

RESPONSE OF INDIAN MUSTARD TO HUMIC ACID AND SULPHUR UNDER VARIABLE WATER REGIMES

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Agronomy

By

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LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

2025

DECLARATION

I, hereby declare that the presented work in the Thesis entitled “**Response of Indian mustard to humic acid and sulphur under variable water regimes**” in fulfilment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision of **Dr. Anaytullah Siddique**, Associate professor in the Department of Agronomy/School of Agriculture, Lovely Professional University, for the award of degree Ph.D. Agronomy.

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We certify that the student's above statement is correct to the best of our knowledge and belief. The Ph.D. Viva-Voce examination of research scholar **Toko Manna** was held on **20/06/2025** and **accepted for the award of Ph.D. Degree.**

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DECLARATION

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CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled “**Response of Indian mustard to humic acid and sulphur under variable water regimes**” 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 **Toko Manna, 12116635**, is bonafide record of 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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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS	DESCRIPTION	ABBREVIATIONS	DESCRIPTION
+	Plus	LAI	Leaf area index
%	Percentage	m	Meter
@	At the rate	m ⁻²	Meter square
*	Significant	Mini	Minimum
μ	Microliter	mg	Milligram
°C	Degree celsius	mg/g FW	Milligrams per gram of fresh weight
°N	Degree north	mg KOH/g	Milligrams of potassium hydroxide per gram
°E	Degree east	Mgm ⁻³	Milligrams per cubic meter
a.i	Active ingredient	meq O ₂ /kg	Milliequivalents of oxygen per kilogram
AA	Antioxidant activity	M ha	Million hectare
ABA	Absciscic acid	mL	Milliter
ANOVA	Analysis of Variance	Max	Maximum
<i>i.e</i>	That is	Mt	Million ton
B: C	Benefit-cost ratio	Mn	Manganese
B. D	Bulk density	Mo	Molybdenum
Bn.	Billion	MUFA	Monounsaturated fatty acids
CD	Critical difference	N	Nitrogen
C.E.C	Cation exchange capacity.	No.	Number
CGR	Crop growth rate	N ₀	Control
cm	Centimeter	N ₁	Humic acid application
Cm ²	Centimeter square	N ₂	Sulphur application

Conc.	Concentration	N ₃	Combined humic acid and sulphur application
DAS	Days after sowing	NS	Non-significant
DAP	Di-Ammonium Phosphate	NUE	Nutrient use efficiency
dsm-1	Deci siemens per meter	OC	Organic carbon
DMA	Dry matter accumulation	P	Phosphorous
E.C	Electrical conductivity	ppm	Parts per million
ET	Evapotranspiration	PUFA	Polyunsaturated fatty acids
Etc.	And so, on	pH	Potential of hydrogen ion concentration
Et al	And co-worker	q	Quintal
FAO	Food and Agriculture Organization	q/ha	Quintal per hectare
gm	Gram	RDF	Recommended dose of fertilizers
g I ₂ /100 g	Grams of iodine per 100 grams	RWC	Relative leaf water content
g/L	Gram per liter	MSI	Membrane stability index
GC-MS	Gas chromatography-mass spectrometry	MDA	Malondialdehyde content
ha	Hectare	Rs	Rupees
ha ⁻¹	Per hectare	Rs/ha	Rupees per hectare
Hrs.	Hours	RPM	Revolutions per minute
Hr.	Hour	R.H	Relative humidity
i.e.,	Which is to say, in other words	ROS	Reactive oxygen species
I	Irrigation depth	R	Replication
I ₀	No post-sowing irrigation	SV	Saponification value
I ₁	One post-sowing irrigation at the vegetative stage	S	Significant
I ₂	Two irrigations at the vegetative and flowering stages	SEm	Standard error of the mean

I ₃	Three irrigations at the vegetative, flowering, and siliqua filling stages	S. No	Serial number
K	Potassium	SPD	Split plot design
kg	Kilogram	t	Tonnes
Kg/ha	Kilogram per hectare	TPC	Total phenolic content
L	Liter	WHO	World Health Organization
LA	Leaf area	WUE	Water use efficiency

The present study investigated the influence of humic acid and sulphur application on growth, physiological responses and yield of Indian mustard (*Brassica juncea* L. cv RLC 3) under diverse water regimes. Two consecutive field experiments were conducted at the Department of Agronomy research farm under the School of Agriculture at Lovely Professional University in Phagwara, Punjab, India, during *rabi* seasons in 2022–2023 and 2023-24. The study focused on identifying the optimal nutrient management strategies in varying irrigation regimes. The experiment was laid out in a split-plot design (SPD) with 16 treatments and 4 replications, with irrigation regimes assigned to the main plot (84.5 m² each) and nutrient management in the subplot (25 m² each). The irrigation regimes applied at critical stages included no post-sowing irrigation (I₀), one post-sowing irrigation at the vegetative stage (I₁), two irrigations at the vegetative and flowering stages (I₂), and three irrigations at the vegetative, flowering, and siliqua filling stages (I₃) meanwhile nutrient management treatments consisted of control (S₀), humic acid application (S₁), sulphur application (S₂), and combined humic acid and sulphur application (S₃). Three post-sowing irrigation (I₃) and combined humic acid and sulphur application (S₃) i.e. I₃S₃ had a significant impact on growth parameters such as plant height, fresh and dry biomass, leaf number and area, and phenological traits like days to emergence, branching, 50 % flowering and maturity. Growth analysis metrics, such as leaf area index (LAI), crop growth rate (CGR), relative growth rate (RGR), and net assimilation rate (NAR) were computed to quantify plant performance. The lowest LAI values were recorded in the I₀ treatment, with measurements of 0.84 at 30 days after sowing (DAS), 3.12 at 60 DAS, and 1.96 at 90 DAS during the 2022-23 season. A similar trend was observed in the following year. Similarly, the moisture content at harvest ranged from 42.54 mm (I₀) to 73.86 mm (I₃) in 2022-23 and 43.25 mm (I₀) to 69.99 mm (I₃). The initial plant population under I₀ was 513.70 plants and I₃ was highest with 518.89 plants per plot. The highest CGR (24.55 g m⁻² day⁻¹) was observed in the I₃S₃ combination and the lowest (9.11 g m⁻² day⁻¹) in I₀N₀. Physiological measurements, such as relative water content (RWC), membrane injury index (MII) and membrane stability index (MSI), and biochemical indicators like chlorophyll content, malondialdehyde content (MDA), total soluble protein and proline, were recorded to evaluate stress responses. The I₃S₃ treatment achieved the highest MSI at 80.24 %, while the I₀S₀ treatment recorded the lowest MSI at 54.21 % at 90 days after sowing in 2023-

24. The treatment with I₃ consistently recorded the highest chlorophyll levels, with values of 2.261 and 2.075 mg g⁻¹ fresh weight (FW) at 60 and 90 DAS, respectively, in 2022-23. The lowest chlorophyll values were recorded in the control I₀ and S₀, with values of 1.851 mg g⁻¹ FW in 2022-23 and 1.867 mg g⁻¹ FW in 2023-24. The lowest MDA content was observed in the I₃, with values of 13.04 µmole/g in 2022-23 and 15.91 µmole/g in 2023-24 at 60 DAS. The lowest MDA levels were observed in the S₃, with values of 13.58 µmole/g in 2022-23 and 18.46 µmol g⁻¹ FW in 2023-24. A similar trend was observed at 90 DAS, where S₀ consistently showed the highest MDA content (27.31 µmol g⁻¹ FW in 2022-23 and 29.81 µmol g⁻¹ FW in 2023-24). The highest MDA content was recorded in the I₀S₀, with values of 30.51 µmol g⁻¹ FW in 2022-23 and 33.67 µmol g⁻¹ FW in 2023-24. Yield and yield attributes, including number of branches, weight and length of siliqua, seed yield, biological yield and test weight, were measured at harvest. The interaction between irrigation regimes and nutrient management positively influenced mustard yield characteristics, with the highest values for siliqua number, siliqua length, test weight, siliqua weight, seed yield, and oil yield recorded under the I₃S₃ treatment, with average siliqua numbers of 416.94 (2022-23) and 462.13 (2023-24) per plant, siliqua length of 6.99 cm, test weight of 4.97 g, siliqua weight of 57.01 g, seed yield of 3663.27 kg ha⁻¹, and oil yield at 44.05%. In contrast, the no irrigation I₀S₀ treatments showed the lowest values across these parameters.

Oil quality parameters such as relative density, saponification value, iodine value, total phenolic content and total antioxidant activity were analysed to assess oil quality. Results showed that increased irrigation reduced antioxidant activity (AA), and total phenolic content (TPC) while increasing iodine value (IV). The I₃ resulted in the lowest AA (25.33%), and the highest saponification SV (189.15 mg KOH g⁻¹ oil), while the no-irrigation regime (I₀) had the highest AA (34.88%), and the lowest SV (147.33 mg KOH g⁻¹ oil). S₃ similarly decreased AA (26.00%) while increasing SV (160.51 mg KOH g⁻¹ oil). I₃N₂ treatment showed the highest SV (201.65 mg KOH g⁻¹ oil). Additionally, iodine and peroxide values decreased with increased irrigation and nutrient application, with the highest iodine value (138.21 g I₂ 100 g⁻¹ oil) in the I₀N₁ treatment and the lowest peroxide value (2.10 meq O₂ kg⁻¹ of oil) in the I₃N₁ treatment. Soil moisture dynamics were monitored across critical growth stages to evaluate water use efficiency and its interaction with nutrient management. In conclusion, S₃, along

with irrigation at critical stages, resulted in the best yield of Indian mustard across both years. This integrated approach is recommended for optimizing mustard production, enhancing oil quality, and improving resource use efficiency. The findings provide valuable insights into sustainable oil seed production, particularly in water-scarce regions, by optimizing nutrient and water management strategies for improved productivity and sustainability.

Keywords: Humic acid; Sulphur; Indian mustard; Oil quality; Irrigation

The present study investigated the influence of humic acid and sulphur application on growth, physiological responses and yield of Indian mustard (*Brassica juncea* L. cv RLC 3) under diverse water regimes. Two consecutive field experiments were conducted at the Department of Agronomy research farm under the School of Agriculture at Lovely Professional University in Phagwara, Punjab, India, during *rabi* seasons in 2022–2023 and 2023-24. The study focused on identifying the optimal nutrient management strategies in varying irrigation regimes. The experiment was laid out in a split-plot design (SPD) with 16 treatments and 4 replications, with irrigation regimes assigned to the main plot (84.5 m² each) and nutrient management in the subplot (25 m² each). The irrigation regimes applied at critical stages included no post-sowing irrigation (I₀), one post-sowing irrigation at the vegetative stage (I₁), two irrigations at the vegetative and flowering stages (I₂), and three irrigations at the vegetative, flowering, and siliqua filling stages (I₃) meanwhile nutrient management treatments consisted of control (S₀), humic acid application (S₁), sulphur application (S₂), and combined humic acid and sulphur application (S₃). Three post-sowing irrigation (I₃) and combined humic acid and sulphur application (S₃) i.e. I₃S₃ had a significant impact on growth parameters such as plant height, fresh and dry biomass, leaf number and area, and phenological traits like days to emergence, branching, 50 % flowering and maturity. Growth analysis metrics, such as leaf area index (LAI), crop growth rate (CGR), relative growth rate (RGR), and net assimilation rate (NAR) were computed to quantify plant performance. The lowest LAI values were recorded in the I₀ treatment, with measurements of 0.84 at 30 days after sowing (DAS), 3.12 at 60 DAS, and 1.96 at 90 DAS during the 2022-23 season. A similar trend was observed in the following year. Similarly, the moisture content at harvest ranged from 42.54 mm (I₀) to 73.86 mm (I₃) in 2022-23 and 43.25 mm (I₀) to 69.99 mm (I₃). The initial plant population under I₀ was 513.70 plants and I₃ was highest with 518.89 plants per plot. The highest CGR (24.55 g m⁻² day⁻¹) was observed in the I₃S₃ combination and the lowest (9.11 g m⁻² day⁻¹) in I₀N₀. Physiological measurements, such as relative water content (RWC), membrane injury index (MII) and membrane stability index (MSI), and biochemical indicators like chlorophyll content, malondialdehyde content (MDA), total soluble protein and proline, were recorded to evaluate stress responses. The I₃S₃ treatment achieved the highest MSI at 80.24 %, while the I₀S₀ treatment recorded the lowest MSI at 54.21 % at 90 days after sowing in 2023-

