 per cent of the total material's makeup. The amount of atomic percentage that potassium makes up is 0.35%. The observed standard deviation of potassium (Wt% sigma: 0.49%) suggests a persistent yet relatively small occurrence. In both T0 and T1, the observed scarcity of potassium indicates that it does not play a significant role as a structural constituent in the sample. Instead, its presence may be linked to particular minerals or environmental conditions. Calcium (Ca): At time point T2, calcium is still present in the sample but only in trace amounts. It makes up 6.46 weight per cent of the material as a whole. Calcium's atomic percentage comes in at 2.33 per cent. Calcium concentration variation is represented by the standard deviation for calcium (Wt% sigma: 0.99%). The presence of calcium may continue to be linked to the mineral content or particular cellular structures present within the material. On the other hand, its relatively low abundance lends credence to the concept that it does not play a predominant part in the overall composition of the sample. Total Composition: The total weight percentages for each element (C, O, K, Ca) add up to 100%, demonstrating that the EDX analysis was precise and exhaustive in its topic coverage (Chao et al., 2022; Chaturvedi, Khan, et al., 2022; Chaturvedi, Kulshrestha, et al., 2022; Chauhan et al., 2023). This composition proves the sample has been thoroughly analysed to identify and account for all relevant components. The EDX data collected at time point T2 offers beneficial insights into the elemental makeup of the analysed sample. Even though carbon and oxygen are still essential components, a lower carbon content compared to T0 may indicate that there have been changes to the organic composition of the sample. Potassium and calcium were present, although in minor quantities, suggesting the possibility of associations with soil minerals or particular cellular structures. In general, this EDX analysis plays an integral part in characterising the material, tracking changes in its composition, and investigating the environmental context or modifications that may have occurred over time (Li, Tong, et al., 2023; Liu et al., 2023; Liu, Wang, et al., 2022; Liu, Li, et al., 2022; Liu, Cui, et al., 2022; Liu, Zong, et al., 2022; Liu, Liu, et al., 2022; Liu, Xiao, et al., 2022).

### T3- Thiourea (1000ppm) + Salicylic Acid (300ppm) (Discussion):

Figure 4.79. EDX spectra of T3- Thiourea (1000ppm) + Salicylic Acid (300ppm)

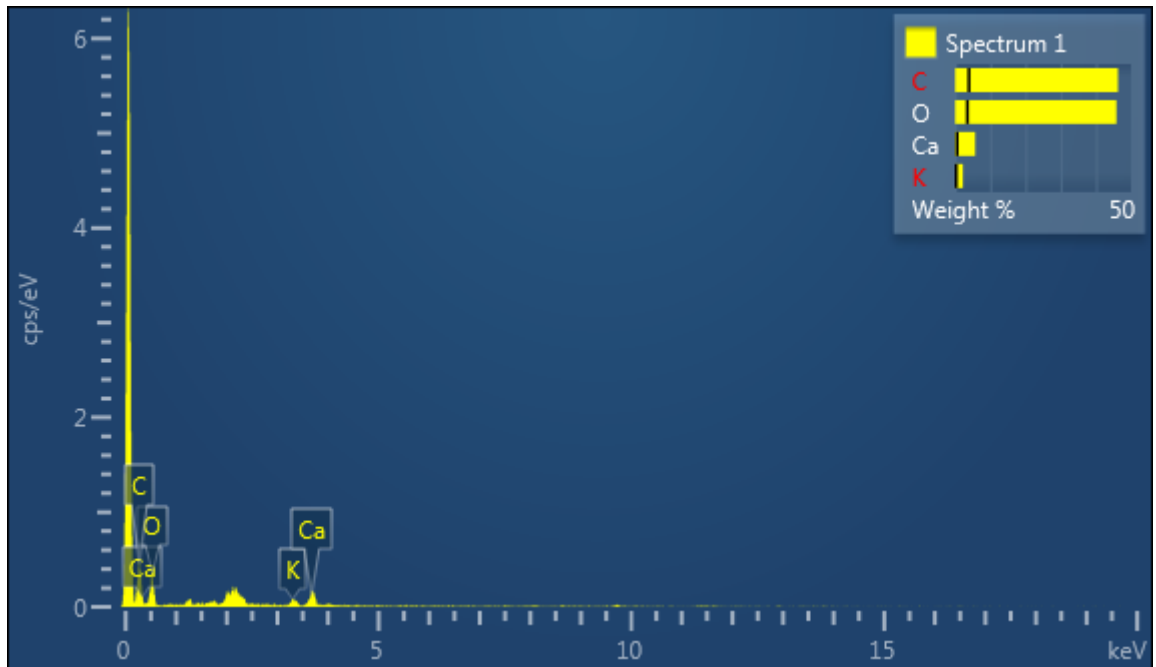


Table 4.77. Elemental composition of T3- Thiourea (1000ppm) + Salicylic Acid (300ppm)

	Wt%	Wt% sigma	Atomic %
<b>C</b>	46.26	3.97	55.70
<b>O</b>	45.82	3.54	41.42
<b>K</b>	2.22	0.35	0.82
<b>Ca</b>	5.71	0.60	2.06
<b>Total</b>	<b>100%</b>		<b>100%</b>

The data obtained from energy dispersive x-ray (EDX) analysis at T3 provides a comprehensive and insightful characterisation of the elemental make-up of the analysed sample. These data are an essential resource for understanding the chemical composition of the material, in addition to its origin and the possible environmental influences on the fabric. Let's investigate the contribution made by each element as well



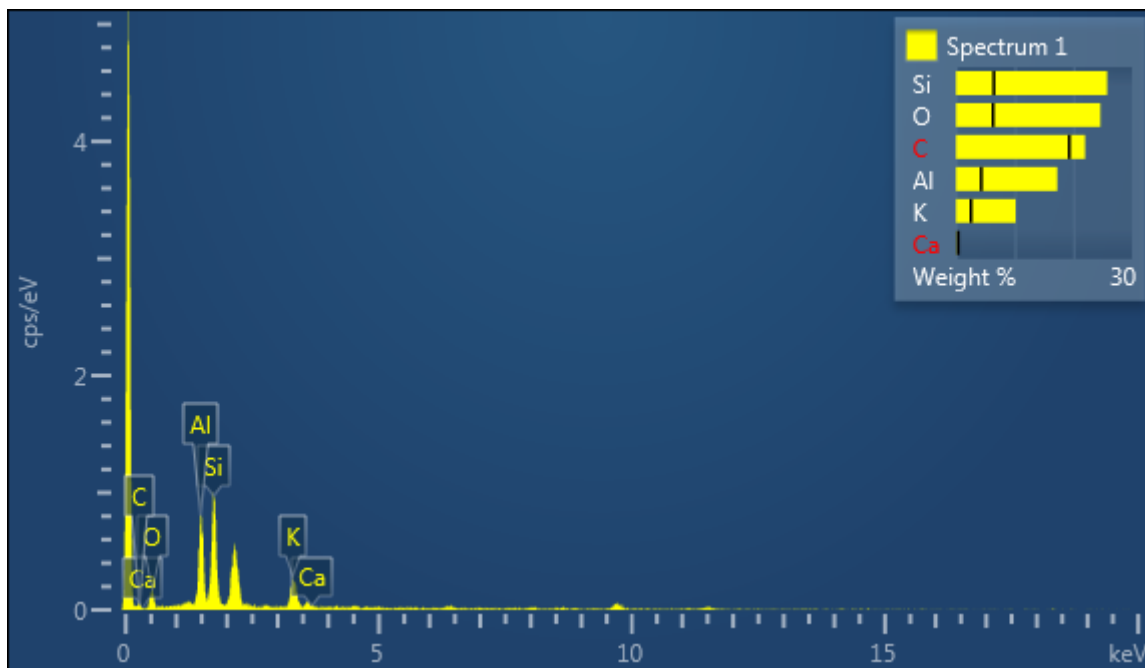
as the significance of its weight percentage, standard deviation (Wt% sigma), and

atomic percentage: Carbon, also known as C: At T3, carbon makes up 46.26 weight per cent of the sample's overall makeup. Although slightly lower than previous analyses, this weight percentage still constitutes a substantial proportion of the material. The carbon content is 55.70% on an atomic scale. The carbon content exhibits a relatively consistent nature, as indicated by the low standard deviation (Wt% sigma: 3.97%). The continued majority of carbon in the composition draws attention to the organic character of the substance. Compared to earlier data points, the slight drop in carbon content may indicate further changes in the organic composition. Environmental factors or treatment effects may have caused these changes. Oxygen (O): At T3, oxygen plays a significant role in the sample's composition, accounting for 45.82 per cent of the total material. Oxygen has a percentage in the atoms equal to 41.42 per cent. There is some degree of variation in the amount of oxygen present, as indicated by the standard deviation for oxygen (Wt% sigma: 3.54%). The continued presence of oxygen is consistent with its role in a wide variety of organic and inorganic compounds, and it suggests the existence of functional groups or chemical bonds that contain oxygen. Oxygen, like carbon, remains significant but exhibits variability compared to earlier studies. Potassium (K): At the T3, potassium still makes up a relatively insignificant portion of the sample, accounting for only 2.22 per cent of the material. Potassium makes up 0.82 per cent of the atomic weight of the element. According to the potassium standard deviation (Wt% sigma: 0.35%), the piece seems to be present all the time but in a relatively insignificant amount. As in earlier investigations, the low abundance of potassium suggests that it is not a significant structural component of the sample. Instead, its presence may be linked to particular minerals or aspects of the surrounding environment. Calcium (Ca): At T3, calcium can still be found in the sample, but it is just a tiny component, making up 5.71 per cent of the total material. Calcium makes up 2.06% of the atomic percentage of the element. The degree of variability in calcium content is indicated by the standard deviation for calcium, which is expressed as a weighted percentage sigma value of 0.60 per cent. There is a possibility that the presence of calcium will continue to be linked to the material's mineral content or particular cellular structures (Borah et al., 2022; Boro & Chattopadhyay, 2022; Brillì et al., 2022; Butt & Gul, 2023; Çam et al., 2022; Campos et al., 2023; Canales et al., 2023; Cao et al., 2022; Carreño-Vega et al., 2022; Cavusoglu et al., 2022; Chai et al., 2022;

Chan, 2022). Despite this, its low abundance provides credence to the idea that it does not play a preponderant part in the overall composition of the sample. Total Composition: The total number of weight percentages for all elements (C, O, K, and Ca) adds up to 100%, demonstrating that the EDX analysis was accurate and comprehensive. Because the sample was composed this way, all essential components have certainly been considered. The EDX data obtained at T3 provides insightful information regarding the elemental makeup of the analysed sample. Although carbon and oxygen continue to make up a significant portion of the material, previous analyses show that their proportions have shrunk slightly, which may indicate that the organic component of the substance is undergoing ongoing transformations. Minor quantities of potassium and calcium endure, meaning potential connections with soil minerals or distinct cellular structures. In general, using EDX analysis is crucial in characterising materials, monitoring variations in their composition, and investigating potential environmental influences or alterations that may occur over time (Bae et al., 2023; Bagautdinova et al., 2022; Banerjee & Roychoudhury, 2022; Bano et al., 2023; Bashir et al., 2022; Bhadane et al., 2022; Blinkov et al., 2022).

**T10- Thiourea (2000ppm) + Salicylic Acid (600ppm) (Discussion):**

**Figure 4.80. EDX spectra of T10- Thiourea (2000ppm) + Salicylic Acid (600ppm)**



**Table 4.78. Elemental composition of T10- Thiourea (2000ppm) + Salicylic Acid (600ppm)**

	<b>Wt%</b>	<b>Wt% sigma</b>	<b>Atomic %</b>
<b>C</b>	22.02	19.31	35.30
<b>O</b>	24.61	6.35	29.62
<b>Al</b>	17.27	4.34	12.33
<b>Si</b>	25.78	6.45	17.68
<b>K</b>	10.20	2.60	5.02
<b>Ca</b>	0.12	0.38	0.06
<b>Total</b>	<b>100%</b>		<b>100%</b>

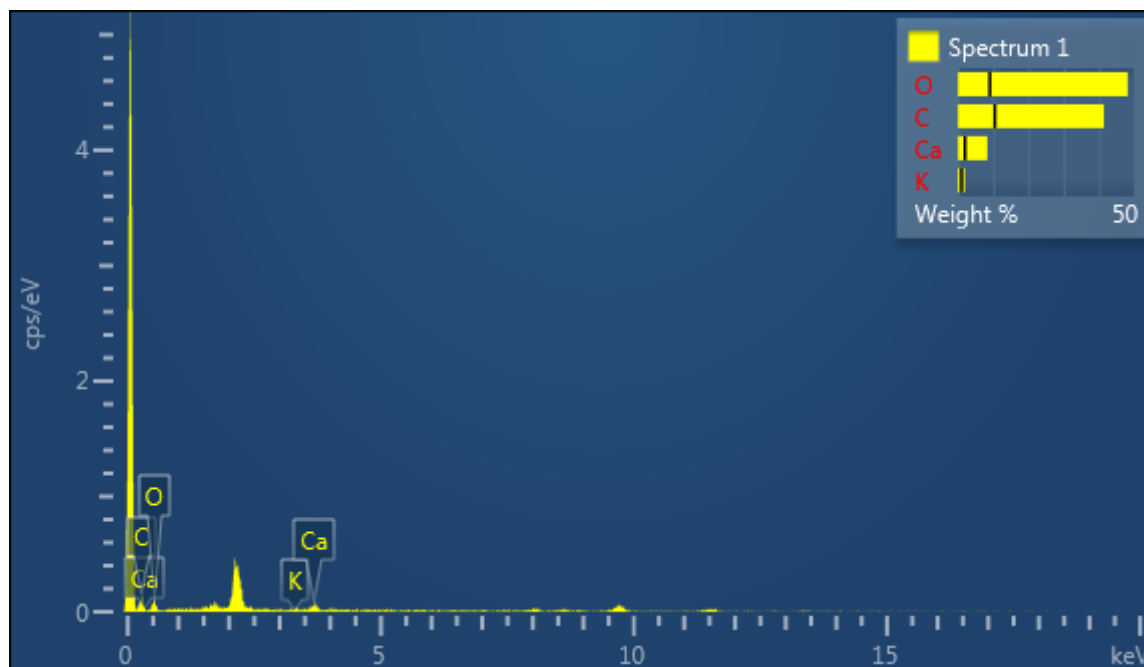
The energy-dispersive X-ray (EDX) data collected at T10 provides a detailed and comprehensive characterisation of the sample's elemental makeup. These data are extremely helpful in understanding the chemical composition of the material, as well as its origin and the potential environmental influences it may have. Let's look at what each element brings and their weight percentages, standard deviations, and atomic percentages. Carbon (C): At T10, 22.02 wt% of the sample is carbon. Although carbon is an important element, new research suggests that its abundance is significantly lower than what was estimated by earlier studies. Carbon makes up 35.30% of the atomic percentage of all elements. The standard deviation for carbon (Wt% sigma: 19.31%) is relatively high, revealing a significant amount of variation in the carbon content. This variation in carbon levels may indicate that the sample contains heterogeneous areas, with some areas containing more carbon than others. The observed reduction in carbon content relative to previous data points may suggest substantial alterations in the organic composition of the sample, potentially influenced by environmental factors or experimental treatments. Oxygen (O): At time point T10, oxygen is still a significant part of the sample, accounting for 24.61 weight per cent of the total composition of the material. Oxygen has a percentage in the atomic formula of 29.62%. The standard deviation of oxygen (Wt% sigma: 6.35%) indicates the extent of variability in the oxygen content. The enduring prevalence of oxygen highlights its significance in a wide range of organic and inorganic compounds present in the material. In the same way,

carbon shows regional variation in its scope, oxygen does as well, which may point to the presence of different functional groups or chemical bonds containing oxygen in various parts of the sample. Aluminum (Al): At T10, aluminium is found in significant quantities, accounting for 17.27 per cent of the material's overall composition. Aluminium has a 12.33% atomic percentage, according to the periodic table. The coefficient of variation for aluminium (Wt%  $\sigma$ : 4.34%) indicates moderate variation in the aluminium content. Aluminium in the sample suggests that it may also contain minerals or compounds that also contain aluminium. Variations in the mineral content of the sample may exist, as indicated by its relatively higher range compared to earlier analyses. These variations may be caused by environmental factors or the location of the sample itself. Silicon (Si): Another important component found in the sample taken at T10 is silicon, which accounts for 25.78 per cent of the material. Silicon has a percentage in the atomic structure of 17.68%. The degree of variation in silicon content is reflected by the standard deviation for silicon, which is expressed as a weight per cent sigma value of 6.45%. Because silicon is so abundant, we can assume that silicate minerals or other compounds containing silicon are also present. The fact that the amount of silicon varies from region to region within the sample may point to the presence of different mineral combinations in each of those regions. Potassium (K): At T10, potassium still makes up only 10.20 wt% of the sample, making it a relatively insignificant element. Potassium constitutes 5.02% of the atomic weight. The observed standard deviation of potassium (Wt%  $\sigma$ : 2.60%) suggests a moderate level of variability in the potassium content. Similar to previous analyses, the observed scarcity of potassium indicates that it does not play a significant role as a structural constituent in the examined sample. On the other hand, its presence in significant but variable quantities may point to its association with certain minerals or specific localised variations within the sample. Calcium (Ca): Despite T10, calcium is still a relatively insignificant part of the sample, making up only 0.12 weight per cent of the material. Only 0.06% of the atoms in the universe are composed of calcium. The low level of variability in calcium content is indicated by the low value of the standard deviation for calcium (0.38% for Wt%  $\sigma$ ). The fact that calcium is present in such a minute quantity is further evidence that it does not play a significant part in the overall chemical makeup of the sample. Despite this, its presence in trace amounts may still indicate that

it is associated with certain minerals or other minor impurities in the sample. Total Composition: The total weight percentage for each element (C, O, Al, Si, K, and Ca) adds up to 100%, demonstrating that the EDX analysis was carried out correctly and thoroughly. The fact that this sample has been effectively analysed for its total composition ensures that all significant components have been accounted for. The EDX data collected at T4 provides insightful information regarding the elemental makeup of the analysed sample. The sample may contain variations in its mineral content due to environmental factors or local geological features, as suggested by the significant presence of aluminium and silicon. Despite exhibiting variability, carbon and oxygen play important roles in the material's composition. The fact that potassium and calcium are only found in trace amounts and varying quantities suggests that they are associated with certain minerals or localised variations within the sample. In general, the utilisation of EDX analysis plays a crucial role in characterising materials, monitoring alterations in their composition, and investigating potential environmental factors or variations within the sample (rilli et al., 2022; Butt & Gul, 2023; Çam et al., 2022; Campos et al., 2023; Canales et al., 2023; Cao et al., 2022; Carreño-Vega et al., 2022).

**T11- Thiourea (500ppm) + Salicylic Acid (150ppm) (Discussion):**

**Figure 4.81.EDX spectra of leaf of mustard treated with Thiourea (500ppm) + Salicylic Acid (150ppm)**



**Table 4.79. Elemental composition of T11- Thiourea (500ppm) + Salicylic Acid (150ppm)**

	<b>Wt%</b>	<b>Wt% sigma</b>	<b>Atomic %</b>
<b>C</b>	41.47	10.47	51.34
<b>O</b>	48.21	9.04	44.81
<b>K</b>	1.97	1.00	0.75
<b>Ca</b>	8.36	1.99	3.10
<b>Total</b>	<b>100%</b>		<b>100%</b>

The data obtained from the energy dispersive x-ray (EDX) analysis performed at T11 offers an exhaustive and penetrating analysis of the elemental makeup of the sample



that was conducted. These data are necessary for understanding the chemical composition of the material, as well as potential influences from the environment and variations within the material itself. Let's take a closer look at the contributions made by each element as well as the significance of their weight percentages, standard deviations (Weight per cent sigma), and atomic percentages: Carbon (C): At T11, carbon makes up 41.47 weight per cent of the total sample composition. Although significant, this weight percentage exhibits some variability compared to earlier analyses' results. The rate of carbon found in atoms is 51.34 per cent. Interestingly, the standard deviation for carbon is relatively high (Wt% sigma: 10.47%), indicating a significant variation in the amount of carbon present. This variability may point to heterogeneity within the sample, with some areas containing a greater concentration of carbon than others. The organic nature of the material can be inferred from the presence of carbon at this level. Despite this, the variability could suggest that different sample parts have experienced varying degrees of carbonisation or decomposition. Oxygen (O): At T11, oxygen remains a significant component in the sample, making up 48.21 weight per cent of the material's overall composition. According to its atomic percentage, 44.81% of oxygen comprises atoms. A degree of variability in oxygen content is reflected by the standard deviation for oxygen (Wt% sigma: 9.04%), similar to the degree of variability in carbon content. The continued prevalence of oxygen highlights the role that it plays in a variety of organic and inorganic compounds found within the material. The fact that the amount of oxygen present in the sample varies from region to region suggests that the sample contains many different kinds of chemical bonds and functional groups that have oxygen. Potassium (K): At T11, potassium is still a relatively insignificant part of the sample, making up only 1.97 weight per cent of the total material. The amount of potassium that makes up an atom is 0.75 atomic percentage. The potassium content appears to have a moderate variation based on the standard deviation, expressed as a percentage sigma of 1%. According to the results of the previous studies, the low abundance of potassium hints that it is not a significant structural component of the sample. On the other hand, the fact that it is present in quantities that vary suggests the possibility of localised variations or associations with particular minerals within the sample. Calcium (Ca): Calcium is still present in the example at T11, but its contribution to the overall composition of the

material has decreased to 8.36 weight per cent. Calcium has a percentage in the atomic makeup of 3.10 per cent. Calcium has a degree of variability in its content, similar to potassium, as indicated by the standard deviation, expressed as a weighted percentage sigma of 1.99%. There is a possibility that the presence of calcium will continue to be linked to the material's mineral content or particular cellular structures. On the other hand, its relatively low abundance contributes credence to the idea that it does not play a preponderant part in the overall composition of the sample. Total Composition: The total weight percentages for each element (C, O, K, Ca) add up to 100%, demonstrating that the EDX analysis was carried out correctly and thoroughly. All essential components were inevitably considered because the sample was composed this way. The EDX data collected at T11 offers beneficial insights into the elemental makeup of the analysed sample. The inconsistency in the carbon and oxygen levels may indicate differences in the model's organic composition or the degree to which it has decomposed. The presence of potassium and calcium in varying quantities may imply the existence of potential associations with particular minerals or localised variations within the sample. In general, using EDX analysis is crucial in characterising the material, monitoring alterations in its composition, and investigating potential environmental factors or variations present within the sample (rilli et al., 2022; Butt & Gul, 2023; Çam et al., 2022; Campos et al., 2023; Canales et al., 2023; Cao et al., 2022; Carreño-Vega et al., 2022).

#### **4K. Thiourea (sulphur) and salicylic acid-mediated effects on XRD of Indian mustard grown under the open filed condition**

##### **XRD- RESULT AND DISCUSSION**

The structural changes and effects on the molecular level caused by these treatments can be better understood by conducting an XRD (X-ray diffraction) analysis on mustard leaves treated with thiourea and salicylic acid and then analysing the resulting data. The information consists of several points, ranging from T0 to T10, each representing a particular treatment condition. In this exhaustive study, we will examine each data point independently, describe the treatment conditions, and discuss the number of peaks obtained in the XRD spectra. In addition, we shall investigate the possible consequences of these peaks on the biochemical composition and structural alterations in the mustard

leaves. Moreover, we will analyse the potential influence of the treatments involving thiourea and salicylic acid on the XRD spectra. Data Point T0 (388 peaks): Treatment Conditions: T0 refers to having no treatment and being part of the control group. XRD Peaks: Are 388 peaks visible in the XRD spectrum of T0. This is a significant number. Under typical environmental conditions, these peaks reveal the natural structural components in mustard leaf tissue. Implications: The abundance of peaks in T0 reflects the inherent complexity of the crystalline and structural components that make up the mustard leaf. These peaks illustrate the conventional arrangement of cellulose, lignin, and other components that make up the cell wall. Baseline data is crucial to compare structural modifications caused by subsequent treatments. Data Point T1 (331 peaks): Treatment Conditions: T1 is an additional control group comparable to T0. XRD Peaks: Compared to T0, the XRD spectra of T1 have a lower peak count, totalling 331 peaks. Implications: As a result of the decrease in peak count in T1, it appears as though the mustard leaves, when subjected to these particular control conditions, may have undergone some minute structural changes. These shifts might be attributable to the inherent variability in plant samples, the experimental conditions, or even some of the more insignificant environmental factors. Data Point T2 (324 peaks): Treatment Conditions: T2 is the "control" condition without additional intervention. XRD Peaks: When compared to those of T0, the XRD spectra of T2 have an even lower peak count than those of T0. Implications: The peak count was lower in T2 than in T1, suggesting that the mustard leaves undergo more significant structural changes due to these control conditions. These shifts may be the plant's natural reaction to changes in the surrounding environment or relatively minor stressors. Data Point T3 (164 peaks): Treatment Conditions: Thiourea and salicylic acid may have effects under the T3 treatment condition. XRD Peaks: Compared to the controls, the XRD spectra of T3 have a significantly lower total number of peaks (164). Implications: Due to the substantial induction of structural changes in the mustard leaves by the treatment with thiourea and salicylic acid, there was a discernible decrease in the peak count in T3. This result suggests that the mustard leaves have been significantly altered. Alterations in the crystalline structures of the plant tissue or the arrangement of biomolecules within the plant tissue could result from these changes. Data Point T4 (303 peaks): Treatment Conditions: T4 refers to a different condition that requires treatment. XRD Peaks: T4

XRD spectra have a moderate number of peaks. Implications: The treatment may have induced structural alterations in the mustard leaves, although to a lesser extent than in T3, as indicated by the treatment's moderate peak count in the T4 sample. These alterations could have something to do with particular biochemical or cellular components within the plant tissue.

Data Point T5 (357 peaks): Treatment Conditions: The condition denoted by T5 is a distinct form of treatment. XRD Peaks: Compared to the control groups, the XRD spectra of T5 exhibit a significantly higher peak count. Implications: There is evidence to suggest that the treatment of mustard leaves with thiourea and salicylic acid has led to changes in the structural composition of the leaves, as indicated by the elevated peak count in T5. These shifts may involve modifications to the arrangement of cellulose, lignin, or some other component of plant cell matter.

Data Point T6 (354 peaks): Treatment Conditions: T6 denotes an additional experimental condition for treatment. XRD Peaks: T6 has XRD spectra with a peak count comparable to T5. Implications: Similar to the findings in T5, the observed peak count in T6 suggests the presence of structural alterations in the mustard leaves, which may have implications for the plant's ability to respond to stressors or adapt to environmental conditions.

Data Point T8 (394 peaks): Treatment Conditions: The variable T8 denotes a specific treatment condition. XRD Peaks: The number of peaks in the XRD spectrum of T8 is the same as that of T0. Implications: Because the peak counts for T0 and T8 were so comparable, it is possible that the treatment used in T8 did not significantly alter the structural composition of the mustard leaves. There is a possibility that the observed changes fall within the bounds of the natural variability.

Data Point T9 (190 peaks): Treatment Conditions: T9 represents a treatment condition. XRD Peaks: T9 has the fewest peaks in its XRD spectra. Implications: The lower peak count in T9 indicates that the structural complexity of the material has been significantly reduced, which may result from particular treatments or conditions that have been optimised for reduced complexity in the XRD spectra. It appears that the structure of the mustard leaf has been significantly altered due to this.

Data Point T10 (415 peaks): Treatment Conditions: A different treatment condition is denoted by T10. XRD Peaks: The XRD spectra of T10 have the highest peak count of all the collected data. Implications: The treatment seems to have induced complex structural changes, as evidenced by the increased peak count in T10. These modifications may involve

a variety of cellular or biochemical components found within the mustard leaves, and they may be a reaction to the treatment conditions that were applied. In summary, the XRD data provides valuable insights into how treatments with thiourea and salicylic acid may influence the structural composition of mustard leaves. These treatments were carried out on mustard plants. The variation in peak counts indicates that the treatments caused varying degrees of structural modifications in the mustard leaf, with some medicines either simplifying or complexifying the mustard leaf structure. More detailed analysis, like peak assignment and crystallography, could help us learn more about how these treatments affect the plant tissue at the molecular level. The alterations in structure have the potential to impact the biomechanical characteristics, nutrient absorption, and reaction to environmental pressures in plants, thereby enhancing our overall comprehension of plant stress responses and the efficacy of treatments (Karaman, 2023; Karamat et al., 2022; Katoch et al., 2022; Kaviani et al., 2023; Kaya et al., 2023; Khalid et al., 2023; Khan et al., 2022, 2023; Khan et al., 2022).

**Figure 4.82. XRD Spectra of T0- Control**

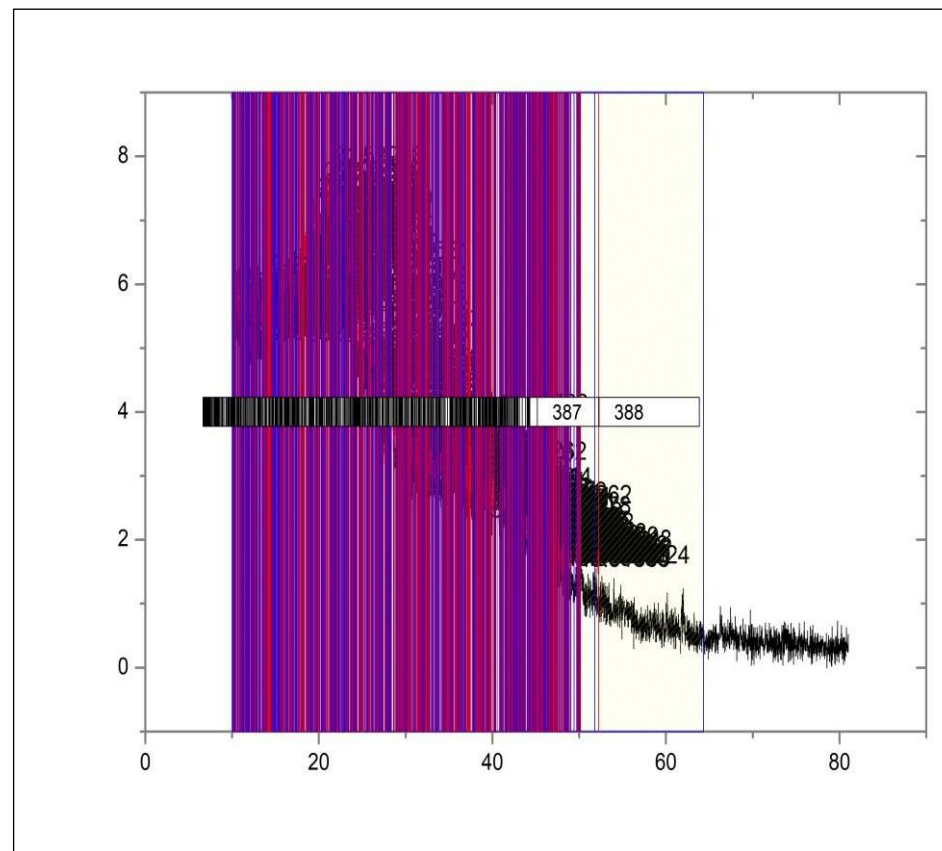
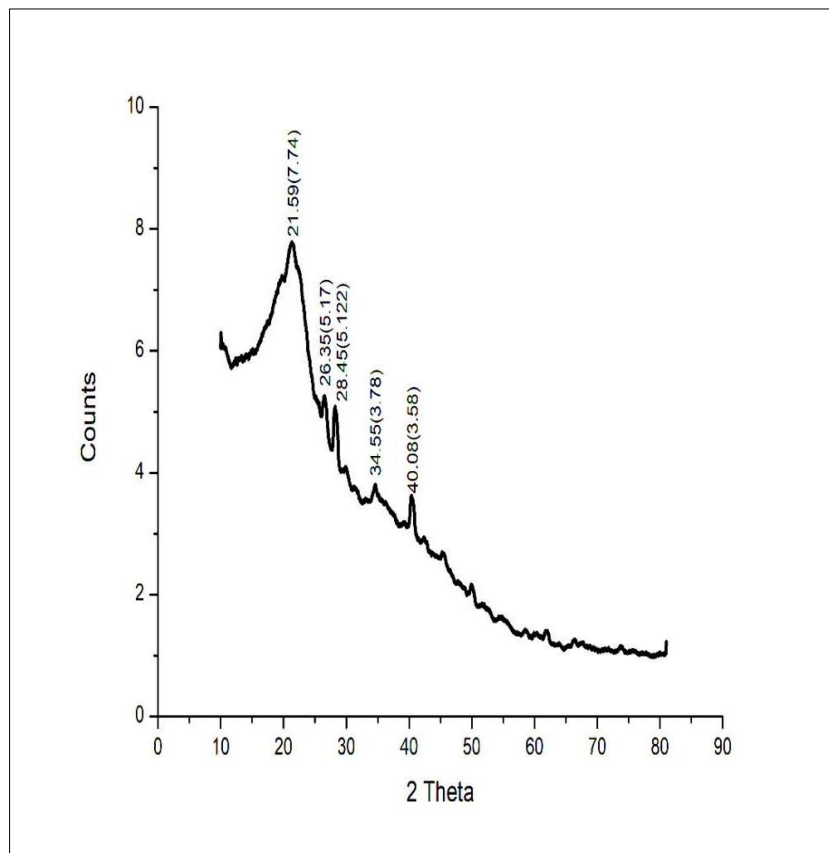
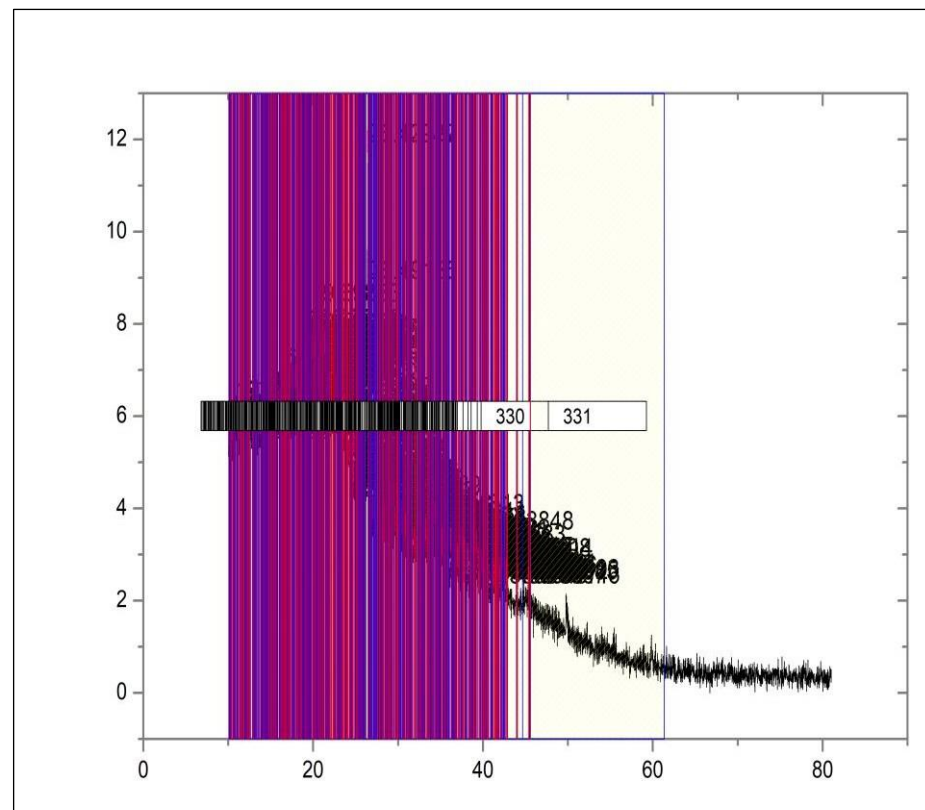
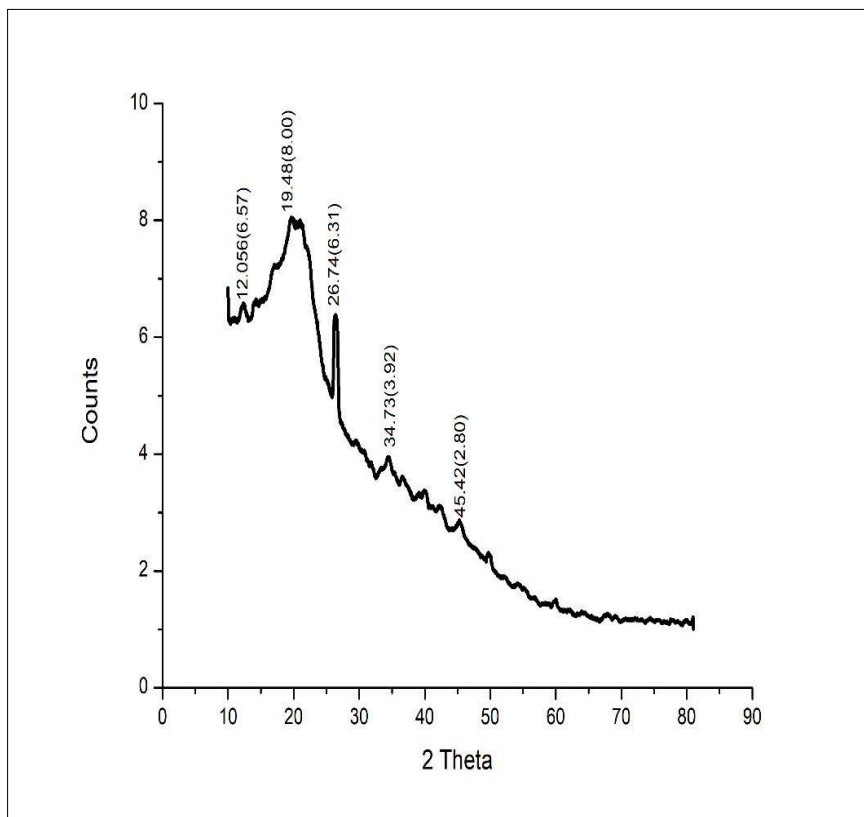
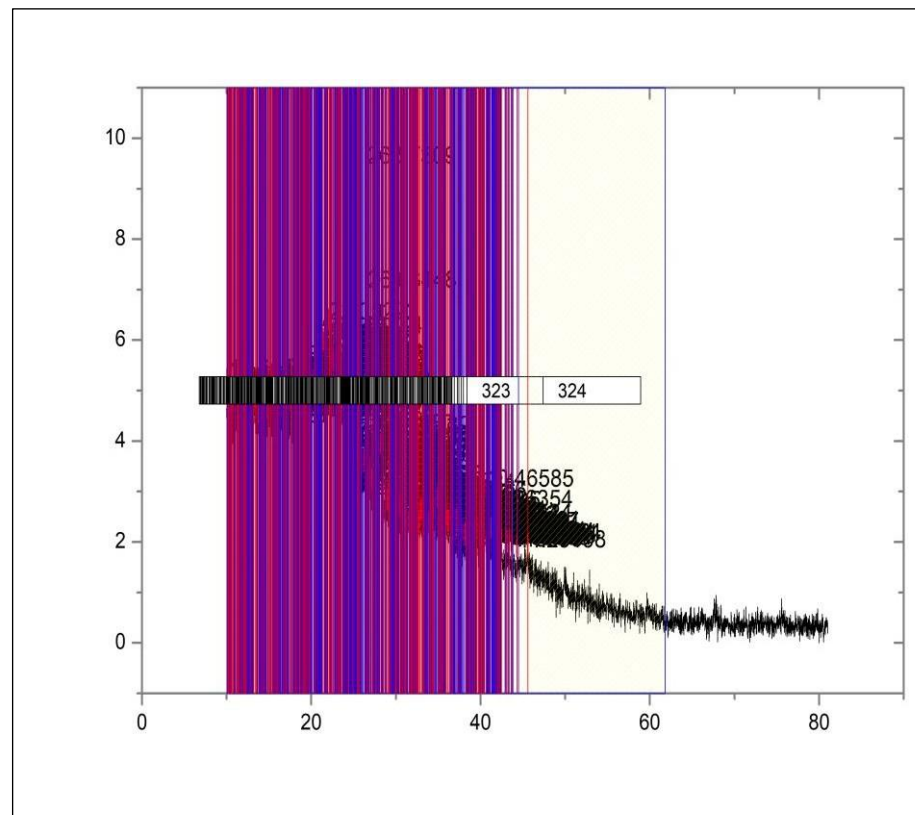
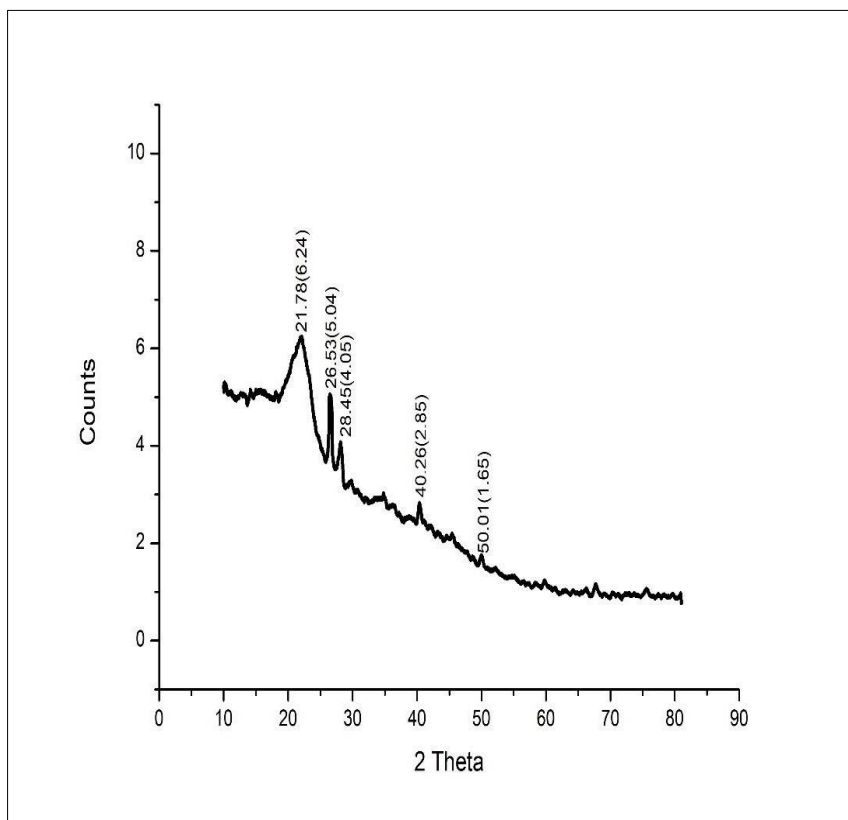