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Keywords: Humic acid; Sulphur; Indian mustard; Oil quality; Irrigation

Brassica spp., prominence as Rapeseed-Mustard, amongst oilseed crops serve as critical function in the Indian agricultural economy by contributing as edible oils, vegetables, condiments and animal feed (Chand *et al.*, 2021; Jat *et al.*, 2019). It is recognized as the third most significant edible oilseed crop globally, following soybean and palm oil, particularly in subtropical and tropical regions. However, Indian mustard (*Brassica juncea* L.) is one of the most significant oilseed crops cultivated in India, amounting for a considerable share of the country's edible oil production (Rai *et al.*, 2022). It plays a substantial role in the agricultural economy, especially in semi-arid and arid territories, where it serves as a key provider of income for farmers (Boomiraj *et al.*, 2010). The cultivation of Indian mustard has been widely acknowledged for its adaptability to various agro-climatic conditions, high oil content, and nutritional value (Thakur *et al.*, 2020). Despite its adaptability, mustard production is often challenged by factors such as nutrient deficiencies, water scarcity, and fluctuating climatic conditions, which collectively impact the crop's growth, yield, and quality (Panjabi *et al.*, 2019). Besides, Oil extracted from Indian mustard known as 'the versatile oil', has been integral to Indian culture for over 4000 years, due to its oil-rich and diverse culinary and health benefits (Jaiswal *et al.*, 2012). It is widely used in North and Northeast India for cooking, salad dressing, and hot oil massages, and as a remedy for various ailments. In 2023-24, India produced a registered 12 MMT of mustard oil, rendering leading producer. However, high domestic consumption prevents India from ranking among the top mustard oil exporters globally. Rajasthan leads the way contributing 40-50% of India's total mustard oil production. Top importers of Indian mustard oil are Australia, United Arab Emirates, and United States. India, a top global producer and consumer of vegetable oils, ranks fourth in the world's oilseed economy. It's the third leading leader of rapeseed-mustard, contributing 8.5% to the world's total (Economic Survey, 2022-2023). In 2023-24, India produced 12 MMT of mustard oil, renouncing it a leading producer. Rajasthan leads the way contributing 40-50% of India's total mustard oil production. However, high domestic consumption by country's growing population and increasing income levels prevents India from ranking amongst the top mustard oil exporters globally (seair.co.in). India meets 60% of its edible oil demand through imports, highlighting its import dependency and the number has been steadily growing. About 80% of India's edible oil is imported from Indonesia and Malaysia. India relies

heavily on edible oil imports as import has grown by 27% during first four months of 2022-23 (Solvent Extractor's Association). The high import dependency of India on edible oils is a major concern, as it makes the country's retail prices vulnerable to international pressures in the global market. The government of India in its Union budget 2024 focused on Atma nirbhar oilseed abhiyaan to cut the imports of edible oil from 60% to 30% so that India can become self-reliant in oilseeds like mustard, groundnut, soybean, and sunflower. Growing Mustard can be an ideal alternative. Despite being the leading edible oil importer, India faces a growing gap between demand and domestic production. Edible oil imports are expected to increase by 3.4% annually until 2030 (Economic Survey, 2022). To reduce reliance on imports, there's a crucial need to enhance oilseed production and crop productivity, especially in the northern plain zone, either as sole crop, or mixed crop in early, timely, late, rain-fed, irrigated and saline or alkaline soils (Choudhary *et al.*, 2014 and Chauhan *et al.*, 2011), where productivity is currently below the global average. Low oilseed yields result from imbalanced fertilizer application, and insufficient nutrient and water management, particularly with limited use of sulfur fertilizers. Therefore, there is a need to explore agronomic practices and inputs that can enhance growth and yield of Indian mustard, especially under water-limited conditions. One promising approach to improving mustard yield under water-stress conditions is the use of humic acid (HA) and sulfur (S) as soil amendments (Imran *et al.*, 2023). Humic acid, a naturally occurring organic compound from decomposed matter, improves soil fertility, water retention, and nutrient absorption. Sulfur is a vital macronutrient critical for plant growth, productivity, and development (Ampong *et al.*, 2022). It forms an integral part of amino acids, vitamins, and proteins, with its deficiency often resulting in significant declines in crop performance (Saady *et al.*, 2020). Multiple studies indicate that the combined application of humic acid and sulfur enhances physiological and biochemical processes in plants, increasing their tolerance to water stress conditions.

Water availability is an important parameter influencing the development and yield of Indian mustard. Water stress, particularly during crucial growth stages, can adversely affect the plant's physiological functions, leading to decreased biomass accumulation, leaf area, and seed yield (Shah *et al.*, 2023). This makes it imperative to investigate the possible benefits of humic acid and sulfur application under various water

regimes to mitigate the negative properties of water scarcity. The response of Indian mustard to these inputs under variable water conditions is not only of scientific interest but also has practical implications for optimizing crop management practices in water-limited environments (Riar *et al.*, 2020). Humic acid has been globally acknowledged for its ability to improve soil structure and boost water retention capacity, and enhance nutrient accessibility. It contains various bioactive groups like carboxyl and phenolic groups, which contribute to its high cation exchange capacity, thereby improving soil fertility (Tiwari *et al.*, 2023). The application of humic acid has been demonstrated to endorse root growth, improved nutrient uptake, and increase crop yield under different conditions. These effects are particularly important for Indian mustard, which has a relatively shallow root system and is more susceptible to water stress. By enhancing soil water retention and nutrient accessibility, humic acid can help mustard plants cope with water deficits and maintain better growth and productivity (Zhou *et al.*, 2019). Sulphur is essential for the growth and progression of oilseed crops such as mustard. It is integral to the production of amino acids, proteins, and fatty acids, while also contributing to chlorophyll formation and photosynthesis, which are crucial for plant development and yield (Zenda *et al.*, 2021). A lack of sulfur in Indian mustard can negatively impact oil content, seed quality, and overall yield. Research has demonstrated that sulfur application can boost plant growth, enhance nutrient absorption, and increase both seed production and oil concentration in mustard. Furthermore, sulfur aids in combating water stress by accelerating the plant's osmotic adjustment and water utilization efficiency, which is particularly important in water-scarce conditions (Meselhy *et al.*, 2021). The integration of humic acid and sulfur is particularly noteworthy, as they can work synergistically to improve crop growth and productivity. Humic acid enhances sulfur availability in the soil by enhancing its solubility and facilitating plant absorption. This can result in improved nutrient assimilation, photosynthetic efficiency, and overall plant growth. Additionally, the joint utilization of humic acid and sulfur enhance a plant's resilience to water stress by optimizing water conservation, nutrient uptake, and physiological functions, ultimately leading to higher yields (Al-Solaimani *et al.*, 2024). Water regimes, which refer to the pattern and availability of water during the crop growth cycle, significantly influence the growth and yield of Indian mustard (Rathore *et al.*, 2019). The crop's response to

varying water regimes is affected by multiple factors, including soil characteristics, climate conditions, and farming practices. Water stress, especially during critical growth phases like flowering and seed development, can result in substantial yield reductions in mustard (Zhang *et al.*, 2013). Therefore, to create strategies for increasing mustard productivity in water-limited places, it is essential to understand how mustard reacts to the application of humic acid and sulphur across differential water regimes. According to recent research, applying sulphur and humic acid can enhance the growth and production of a variety of crops while they are under drought condition (Bolhassani *et al.*, 2024). Comprehensive data on the combined impact of these inputs on Indian mustard under various water regimes is, nevertheless, lacking. Investigating the potential of sulphur and humic acid as soil supplements to improve mustard growth, yield, and water use efficiency under various water regimes is crucial given the rising frequency of droughts and water scarcity in many mustard-growing countries.

The study "Response of Indian Mustard to Humic Acid and Sulfur under Variable Water Regimes" explores the effects of combining humic acid and sulphur on Indian mustard (*Brassica juncea* L.) growth, yield, and quality in differential water conditions. Indian mustard, a crucial oilseed crop in semi-arid and arid areas, faces significant production challenges due to water scarcity. This research uniquely examines the combined influence of humic acid, sulphur, and varying water regimes on Indian mustard performance. While these elements have been studied separately, their interactive effects are not well understood. The investigation focuses on how these inputs affect various growth parameters, physiological processes, yield attributes, and water utilization efficiency in Indian mustard. The researchers hypothesize that applying humic acid and sulfur together may boost mustard growth and yield by enhancing nutrient availability, water retention, and stress tolerance, particularly in water-limited environments. This study seeks to support to the advancement of sustainable crop management practices for Indian mustard, with potential implications for boost productivity and water use efficiency in water-scarce regions. By understanding the interactions between humic acid, sulfur, and water regimes, this research offers valuable insights into optimizing nutrient management for Indian mustard cultivation. It also aims to develop approaches to strengthen mustard crop resilience to water stress, a growing critical factor amid climate change and water

scarcity. The findings may have broader implications for cultivating other oilseed crops in regions with water availability is a main constraint. The study's focus on Indian mustard's response to humic acid and sulfur under variable water regimes is of significant agronomic importance, potentially improving crop productivity, sustainability, and resilience to water stress. This comprehensive research provides insights into how humic acid and sulfur can enhance mustard growth and yield under different water conditions, aiding to the advancement of effective crop management technique for water-limited environments. By incorporating humic acid and sulfur into mustard cultivation practices, farmers may improve crop performance, optimize water use, and enhance the overall sustainability of mustard production systems.

Hence, considering all these factors, the current study entitled “Response of Indian mustard to humic acid and sulphur under variable water regime” has been planned to overcome the issues related to mustard crop by considering the following objectives.

1.1 Objectives:

1. To correlate the growth and yield of Indian mustard with different water regimes
2. To study the effect of humic acid and sulphur application on the growth and yield of Indian mustard under variable moisture regimes
3. To study the effect of moisture deficit on oil content and quality
4. To study the effect of humic acid and sulphur on oil content and quality

Indian mustard (*Brassica juncea* L.) is commonly cultivated on less fertile marginal lands, primarily relying on rainfed conditions in India. Achieving higher quality yields necessitates strategic management of irrigation and fertilizers. Numerous researchers have investigated the impact of irrigation and nutrient concentrations on Indian mustard (*Brassica juncea* L.). However, there is limited available literature addressing the correlation between Indian mustard and irrigation practices, as well as the synergistic influence of sulphur and humic acid. This chapter aims to review relevant existing literature pertaining to the current research study entitled “***Response of Indian mustard to humic acid and sulphur under variable water regimes***”

This chapter reviews the literature on Indian mustard (*Brassica juncea*), focusing on its agricultural, nutritional, and economic importance. It highlights the crop's global significance as a major oilseed and its contribution to food security. The role of humic acid and sulphur in enhancing crop growth and yield is discussed, particularly in improving nutrient bioavailability and soil quality. The chapter explores how water availability impacts oilseed crops, emphasizing the impact of moisture stress on crop development, yield, and oil content. It also delves into the chemical properties of humic acid and its mechanisms for promoting plant development, such as improving nutrient uptake and root growth.