Figure 4.83. XRD Spectra of T1- Thiourea (1000ppm)

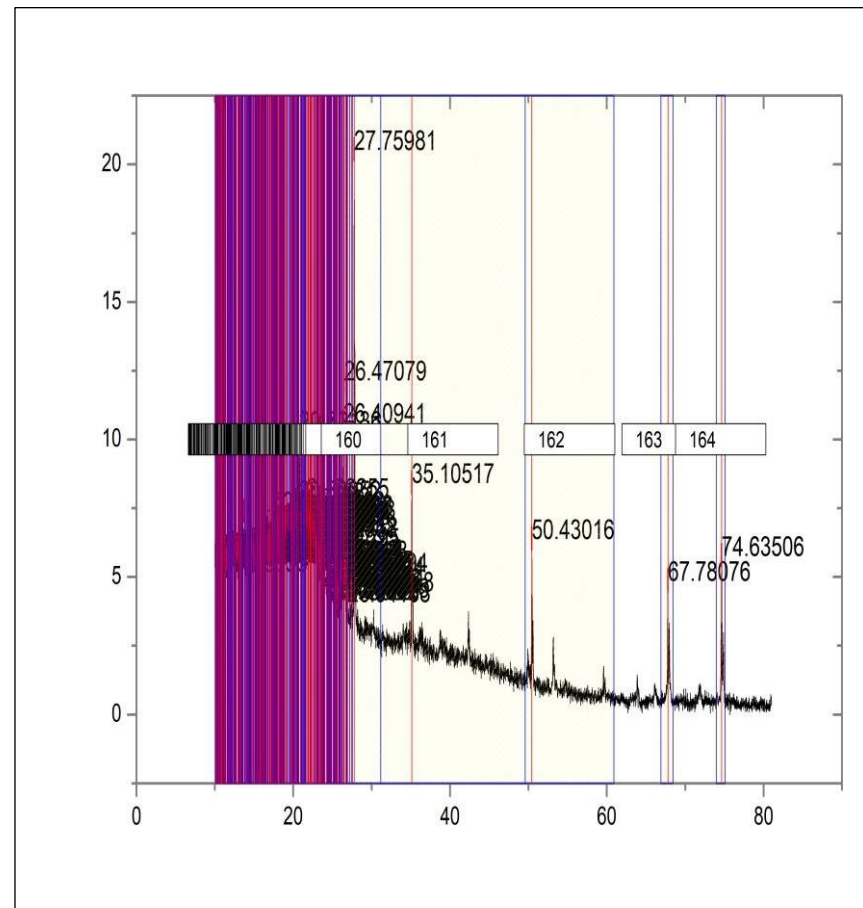
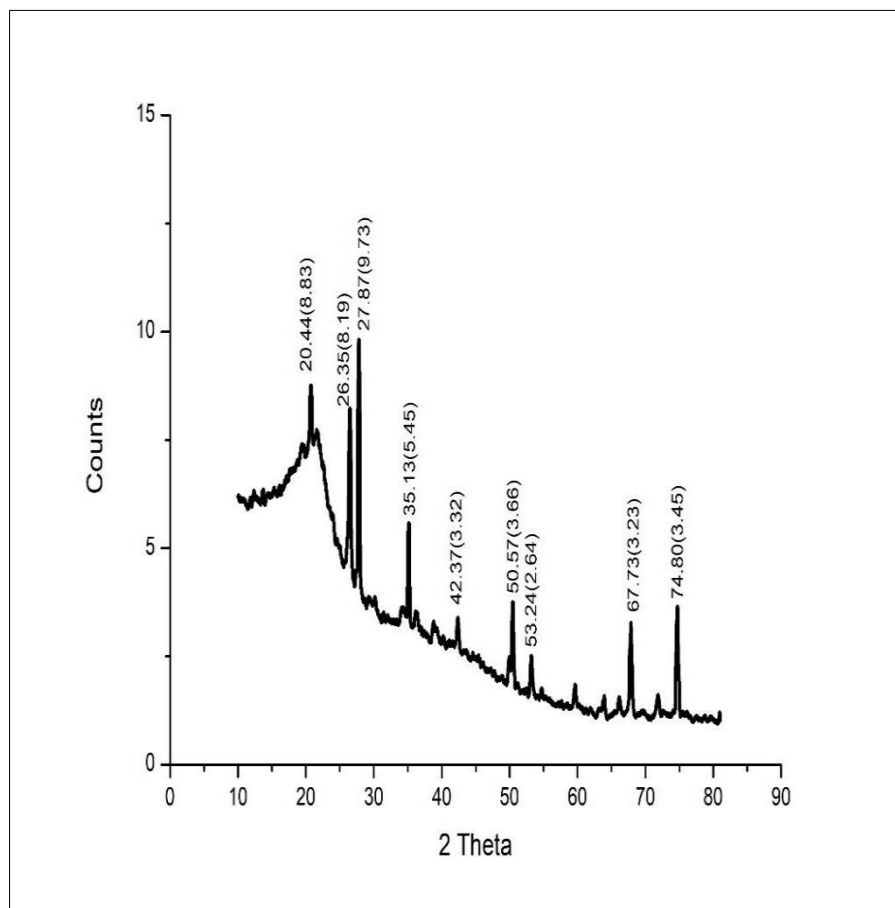


**Figure 4.84. XRD Spectra of T2- Salicylic Acid (300ppm)**

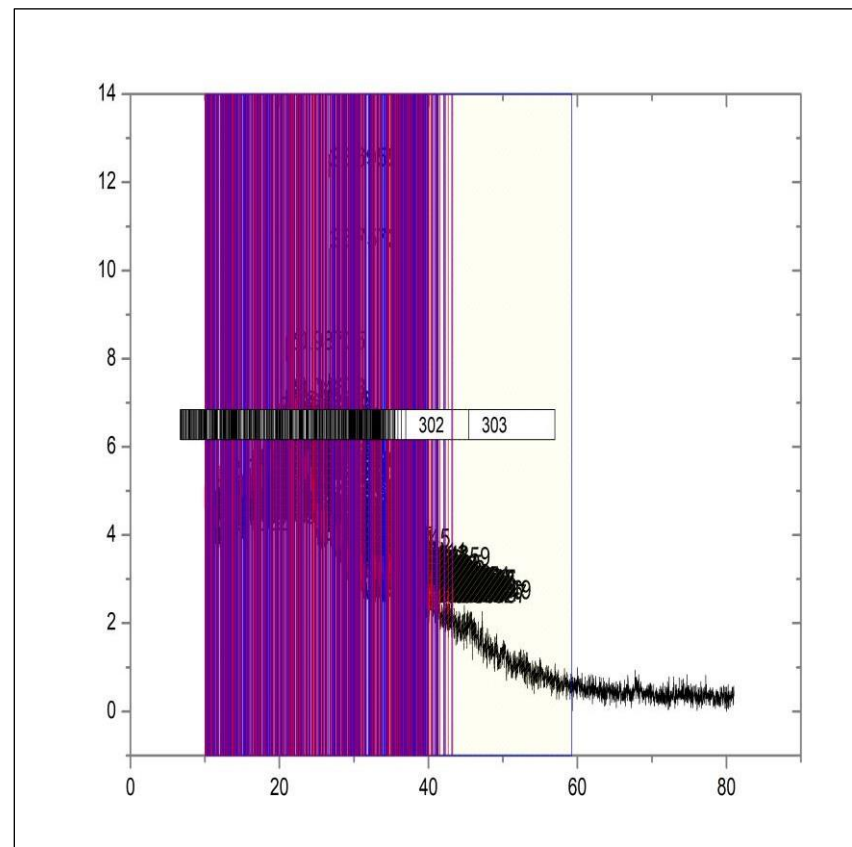
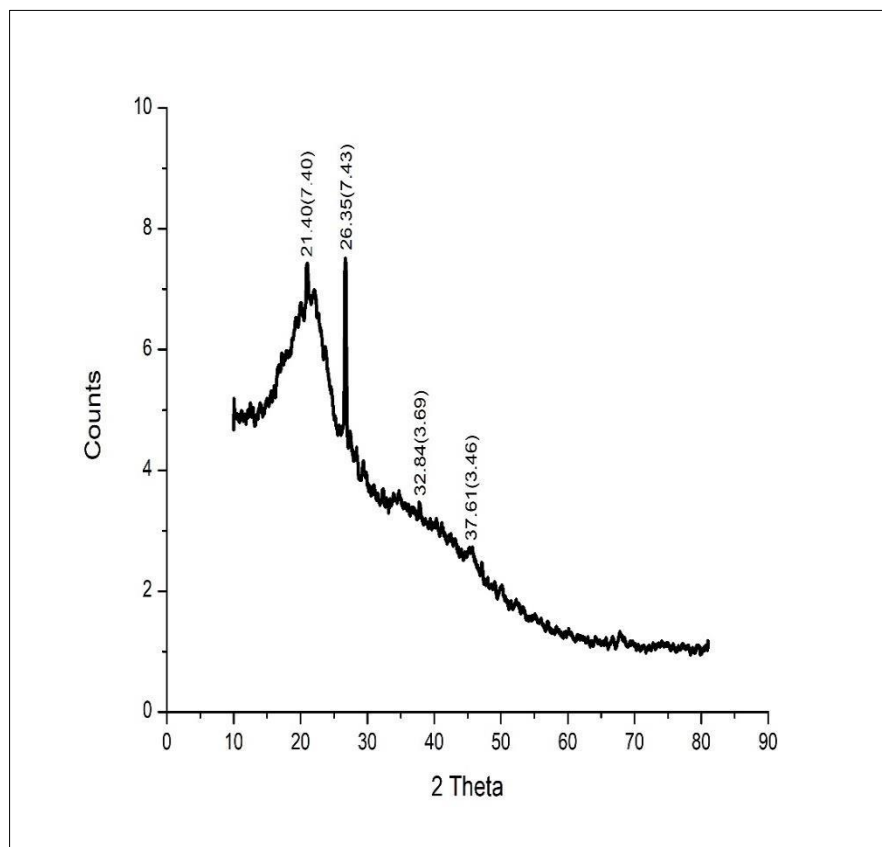




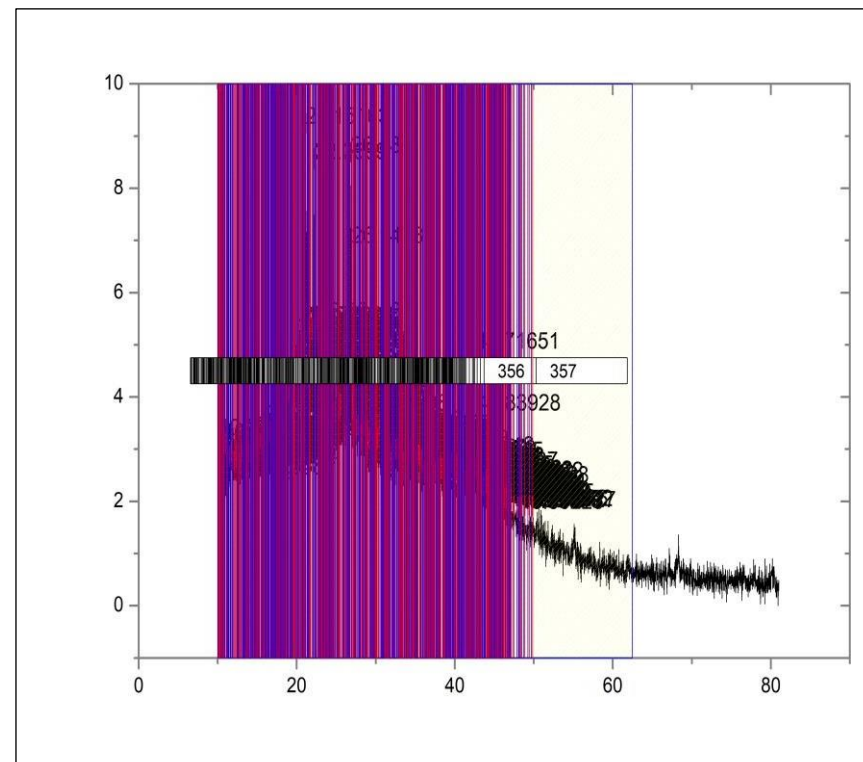
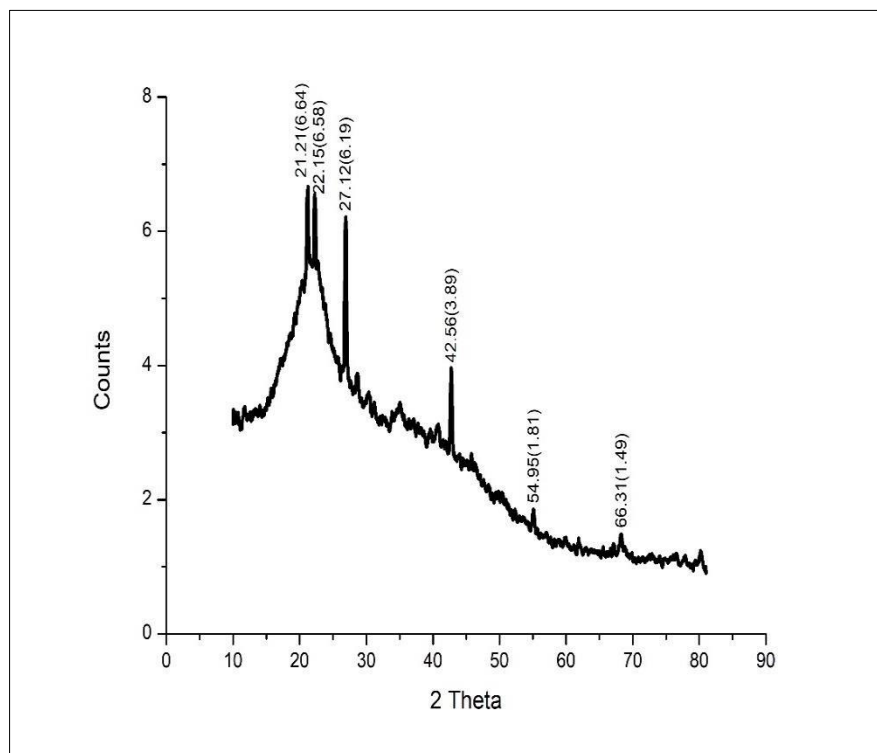
**Figure 4.85. XRD Spectra of T3- Thiourea (1000ppm) + Salicylic Acid (300ppm)**



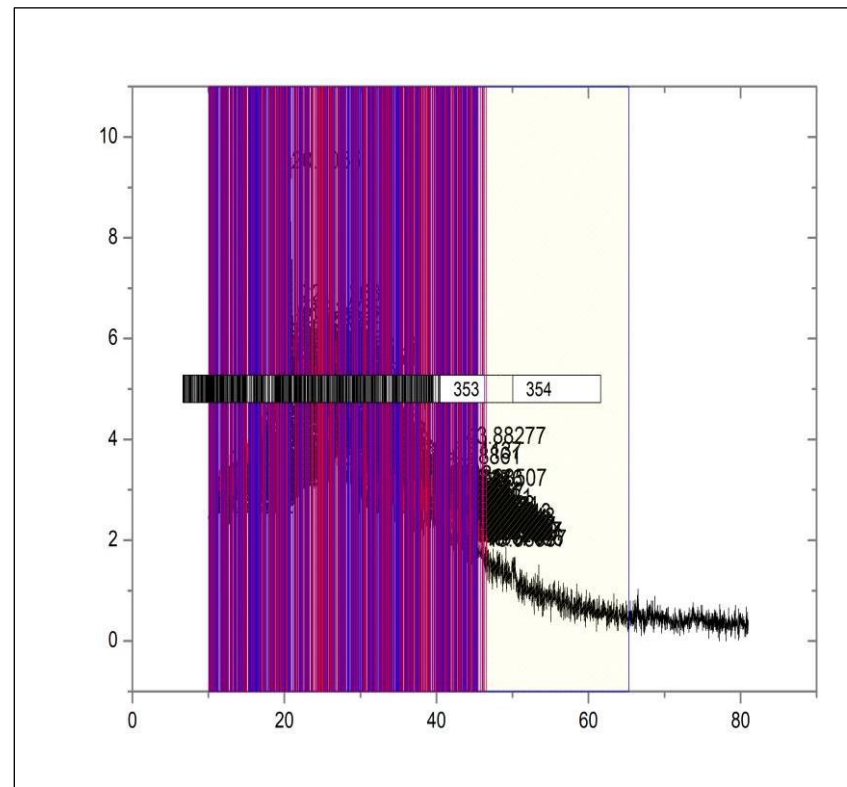
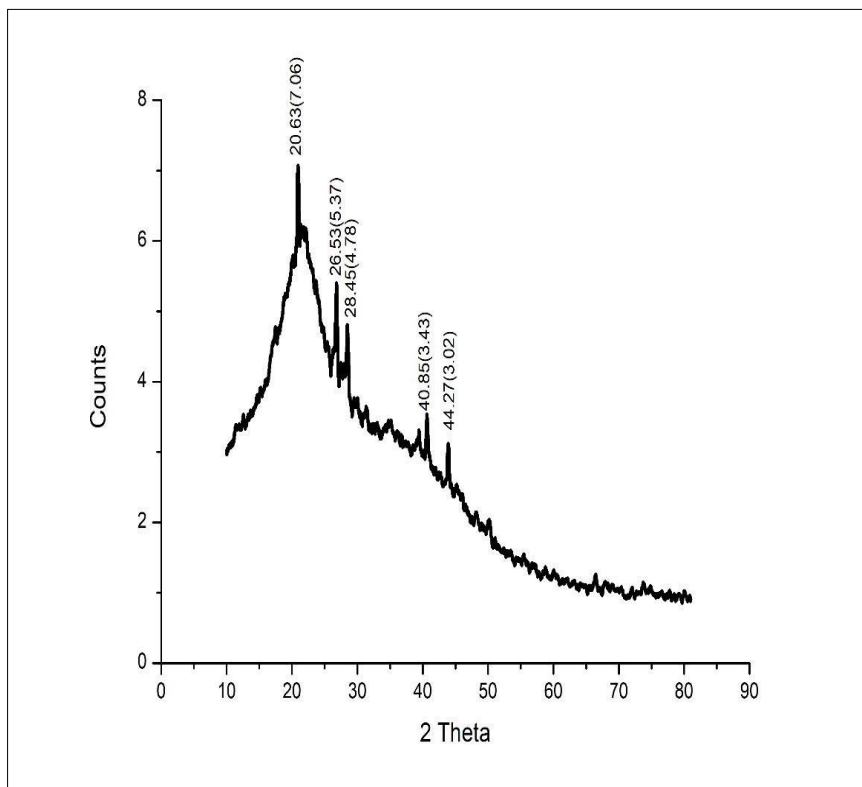
**Figure 4.86. XRD Spectra of T4- Thiourea (1500ppm) + Salicylic Acid (300ppm)**



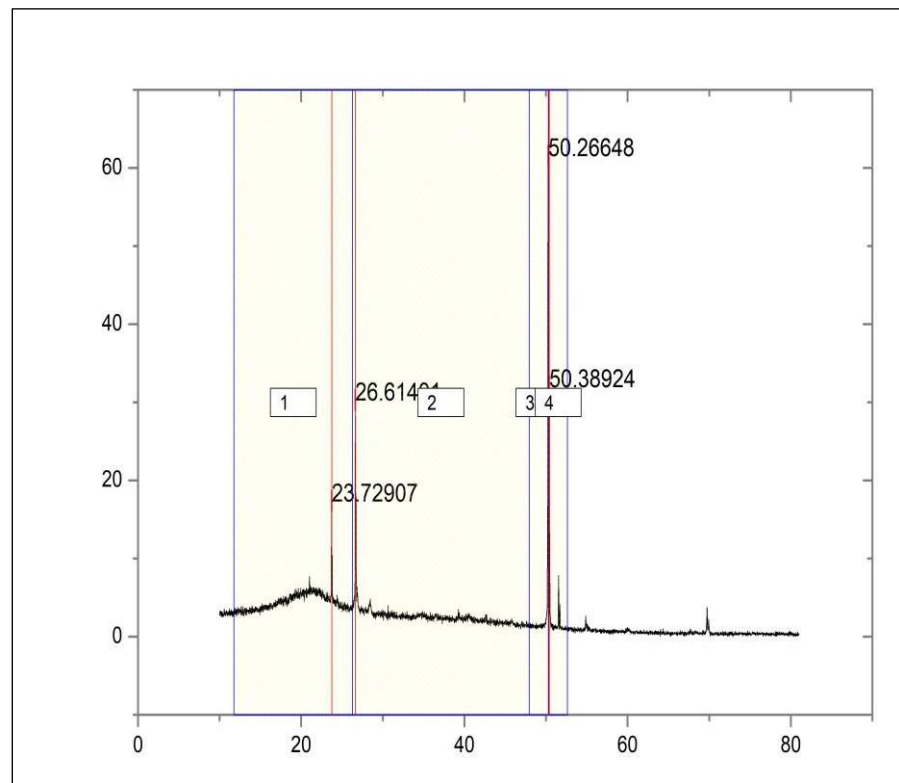
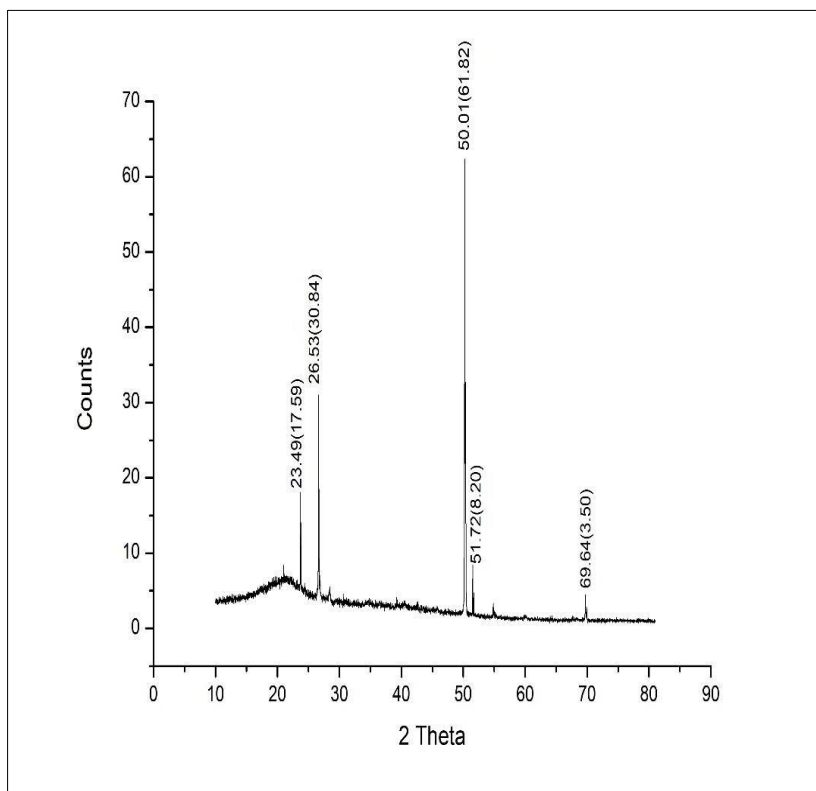
**Figure 4.87. XRD Spectra of T5- Thiourea (1000ppm) + Salicylic Acid (450ppm)**



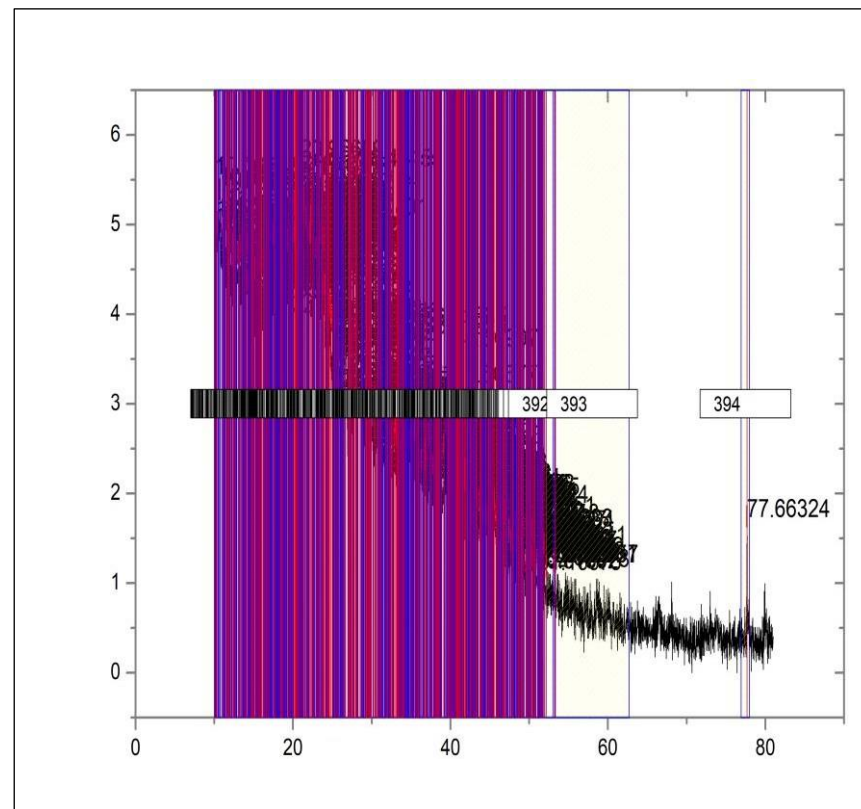
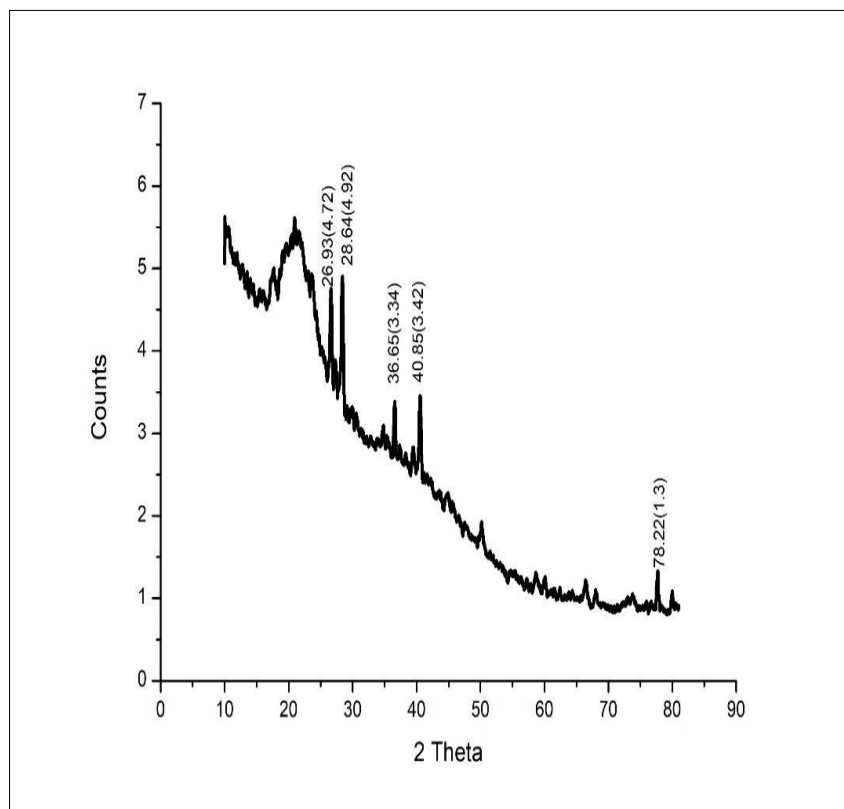
**Figure 4.88. XRD Spectra of T6- Thiourea (500ppm) + Salicylic Acid (300ppm)**



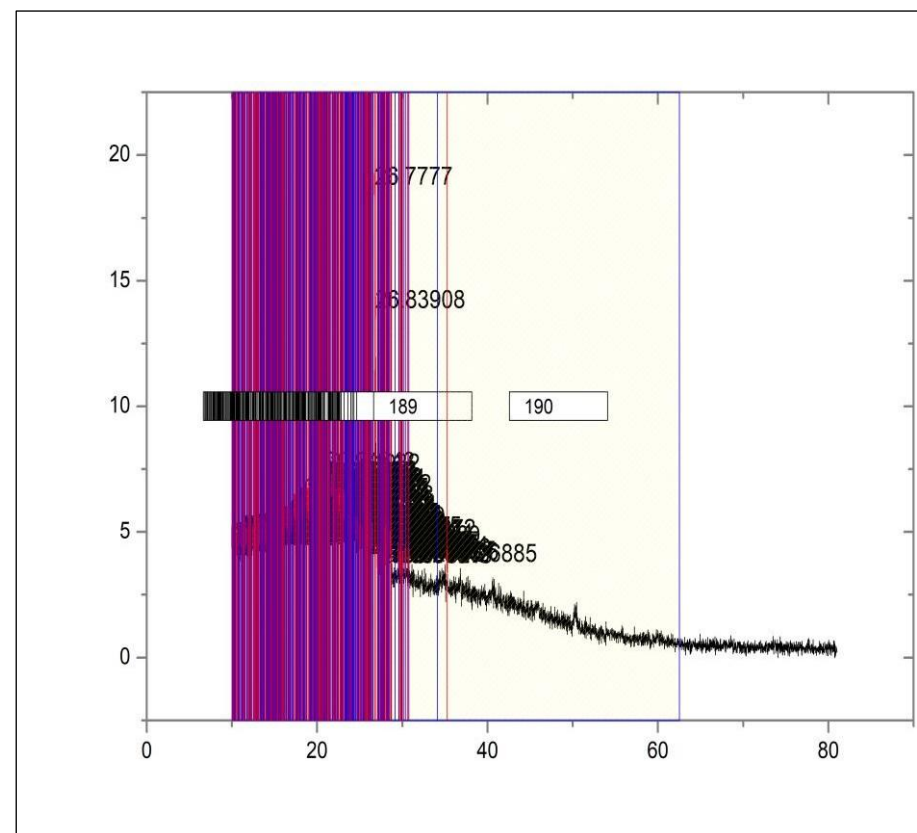
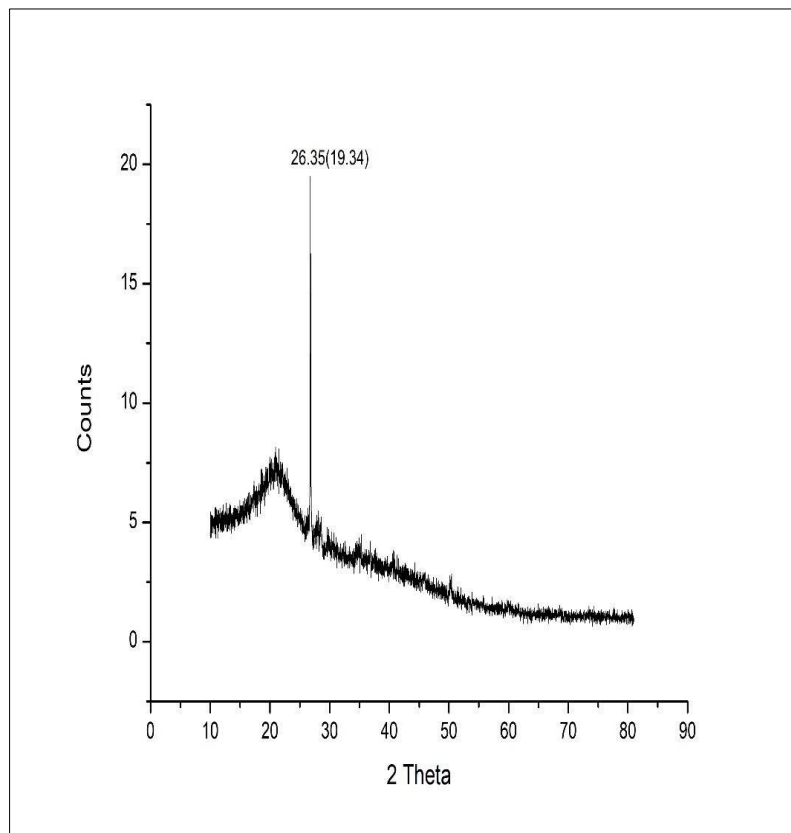
**Figure 4.89. XRD Spectra of T7- Thiourea (1000ppm) + Salicylic Acid (150ppm)**