The role combined effect of sulphur in plant nutrition is addressed, particularly its importance in protein biosynthesis, enzymatic activation, and chlorophyll production. The consequence of both humic acid and sulphur on mustard growth, oil content, and seed protein quality is evaluated, along with their synergistic benefits under different water regimes. The chapter also examines mustard's drought tolerance mechanisms and how yield responds to variable water availability. Finally, the response of humic acid and sulphur on the fatty acid profile of mustard seed oil is reviewed, providing insights into their potential for improving oil quality.

2.1 Importance of Indian mustard (*Brassica juncea* L.) in global agriculture

The global demand for edible oil continues to rise, with Indian mustard playing a crucial role in the oilseed industry. Mustard oil is extensively utilized in cooking, while residual meal after oil extraction serves as a valuable source of protein-rich animal feed (Yılmaz *et al.*, 2024). Over the last decade, studies have highlighted mustard's importance as an economical oilseed crop in India, where it contributes nearly 30% of the country's total oilseed production (Sachan *et al.*, 2024). Globally, Indian

mustard holds a strong position in the oilseed market, contributing to food security, rural livelihoods. Indian mustard is known for its rich nutritional profile. Mustard oil is high in monounsaturated and polyunsaturated fatty acids, which are beneficial for cardiovascular health (Poddar *et al.*, 2022). It also contains essential omega-3 fatty acids and antioxidants such as tocopherols, which have anti-inflammatory properties and help in reducing cholesterol levels. Studies from the past decade have confirmed the positive health impacts of mustard oil consumption, contributing to its growing global demand as a healthier oil alternative (Sharma *et al.*, 2022). In addition, mustard seeds are an admirable source of protein, dietary fiber, vitamins like B-complex and vitamin E, and essential minerals like calcium, magnesium, potassium, and iron (Rai *et al.*, 2022). This nutritional richness not only supports human nutrition but also has industrial applications in food products, bio fortification strategies, and functional food development (Rahman *et al.*, 2024).

Within the framework of sustainable agriculture, Indian mustard is considered an environmentally resilient crop due to its ability to endure climatic conditions, like drought, salinity, and high temperatures. Studies have emphasized the importance of mustard cultivation in areas affected by climate change, where it acts as a reliable crop for food and oil production under sub-optimal conditions (Panjabi *et al.*, 2019). Lately studies have highlighted Indian mustard's potential in water- partial environments. Studies indicate that Indian mustard (*Brassica juncea*), has shown remarkable drought tolerance by adjusting root depth and osmotic potential, enabling them to flourish in water-deficit conditions (Ranjit *et al.*, 2016). This adaptability makes mustard crop for semi-arid and arid regions where water accessibility poses a main challenge. Indian mustard plays a key role in improving soil health and reducing soil erosion. It is often used in crop rotation systems with cereals and legumes, helping to break pest cycles and enhance soil fertility. Recent studies have reported the benefits of mustard in improving soil organic matter and nitrogen content when used in rotation with crops like wheat. This practice contributes to better soil and sustainability in farming systems, minimizing reliance on synthetic fertilizers (Sachan *et al.*, 2024).

2.2 Nutritional and Economic Importance of Indian Mustard

Indian mustard (*Brassica juncea*) holds a pivotal position in both the nutritional and economic spheres, especially in areas like South Asia, wherein it is a key oilseed crop. Nutritionally, mustard seeds and oil are packed with essential nutrients. Mustard oil,

derived from the seeds, is rich in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), which are crucial for heart health (Podder *et al.*, 2022). It also contains omega-3 and omega-6 fatty acids that are beneficial in reducing inflammation, improving brain function, and protecting against cardiovascular diseases. The oil has abundant antioxidants, particularly tocopherols, which help in neutralizing free radicals and promoting overall well-being. Mustard seeds are a valuable source of protein, dietary fiber, and essential minerals viz. calcium, magnesium, potassium, and iron (Youssef *et al.*, 2014). Additionally, mustard leaves are consumed as green vegetables are plentiful source of vitamins A, C, and K, further contributing to human nutrition (Favela-González *et al.*, 2020). Economically, Indian mustard is crucial to the agricultural economy of India and other mustard-growing countries. It is the 2nd largest oilseed crop in India, providing both edible oil and protein-rich seed meal, which is used as an animal feed. The cultivation of mustard contributes significantly to rural livelihoods, especially in semi-arid regions where mustard can thrive with minimal water (Sachan *et al.*, 2024). Recent studies highlight its economic importance in terms of its contribution to edible oil production and its role in global oilseed markets (Hagos *et al.*, 2020). The increasing demand for healthier oils has also bolstered mustard's market value, particularly due to its higher unsaturated fat content compared to other commonly used cooking oils. Mustard is also being explored for its potential in biodiesel production, adding to its economic importance in the renewable energy sector (Grygier, 2023).

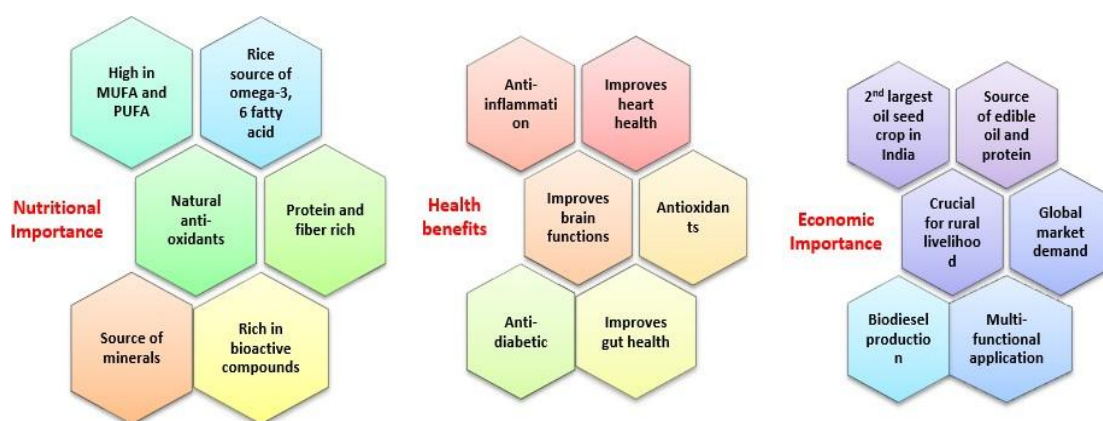


Figure 2.1. Nutritional importance, health benefits, and economic importance of Indian mustard

2.3 Overview of Humic Acid and Sulphur in Crop Nutrition

Two essential substances (humic acid and sulphur) that are important for improving soil fertility and improving crop nutrition are sulphur (S) and humic acid (HA). It is a naturally occurring substance created by organic matter, primarily plant deposits, breaks down in the soil. Because escalates soil structure, water conservation, and nutrient bioavailability, it is an essential part of sustainable agriculture (Hayes and Swift, 2020). By binding vital elements like potassium, phosphorus, and nitrogen, humic acid increases the accessibility of nutrients to crop roots and improves nutrient uptake. Moreover, it enhances soil aeration and water-holding capacity, especially in sandy or damaged soils, which supports crop growth under challenging environmental circumstances (Izhar Shafi *et al.*, 2020). Furthermore, humic acid acts as a natural growth stimulant, encouraging root architecture, which leads to a better nutrient absorption system (Abbas *et al.*, 2022). Research had reflected that HA application can expand plant tolerance to abiotic stresses including drought and salinity, further contributing to increased yield potential and crop productivity. Additionally, HA promotes root elongation and branching, which results in a more effective nutrient absorption system, acting as a natural growth stimulant (Abbas *et al.*, 2022). Applying humic acid to plants reflected increase their tolerance to abiotic conditions including drought and salinity, which further boosts crop productivity and yield potential. Sulphur is another vital nutrient for plant growth, particularly in the formation of proteins, enzymes, and other essential compounds (Zenda *et al.*, 2021). Sulphur is a key element of certain amino acids, such as cysteine and methionine, which are crucial for protein synthesis in plants (Narayan *et al.*, 2023). It has a key central role in chlorophyll formation, aiding in the process of photosynthesis, which is important for plant growth and biomass accumulation (Shah *et al.*, 2022). Sulphur deficiency can cause decline in crop yield, delayed maturity, and poor quality, as it affects protein content and oil quality in oilseed crops like mustard (Rehman *et al.*, 2013). In cereals and legumes, sulphur is instrumental in enhancing grain quality, nitrogen fixation, and overall productivity. Adequate sulphur supply in crops has been shown to improve disease resistance and promote better overall plant health. Recent studies have shown that the combination of humic acid and sulphur creates synergistic effects that enhance nutrient absorption, root development, and plant stress tolerance (Osman *et al.*, 2012). Humic acid helps to solubilize sulphur in the soil, increasing its bioavailability for plants (Imran *et al.*, 2023).

This collective application has resulted in improved crop yields, oil content, and overall crop quality in several agricultural trials. The interaction of these two components is particularly beneficial in nutrient-deficient soils or areas with water scarcity, where improving nutrient uptake and water retention is critical for crop survival (Kumari *et al.*, 2020). Collective application of humic acid and sulphur enhanced root growth, nutrient uptake, and seed yield, particularly in drought conditions in Indian mustard. Furthermore, Sulphur application significantly improved the oil content and enhanced the fatty acid composition. Thus, sulphur can be effectively used for the plant-growth development (Imran *et al.*, 2023).

2.4 Impact of Water Availability on Oilseed Crops

The growth, yield, and quality of oilseed crops are significantly influenced by water availability. Oilseed crops require an adequate and consistent water supply to ensure proper germination, vegetative growth, and seed development (Ebrahimian *et al.*, 2019). Insufficient water during key growth stages, particularly during flowering and seed formation, may cause significant yield deduction and diminished oil quality (Sehgal *et al.*, 2018). Research over the past decade has highlighted that oilseed crops are highly sensitive to water stress, especially during the reproductive stage, where drought conditions can result in fewer flowers, reduced seed weight, and lower oil content (Hussain, *et al.*, 2018). Conversely, excess water, caused by poor drainage or heavy rainfall, can lead to root suffocation, reduced nutrient uptake, and increased susceptibility to diseases, further affecting yield (Akhtar *et al.*, 2025). Water scarcity is a growing challenge in many regions, especially in arid and semi-arid areas, where oilseed crops are increasingly cultivated due to their drought-tolerant characteristics. However, despite this resilience, prolonged water deficits can significantly deduct in crop productivity (Rajanna *et al.*, 2018). Few studies unveiled that drought-tolerant varieties of oilseeds, such as Indian mustard, are better adapted to water-limited conditions due to their deep root systems, which enable them to access moisture from deeper soil layers (Raza *et al.*, 2023).