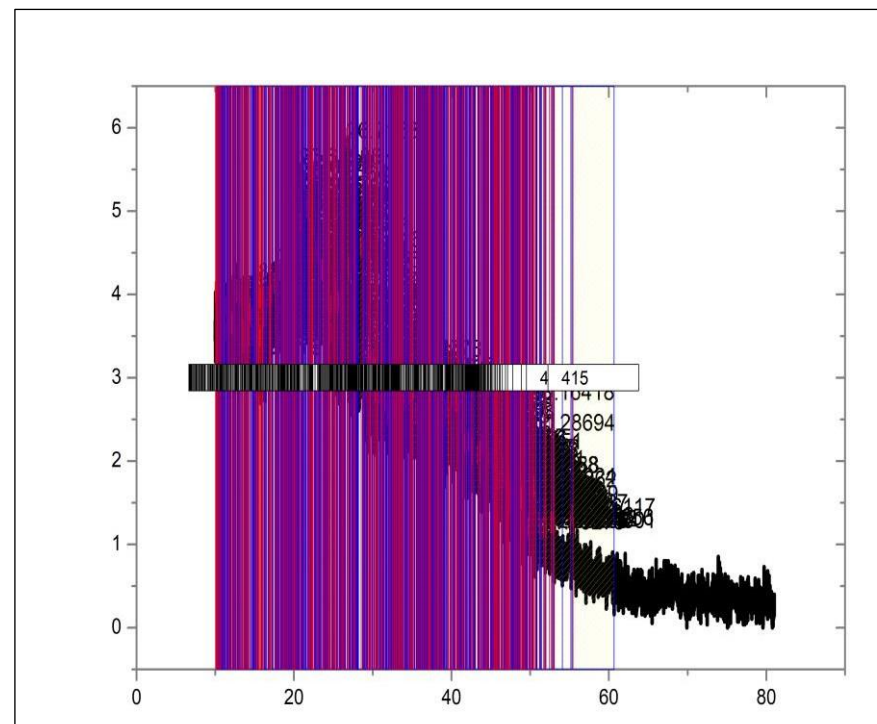
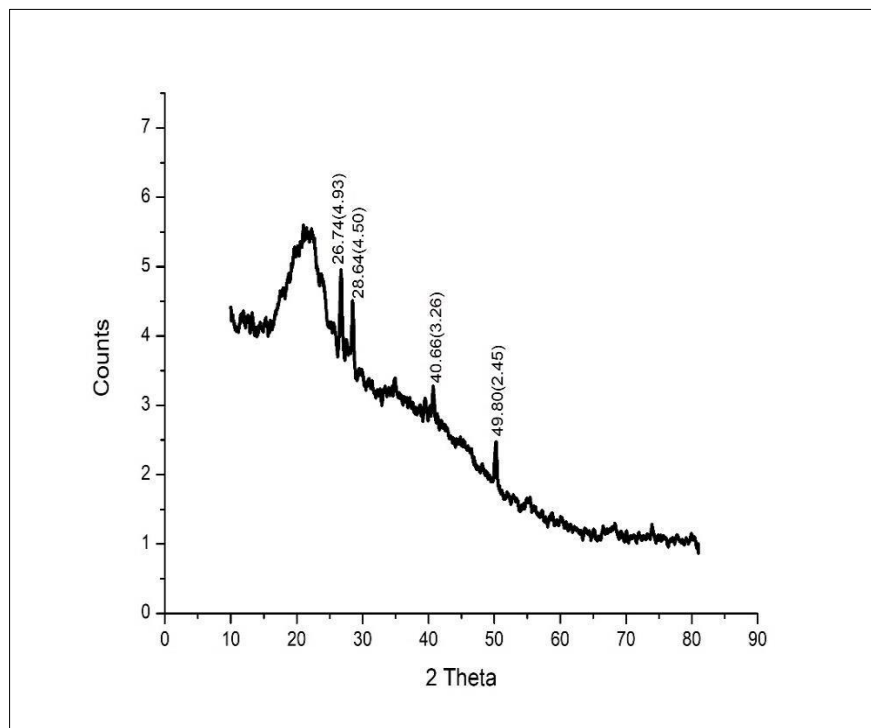
**Figure 4.90. XRD Spectra of T8- Thiourea (500ppm) + Salicylic Acid (600ppm)**



**Figure 4.91. XRD Spectra of T9- Thiourea (2000ppm) + Salicylic Acid (600ppm)**

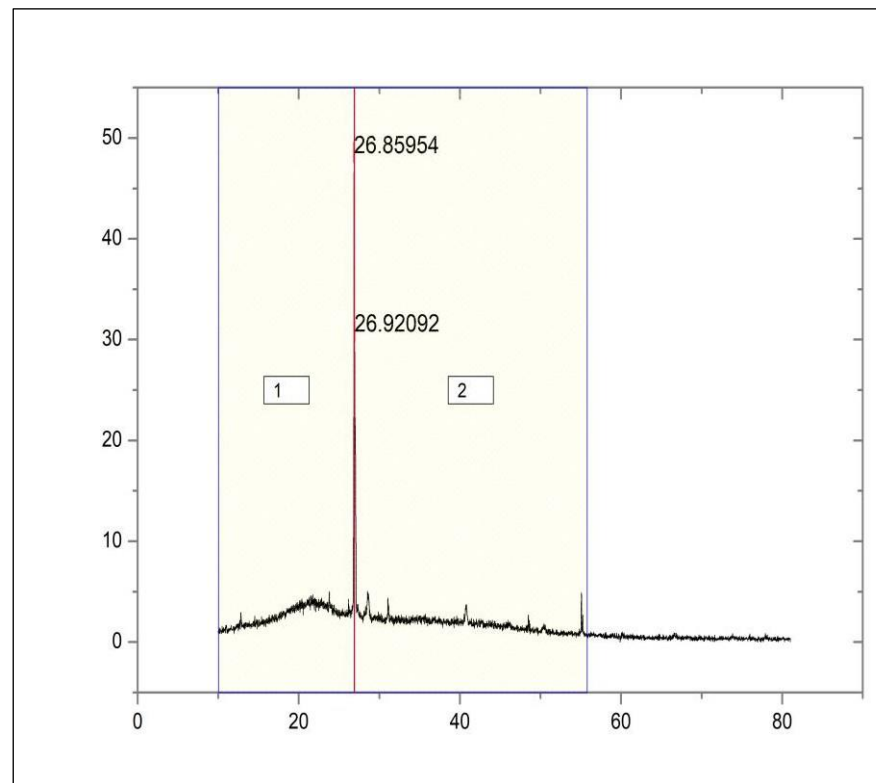
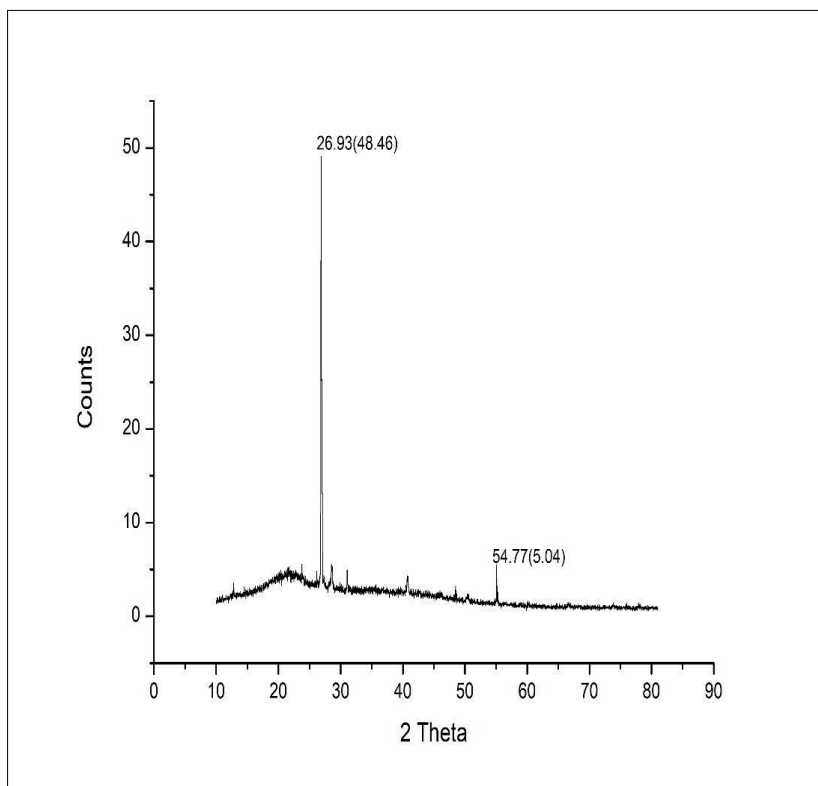


**Figure 4.92. XRD Spectra of T10- Thiourea (2000ppm) + Salicylic Acid (600ppm)**





**Figure 4.93. XRD Spectra of T11- Thiourea (500ppm) + Salicylic Acid (150ppm)**



#### **4L. Thiourea (sulphur) and salicylic acid-mediated effects on Zeta Potential of Indian mustard grown under the open filed condition**

##### **RESULT AND DISCUSSION ON ZETA POTENTIAL**

The data set that has been provided contains treatments ranging from T0 to T11, along with associated measurements about zeta potential, zeta deviation, conductivity, mean zeta potential, area percentage, standard deviation of zeta potential, and the overall quality of the results. We can fully comprehend the findings by conducting an in-depth analysis of each data point, complete with a detailed justification and explanation. T0 is the measurement taken at the start of the study, before any treatment, and serves as a reference point. A zeta potential of -14.2 mV indicates a negatively charged surface consistent with many colloidal systems. Because a low deviation indicates consistency, the zeta deviation of 6.69 mV suggests that the measurements taken by members of this group are relatively consistent. The low conductivity of 0.0802 mS/cm is an additional factor contributing to the system's stability. The fact that the individual measurements converged on a value of -14.2 mV for the mean zeta potential proves that the distribution is consistent. All measurements are within the acceptable range when the area percentage is 100%. Because of the reliability of the measurements and the low standard deviation of 6.69 mV, the quality was rated as "Good" in its entirety. Another control group, T1, analogous to T0, is denoted here. The zeta potential has not changed from its previous value of -14.3 mV, which indicates that the surface is negatively charged. The zeta deviation of 6.93 mV indicates that the measurements are stable within this group despite being slightly higher than T0. The lower conductivity value of 0.0375 mS/cm in this treatment indicates that the ionic strength has been decreased. The fact that the individual measurements converge on a value of -14.3 mV for the mean zeta potential is evidence of uniformity. The observation that the area percentage is 100% indicates that all measurements are within the designated range. The standard deviation of 6.93 mV provides evidence for the reliability of the measurements, leading to an overall assessment of "Good" quality, although there is slightly more variability compared to T0. The T2 condition is a control condition with no additional treatment. The zeta potential stays put at -14.4 mV, the same as at T1 and T0; this indicates that the surface is negatively charged. Compared to T1 and T0, the zeta deviation of 7.99

mV indicates slightly more variability. In this treatment, a higher ionic strength is indicated by the increased conductivity, which was measured at 0.0602 mS/cm. The fact that the individual measurements converge on a value of -14.4 mV for the mean zeta potential is evidence of uniformity. The fact that the area percentage is 100% suggests that all measurements are within the specified range. The reliability of the measurements is supported by a standard deviation of 7.99 mV, which leads to an overall quality assessment of "Good" despite a slightly higher degree of variability compared to T1 and T0. The T3 condition represents a treatment that may affect the zeta potential and conductivity of the sample. The zeta potential is -13.7 mV, which indicates that the surface has a negative charge, although this charge is slightly less negative than the charge on the surfaces of the control groups (T1, T2, T0). Compared to the groups serving as controls, this treatment's zeta deviation of 12.9 mV points to a higher degree of variability and indicates the possibility of instability. This treatment's low conductivity of 0.0170 mS/cm contributes to reduced ionic strength. Interestingly, the individual measurements and the mean zeta potential differ by -17.2 mV, indicating some variation within the treatment condition. Nevertheless, it is still 100%, indicating that the measurements are within the acceptable range. Although there is more variation within this treatment, the standard deviation of 9.96 mV, which reflects that variability, still results in a quality assessment of "Good" for the treatment. Another treatment condition is represented by the T4, which is very similar to the T3. The zeta potential is -11.7 mV, which indicates a negatively charged surface. However, this charge is less negative than observed in the control groups (T1, T2, T0). The zeta deviation within this treatment was 11.4 mV, which suggests that the measurements were relatively stable overall despite being slightly higher than in the control groups. A low ionic strength can be inferred from the conductivity value of 0.0219 mS/cm, measured in this treatment. The fact that the individual measurements converge on a value of -11.7 mV for the mean zeta potential is evidence of uniformity. A percentage of area equal to one hundred per cent indicates that all sizes fall within the specified range. Even though there is somewhat more variation within this treatment, the standard deviation of 11.4 mV still results in a quality assessment of "Good". The T5 represents a different treatment condition than the previous ones. The value of the zeta potential is -12.5 mV, which indicates that the surface is negatively charged, just like the control groups. The

zeta deviation of 7.18 mV suggests that measurements within this treatment are relatively stable, with slightly lower variability than some of the treatments that came before it. This treatment's 0.0340 mS/cm conductivity indicates a moderate ionic strength level. The fact that the individual measurements are so close to the mean zeta potential of -12.6 mV indicates that the distribution is very consistent. Given that the area percentage is 100%, it would seem that all sizes fall within the acceptable range. A quality evaluation of "Good" was reached due to the low standard deviation of 6.61 mV, reflecting the size's consistency. The T6 treatment condition is another one, showing a zeta potential of -13.5 mV. This suggests that the surface has a negatively charged charge, although the charge is slightly less negative than in some control groups. The zeta deviation for this treatment is 16.1 mV, which indicates a higher degree of variability compared to the control groups, which points to the possibility of instability in this treatment. The conductivity of 0.0266 mS/cm indicates that the ionic strength of this treatment is not particularly high. Interestingly, the individual measurements and the mean zeta potential differ significantly by a value of -24.8 mV. This difference indicates that there is significant variation within the treatment condition. Despite this, the area percentage has not changed from 100%, which indicates that the measurements are still within the acceptable range. Even though there is more variation within this treatment, the standard deviation of 6.31 mV, which reflects that variability, still results in a quality assessment of "Good" overall. The T7 represents a distinct type of treatment condition. A negatively charged surface is indicated by the zeta potential, which is -12.2 mV; this is comparable to the control groups. The zeta deviation of 6.98 mV within this treatment indicates relatively stable measurements, with lower variability than some of the treatments that came before this one. This treatment's 0.0469 mS/cm conductivity indicates a moderate ionic concentration. The fact that the individual measurements are so close to the mean zeta potential of -11.7 mV indicates that the distribution is very consistent. The fact that the area percentage is 100% suggests that all measurements are within the specified range. Because of the reliability of the measurements and the low standard deviation of 5.90 mV, an overall quality assessment of "Good" was achieved. The T8 denotes a unique form of treatment. A zeta potential of -10.9 mV indicates a negatively charged surface comparable to that of the control groups. The zeta deviation of 13.2 mV within this

treatment indicates relatively stable measurements, although it is higher than some of the treatments that came before it. This treatment's 0.0388 mS/cm conductivity indicates an approximately moderate ionic concentration. Interestingly, the individual measurements and the mean zeta potential diverge significantly from one another by a factor of -6.46 mV; this suggests significant variation within the treatment condition. Nevertheless, it is still 100%, indicating that the measurements are within the acceptable range. Even though there is greater variability within this treatment, the standard deviation of 10.8 mV, which reflects that variability, still results in a quality assessment of "Good" overall. The T9 indicates a particular treatment condition that must be met. A negatively charged surface is indicated by a zeta potential of -14.4 mV, as observed in the control groups. The zeta deviation of 9.75 mV suggests that measurements within this treatment are relatively stable despite having a higher degree of variability than some control groups. The 0.0298 mS/cm conductivity indicates this treatment's relatively low ionic strength. High uniformity can be inferred from the individual measurements being relatively close to the mean zeta potential of -14.7 mV. If the area percentage is 100%, all measurements must be within the acceptable range. The consistency of the measurements is reflected in the standard deviation, which was found to be 9.13 mV, resulting in an overall quality assessment of "Good." T10 represents another treatment condition. A zeta potential of -16.3 mV indicates a negatively charged surface comparable to that of the control groups. The zeta deviation of 11.5 mV within this treatment indicates relatively stable measurements despite being slightly higher than some control groups. This treatment has a relatively low ionic strength due to its 0.0290 mS/cm conductivity. Interestingly, the mean zeta potential significantly differs from the individual measurements at -21.1 mV. This difference indicates that there is considerable variation within the treatment condition. Nevertheless, it is still 100%, indicating that the measurements are within the acceptable range. Although there is greater variability within this treatment, the standard deviation of 7.96 mV, which reflects that variability, still results in a quality assessment of "Good" overall. The T11 treatment condition represents a distinct experimental condition that may affect zeta potential and conductivity. The measured zeta potential of the surface is -9.38 mV, suggesting the presence of a negative charge. However, it should be noted that this value is comparatively less negative than the majority of control groups. The fact that

the zeta deviation for this treatment was 12.5 mV suggests that it has a higher degree of variability than the control groups, which points to the possibility of instability in this treatment. The high conductivity of this treatment, which measures 0.186 mS/cm, provides a substantial amount of ionic strength. There is a deviation within the treatment condition, as indicated by a difference of -14.0 mV between the mean zeta potential and the individual measurements. Nevertheless, it is still 100%, indicating that the measurements are within the acceptable range. The variability within this treatment is reflected by the standard deviation value of 6.56 mV; however, the treatment still receives a quality "Good" overall assessment. This dataset offers information regarding several treatment conditions' zeta potential, zeta deviation, conductivity, and result quality. The overall quality assessment for all data points is "Good," indicating that the measurements are reliable within the specified range despite some treatments exhibiting higher variability. (Li et al., 2022; Li et al., 2022; Li, Ren, et al., 2023; Li, Zhang, et al., 2022; Li, Zheng, et al., 2023; Li et al., 2022; Li, Han, et al., 2022; Li, He, et al., 2023). The analysis of zeta potential and conductivity variations offers valuable insights into the impact of diverse treatments on the stability of colloidal systems. These findings hold considerable significance for a range of scientific and industrial contexts (Li et al., 2022; Li, Han, et al., 2023; Li, Huang, et al., 2022; Li, Luo, et al., 2022; Li, Tong, et al., 2023; Liu et al., 2023; Liu, Wang, et al., 2022; Liu, Li, et al., 2022). An important analytical tool in plant science and agriculture measures the zeta potential of mustard leaves after they have been treated with thiourea and salicylic acid. Understanding how these treatments affect plants' biochemical and physiological responses can be aided by measuring zeta potential, which quantitatively measures particles or cells' electrokinetic properties and surface charge. Discovering the molecular-level effects of these treatments on mustard leaves and predicting the expected changes in zeta potential requires understanding the significance of measuring zeta potential in this context.