Indian mustard exhibit mechanisms such as osmotic, which helps them sustain cell turgor and survive under water stress. Osmotic change is a key mechanism that allows oilseed crops to cope with water deficit by accumulating osmolyte, which lower osmotic potential and help retain water, thereby maintaining cell turgor and physiological functions. This process improves water uptake and can protect cellular

structures during drought, sometimes even enhancing oil accumulation under moderate stress conditions. As a result, osmotic adjustment supports crop adaptability and productivity in water-scarce environments (Batool *et al.*, 2022). Despite these adaptations, significant yield reductions still occur under severe drought conditions. On the other hand, water management practices, like that of controlled irrigation and the use of water-efficient technologies like drip irrigation, have proven effective in optimizing water use in oilseed cultivation (Tiwari *et al.*, 2023). Controlled water supply, especially during critical growth periods helps improve seed yield and oil content. Furthermore, mulching and conservation tillage practices have been adopted for improving soil moisture retention, prevent water loss, and enhance oilseed crop performance under sub-optimal water conditions. While oilseed crops display some level of resilience to water stress, ensuring optimal water availability is essential for maximizing yields and maintaining oil quality (Mallareddy *et al.*, 2023). In this context, the impact of water availability on oilseed crops has been widely studied, revealing significant effects on yield, oil content, and overall crop quality. Drought conditions during critical growth stages, such as flowering, can drastically reduce crop yields and oil content, as observed in sunflower (*Helianthus annuus*), where drought led to significant yield losses and reduced oil content (Harsányi *et al.*, 2021). Comparable results were noted in soybean (*Glycine max*), where severe water stress caused a 25% reduction in oil content, thereby impacting seed quality (Mertz-Henning *et al.*, 2017). Drought tolerance mechanisms in crops like Indian mustard (*Brassica juncea*) have shown that drought-tolerant varieties exhibit better root growth and osmotic adjustment, with less yield reduction (Aneja *et al.*, 2015). Controlled irrigation has proven to be a beneficial practice for crops like rapeseed (*Brassica napus*), leading to improved yield, oil quality, and optimal water use efficiency. Water management practices, particularly in mustard cultivation, have also been highlighted. Mulching and drip irrigation were found to improve soil moisture retention, which in turn enhanced seed yield and oil content in Indian mustard (Das *et al.*, 2020). In canola (*Brassica napus*), irrigation deficiency resulted in a 20% reduction in seed weight and oil content, further demonstrating the importance of adequate water supply (Naghavi *et al.*, 2015). Water stress during flowering in groundnut (*Arachis hypogea*) caused a 30% reduction in seed yield and a decrease in oil content, emphasizing the crop's sensitivity to water availability during critical growth stages (Puppala *et al.*, 2023). Even under water stressed regions,

some crops like safflower (*Carthamus tinctorius*) showed an improvement in water use efficiency despite reduced economic yield and oil concentration (Bortolheiro and Silva, 2017). Castor bean (*Ricinus communis*) exhibited a moderate decline in yield under water deficiency, though the oil quality remained relatively unaffected (Sadeghi-Bakhtavari and Hazrati, 2021). Conversely, water logging had a detrimental effect on Indian mustard, leading to a significant reduction in plant growth and seed formation, which adversely impacted yield (Xu *et al.*, 2015). Thus, these findings emphasized on the importance of water availability and management practices in oilseed crops, showing that both water scarcity and excess can negatively affect crop productivity, oil content, and quality. Adequate irrigation, water conservation techniques, and the selection of drought-tolerant varieties are critical strategies to mitigate these impacts and ensure optimal yields.

2.5 Humic Acid (HA): Composition and Agricultural Relevance

HA is a naturally occurring organic compound that plays an important role in soil fertility and crop development. It is formed through the breakdown of plant and animal matter over time and is a key component of humus, the organic part of soil (Lawrence, 2017). These groups give HA its unique ability to interact with soil particles and nutrients, acting as a natural chelator that binds essential elements like N, P, K (Qiao *et al.*, 2024). This binding mechanism increases the nutrients' availability to plants by stopping them from evaporating, which is crucial in sandy or nutrient-poor soils (Tahoun *et al.*, 2022). According to Rashad *et al.* 2022, HA is extremely important in agriculture because it improves soil structure, increases water retention enhances nutrient bioavailability—all of which are critical for promoting plant development and yields.

By improving soil porosity, humic acid helps create stable aggregates that allow for better root architecture and soil aeration, reducing lumpiness and better plant access to nutrients and water. One of its critical functions is enhancing the soil's water retention capacity, making moisture more available to plants, especially in arid or drought-prone regions (Al-Maliki *et al.*, 2018). This property makes humic acid an important input for sustainable agriculture in areas facing water scarcity. Humic acid also stimulates root growth by promoting cell division and extension in root tissues, resulting in larger and more efficient root systems (Ghadirnezhad Shiade *et al.*, 2023). This allows plants to access a broader range of soil nutrients and moisture, improving overall plant health

and resilience. Additionally, humic acid enhances plants' ability to resist environmental stresses; salinity, and extreme temperatures by helping maintain higher rates of photosynthesis and metabolic activity (Chen *et al.*, 2022). Its ability to increase nutrient uptake efficiency means that less fertilizer is needed, reducing the environmental effect of farming practices (Shukry *et al.*, 2023). Thus, humic acid is an essential tool for sustainable agriculture, offering benefits that include improved soil structure, enhanced water and nutrient retention, and better stress tolerance in plants. It not only supports higher crop yields but also pays for more efficient and environmentally friendly farming systems, making it a key input for modern agriculture.

2.5.1 Definition and Natural Sources of Humic Acid

The natural breakdown of plant and animal material in soil and aquatic environments produces humic acid, a complex chemical molecule. It contributes significantly to humus, the organic matter in soil, and is crucial for increasing nutrient availability, boosting plant development, and improving soil fertility (Lawrence, 2017). Humic acid is an essential part of sustainable agriculture since it helps plants retain water, promotes root development, and improves their general health (da Silva *et al.*, 2021).

Humic acid is naturally derived from various organic sources and one of the primary sources is peat, which is formed from partially decomposed plant matter in wetland environments (Paleckiene *et al.*, 2021). Over time, the accumulation and slow decomposition of this organic material led to the formation of humic substances, including humic acid. Soil itself is another key source, as organic matter like dead plant roots, leaves, and animal residues decompose through microbial activity, contributing to the soil's humic content (Paleckiene *et al.*, 2021). Compost, which is a product of the controlled decomposition of organic residues such as plant waste, manure, and food scraps, also contains humic acid, especially when the composting process is well managed. Lignite, a type of soft coal, is a geological source of humic acid, as it is formed from ancient plant material that has undergone low-grade coalification, preserving its humic content. In addition, humic acids can also be found in freshwater and marine sediments, where organic matter accumulates and decomposes over long periods, contributing to the formation of humic substances in these environments (Zavarzina *et al.*, 2021).

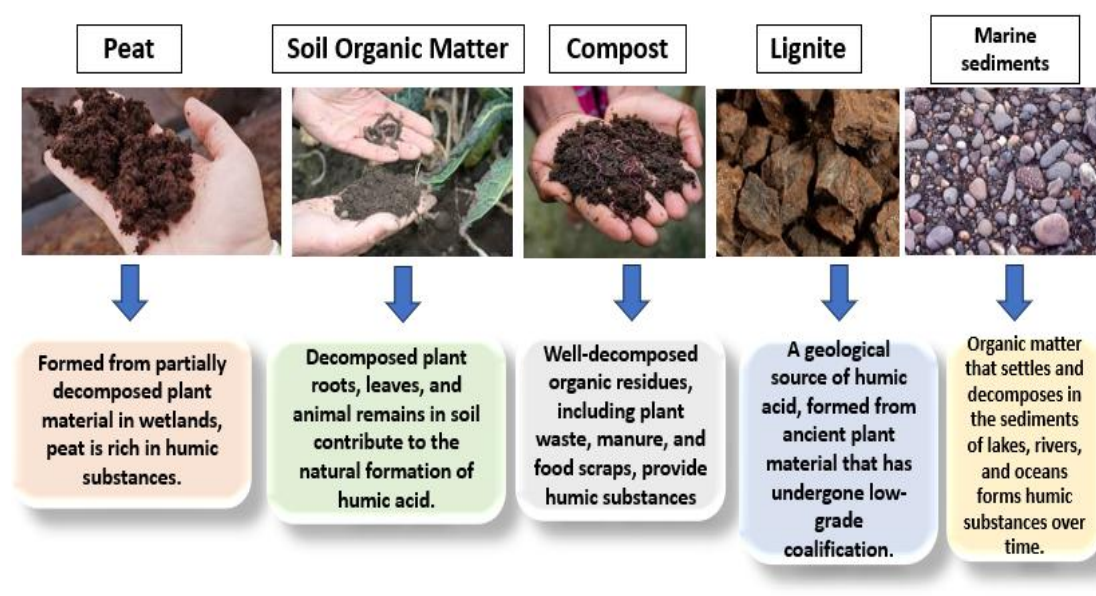


Figure 2.2. Different natural sources of humic acid and their description

2.5.2 Chemical Structure and Properties of Humic Acid

A complex organic molecule with an extensive heterogeneous molecular structure, humic acid is essential to plant nutrition and soil chemistry. Carbon, hydrogen, oxygen, and nitrogen make up the majority of its composition, with trace amounts of additional elements including phosphorus and sulphur (Tiwari *et al.*, 2023). The structure of humic acid includes both aliphatic (open-chain) and aromatic (ring-based) components, making it highly reactive. It contains various functional groups, including carboxyl (-COOH), hydroxyl (-OH), methoxyl (-OCH₃), and phenolics, which give humic acid its unique properties and ability to interact with soil particles, nutrients, and plant roots (de Melo *et al.*, 2016). These functional groups are responsible for its elevated cation exchange capacity (CEC), allowing HA to bind with essential plant nutrients, viz., calcium, magnesium, potassium, and trace metals, making them more available to plants while preventing nutrient leaching (Ampong *et al.*, 2022). Humic acid is mildly acidic, attributable to the presence of carboxyl and phenolic groups, which can dissociate in water to release hydrogen ions (H⁺). This property helps buffer soil pH, improving the bioavailability of nutrients and ensuring stable environment for crop growth (Boguta *et al.*, 2019).

Another important property of HA is its ability to elevate soil structure and water retention. The large, porous molecules can trap water, making it more available to plants during periods of drought, particularly in sandy or nutrient-poor soils.

Additionally, humic acid promotes the development of soil aggregates, enhancing aeration and root development (Morales *et al.*, 2018). One of the key functions of HA is its role as a natural chelator, binding metal ions such as iron, zinc, and copper, which are crucial for plant growth. This chelation process not only makes these micronutrients more bioavailable to plants but also helps reduce the toxic effects of harmful metals like aluminum (Rahale *et al.*, 2021). Furthermore, humic acid stimulates microbial activity in the soil by providing an energy source for soil microbes, which enhances organic matter decomposition and nutrient cycling. Its hormone-like effects promote root elongation and branching, resulting in improved nutrient uptake and overall plant growth (Condrón *et al.*, 2010). Humic acid also has a buffering capacity, stabilizing soil pH and protecting plants from stress caused by fluctuations in soil acidity or alkalinity. These combined properties make humic acid a critical component for improving soil fertility, supporting plant health, and promoting sustainable agricultural practices (Biswas and Kole, 2017).

2.5.3 Mechanism of Humic Acid Action in Plant Growth

Humic acid promotes crop development through different mechanisms that enhance nutrient availability, root development, water retention, microbial activity, and plant hormone regulation (Canellas *et al.*, 2014). One of the primary ways humic acid benefits plants is by improving nutrient availability. It acts as a natural chelator, binding essential nutrients and trace elements like iron, zinc, and copper, production them more accessible to plants (Canellas and Olivares, 2014). This benefit checks nutrient leaching and ensures a steady supply of nutrients to plant roots, which supports metabolic processes and boosts overall growth. In addition to enhancing nutrient availability, humic acid stimulates root development. It interacts with plant hormones such as auxins, which promote root elongation and branching (Mora *et al.*, 2012). This leads to a more extensive root system, allowing plants to access water and plant nutrients from a larger soil area. The formation of additional root hairs increases the surface area for nutrient absorption, enabling the plant to take up nutrients more efficiently (Nardi *et al.*, 2021).

Additionally, a strong root system increases the crop tolerance to environmental stressors including drought and nutrient shortages. Additionally, humic acid is essential for enhancing soil water retention. By enhancing soil structure and forming stable aggregates, humic acid increases the ability of soil to hold water (Zhou

et al., 2019). This is mainly beneficial in dry or sandy soils, where water retention is typically low. The increased water-holding capacity helps plants maintain access to moisture during dry periods, promoting consistent growth and improving drought tolerance (Zheng *et al.*, 2018). Moreover, humic acid stimulates microbial activity in the soil, fostering a healthy soil ecosystem. It provides an energy source for beneficial microorganisms that break down biological matter, releasing essential plant nutrients (Hriciková *et al.*, 2023).