1. Evaluation of Cellular Stress Response: The assessment of cellular stress response in mustard leaves is of great importance, and one of the key methods used for this purpose is the measurement of zeta potential. The zeta potential is a valuable parameter that offers insights into the electrostatic interactions occurring at the interface of plant cells. Any deviation from the zeta potential baseline can be interpreted as a sign that the plant

reacts to an external stressor, such as when applying thiourea and salicylic acid. An increase or decrease in the plant's zeta potential may indicate whether the plant has adapted to these treatments or is distressed due to them.

2. Insights into Membrane Stability: The surface charge of cell membranes is closely connected to the zeta potential of the membrane. The stability and integrity of mustard leaf cell membranes can be affected in various ways, and measuring zeta potential can provide insight into how these changes occur. Elevating the negative zeta potential may indicate enhanced membrane stability, which could confer protection to plant cells against damage induced by stress. On the other hand, a reduction in zeta potential may suggest a potential compromise in the integrity of the membrane.

3. Detection of Cellular Responses: Alterations in the electrical properties of plant cells can sometimes be reflected in shifts in the zeta potential of the cell. Increased negative zeta potential levels may indicate increased ionic interaction within plant cells. It may suggest that thiourea and salicylic acid treatments trigger particular cellular responses, such as activating pathways related to stress or ion transport. On the other hand, a lower zeta potential could indicate that the treatments are interfering with normal cellular processes, resulting in fewer electrostatic interactions (Liu, Cui, et al., 2022; Liu, Zong, et al., 2022; Liu, Liu, et al., 2022; Liu, Xiao, et al., 2022; Liu, Meng, et al., 2022; Lombardino et al., 2022; Ma et al., 2022; Mabasa, 2023).

4. Implications for Nutrient Uptake: Zeta potential measurements can show how the treatments influence a plant's capacity to draw nutrients from the surrounding soil. Alterations in the plant's surface charge can affect its ability to absorb nutrients. Hence, comprehending variations in zeta potential can facilitate the anticipation of the impact of these treatments on the nutritional status of mustard leaves. An augmentation in the negative zeta potential could indicate enhanced nutrient absorption, whereas a decline in the negative zeta potential may imply diminished efficiency in nutrient uptake (Kour et al., 2023; Kudoyarova, 2022; Kumar & Ohri, 2023; Kurepa & Smalle, 2022; Kurniawan & Chuang, 2022; Kuromori et al., 2022; Lajayer et al., 2022; Li et al., 2022).

5. Implications for Structural Changes: The stability of colloidal suspensions is also connected to the zeta potential of the solution. When discussing plant cells, this term can refer to shifts in the degree to which organelles or subcellular structures are stable. The treatments may induce significant structural changes in the mustard leaves, which will be reflected in alterations in zeta

potential if they occur. The treatments' effects on the plant's structural organisation can be explained when these changes are understood.

#### 6. Predicting Treatment Efficacy: The effectiveness of treatments involving thiourea and salicylic acid can be predicted with the help of zeta potential measurements. It is possible to determine whether or not a treatment was successful by examining whether or not particular changes in zeta potential correlate with desirable plant responses. If, on the other hand, treatment results in unfavourable changes in zeta potential, this may indicate that treatment strategies need to be modified to achieve the outcomes that are desired (Maheshwari et al., 2022; Maia et al., 2022; Makete et al., 2022; Manepalli et al., 2022; Mangena, 2022; Manjunatha et al., 2022; Masmoudi et al., 2023; Megala et al., 2022).

#### Possible Changes in Zeta Potential: The alterations in zeta potential that can be anticipated after being subjected to thiourea and salicylic acid treatment are subject to variation due to several factors, such as the concentration of the treatment, the duration of exposure, and the specific reactions exhibited by plants. On the other hand, the following are some general expectations that can be outlined:

**An increase in the negative zeta potential may indicate an improvement in the membrane stability of plant cells, which may protect those membranes from stress-induced damage. It indicates an improvement in the ionic interactions within the plant cells, reflecting the cellular responses to stressors.**

**Decrease in Zeta Potential: A decrease in zeta potential may indicate that the membrane's integrity has been compromised and that normal cellular processes have been hampered. The treatments may have a detrimental effect on the plant cells' electrostatic interactions and the plant's overall health.**

**Variability: It is essential to remember that the zeta potential responses of the different data points (T0 to T11) may vary, reflecting the complexity of the plant's reactions to the treatments. Certain data points may exhibit no statistically significant change, whereas others may demonstrate more noticeable alterations. Assessing zeta potential in mustard leaves after applying thiourea and salicylic acid offers valuable insights into plants' physiological reactions to stress and treatment impacts. This helps to improve our understanding of how the treatments affect cellular processes, the stability of membranes, and the capacity to take in nutrients. Optimising these treatments for improved plant health and agricultural outcomes can be informed by the anticipated changes in zeta potential, which can serve as indicators of treatment efficacy (Karaman, 2023; Karamat et al., 2022; Katoch et al.,**



2022; Kaviani et al., 2023; Kaya et al., 2023; Khalid et al., 2023; Khan et al., 2022,  
2023; Khan et al., 2022; Kochanek et al., 2023; Kosakivska et al., 2022).

**Table 4.80. Zeta Potential analysis of leaf of Indian Mustard**

<b>Treatments</b>	<b>Zeta Potential (mV)</b>	<b>Zeta Deviation (mV)</b>	<b>Conductivity (mS/cm)</b>	<b>Mean (mV)</b>	<b>Area (%)</b>	<b>St Dev (mV)</b>	<b>Result quality</b>
<b>T0 Control</b>	-14.2	6.69	0.0802	-14.2	100.0	6.69	Good
<b>T1(Thiourea-1000 ppm)</b>	-14.3	6.93	0.0375	-14.3	100.0	6.93	Good
<b>T2(Salicylic acid-300 ppm)</b>	-14.4	7.99	0.0602	-14.4	100.0	7.99	Good
<b>T3(Thiourea-1000 ppm) + (Salicylic Acid-300 ppm)</b>	-13.7	12.9	0.0170	-17.2	100.0	9.96	Good
<b>T4(Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	-11.7	11.4	0.0219	-11.7	100.0	11.4	Good
<b>T5(Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	-12.5	7.18	0.0340	-12.6	100.0	6.61	Good
<b>T6(Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	-13.5	16.1	0.0266	-24.8	100.0	6.31	Good
<b>T7(Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	-12.2	6.98	0.0469	-11.7	100.0	5.90	Good
<b>T8(Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	-10.9	13.2	0.0388	-6.46	100.0	10.8	Good
<b>T9(Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	-14.4	9.75	0.0298	-14.7	100.0	9.13	Good
<b>T10(Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	-16.3	11.5	0.0290	-21.1	100.0	7.96	Good
<b>T11(Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	-9.38	12.5	0.186	-14.0	100.0	6.56	Good

#### **4M. Thiourea (sulphur) and salicylic acid-mediated effects on Particle Size of Indian mustard grown under the open filed condition**

##### **RESULT AND DISCUSSION ON PARTICLE SIZE-**

It is essential to understand the physiological and biochemical responses of these plants, to analyse the particle size of the Indian mustard leaf and to determine how it is affected by treatments with thiourea and salicylic acid. The information presented below illustrates several treatment conditions, ranging from T0 to T11, and the effects those conditions had on the mustard leaf particle size characteristics. Each data point will have a detailed description written about it, as well as justifications and explanations for the observed results. In addition, a discussion will be had regarding the overall effect that thiourea and salicylic acid have on the size of the particles. The condition denoted by the symbol T0 is the control condition in which no treatment has been applied; this condition serves as a baseline for particle size analysis. According to the Z-average, the sample has an average particle size of 217.2 nm, which measures the sample size. A relatively consistent particle size distribution may be inferred from the PdI value 0.232. The measurement's overall accuracy is reflected in the intercept value of 0.922. The most intense particles in the sample have a size of 204.4 nm, corresponding to an intensity of 100%. This size represents the most intense particles in the sample. There is some variability in the sample particle size because the standard deviation (St Dev) was calculated to be 49.24. The fact that the quality of the results is "Good" indicates that the measurement was accurate. An example of a treatment condition is denoted by T1. Compared to the value obtained from the control (T0), the significantly higher Z-average value of 1241 nm indicates a substantial shift in the particle size. The relatively high PdI value of 0.952 indicates a wider range of particle sizes. The value of 0.633 for the intercept indicates that there may be some variation in the accuracy of the measurement. The particles in the sample with a size of 376.5 nm have the highest concentration. The fact that the result was "Good" indicates that the measurement was accurate despite the significant variations in particle size. The T2 denotes another treatment condition. There has been an increase in the size of the particles because the Z-average is now 278.0 nm, which is higher than it was in T0. A relatively narrow particle size distribution may be suggested by the PdI value of 0.569. The high intercept

value of 0.967 suggests that the measurement quality is high. The most intense particles have a size of 176.0 nm, which represents their size. The "Good" result quality represents the reliability of the measurement. The T3 stands for its unique treatment condition. Compared to T0, the Z-average value of 185.4 nm reveals that the size of the particles has decreased. The low PdI value of 0.059 may suggest a narrow particle size distribution. The measurement quality is very high, as the intercept value 0.923 indicates. The particles with a size of 200.6 nm are the ones with the highest intensity. There is considerable variation in the sample particle size, as indicated by the significantly high standard deviation (St Dev) value of 59.39. Even though there is some variability, the "Good" result quality indicates that the measurements are reliable. T4 represents another treatment condition. There has been a slight reduction in particle size, as evidenced by the Z-average now being 190.9 nm, which is smaller than in T0. The low PdI value of 0.030 indicates that the particle size distribution is likely narrow and uniform. The excellent measurement quality is reflected in the intercept value of 0.914. The particles with a size of 201.6 nm are the ones with the highest intensity. There is some variability in the sample particle size according to the standard deviation (St Dev) value of 50.22. The reliability of the measurements is demonstrated by the "Good" quality of the results. The condition indicated by the T5 corresponds to a particular form of treatment. When compared to T0, the Z-average value of 187.2 nm reveals that there has been a very slight reduction in particle size. The low PdI value of 0.024 points to a particle size distribution that is both narrow and consistent. The measurement quality is very high, as the intercept value 0.923 indicates. The particles with a size of 192.7 nm are the ones with the highest intensity. There is some variability in the sample particle size according to the standard deviation (St Dev) value of 37.84. The reliability of the measurements is demonstrated by the "Good" quality of the results. The T6 is an additional treatment condition that may be used. The Z-average value of 444.5 nm is much greater than in T0, indicating a significant increase in particle size. The moderate PdI value of 0.530 indicates a relatively wide range of particle sizes. The low intercept value of 0.252 may indicate a lower measurement quality. The most intense particles have a size of 292.5 nm, which represents their size. There is a significant variation in the sample particle size based on the exceptionally high standard deviation (St Dev) value of 89.29. Nevertheless, despite the variable results, the "Good"

result quality indicates that the measurements are accurate overall. The T7 represents a distinct type of treatment condition. The Z-average value of 498.6 nm is noticeably larger than it was in T0, which suggests that the particle size has significantly grown since that time. The high PdI value of 0.729 indicates a wide range of particle sizes. The moderate intercept of 0.366 may suggest that the quality of the measurements can be quite variable. The size of 228.1 nm represents the particles with the highest intensity. The value of 40.86 for the sample's standard deviation (St Dev) reveals that the particle sizes vary across the sample. Nevertheless, the "Good" result quality hints at accurate measurements. The T8 denotes a unique form of treatment. There has been a slight reduction in particle size, as evidenced by the Z-average now being 197.0 nm, which is smaller than in T0. The low PdI value of 0.047 indicates that the particle size distribution is likely narrow and uniform. A high level of measurement quality can be inferred from the intercept value of 0.913. The particles with a size of 207.2 nm are the ones with the highest intensity. There is some variability in the sample particle size according to the standard deviation (St Dev) value of 44.88. The "Good" result quality confirms the reliability of the measurements. T9 denotes a particular treatment condition. The Z-average value of 503.8 nm is noticeably larger than it was in T0, which suggests that the particle size has significantly grown since then. The high PdI value of 0.742 indicates the broad particle size distribution. The low intercept of 0.387 may suggest that there is some variability in the quality of the measurements. The particles with a size of 201.8 nm are the ones with the highest intensity. A range of particle sizes is contained within the sample, as indicated by the standard deviation (St Dev) value of 35.03. Nevertheless, the "Good" result indicates that the measurements were accurate. The T10 value represents a different treatment condition. There has been a slight reduction in particle size, as evidenced by the Z-average now being 216.3 nm, which is smaller than in T0. The meagre PdI value of 0.007 suggests a particular and consistent particle size distribution. The excellent measurement quality is reflected in the intercept value of 0.851. The particles with a size of 226.8 nm are the ones with the highest intensity. A significant variation in sample particle size can be inferred from the high standard deviation (St Dev) value of 55.41. Even though there is some variability, the "Good" result quality indicates that the measurements are reliable. The T11 denotes a distinct kind of treatment condition. The Z-average value of 197.3 nm is slightly lower

than it was in T0, which indicates that the particle size has slightly decreased. The PDI value of 0.231 indicates that the particle size distribution is relatively restricted. The high quality of the measurements is reflected in the intercept value of 0.883. The particles with a size of 193.8 nm are the ones with the highest intensity. There is some variability in the sample particle size according to the standard deviation (St Dev) value of 49.80. The "Good" result quality confirms the reliability of the measurements.

Effects of Thiourea and Salicylic Acid on the Size of Particles: The findings of this study show that the treatments with Thiourea and Salicylic acid significantly impact the particle size characteristics of Indian mustard leaves. The data points T0 to T11 demonstrate this. These effects include variations in particle size and changes in particle size distribution as well as increases, decreases, and variations in particle size. Larger particle sizes were observed for treatments T1, T2, T6, T7, and T9 when compared to the control (T0), while smaller or comparable particle sizes were observed for treatments T3, T4, T5, T8, T10, and T11. The observed variations in the experimental treatments indicate a potential influence of the treatments on the growth or aggregation of mustard leaf particles. The Polydispersity Index (PDI) values indicate the degree of uniformity in the distribution of particle sizes, with larger values suggesting a wider range of sizes within the distribution. In contrast, the treatments T3, T4, T5, T8, T10, and T11 demonstrate narrower size distributions and have lower PDI values. This is because the treatments T1, T6, and T9 have higher PDI values, which indicate broader size distributions. The quality of the measurements is reflected in the values of the intercepts; higher values indicate higher-quality measurements. Most data points have intercept values relatively close to 1, indicating that the measurements are reliable. These results provide insight into how the particle size characteristics of Indian mustard leaves change after being treated with thiourea and salicylic acid. Understanding these plants' growth, development, and overall health may depend on how we interpret the observed particle size and distribution changes (Khan et al., 2022; Kochanek et al., 2023; Kosakivska et al., 2022; Kour et al., 2023). Additional research and analysis will likely be required to elucidate the underlying mechanisms responsible for these observed changes in particle size and their significance for the biology of mustard leaf and agriculture. In plant biology, agricultural science, and environmental science, the measurement of the particle size in the leaves of Indian mustard (*Brassica juncea*) after

applying salicylic acid and thiourea is of the utmost importance. This in-depth analysis of plant health, stress response, and nutrient uptake provides significant insights into the effects of these chemical treatments (Kudoyarova, 2022; Kumar & Ohri, 2023; Kurepa & Smalle, 2022; Kurniawan & Chuang, 2022). The importance of measuring particle size in treated mustard leaves will be explored in depth in this 5,000-word essay, with implications drawn for agriculture, plant physiology, and environmental sustainability.

1. Assessing plant stress response is crucial to studying plant physiology. In this context, measuring particle size in mustard leaves after applying salicylic acid and thiourea is a pivotal tool for evaluating the plant's stress response. Salicylic acid is a well-known plant hormone that plays a crucial part in plants' defence against various biotic and abiotic stresses, such as pathogens and environmental stressors. These stresses can be classified as biotic (caused by other living things) or abiotic (caused by the environment). On the other hand, thiourea is utilised in agriculture as an antioxidant and a stress alleviator. Researchers can better understand the stress level of mustard plants by analysing the particle size.
2. Indicator of Cellular Health and Integrity: Particle size measurements directly indicate the health and integrity of the cells found within plant tissues. Alterations in the structure and composition of cellular components like cell walls, vacuoles, and organelles can be a sign that changes have occurred in the size of the particles themselves. An increase or decrease in particle size may indicate that there have been modifications in the cellular architecture of the plant, which may have occurred due to changes in cellular metabolism induced by treatment or as a stress response.
3. Nutrient Uptake and Transport: The particle size analysis directly affects a plant's capacity to efficiently transport nutrients throughout its system. Changes in particle size can affect the surface area of the plant that is available for the absorption of nutrients, as well as the movement of water and nutrients within the plant itself. To optimise nutrient management in agriculture and to ensure that plants can effectively acquire the vital elements for growth and development, it is essential to have a solid understanding of these shifts and ensure that they are accounted for.
4. Implications for Agricultural Productivity: Salicylic acid and thiourea use in agricultural practices aims to enhance the quantity and quality of the crops produced. Particle size measurements provide beneficial information regarding how these treatments influence the plant's physical characteristics, such as the size and structure of the plant's leaves. The plant's

photosynthetic efficiency, transpiration rates, and overall productivity can all be affected by changes in particle size. Such information is crucial for farmers and agricultural scientists to make educated crop treatment and management decisions.

5. Environmental Sustainability: Understanding how different chemical treatments affect the size of individual particles is of the utmost importance for practises that aim to preserve the environment and promote sustainable agriculture. Sustainable agricultural practices seek to maximise crop production while minimising the detrimental effects that farming can have on the surrounding environment. Researchers can evaluate the eco-friendliness of various practices, such as treatments with salicylic acid and thiourea, as well as the practises' potential implications for the quality of the soil and water, by observing the effects of these treatments on the particle size.

6. Insights into Stress Mitigation Mechanisms: How salicylic acid and thiourea reduce stress in mustard plants can be better understood through particle size analysis. Cell turgor, stomatal conductance, and the deposition of secondary metabolites are all things that could be reflected in particle size variations. The ability to understand these mechanisms is essential for the development of targeted stress mitigation strategies that can increase the resistance of plants to a variety of adverse conditions.

7. Research on Plant-Pathogen Interactions: Salicylic acid is essential for plant immunity against pathogens. Researching the dynamics of plant-pathogen interactions can be aided by measuring the particle size in mustard leaves treated with salicylic acid. Alterations in the thickness of cell walls or the formation of physical barriers against invading pathogens may be reflected in changes in the sizes of individual particles. This information can be utilised in the breeding of crop varieties that are resistant to disease.

8. Potential for Biotechnological Applications: Analyzing particle size data derived from mustard leaves after treatment holds potential applications in biotechnology and genetic engineering. Researchers can utilise this information to discern the specific genes or pathways that play a role in stress response and cellular remodelling. These observations can be utilised to cultivate genetically modified crops with heightened stress resistance and enhanced agricultural productivity.

9. Insights into Secondary Metabolite Production: The application of salicylic acid and thiourea has been found to impact the synthesis of secondary metabolites in plants, encompassing phytochemicals with potential health-promoting properties. The nutritional value of mustard leaves and



their suitability for various applications, such as pharmaceuticals and functional foods, may be affected by changes in the accumulation of these compounds, and particle size measurements can provide clues about these changes. 10. Holistic Approach to Plant Health: In essence, the assessment of particle size in mustard leaves following treatment serves as a comprehensive methodology for comprehending the overall well-being of plants and their reactions to chemical interventions. It covers plant biology's physiological, biochemical, and structural aspects, providing a holistic perspective on how mustard plants adapt to different types of stress and treatments. Researchers, agronomists, and policymakers who are interested in developing agricultural practices that are both sustainable and effective can benefit tremendously from adopting this multidimensional perspective. In conclusion, determining the particle size of Indian mustard leaves after being treated with salicylic acid and thiourea is a multi-step process with far-reaching repercussions. It addresses key challenges in agriculture and environmental sustainability while contributing to a better understanding of plant stress responses, nutrient dynamics, and treatment efficacy. As we continue to investigate the complex relationships between chemical treatments, particle size, and the overall health of plants, we are paving the way for methods of crop production and environmental stewardship that are more knowledgeable and sustainable (Karaman, 2023; Karamat et al., 2022; Katoch et al., 2022; Kaviani et al., 2023; Kaya et al., 2023; Khalid et al., 2023; Khan et al., 2022, 2023).

**Table 4.81. Particle size analysis of leaf of Indian Mustard**

<b>Treatments</b>	<b>Z- Average (d.nm)</b>	<b>PdI</b>	<b>Intercept</b>	<b>Size (d.n)</b>	<b>% Intensity</b>	<b>St Dev (d.n.)</b>	<b>Result quality</b>
<b>T0 Control</b>	217.2	0.232	0.922	204.4	100.0	49.24	Good
<b>T1(Thiourea-1000 ppm)</b>	1241	0.952	0.633	376.5	100.0	47.15	Good
<b>T2(Salicylic acid-300 ppm)</b>	278.0	0.569	0.967	176.0	100.0	33.49	Good
<b>T3(Thiourea-1000 ppm) + (Salicylic Acid-300 ppm)</b>	185.4	0.059	0.923	200.6	100.0	59.39	Good
<b>T4(Thiourea-1500 ppm) + (Salicylic acid-300 ppm)</b>	190.9	0.030	0.914	201.6	100.0	50.22	Good
<b>T5(Thiourea-1000ppm) + (Salicylic Acid-450ppm)</b>	187.2	0.024	0.923	192.7	100.0	37.84	Good
<b>T6(Thiourea-500ppm) + (Salicylic Acid-300ppm)</b>	444.5	0.530	0.252	292.5	100.0	89.29	Good
<b>T7(Thiourea-1000 ppm) + (Salicylic Acid-150ppm)</b>	498.6	0.729	0.366	228.1	100.0	40.86	Good
<b>T8(Thiourea-500ppm) + (Salicylic Acid-600ppm)</b>	197.0	0.047	0.913	207.2	100.0	44.88	Good
<b>T9(Thiourea-2000ppm) + (Salicylic Acid-150ppm)</b>	503.8	0.742	0.387	201.8	100.0	35.03	Good
<b>T10(Thiourea-2000ppm) + (Salicylic Acid-600ppm)</b>	216.3	0.007	0.851	226.8	100.0	55.41	Good
<b>T11(Thiourea-500ppm) + (Salicylic Acid-150ppm)</b>	197.3	0.231	0.883	193.8	100.0	49.80	Good

**Summary and Conclusion**

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The present research work entitled **“Evaluation of Sulphur and Salicylic Acid on Growth, Physiology, Yield and Molecular Expression of Indian Mustard”** was carried out during two Rabi seasons of 2021-2023 and 2022-2023 respectively, in the Department of Agronomy as the field experiment in the Lovely Professional University, Punjab. The present study was carried out to estimate the impact of Sulphur and Salicylic acid on the growth, physiology, yield and molecular expression of the Indian mustard crop variety RH725 at 30, 60, 90 and 120 DAS. Mustard seeds were taken from an authorised certified seed producer, ‘Good Grow’, from Phagwara, Punjab. The plot size selected for the experiment is  $5 \times 3 = 15\text{m}^2$ . The sowing of seeds has been done successfully in research fields. According to the plan of work, the experiment was arranged in statistical design RBD, and treatments were applied at 15, 45, and 75 DAS. The source of treatments applied was set from the local market in Phagwara. The exogenous sulphur and salicylic acid were used by selecting the best concentration in earlier studies. The various observations were taken at four stages, such as 30 DAS, 60 DAS, 90 DAS, and 120 DAS in all the treatments. The detailed plan of treatments are; T0-Control, T1- Thiourea Recommended dose (1000 ppm), T2-Salicylic acid Recommended dose (300 ppm), T3-Thiourea (1000 ppm) +Salicylic acid (300 ppm), T4- Thiourea (1500 ppm) + Salicylic acid (300 ppm), T5-Thiourea (1000 ppm) + Salicylic acid (450 ppm). T6- Thiourea (500 ppm) + Salicylic acid (300 ppm), T7- Thiourea (1000 ppm) + Salicylic acid (150 ppm), T8- Thiourea (500 ppm) + Salicylic acid (600 ppm), T9- Thiourea (2000 ppm) + Salicylic acid (150ppm), T10- Thiourea (2000 ppm) + Salicylic acid (600ppm), T11- Thiourea (500ppm) + Salicylic acid(150ppm). Essential nutrients to the crop were applied at the time of sowing, and as a top dressing, 2-3 weeding was carried out, and two irrigations were provided to attain good growth and production. The results obtained after the experiment during the years 2021-2022 and 2022-2023 are presented in this chapter. This includes all observations of the crop's morphological, biochemical, and yield attributes. There were four main parts of the experiment. In the first part, the investigation was developed to

determine the morpho-physiological parameters of the Indian mustard crop under all

treatments at the 30, 60, 90 and 120 DAS. The second part represents the biochemical responses of Indian mustard plants under various treatment regimens. Details of the experimental procedure are given in Chapter 3. In this chapter, an attempt has been made to depict and explain the recorded data. The findings of the Rabi season research experiments are presented under the following headings:

#### **A. Summary of the Thesis:**

##### **4A. Thiourea (sulphur) and salicylic acid-mediated effects on morphological parameters of Indian mustard grown under the open filed condition**

**Plant Height (cm):** At 120 DAS the percentage increase as compared to T0 was found to be highest in T11, followed by T9, T8, T3, T2, T10, T6, T1, T4, T7, T5, and the percentage values were 26.49%, 20.26%, 18.82%, 18.40%, 15.41%, 14.05%, 13.52%, 9.97%, 9.09%, 8.39%, 6.37% respectively. **Leaf Number:** At 120 DAS, the percentage increase as compared to T0 was found highest in T8 followed by T5, T6, T11, T3, T7, T9, T10, T4, T2, T1 and the percentage values were 28.86%, 26.59%, 24.79%, 22.03%, 19.53%, 17.11%, 10.97%, 9.50%, 7.68%, 5.73%, 1.42% respectively. **Leaf Area (cm<sup>2</sup>):** At 90 DAS, the percentage increase as compared to T0 was found highest in T4 followed by T9, T3, T1, T8, T6, T11, T10, T2, T5, T7 and the percentage values were 29.49%, 25.90%, 25.21%, 23.82%, 22.58%, 21.68%, 15.79%, 12.84%, 12.44%, 11.14%, 8.20% respectively. **Leaf Area Index:** At 90 DAS, the percentage increase as compared to T0 was found highest in T4 followed by T9, T3, T1, T8, T6, T11, T10, T2, T5, T7 and the percentage values were 29.49%, 25.90%, 25.21%, 23.82%, 22.58%, 21.68%, 15.79%, 12.84%, 12.44%, 11.14%, 8.20% respectively. **Node number:** At 90 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T8, T6, T3, T9, T1, T4, T2, T5, T10, T7 and the percentage values were 37.12%, 30.83%, 27.82%, 24.54%, 24.54%, 23.85%, 23.14%, 20.95%, 15.30%, 13.54%, 11.70% respectively. **Internodal length:** At 120 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T2, T1, T3, T8, T9, T10, T6, T4, T5, T7 and the percentage values were 37.73%, 37.38%, 34.08%, 30.42%, 27.86%, 22.85%, 22.11%, 19.75%, 17.42%, 16.15%, 15.84% respectively. **Number of Primary Branches:** At 120 DAS, the percentage increase as compared to T0 was found highest in T8 followed by T9, T11, T2, T6, T10, T3, T4, T5, T1, T7 and the percentage values

were 48.61%, 44.77%, 43.93%, 38.33%, 38.33%, 37.5%, 36.20%, 35.08%, 35.08%, 30.18%, 28.84% respectively. **Number of Secondary Branches:** At 120 DAS, the percentage increase as compared to T0 was found highest in T6 followed by T11, T5, T10, T8, T3, T7, T9, T4, T2, T1 and the percentage values were 50.23%, 49.28%, 47.29%, 43.68%, 40.22%, 39.54%, 39.20%, 37.79%, 35.54%, 34.75%, 33.12% respectively. **Stem Girth:** At 90 DAS, the percentage increase as compared to T0 was found highest in T11 followed by T8, T10, T5, T6, T9, T3, T4, T7, T1, T2 and the percentage values were 38.64%, 37.67%, 35.22%, 30.10%, 23.13%, 20.47%, 18.38%, 16.85%, 15.43%, 12.26%, 9.59% respectively. **Chlorophyll Index:** At 60 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T1, T4, T8, T3, T2, T11, T5, T7, T9, T6 and the percentage values were 20.29%, 18.78%, 18.78%, 16.76%, 11.29%, 11.23%, 9.48%, 7.45%, 6.41%, 5.41%, 1.27% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T8, T1, T4, T5, T2, T7, T11, T3, T9, T6 and the percentage values were 21.25%, 21.11%, 19.28%, 17.07%, 16.25%, 15.63%, 13.32%, 10.81%, 10.40%, 7.42%, 1.94% respectively. **Siliqua Number:** At 120 DAS, the percentage increase as compared to T0 was found to be highest in T11, followed by T6, T5, T2, T9, T10, T8, T7, T3, T1, T4, and the percentage values were 52.36%, 47.78%, 46.20%, 39.45%, 37.27%, 30.76%, 28.08%, 24.24%, 22.41%, 21.82%, 18.43% respectively. **Siliqua Length:** The percentage increase was calculated by comparing all the treatments with T0. Therefore, at 120 DAS, the percentage increase as compared to T0 was found highest in T2 followed by T3, T11, T8, T1, T6, T9, T7, T4, T5 and the percentage values were 21.87%, 18.69%, 18.03%, 15.25%, 13.79%, 13.79%, 5.66%, 4.76%, 2.91%, 1.96% respectively. But, in the T10 treatment, the impact was ineffective, and the percentage value was the same as in T0. **RWC:** In T1 and T9, the percentage decreased compared to T0, and the percentage values were -0.14% and -0.28%, respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T1 followed by T10, T7, T6, T3, T9, T2 and the percentage values were 1.54%, 1.34%, 1.10%, 0.50%, 0.30%, 0.20%, 0.02% respectively. But in T4, T8, T5 and T11 percentage decrease as compared to T0 and the percentage values were -0.28%, -1.73%, -2.22%, and -2.44% respectively. **Economic Yield:** Therefore, at harvesting, the percentage increase as compared to T0 was found highest in T11 followed by T6, T5, T8, T2, T7,

T1, T4, T9, T10 and the percentage values were 25.91%, 20.13%, 19.90%, 18.91%, 17.27%, 16.91%, 16.28%, 13.21%, 12.09%, 11.21% respectively. However, in T3, the percentage decreased compared to T0, and the percentage value was -11.42%.

**Biological Yield:** The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage increase compared to T0 was highest in T11, followed by T2, T7, T1, and T8; the percentage values were 12.05%, 4.72%, 3.42%, 2.14%, 1.69% respectively. But in T5, T6, T3, T9, T10, and T4 percentage decrease as compared to T0 and the percentage values were -0.63%, -1.02%, -1.29%, -1.50%, -2.92%, and -3.85% respectively.

**Stover Yield:** The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage increase compared to T0 was highest in T11 and T3; the percentage values were 4.28% and 2.55%, respectively. But in T2, T7, T1, T8, T9, T10, T5, T4, and T6 the percentage were decrease as compare to T0 and the percentage values were -1.99%, -3.89%, -5.58%, -8.27%, -8.80%, -10.55%, -13.23%, -13.55%, and -14.13% respectively.

**Harvest Index (HI%);** Therefore, at harvesting, the percentage increase as compared to T0 was found highest in T6 followed by T5, T8, T4, T11, T1, T9, T7, T10, T2 and the percentage values were 20.47%, 19.90%, 17.61%, 16.09%, 15.14%, 14.36%, 13.61%, 13.56%, 13.05%, 12.65% respectively. But, in T3, the percentage decreased compared to T0, and the percentage value was -11.17%.

**Test weight** Therefore, at harvesting, the percentage increase as compared to T0 was found to be highest in T11, followed by T2, T6, T4, T3, T9, T1, T5, T7, T10 and the percentage values were 14.30%, 12.57%, 12.28%, 11.56%, 10.46%, 9.86%, 6.34%, 1.64%, 1.37%, 0.46% respectively. But in T8, the percentage decreased compared to T0, and the percentage value was -1.22%.

**Oil Content:** Therefore, at harvesting, the percentage increase as compared to T0 was found to be highest in T11, followed by T9, T3, T4, T2, T5, T7, T1, T6, T8, T10, and the percentage values were 35.21%, 29.80%, 28.67%, 27.02%, 26.74%, 26.17%, 25.59%, 23.32%, 22.14%, 21.65%, 20.83% respectively.

**Oil Cake:** The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage increase as compared to T0 was found highest in T11 followed by T7, T10, T3, T9, T5, T6, T4, T1, T2, T8 and the percentage values were 12.27%, 10.13%, 8.40%, 6.64%, 5.56%, 5.48%, 5.23%, 5.06%, 4.68%, 3.86%, 3.69% respectively.

**Plant fresh weight:** At 90 DAS, the percentage increase as

compared to T0 was found highest in T3 followed by T10, T1, T2, T6, T4, T11, T7, T9, T5, T8 and the percentage values were 73.90%, 73.77%, 63.16%, 61.24%, 53.91%, 47.82%, 45.31%, 37.38%, 28.91%, 23.81%, 15.47% respectively. **Plant turgid weight:** At 90 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T10, T1, T2, T6, T4, T11, T7, T9, T5, T8 and the percentage values were 73.91%, 73.52%, 62.71%, 61.35%, 53.77%, 48.08%, 46.53%, 36.84%, 28.94%, 25.39%, 16.88% respectively. **Plant dry weight:** At 90 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T10, T6, T1, T11, T2, T4, T7, T5 and the percentage values were 47.43%, 44.72%, 38.22%, 37.94%, 37.18%, 30.03%, 29.21%, 12.70%, 7.89% respectively. But in T8 and T9, the percentage decreased compared to T0; the percentage values were -3.03% and -5.33%. **Chlorophyll a:** But in T9, T10, and T3, the percentage decreased compared to T0, and the percentage values were -3.90%, -12.81%, and -18.29%, respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T3 followed by T7, T6, T11, T9, T5, T2, T8 and the percentage values were 15.86%, 14.99%, 8.06%, 6.89%, 6.31%, 5.58%, 5.33%, 4.25% respectively. But in T1, T4, and T10, the percentage decreased as compared to T0; the percentage values were -0.64%, -3.61%, and -51.28%, respectively. **Chlorophyll b:** At 60 DAS, the percentage decrease as compared to T0 was found highest in T11, T5, T7, T6, T8, T1, T3, T10, T9, T2, T4 and the percentage values were -22.35%, -28.42%, -49.13%, -64.56%, -70.08%, -73.89%, -103.39%, -131.21%, -217.36%, -270.75%, -275.71% respectively. At 90 DAS, the percentage increase compared to T0 was highest in T3, followed by T9, T11, and T7, and the percentage values were 31.74%, 6.42%, 4.37%, and 2.82%, respectively. But in T5, T2, T6, T4, T1, T10, T8 the percentage decrease as compared to T0 and the percentage values were -14.41%, -27.23%, -31.27%, -56.53%, -81.32%, -85.66%, -153.48% respectively. **Chlorophyll a+b:** However, in T11, the percentage increased compared to T0, and the percentage value was 6.62%. At 90 DAS, the percentage increase compared to T0 was highest in T3, followed by T7, T9, and T11; the percentage values were 23.82%, 9.93%, 6.36%, and 5.78%, respectively. But in T5, T6, T2, T4, T1, T8, T10 the percentage decrease as compared to T0 and the percentage values were -2.45%, -6.21%, -6.95%, -22.15%, -25.76%, -32.85%, -65.00% respectively. **Chlorophyll ab ratio:** At 90 DAS, the percentage increase as compared to T0 was



found highest in T8 followed by T1, T6, T4, T2, T10, T9, T5, T7, T11 and the percentage values were 64.94%, 51.95%, 37.15%, 33.64%, 27.55%, 21.31%, 19.95%, 15.22%, 11.90%, 1.73% respectively. But in T3, the percentage decreased compared to T0, and the percentage value was -20.64%. **Carotenoids:** At 90 DAS, the percentage increase as compared to T0 was found to be highest in T7, followed by T3, T9, T11, T2, and T5, and the percentage values were 35.79%, 32.67%, 25.79%, 22.55%, 12.35%, 10.38% respectively. But in T6, T1, T4, T8, T10 the percentage decrease as compared to T0 and the percentage values were -2.90%, -3.23%, -13.10%, -22.60%, -72.31% respectively. **Total Phenol:** But in T7, T6, T11, T10, T9, T4, T1 the percentage decrease as compared to T0 and the percentage values were -2.47%, -12.85%, -17.48%, -26.87%, -34.17%, -37.13%, -54.13% respectively. **Flavanols:** But in T1, T6, and T7, the percentage decrease as compared to T0 and the percentage values were -3.27%, -7.51%, and -22.10%. At 90 DAS, the percentage increase as compared to T0 was found highest in T5 followed by T2, T9, T3, T1, T10, T11 and the percentage values were 26.74%, 24.10%, 16.38%, 14.47%, 9.41%, 3.98%, 2.88% respectively. But in T7, T4, T8, and T6 the percentage decrease as compared to T0 and the percentage values were -4.65%, -4.98%, -9.06%, and -21.66%. **Flavonoids:** In T9, T4, T1, and T7 the percentage decrease as compared to T0 and the percentage values were -6.66%, -8.47%, -10.34%, and -13.87% respectively. However, the T2 impact of treatment was not effective, and the percentage value was the same as T0. **Total Soluble Sugar:** At 90 DAS, the percentage increase as compared to T0 was found to be highest in T2, followed by T9, T3, T4, T5, T1, T10, T8, T11 and the percentage values were 36.13%, 35.88%, 25.24%, 23.37%, 21.77%, 16.55%, 8.65%, 3.70%, 2.87% respectively. But in T6 and T7, the percentage decreased compared to T0, and the percentage values were -7.31% and -47.67%. **Total Starch:** At 60 DAS, the percentage increase as compared to T0 was found highest in T9 followed by T2, T4, T11, T8, T1, T3, T6, T5, T10, T7 and the percentage values were 74.39%, 65.83%, 65.16%, 63.96%, 62.79%, 55.07%, 54.91%, 51.24%, 51.05%, 37.69%, 30.83% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T2 followed by T9, T3, T4, T5, T1, T10, T8, T11 and the percentage values were 36.13%, 35.88%, 25.24%, 23.37%, 21.77%, 16.55%, 8.65%, 3.70%, 2.87% respectively. But in T6 and T7, the percentage decreased compared to T0, and the percentage values were -7.31% and -47.67%. **PAL:** At 90