The enhanced microbial activity improves soil fertility and nutrient cycling, particularly for N and P, those are critical for plant development. Finally, humic acid interacts with plant hormones, influencing growth and development (Hriciková *et al.*, 2023). It mimics auxins, promoting root growth and cell elongation, while also enhancing the activity of gibberellins and cytokinins, which regulate stem elongation, seed germination, and shoot development. By modulating these hormonal pathways, humic acid accelerates plant growth, improves biomass production, and enhances crop yields. Additionally, humic acid helps plants adapt to environmental stresses such as drought and salinity by supporting antioxidant defense mechanisms and improving overall plant resilience. These combined actions make humic acid a vital component in sustainable agriculture, improving plant health and productivity (Nard *et al.*, 2021).

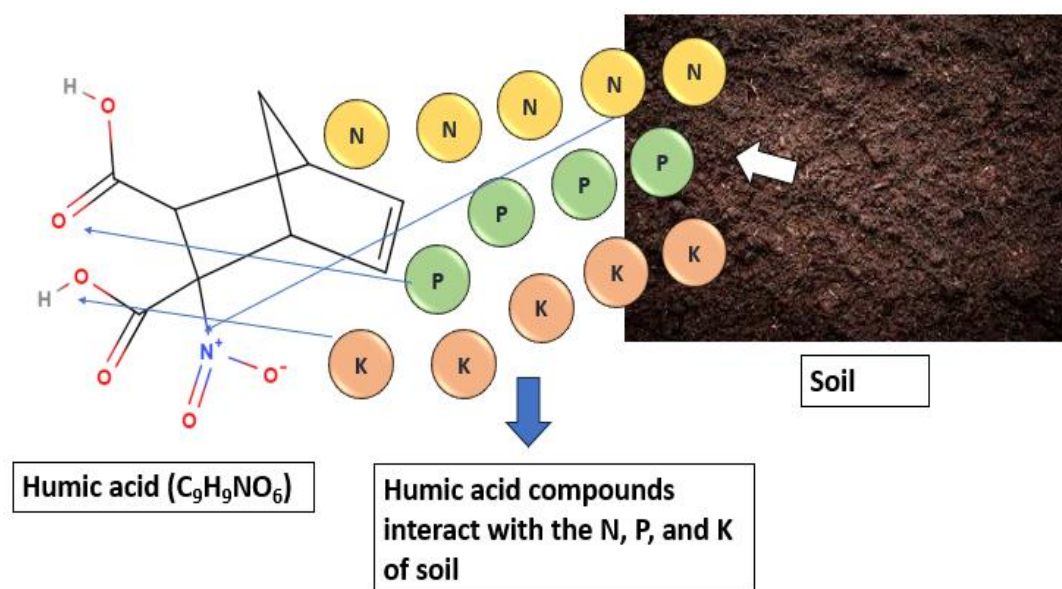


Figure 2.3. Binding interaction of humic acid compounds with nitrogen, phosphorus, and potassium of soil

2.5.3.1 Hormone-like Effects of Humic Acid

Humic acid exerts hormone-like effects on plant growth, acting in a way similar to natural plant hormones, particularly auxins, cytokinins, and gibberellins. These effects have been widely studied and are considered one of the key benefits of humic acid in agriculture. Humic acid stimulates root growth by interacting with auxins, the hormones responsible for cell elongation and root development (Savy *et al.*, 2017). This results in enhanced root branching, elongation, and the formation of root hairs, which increases the surface area for nutrient and water absorption. A more developed root system allows plants to access more resources from the soil, leading to improved growth and stress tolerance. In addition to its auxin-like activity, humic acid also influences other plant hormones, such as cytokinins and gibberellins. Cytokinins are responsible for promoting cell division and shoot development, leading to increased biomass and better overall plant growth (Souza *et al.*, 2022). Humic acid enhances cytokinin activity, which encourages shoot formation and the expansion of leaves, thus increasing photosynthetic capacity. Similarly, humic acid has gibberellin-like effects, which help in stem elongation, seed germination, and flowering (de Moura *et al.*, 2023). These hormonal effects accelerate the plant's growth cycle, leading to faster development and potentially higher yields.

Moreover, humic acid helps balance hormonal levels in plants, allowing for better growth regulation under different environmental conditions. By influencing these hormone pathways, humic acid contributes to improved plant vigor, enhanced resilience to stress, and increased productivity, making it an essential tool for sustainable agricultural practices (de Moura *et al.*, 2023). These hormone-like actions, combined with its role in nutrient availability and water retention, make humic acid a powerful agent in promoting healthy and vigorous plant growth.

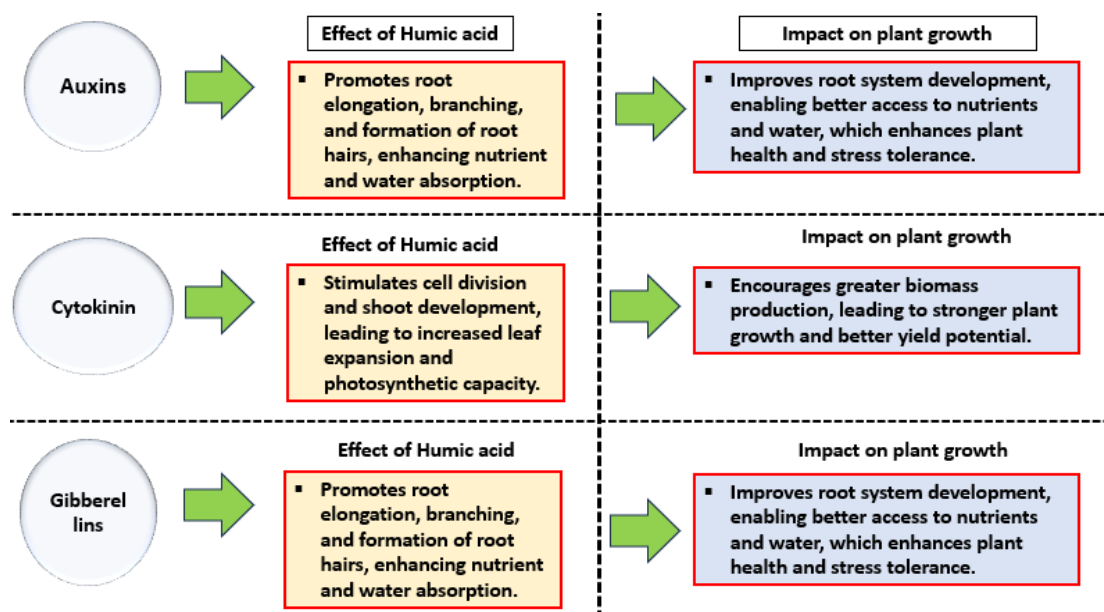


Figure 2.4 Effect of humic acid as a plant growth hormone and its impact on plant growth.

2.5.3.2 Enhancing Nutrient Availability and Soil Microbiology

Humic acid plays a vital role in enhancing nutrient availability and promoting soil microbiology, which are essential for improving plant growth and soil health. One of its primary functions is its ability to chelate essential nutrients and micronutrients (Maji *et al.*, 2017). Through its carboxyl and phenolic functional groups, humic acid binds to these nutrients, preventing them from leaching out of the soil or becoming insoluble. This process keeps nutrients in a bioavailable form, ensuring that plant roots can easily access them. By improving nutrient retention and facilitating slow-release mechanisms, humic acid reduces the need for frequent fertilizer applications, making nutrient uptake more efficient and sustainable (Nardi *et al.*, 2017). This leads to better plant nutrition, stronger growth, and higher yields. In addition to enhancing nutrient availability, humic acid has a profound effect on soil microbiology. It provides a source of energy for beneficial soil microorganisms, encouraging their proliferation.

Microbial communities are vital for nutrient cycling, organic matter decomposition, and overall soil fertility. As humic acid promotes microbial activity, it helps break down organic materials in the soil, releasing essential nutrients like nitrogen and phosphorus that are otherwise locked in organic matter (Jing *et al.*, 2020). This creates a more balanced and healthy soil ecosystem, fostering beneficial relationships between plants

and microbes. Furthermore, enhanced microbial activity improves soil structure by forming aggregates, which increases soil porosity, aeration, and water retention, further supporting plant growth. In this way, humic acid not only improves nutrient availability but also supports a thriving microbial environment, creating more fertile and resilient soils (Erro *et al.*, 2016).

2.6 Polyelectrolytic properties of humic acid

Humic acid, a key component of organic matter found in soils, exhibits polyelectrolytic properties due to its ionizable functional groups, such as carboxyl, hydroxyl, and phenolic groups. These groups dissociate in aqueous solutions, resulting in a negatively charged macromolecule, similar to anionic polyelectrolytes (Ampong *et al.*, 2022). The degree of ionization and polyelectrolytic behavior of humic acid is influenced by environmental factors like pH, with higher pH levels leading to greater ionization. As a polyelectrolyte, humic acid interacts with metal ions through electrostatic forces and complexation, enabling it to bind essential nutrients (Li *et al.*, 2022). This ability to form stable complexes plays a vital role in nutrient availability for plants, improving soil structure and supporting plant growth, particularly in nutrient-deficient conditions. The polyelectrolytic nature of humic acid also makes it valuable in environmental applications, such as water treatment, where it binds to pollutants and heavy metals, aiding in their removal. Overall, the relationship between humic acid and polyelectrolytes is central to its function in soil chemistry, nutrient cycling, and environmental sustainability (Yang and Antonietti, 2020).

2.7 Impact of Humic Acid (HA) on Soil Health and Fertility

2.7.1 Improvement of Soil Structure and Water Retention

Humic acid has a important impact on soil structure and enhancing water retention, both of which are critical for sustainable agriculture and healthy plant growth. One of the key ways humic acid affects soil structure is by improving the development of soil aggregates (Gerke, 2018). These aggregates are clusters of soil particles bound together by organic matter, creating a more stable soil environment. When humic acid is present in the soil, its large, complex molecules bind with minerals and organic matter, forming stable aggregates. This improves soil porosity, aeration, and drainage, which is especially important in compacted or clay-heavy soils where poor structure can restrict root growth and nutrient uptake (Hayes, & Swift, 2020). Improved soil structure also

enhances the ability of plant roots to penetrate the soil, allowing for better access to water and nutrients.

In addition to its impact on soil structure, humic acid significantly increases the soil's capacity to retain water. The complex molecular structure of humic acid allows it to absorb and hold water, which is particularly beneficial in sandy or arid soils that typically have low water-holding capacity. By improving the soil's ability to retain moisture, humic acid ensures that plants have access to water during dry periods, reducing the effects of drought and improving overall plant resilience (Zhou *et al.*, 2019). This water retention capability not only helps in maintaining consistent soil moisture levels but also reduces the frequency of irrigation needed, making it an important factor in water conservation efforts in agriculture. The combination of improved soil structure and enhanced water retention provided by humic acid leads to healthier soils, more vigorous plant growth, and better crop yields (Kandra *et al.*, 2024).

2.8 Humic Acid in Indian Mustard Cultivation

2.8.1 Influence on Root Development and Nutrient Uptake

Humic acid plays a crucial role in Indian mustard cultivation, particularly by enhancing root development and nutrient uptake, which are vital for reaching optimal crop development and yield attributes. In the soil, humic acid acts as a growth promoter, influencing the root system of Indian mustard through its hormone-like activity. By interacting with plant hormones, such as auxins, humic acid stimulates root elongation and branching, leading to a more extensive and deeper root system (Jindo *et al.*, 2020). This increased root surface area allows Indian mustard plants to access water and nutrients more efficiently, even from deeper soil layers, which is particularly beneficial in nutrient-poor or drought-prone soils. As a result, the plant can develop stronger root structures that contribute to its overall vigor and stress tolerance (Arif, 2020).

Humic acid also enhances nutrient uptake in Indian mustard by improving the bioavailability of essential nutrients as well as micronutrients like iron, zinc, and copper. Its chelating properties enable humic acid to bind with these nutrients, preventing them from becoming fixed in the soil or lost through leaching (Kalsi *et al.*, 2016). This process ensures that nutrients remain available in a soluble form, ready for absorption by the plant roots. Moreover, the improved root architecture facilitated by humic acid allows Indian mustard to better absorb these nutrients, leading to more efficient nutrient uptake and utilization (Rathor *et al.*, 2023). This results in improved

plant health, higher biomass production, and enhanced seed yield and oil content, which are crucial for the economic success of mustard cultivation. By promoting both root development and nutrient uptake, HA serves as a valuable tool for improving development and sustainability of Indian mustard farming.

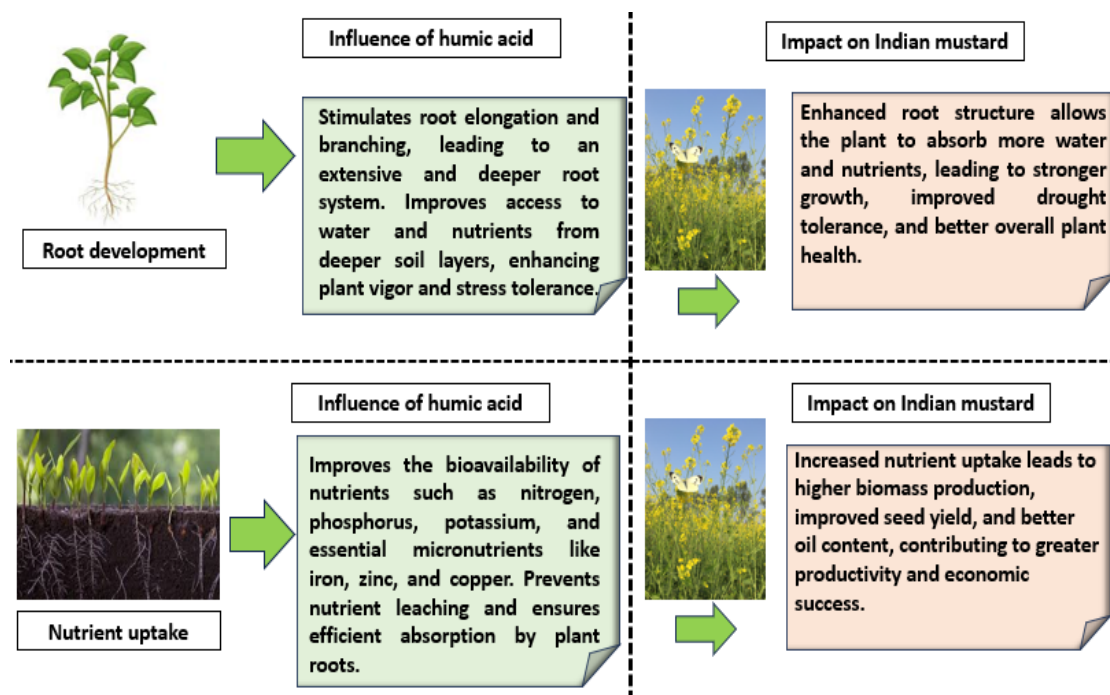


Figure 2.5 Effect of humic acid on the root development and nutrient uptake on Indian mustard

2.8.2 Effect on Chlorophyll Content and Photosynthesis

Humic acid has a notable impact on chlorophyll content and photosynthesis in Indian mustard, playing a crucial role in enhancing the plant's overall growth and productivity. Photosynthesis, the process by which plants transform light energy into chemical energy to power their development, depends on chlorophyll, the green pigment found in plant leaves (Mandal, & Dutta, 2020). Humic acid positively influences chlorophyll content by improving nutrient uptake, particularly nitrogen, magnesium, and iron, which are essential elements for chlorophyll synthesis (Turan *et al.*, 2022). By making these nutrients more readily available to the plant, humic acid ensures that Indian mustard can produce higher levels of chlorophyll, leading to more efficient photosynthesis. Increased chlorophyll content allows the plant to capture more sunlight and enhance the photosynthetic process, resulting in improved energy production (Rathor *et al.*, 2023).

This, in turn, supports greater biomass accumulation, stronger growth, and better crop yields. Additionally, the enhanced photosynthesis enabled by humic acid contributes to better carbon assimilation, which is vital for developing key plant tissues and supporting overall physiological functions. Research showed that plants treated with humic acid exhibit higher rates of photosynthesis (Chen *et al.*, 2022). By boosting chlorophyll production and photosynthetic efficiency, humic acid directly contributes to higher productivity in Indian mustard cultivation. This leads to improved seed yield, enhanced oil content, and better overall crop quality, making humic acid a valuable input for optimizing the growth and economic performance of Indian mustard in both traditional and sustainable farming systems (Hemati *et al.*, 2022).

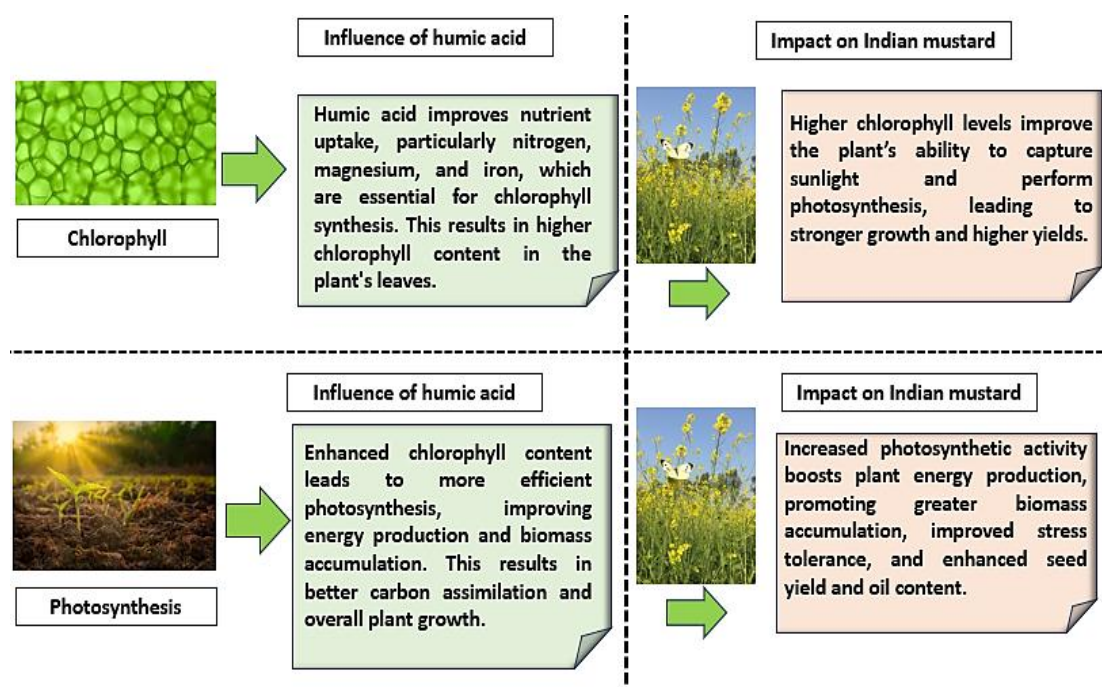


Figure 2.6 Effect of humic acid on chlorophyll content and photosynthesis of Indian mustard

2.8.3 Yield and Quality Improvements in Mustard Treated with Humic Acid

Humic acid has been shown to significantly improve both the yield and quality of mustard, making it a valuable input in modern agricultural practices. Profits of HA is its capability to enhance nutrient availability and uptake, which directly influences plant growth and seed yield (Tahoun *et al.*, 2022). By chelating essential nutrients and trace elements like iron and zinc, humic acid ensures these nutrients are readily

available to the plant throughout the growing season. This improved nutrient uptake leads to more vigorous plant growth, increased biomass, and a greater number of siliquae (seed pods), resulting in a higher seed yield (Dhaliwal *et al.*, 2024). The increased root development fostered by humic acid further supports efficient nutrient absorption and contributes to the overall productivity of the plant. In addition to boosting yield, humic acid has been found to enhance the oil content of mustard seeds, a critical factor for oilseed crops. By increasing the availability of nutrients like phosphorus and sulfur, which are key in fatty acid synthesis, humic acid promotes higher oil production in the seeds (Olivares *et al.*, 2017).

Research showed that mustard plants treated with humic acid produce seeds with improved fatty acid composition, leading to higher oil content and better oil quality. This includes improvements in important oil quality parameters such as the iodine value, which is essential for assessing oil usability and market value. Humic acid also enhances the overall quality of mustard seeds by increasing protein content and improving the plant's resistance to environmental stressors like drought or nutrient-poor soils (Hemat *et al.*, 2022). This increased resilience helps maintain consistent and high-quality yields even under challenging growing conditions. By contributing to both yield improvement and better seed and oil quality, humic acid enhances the economic value of mustard crops, making it an indispensable tool for farmers aiming to optimize mustard production and improve profitability (Jing *et al.*, 2020).

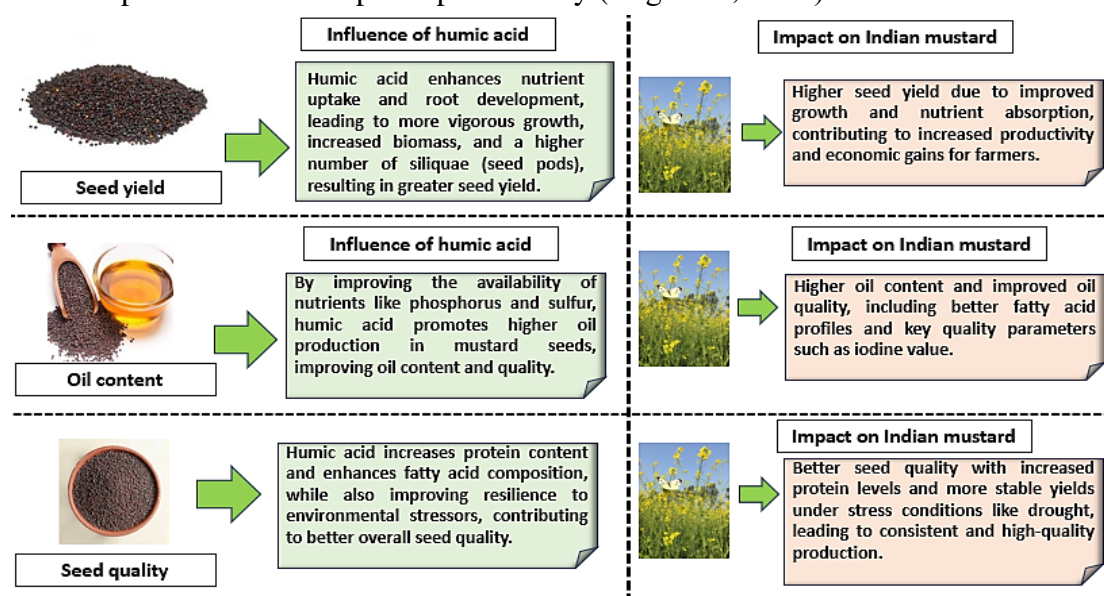


Figure 2.7 Effect of humic acid on seed yield, oil content and seed quality of Indian mustard

2.9 Sulphur: Essentiality in Oilseed Crops

2.9.1 Importance of Sulphur in Crop Nutrition

Sulphur is an essential macronutrient in crop nutrition, playing a vital role in protein synthesis, chlorophyll formation, and overall plant growth. It is a key component of amino acids like cysteine and methionine, which are building blocks of proteins and critical for enzyme activity and structural development in plants (Zenda *et al.*, 2021). Sulphur is also essential for chlorophyll production, enabling efficient photosynthesis, which drives plant growth and carbohydrate production. In oilseed crops such as mustard and soybean, sulphur improves oil content and seed quality by enhancing fatty acid synthesis (Anjum *et al.*, 2011). Additionally, sulphur boosts the uptake of other nutrients promoting balanced growth and resilience against environmental stresses like drought. It helps to enhance soil fertility by improving microbial activity and nutrient cycling, ultimately leading to higher crop yields and better-quality produce. Ensuring adequate sulphur levels is crucial for maximizing crop productivity and maintaining soil health (Kaya *et al.*, 2020).