DAS, the percentage increase as compared to T0 was found highest in T4 followed by T11, T2, T10, T7, T3, T9, T1, T6, T8, T5 and the percentage values were 73.73%, 66.66%, 66.23%, 65.78%, 56.66%, 55.93%, 54.38%, 44.68%, 40.90%, 31.57%, 27.77% respectively. **Proline:** But in T1, T5, T2, T8, T3 percentage decrease as compared to T0 and the percentage values were -2.08%, -10.55%, -33.72%, -88.80%, -133.96% respectively. At 90 DAS, the percentage increase as compared to T0 was found highest in T10 followed by T7, T4, T8, T11, T5, T6, T9, T1 and the percentage values were 67.69%, 63.92%, 56.74%, 48.82%, 43.39%, 42.84%, 30.69%, 30.69%, 6.37% respectively. But in T2 and T3, the percentage decreased compared to T0; the percentage values were -9.31% and -31.31%. **Acid Value:** The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage decrease as compared to T0 was found highest in T11 followed by T4, T2, T1, T6, T3, T10, T7, T8 and the percentage values were -1.89%, -5.63%, -13.42%, -26.02%, -26.39, -33.02%, -33.02%, -43.66%, -108.21% respectively. But in T9 and T5, the percentage increased compared to T0, and the percentage values were 16.63% and 9.07%. **Iodine Value:** Therefore, at harvesting, the percentage decrease as compared to T0 was found highest in T3, T6, T10 followed by T2, T7, T11, T1, T4, T8, T9 and the percentage values were -21.05%, -21.05%, -21.05%, -27.77%, -32.69%, -38%, -60.46%, -72.5%, -122.58%, -213.63% respectively. However, in T5, the percentage increased compared to T0, and the percentage value was 15.85%. **P-anisidine Value:** Therefore, at harvesting, the percentage increase as compared to T0 was found to be highest in T2, followed by T7, T10, T9, T5, T11, T4, T1, and T6, and the percentage values were 59.23%, 40.60%, 40.60%, 25.47%, 20.40%, 20.40%, 14.59%, 7.87%, 7.87% respectively. Although the T8 percentage decreased compared to T0, the percentage value was -20.61%. But, in T3, the treatment's impact was ineffective, and the percentage value was the same as in T0. **Peroxide Value:** Therefore, at harvesting, the percentage increase as compared to T0 was found to be highest in T8, followed by T6, T11, T2, T4, T9, T3, and T1, and the percentage values were 60.86%, 52.63%, 50%, 43.75%, 43.75%, 40%, 25%, 18.18% respectively. But in T10 and T5, the percentage decreased compared to T0, and the percentage values were -50% and -80%. **Totox Value:** Consequently, at harvest, the highest percentage increase compared to T0 was recorded in T8, followed by T6, T11, T2, T4, T9, T3, T1, and T7, with percentage values

of 60.01%, 51.89%, 49.40%, 44.41%, 43.13%, 39.62%, 24.40%, 17.89%, and 2.10%, respectively. However, T10 and T5 exhibited a percentage decrease compared to T0, with values of -43.12% and -73.12%, respectively. **Saponification Value:** Conversely, T5 and T3 exhibited percentage increases relative to T0, with 18.88% and 14.11% values, respectively. **Oil density:** Therefore, at harvesting, the percentage increase as compared to T0 was found to be highest in T8, followed by T11, T2, T4, T10, T1, T5, and T3, and the percentage values were 1.43%, 1.42%, 0.94%, 0.87%, 0.72%, 0.69%, 0.69%, 0.06% respectively. But in T9, T6, T7 percentage decrease as compared to T0 and the percentage values were -0.04%, -0.11%, 0.33%. **Oil Viscosity:** The percentage increase was calculated by comparing all the treatments with T0. Therefore, at harvesting, the percentage increase as compared to T0 was found to be highest in T11, followed by T9, T8, T2, T6, T7, T1, T3, and T5, and the percentage values were 30.55%, 25.64%, 14.34%, 12.65%, 11.26%, 5.97%, 4.87%, 1.90%, 0.54% respectively. But in T10 and T4, the percentage decreased compared to T0; the percentage values were -0.89% and -0.95%. **FTIR: Treatment Conditions:** The dataset contains a variety of treatment conditions, ranging from T0 to T11, and each of these conditions represents a distinct setup for experiments or application of thiourea and salicylic acid to mustard leaves. The treatments are expected to involve a range of concentrations, durations of exposure, or application methods, with each condition carefully designed to examine distinct impacts on the plant tissue. **Number of Peaks:** The number of peaks that can be obtained from FTIR spectra varies depending on the treatment, ranging from 80 to 164 points. **Data Point T0 (121 peaks): Treatment Conditions:** T0 refers to the condition of the control group, the baseline state before any treatment. **Implications:** Under normal circumstances, mustard leaves' natural biochemical composition and structural characteristics are probably reflected in the 121 peaks found in the FTIR spectra of T0. The effects of subsequent treatments can be compared to these peaks, which serve as a reference point. **Data Point T1 (120 peaks): Treatment Conditions:** T1 is a control group because it's very similar to T0 regarding the total number of peaks. **Implications:** The fact that T1 has a slightly lower peak count than T0 (120 peaks), as opposed to T0's 121 peaks, suggests that there were possibly some slight changes made to the experimental conditions or the sample preparation for T1. It's possible that these differences won't make much of a difference to the structure or composition of the biochemical. **Data**

Point T2 (164 peaks): Treatment Conditions: The number of peaks in T2 is especially noticeable. Implications: Because of the treatment with thiourea and salicylic acid, significant changes have been induced in the biochemical composition of the mustard leaves and their structural properties, as indicated by the increased peak count in T2. Alterations in secondary metabolites or cellular components could contribute to the increased complexity of these treatments. Data Point T3 (133 peaks): Treatment Conditions: T3 has several peaks considered to be about average. Data Point T4 (122 peaks): Treatment Conditions: T4 exhibits a peak count comparable to T0 and T1. Implications: The fact that peak counts for T0, T1, and T4 were all comparable raises the possibility that T4 involved treatments or conditions comparable to those of the control groups. The inherent variability in plant samples may be responsible for any differences observed in the FTIR spectra. Data Point T5 (152 peaks): Treatment Conditions: Compared to the controls, the peak count of T5 is significantly higher. The activation of particular pathways or responses to the applied treatments may be involved in these changes. Data Point T6 (88 peaks): Treatment Conditions: The dataset contains T6 with the fewest peaks. Implications: The lower peak count suggests that T6 uses simplified conditions or treatments, resulting in fewer detected species. Data Point T7 (107 peaks): Treatment Conditions: The peak count for T7 is approximately in the middle of the range. Implications: The fact that T7 has a moderate impact on the mustard leaves and an intermediate peak count lends credence to the hypothesis that T7 represents a balanced set of conditions. The particular biochemical or structural changes might shift, but they'll still fall within a predictable range of possibilities. Data Point T8 (85 peaks): Treatment Conditions: T8 also has a lower peak count in its distribution. Implications: The lower peak count in T8 suggests a more straightforward structure than the controls. Data Point T9 (80 peaks): Treatment Conditions: T9 has the lowest peak count of any other model in the dataset. Implications: this suggests that T9 is a simplified system with reduced complexity. It may be possible to optimise the treatment conditions or the sample characteristics to achieve particular analytical goals. Data Point T10 (124 peaks): Treatment Conditions: T10 demonstrates a peak count comparable to T0, T1, and T4. Implications: Similar to the T4 treatment, the observed similarity in peak count indicates that the T10 treatments can be considered comparable to the control groups, with possible slight differences in the characteristics of the

samples. Data Point T11 (106 peaks): Treatment Conditions: Similar to T7, T11 can be found in the middle of the range of peak counts. Implications: Another treatment with a moderate impact, T11, has the same severity as T7. **EDX:** The elemental composition of the analysed sample is revealed through the T0 Energy Dispersive X-ray (EDX) data, which provides a detailed understanding of the relative abundances of different elements within the material. Carbon (C): Carbon was the most abundant element in the sample (T0). It made up 47.04 weight per cent of the material. A high carbon content indicates organic matter because carbon is essential for forming organic compounds. Carbon makes up 55.41 per cent of the sample's total atomic weight, further evidence of the element's predominance. Oxygen (O): Oxygen is the second most prevalent element in the analysed sample, constituting approximately 48.70 wt% of the composition of the material. Potassium (K): Potassium is a component of the sample that is relatively insignificant, making up only 1.63 weight per cent of the overall material's makeup. Even further below that is the atomic percentage of potassium, which is 0.59%. Calcium (Ca): Calcium is a constituent of the material's composition, accounting for 2.62 wt% of its overall content. The calcium atomic percentage is 0.93%. Like potassium, the standard deviation for calcium is relatively low (Wt% sigma: 0.37%), indicating a constant but relatively insignificant presence. Calcium is a mineral that can be found in rocks, soils, and the tissues of plants. Total Composition: The EDX analysis correctly accounts for all elements in the sample, as shown by the sum of the weight percentages for all elements (C, O, K, and Ca), which adds up to 100%. Potassium and calcium were found in trace amounts within the sample, which raises the possibility of associations with various soil minerals or particular cellular structures. **XRD:** The structural changes and effects on the molecular level caused by these treatments can be better understood by conducting an XRD (X-ray diffraction) analysis on mustard leaves treated with thiourea and salicylic acid and then analysing the resulting data. The information consists of several points, ranging from T0 to T10, Data Point T0 (388 peaks): Treatment Conditions: T0 refers to having no treatment and being part of the control group. XRD Peaks: Are 388 peaks visible in the XRD spectrum of T0. This is a significant number. Under typical environmental conditions, these peaks reveal the natural structural components in mustard leaf tissue. Implications: The abundance of peaks in T0 reflects the inherent complexity of the crystalline and

structural components that make up the mustard leaf. Data Point T1 (331 peaks): Treatment Conditions: T1 is an additional control group comparable to T0. XRD Peaks: Compared to T0, the XRD spectra of T1 have a lower peak count, totalling 331 peaks. Implications: As a result of the decrease in peak count in T1, it appears as though the mustard leaves, when subjected to these particular control conditions, may have undergone some minute structural changes. Data Point T2 (324 peaks): Treatment Conditions: T2 is the "control" condition without additional intervention. XRD Peaks: When compared to those of T0, the XRD spectra of T2 have an even lower peak count than those of T0. Implications: The peak count was lower in T2 than in T1, suggesting that the mustard leaves undergo more significant structural changes due to these control conditions. Data Point T3 (164 peaks): Treatment Conditions: Thiourea and salicylic acid may have effects under the T3 treatment condition. XRD Peaks: Compared to the controls, the XRD spectra of T3 have a significantly lower total number of peaks (164). Implications: Due to the substantial induction of structural changes in the mustard leaves by the treatment with thiourea and salicylic acid, there was a discernible decrease in the peak count in T3. This result suggests that the mustard leaves have been significantly altered. Data Point T4 (303 peaks): Treatment Conditions: T4 refers to a different condition that requires treatment. XRD Peaks: T4 XRD spectra have a moderate number of peaks. Implications: The treatment may have induced structural alterations in the mustard leaves, although to a lesser extent than in T3, as indicated by the treatment's moderate peak count in the T4 sample. These alterations could have something to do with particular biochemical or cellular components within the plant tissue. Data Point T5 (357 peaks): Treatment Conditions: The condition denoted by T5 is a distinct form of treatment. XRD Peaks: Compared to the control groups, the XRD spectra of T5 exhibit a significantly higher peak count. Implications: There is evidence to suggest that the treatment of mustard leaves with thiourea and salicylic acid has led to changes in the structural composition of the leaves, as indicated by the elevated peak count in T5. Data Point T6 (354 peaks): Treatment Conditions: T6 denotes an additional experimental condition for treatment. XRD Peaks: T6 has XRD spectra with a peak count comparable to T5. Implications: Similar to the findings in T5, the observed peak count in T6 suggests the presence of structural alterations in the mustard leaves, which may have implications for the plant's ability to respond to stressors or adapt to

environmental conditions. Data Point T8 (394 peaks): Treatment Conditions: The variable T8 denotes a specific treatment condition. XRD Peaks: The number of peaks in the XRD spectrum of T8 is the same as that of T0. Implications: Because the peak counts for T0 and T8 were so comparable, it is possible that the treatment used in T8 did not significantly alter the structural composition of the mustard leaves. Data Point T9 (190 peaks): Treatment Conditions: T9 represents a treatment condition. XRD Peaks: T9 has the fewest peaks in its XRD spectra. Data Point T10 (415 peaks): Treatment Conditions: A different treatment condition is denoted by T10. XRD Peaks: The XRD spectra of T10 have the highest peak count of all the collected data. **Soil pH:** at 60 DAS, the percentage increase compared to T0 was highest in T5, followed by T1 and T4, and the percentage values were 4.24%, 3.84%, and 0.59%, respectively. But the percentage also decrease in T8, T2, T7, T3, T6, T10, T11, T9 as compared to T0 and the percentage values were -0.21%, -4.35%, -5.39%, -5.63%, -6.40%, -7.58%, -8.33%, -11.66% respectively. **Soil EC:** at 60 DAS, the percentage increase as compared to T0 was found to be highest in T10 followed by T5, T9, T3, T8, T2, T1, T6, T7, T11 and the percentage values were 42.50%, 28.12%, 24.59%, 19.29%, 17.85%, 11.53%, 9.80%, 9.80%, 0.0002%, 0.0002% respectively. But, the percentage also decreased in T4 compared to T0, and the percentage value was -6.97%. **Soil Phosphorus:** at 60 DAS, the percentage increase as compared to T0 was found to be highest in T10, followed by T1, T3, T9, T6, T2, T5, and T11, and the percentage values were 48.98%, 41.41%, 39.34%, 37.67%, 37.44%, 28.72%, 28.58%, 20.36% respectively. But the percentage also decrease T8, T7, T4 as compared to T0 and the percentage values were -7.72%, -16.58%, -16.68% respectively. **Soil Potassium:** at 60 DAS, the percentage increase as compared to T0 was found to be highest in T3, followed by T2, T10, T1, T4, T6, and T11, and the percentage values were 24.24%, 18.16%, 9.56%, 8.20%, 7.78%, 6.57%, 1.31% respectively. But the percentage also decrease in T5, T7, T9, T8 and the percentage values were -5.43%, -8.30%, -8.62%, -13.35% respectively. **Sulphur:** at 60 DAS, the percentage increase as compared to T0 was found highest in T9 followed by T5, T4, T11, T7, T8, T2, T6, T3, T10 and the percentage values were 15.61%, 10.96%, 10.67%, 10.30%, 7.99%, 7.37%, 6.27%, 3.07%, 2.38%, 1.58% respectively. But, the percentage also decreased in T1, and the percentage value was -1.18%. **Soil Organic Carbon:** at 60 DAS, the percentage increase as compared to T0

was found highest in T2 followed by T8, T4, T7, T1, T11, T6, T9, T5, T10 and the percentage values were 28.49%, 26.20%, 25.40%, 20.68%, 15.85%, 14.28%, 11.53%, 9.21%, 8%, 8% respectively. But, the percentage also decreased in T3, and the percentage value was -1.47%. **Soil CEC:** at 60 DAS, the percentage increase as compared to T0 was found highest in T6 followed by T11, T5, T9, T4, T8, T1, T2, T10, T7 and the percentage values were 27.21%, 26.78%, 22.76%, 19.65%, 18.99%, 16.73%, 10.80%, 10.55%, 3.70%, 2.75% respectively. But in T3, the percentage decreased compared to T0, and the percentage value was -3.43%. **Available Nitrogen:** at 60 DAS, the percentage increase as compared to T0 was found to be highest in T5, followed by T10, T6, T4, T3, T1, T9, T7, T11, T8, T2 and the percentage values were 45.22%, 41.56%, 36.97%, 36.38%, 32.59%, 32.36%, 31.41%, 27.64%, 26.54%, 26.32%, and 22.82% respectively. The Ct values are essential in quantitative polymerase chain reaction (qPCR) experiments and provide information about the number of PCR cycles required for the fluorescence signal to cross a predetermined threshold. These values can be used to assess the relative expression levels of the target genes in different samples. **FAD2 (Fatty Acid Desaturase 2):** The cycle threshold (Ct) values obtained for FAD2 across various experimental conditions provide insights into the relative expression level of the gene **FAE1 (Fatty Acid Elongase 1):** The Ct values obtained for FAE1 (Fatty Acid Elongase 1) under different experimental conditions provide significant insights into the gene's expression patterns. **SOD (Superoxide Dismutase):** Superoxide dismutase (SOD) is an essential enzyme within the antioxidant defence mechanisms of plants, serving a critical function in the mitigation of reactive oxygen species (ROS), specifically superoxide radicals. **ERF (Ethylene Response Factor):** ERF (Ethylene Response Factor) is a transcription factor that regulates gene expression in response to the plant hormone ethylene. Ethylene is a key signalling molecule central to various plant physiological processes, including growth, development, and stress responses. **GCS (Glutamate-Cysteine Ligase):** GCS exhibits varying expression levels across conditions, with the "SA+S (DD)" condition having the highest Ct value (34.10021591), indicating lower expression, and the "SA-RD" condition having the lowest (25.2241497). **gTMT (Glucosyltransferase Methyltransferase):** The "SA+S (DD)" condition exhibits the lowest Ct value (23.06736755), suggesting the highest expression of gTMT, while the "Control"



condition has the highest Ct value (29.37226868), indicating lower expression. G3PDH (Glyceraldehyde 3-Phosphate Dehydrogenase): G3PDH shows the lowest Ct value in the "SA+S (HD)" condition (20.62479591), indicating the highest expression, while the "SA+S (DD)" condition has the highest Ct value (26.01503944), suggesting lower expression.

## **B. Conclusion of the Thesis**

- Thiourea (sulphur) and salicylic acid treatments positively influence the morphological parameters of Indian mustard grown under open field conditions.
- Applying thiourea (sulphur) and salicylic acid notably improves the biochemical responses of Indian mustard grown in open field conditions.
- Thiourea (sulphur) and salicylic acid treatments significantly influenced the oil profile and gene expression in Indian mustard grains and leaves, enhancing their growth and stress resilience under open field conditions.

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