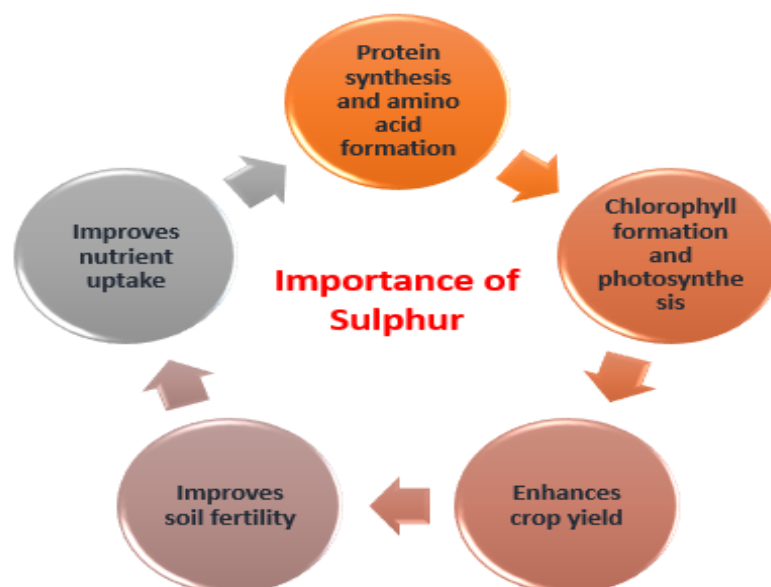


Figure 2.8 Importance of Sulphur on plant growth

2.9.2 Biochemical Role of Sulphur in Plants

Sulphur plays a crucial biochemical role in plants, being integral to several key processes that support growth and development. One of its primary functions is in the synthesis of amino acids, particularly cysteine and methionine, which are essential

building blocks of proteins. These sulfur-containing amino acids are not only vital for protein structure but also for the formation of enzymes that drive metabolic reactions in plants (Yi *et al.*, 2010). Sulphur is also involved in the formation of coenzymes like coenzyme A and certain vitamins, such as biotin and thiamine, which are necessary for various biochemical reactions. Another important role of sulphur is in chlorophyll production, which is essential for photosynthesis (Francioso *et al.*, 2020). Without sufficient sulphur, chlorophyll synthesis is impaired, leading to reduced photosynthetic capacity, lower energy production, and stunted plant growth. Sulphur also contributes to the formation of secondary metabolites, such as glucosinolates in brassicas, which help plants defend against pests and diseases. Furthermore, sulphur enhances the efficiency of nitrogen utilization, working synergistically with nitrogen to promote balanced plant growth and improve protein synthesis (Tiwari *et al.*, 2020). Additionally, sulphur plays a role in regulating redox reactions and protecting plants from oxidative stress. It is involved in the synthesis of glutathione, a powerful antioxidant that helps detoxify reactive oxygen species (ROS), protecting cells from damage caused by environmental stressors like drought, heat, or pollution. Through these biochemical functions, sulphur is essential for maintaining healthy plant metabolism, improving stress resilience, and supporting overall growth and productivity (Capaldi *et al.*, 2015).

2.9.3 Protein Synthesis and Amino Acid Formation (Cysteine and Methionine)

Sulphur is essential for protein synthesis and amino acid formation in plants, serving as a key element in the production of sulfur-containing amino acids such as cysteine, methionine, and homocysteine (Li *et al.*, 2020). These amino acids are critical components of proteins, which are vital for plant structure, enzyme function, and metabolism. Cysteine, in particular, plays a central role in protein structure by forming disulfide bonds that stabilize the three-dimensional shape of proteins, while methionine acts as a precursor for several important molecules and is involved in initiating protein synthesis (Ahmad *et al.*, 2017). Sulphur's involvement in amino acid formation begins with its incorporation into cysteine, which is synthesized through the assimilation of sulfate from the soil. Once formed, cysteine serves as a precursor for methionine and other important biomolecules. Methionine is crucial not only for protein structure but also as a methyl group donor in various metabolic reactions, influencing processes such as DNA methylation and cellular metabolism. The availability of sulphur directly affects the production of these amino acids, and consequently, the synthesis of proteins

necessary for plant growth and development (Froese *et al.*, 2019). In addition to being vital for protein formation, sulphur-containing amino acids play a role in the formation of coenzymes and other essential compounds that regulate enzymatic activity and stress responses. A deficiency in sulphur can lead to reduced amino acid and protein synthesis, which in turn negatively affects plant growth, leading to symptoms such as chlorosis (yellowing of leaves), stunted growth, and reduced yield. Therefore, sulphur is indispensable for ensuring efficient protein synthesis and maintaining healthy plant development (Zenda *et al.*, 2021).

2.9.4 Role of Sulphur in Chlorophyll and Enzyme Activation

For plants to develop and be productive, sulphur is necessary for the synthesis of chlorophyll and the activation of enzymes. Chlorophyll, the green pigment responsible for photosynthesis, is vital for converting light energy into chemical energy, which plants use to produce carbohydrates and sustain growth. Sulphur is involved in the synthesis of key amino acids which are essential for the formation of proteins that directly participate in chlorophyll synthesis (Simkin *et al.*, 2022). A deficiency in sulphur leads to reduced chlorophyll production, resulting in chlorosis, where plant leaves turn pale or yellow, negatively impacting photosynthesis and overall plant health. In addition to its role in chlorophyll formation, sulphur is crucial for enzyme activation. Many enzymes that drive metabolic processes in plants rely on sulphur-containing amino acids like cysteine for their proper functioning (Tiwari *et al.*, 2020).

Sulphur helps activate enzymes involved in nitrogen metabolism, such as nitrate reductase and nitrite reductase, which are responsible for converting nitrogen into usable forms for plant growth (Rizwan *et al.*, 2019). Sulphur also supports the activation of enzymes involved in sulfur metabolism, carbohydrate metabolism, and the synthesis of coenzymes and vitamins like biotin and thiamine. Overall, sulphur's role in both chlorophyll synthesis and enzyme activation is fundamental to plant metabolism, photosynthesis, and nutrient cycling (Francioso *et al.*, 2020). Adequate sulphur availability ensures that plants can efficiently produce energy through photosynthesis and regulate essential enzymatic processes that support growth, yield, and stress tolerance.

2.10 Effect of Sulphur on Indian Mustard Growth and Yield

2.10.1 Sulphur Fertilization and Mustard Biomass Production

Sulphur plays a vital role in enhancing the growth and yield of Indian mustard, as it is an essential nutrient for various physiological processes that directly impact the plant's productivity. Sulphur fertilization in mustard cultivation has been shown to significantly improve biomass production, seed yield, and oil content (Zenda *et al.*, 2021). This is primarily because sulphur is involved in protein synthesis, enzyme activation, and the formation of essential amino acids like cysteine and methionine, all of which are crucial for the growth and development of mustard plants. When mustard plants receive adequate sulphur, they exhibit increased vegetative growth, characterized by a greater number of leaves, improved leaf area, and enhanced root development (Mir *et al.*, 2021). These factors contribute to higher biomass accumulation, as the plant is able to photosynthesize more efficiently due to the increased chlorophyll content supported by sulphur. Moreover, sulphur aids in the assimilation of nitrogen, improving nitrogen use efficiency and further enhancing the plant's growth potential. In terms of yield, sulphur fertilization has been shown to increase the number of siliquae (seed pods) per plant, the number of seeds per silique, and the overall seed weight, all of which contribute to higher seed yields (Mustafa *et al.*, 2022).

The oil content of mustard seeds is also significantly influenced by sulphur, as the nutrient is involved in fatty acid synthesis, leading to better oil quality and quantity. Research has consistently demonstrated that mustard plants treated with optimal levels of sulphur produce higher yields with better oil content compared to those grown in sulphur-deficient conditions. Overall, sulphur fertilization is crucial for maximizing biomass production and yield in Indian mustard, as it supports the plant's metabolic functions, improves nutrient use efficiency, and enhances the production of high-quality seeds and oil (Waraich *et al.*, 2022).

2.10.2 Sulphur and Mustard Seed Oil Content

Sulphur plays a critical role in enhancing the oil content and quality of mustard seeds. As a key nutrient involved in the synthesis of fatty acids, sulphur significantly influences the amount and composition of oil in mustard seeds. Adequate sulphur nutrition ensures that the biochemical processes necessary for oil production are optimized, leading to an increase in both the quantity and quality of the oil extracted from the seeds (Zenda *et al.*, 2021). One of the primary ways sulphur affects mustard seed oil content is through its involvement in enzyme activation and protein synthesis. Sulphur-containing enzymes are essential for the metabolic pathways responsible for

the synthesis of fatty acids, which are the building blocks of oil in seeds (Bouranis, & Chorianopoulou, 2023). By supporting these enzymatic functions, sulphur enables mustard plants to produce a greater amount of oil during seed development. Additionally, sulphur improves the synthesis of sulfur-containing amino acids like methionine and cysteine, which play a role in enhancing the overall seed quality. Research shows that when mustard plants receive sufficient sulphur, the oil content in seeds can increase significantly, with improvements in key oil quality parameters such as iodine value and fatty acid profile (Chen *et al.*, 2023).

This is particularly important for mustard oil, which is highly valued for its healthy fatty acid composition, including a high concentration of monounsaturated and polyunsaturated fats. Sulphur deficiency, on the other hand, can lead to reduced oil content and poorer seed quality. Insufficient sulphur disrupts fatty acid synthesis, resulting in lower oil accumulation in the seeds and an unfavorable fatty acid composition (Sharma *et al.*, 2022). This can negatively affect the economic value of the crop, as oil content and quality are critical factors in mustard's marketability. In summary, sulphur is essential for maximizing mustard seed oil content and quality by supporting fatty acid synthesis and enzymatic processes. Ensuring adequate sulphur availability through proper fertilization is crucial for producing high-yielding, high-quality mustard crops with superior oil characteristics (Pramanick *et al.*, 2023).

2.10.3 Impact of Sulphur on Mustard Seed Protein Content and Quality

Sulphur has a significant impact on the protein content and quality of mustard seeds, playing a crucial role in improving both the economic and nutritional value of the crop. As a key element in the synthesis of sulfur-containing amino acids, such as cysteine and methionine, sulphur directly contributes to the formation of proteins in mustard seeds. These amino acids are essential building blocks for proteins and are critical for many physiological processes that support plant growth and seed development (Perkowski, & Warpeha, 2019). Adequate sulphur availability ensures that mustard plants can synthesize these vital amino acids, leading to higher protein content in the seeds. Sulphur also influences the overall quality of the protein by enhancing the balance of amino acids, making the protein profile more complete and nutritionally valuable (Monda *et al.*, 2022). This is especially important in oilseed crops like mustard, where protein content is a key indicator of seed quality and market value. Sulphur -deficient mustard plants tend to have lower protein content and a less

favorable amino acid profile (Patel *et al.*, 2022).

This deficiency impairs the synthesis of different amino acids, which are critical not only for protein formation but also for the structural integrity and functionality of the proteins in the seeds. As a result, the overall protein quality of the mustard seeds is diminished, potentially lowering the crop's nutritional value for both human consumption and animal feed. In addition to increasing protein content, sulphur also enhances the processing qualities of mustard seeds (Shah *et al.*, 2022). Sulphur-containing proteins contribute to the functional properties of mustard meal, which is a byproduct of oil extraction and widely used in animal feed. Higher protein content and better amino acid composition improve the feed's digestibility and nutritional value, making it more beneficial for livestock. In conclusion, sulphur is essential for maximizing both the protein content and quality of mustard seeds. By ensuring proper sulphur fertilization, farmers can significantly enhance the nutritional value of their crops, improve seed quality, and increase the economic returns from mustard cultivation (Zenda *et al.*, 2017).

2.11 Water Regimes in Indian Mustard Cultivation

2.11.1 Water Requirements for Mustard Growth and Development

Mustard has specific water requirements that are essential for its growth and development, with water availability playing a critical role at different growth stages. While mustard is relatively drought-tolerant, adequate and timely water supply is crucial for optimizing yield and seed quality. During the germination and early seedling stage, sufficient soil moisture is needed to ensure proper seed germination and strong root establishment. A lack of water at this stage can lead to poor seedling emergence and weak plants (Li *et al.*, 2013). As mustard enters the vegetative growth phase, moderate water is required to support leaf and root development, enabling the plant to photosynthesize efficiently and absorb nutrients. Water stress during this period can limit plant size and reduce foliage, negatively affecting growth. The flowering stage is particularly sensitive to water availability (Singh *et al.*, 2019). Moisture stress during this time can cause flower drop, reduce pollination, and result in fewer siliquae (seed pods), ultimately leading to lower yield potential. Mustard requires consistent moisture during this stage to ensure successful flower formation and seed development (Ahmad *et al.*, 2022). Similarly, during the pod development and seed filling stage, adequate water is essential for proper siliquae formation and seed filling. Water stress at this

stage can result in smaller seeds, reduced oil content, and lower overall yields. Ensuring sufficient moisture during seed filling is vital for achieving good-quality seeds and maximizing production (Oguz *et al.*, 2022). In areas with low rainfall, supplemental irrigation is necessary to maintain adequate water supply during critical growth stages. Potential irrigation methods like drip or furrow irrigation, can enhance water use and minimize wastage, while mulching can help conserve soil moisture (Zahoor *et al.*, 2019). Although mustard can tolerate short periods of drought, maintaining consistent water availability, especially during germination, flowering, and seed filling, is key to achieving optimal growth, higher yields, and improved seed and oil quality.

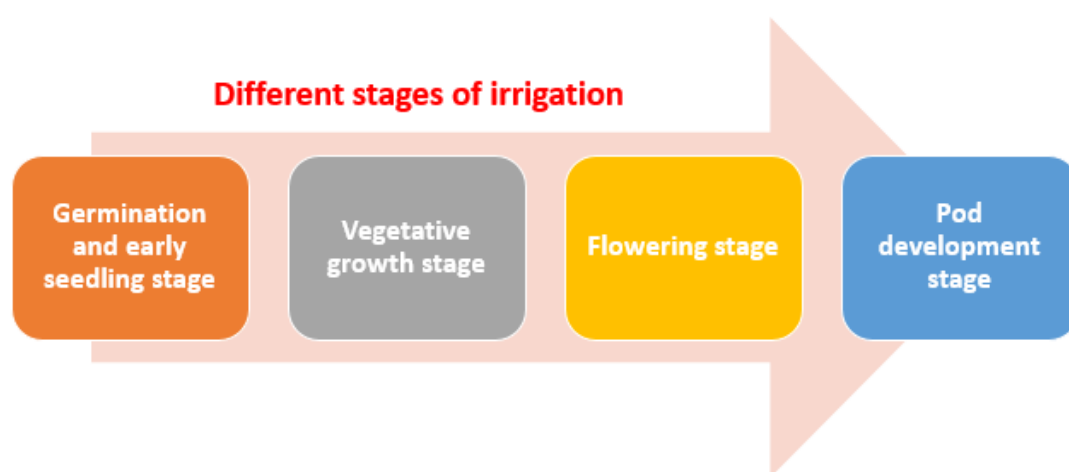


Figure 2.9 Different stages of irrigation of mustard crop

2.11.2 Effects of Water Stress on Mustard Physiology

Water stress pointedly affects the physiology of mustard, impacting its growth, development, and yield potential. Mustard is moderately drought-tolerant, but prolonged or severe water stress during critical stages of growth can cause substantial physiological changes, leading to reduced productivity and poor seed quality (Bandeppa *et al.*, 2019). One of the most immediate effects of water stress on mustard physiology is reduced cell turgor, which limits cell expansion and results in stunted plant growth. This is particularly evident during the vegetative phase when limited water availability can cause smaller leaf size, reduced root development, and decreased overall plant biomass. Water stress also affects photosynthesis in mustard plants by reducing stomatal conductance (Ge *et al.*, 2012). Under drought conditions, plants which limits the intake of carbon dioxide (CO₂) and subsequently reduces photosynthetic activity. This reduction in photosynthesis leads to lower energy

production, impairing the plant's ability to generate the carbohydrates needed for growth and development (Carmo-Silva *et al.*, 2012). Chlorophyll degradation is another common physiological response to water stress, causing yellowing of leaves (chlorosis) and further reducing the plant's photosynthetic efficiency. In addition to its effects on vegetative growth, water stress during the flowering stage can have severe consequences on mustard's reproductive development (Wu *et al.*, 2022).

Drought conditions often lead to flower abortion and reduced pollination, which diminishes the number of siliquae (seed pods) and the seeds per pod. Water stress during the seed filling stage can lead to smaller, poorly developed seeds with reduced oil content, negatively affecting the economic value of the crop. At the biochemical level, water stress causes the accumulation of reactive oxygen species (ROS), which can harm cellular structures, proteins, and lipids (Sharma, 2020). Mustard plants under water stress may produce antioxidant compounds to mitigate oxidative damage, but prolonged exposure to drought can overwhelm the plant's defense mechanisms, leading to cell death and reduced plant viability. The plant's ability to uptake essential nutrients, such as nitrogen, phosphorus, and potassium, is also compromised under water stress, further limiting growth and yield (Mostafaei *et al.*, 2018). In summary, water stress has profound effects on mustard physiology, including stunted growth, reduced photosynthesis, impaired reproductive development, and diminished seed quality. These physiological changes underscore the importance of adequate water management, particularly during critical growth stages, to maintain plant health and optimize mustard yield.

2.11.3 Impact of Water Stress on Seed Formation and Oil Content

Water stress has a profound impact on seed formation and oil content in mustard, significantly reducing both yield and the quality of the seeds. During the critical stages of flowering and seed filling, water availability is essential for proper reproductive development and seed formation. Water stress during these stages can severely hinder the plant's ability to develop siliquae (seed pods) and fill seeds, ultimately reducing the number of seeds per pod and the overall seed size (Ahmad *et al.*, 2022). This leads to a significant decline in seed yield and the economic value of the crop. One of the primary effects of water stress on seed formation is flower and pod abortion. Mustard plants subjected to drought conditions during flowering often experience reduced pollination and increased flower drop, resulting in fewer siliquae

and seeds (Farooq *et al.*, 2017). Additionally, water stress during seed filling leads to inadequate translocation of nutrients and photosynthates from the leaves to the developing seeds, causing incomplete seed development and smaller, underdeveloped seeds. This reduction in seed size directly correlates with lower overall yields. In terms of oil content, water stress negatively impacts the biochemical processes responsible for oil synthesis in mustard seeds (Kumar *et al.*, 2020).

Fatty acid synthesis, which is crucial for oil accumulation in seeds, is highly sensitive to water availability. Water stress reduces the availability of carbon compounds needed for fatty acid production, resulting in lower oil content in seeds. Studies have shown that water deficit during the seed filling stage can lead to a significant reduction in oil content, as well as alterations in the fatty acid composition, which can affect the quality of the oil produced. The seeds may contain a higher proportion of saturated fats and fewer polyunsaturated fats, reducing the oil's nutritional value and marketability (de Araújo Silva *et al.*, 2021). Moreover, water stress-induced physiological changes, such as reduced photosynthetic activity and nutrient uptake, further diminish the plant's ability to accumulate oil in the seeds. As the plant struggles to maintain basic metabolic processes under drought conditions, resources are diverted away from oil production, leading to suboptimal seed quality (Xiong *et al.*, 2018). In severe cases, water stress can also cause seeds to mature prematurely, further reducing oil content and affecting the overall quality of the crop. In conclusion, water stress during critical stages of mustard growth, particularly flowering and seed filling, significantly impacts seed formation and oil content (Ali *et al.*, 2010).

It leads to reduced seed yield, smaller seeds, and lower oil content, all of which negatively affect the crop's economic value. Effective water management during these stages is crucial for ensuring optimal seed development and maximizing oil production in mustard cultivation. The impact of water stress on mustard yield and oil quality has been extensively studied, revealing significant detrimental effects on various stages of plant growth and seed development. Singh *et al.* (2021) demonstrated that water stress during critical periods like flowering and seed filling substantially reduced both seed yield and oil content, leading to smaller seed sizes and inferior oil quality. Similarly, Kumari *et al.* (2020) found that drought during flowering stages caused increased flower drop and impaired pollination, which in turn led to fewer siliquae and

underdeveloped seeds. This underscores the vulnerability of mustard plants to water deficits during key reproductive stages. Further exploration into water deficits during seed filling by Chauhan *et al.* in 2007 revealed a marked reduction in oil content, along with alterations in the fatty acid composition, thus diminishing the oil's nutritional value. These findings align with the work of Enjalbert *et al.* in 2013, who reported that severe water stress not only reduced seed size but also increased the saturated fat content of the oil, negatively affecting its quality and market appeal. The effects of water stress on mustard flowering and pod development were highlighted by Sodani *et al.* in 2017, who observed that water scarcity during pod development resulted in premature seed maturation, smaller pods, and reduced oil accumulation. This is further supported by Pradhan *et al.* in 2014, whose research found that water stress during germination led to reduced seedling establishment and stunted early growth, ultimately compromising the final yield potential of the crop. Shah *et al.* in 2023 focused on the relationship between water availability and oil synthesis, finding that reduced water availability during oil synthesis phases led to lower oil accumulation and compromised seed filling, resulting in diminished oil content. Long-term drought effects on mustard seed quality were also investigated by Jankowsk *et al.* in 2020, who reported that prolonged exposure to drought significantly reduced seed quality and altered the overall nutritional profile of mustard seeds. Aneja *et al.* in 2015 examined the impact of drought-induced stress on mustard oil composition, noting that such stress decreased the proportion of polyunsaturated fatty acids, which are essential for maintaining the oil's nutritional value. Collectively, these studies highlight the critical influence of water availability on the yield, quality, and nutritional composition of mustard seeds and oil, emphasizing the need for effective water management strategies to mitigate the adverse effects of drought on mustard cultivation.

2.12 Drought Tolerance Mechanisms in Indian Mustard

Indian mustard has developed several drought tolerance mechanisms that enable it to survive and perform under water-limited conditions. One of the primary adaptations is its deep and extensive root system, which allows the plant to access water from deeper soil layers (Kumari *et al.*, 2020). This enhanced root architecture helps the plant maintain water uptake even when surface moisture is scarce. Additionally, Indian mustard regulates its stomatal openings to minimize water loss through transpiration. By closing its stomata during periods of water stress, the plant conserves moisture while

balancing the need for carbon dioxide intake for photosynthesis (Shah *et al.*, 2023). Osmotic adjustment is another key mechanism, where the plant accumulates compatible solutes like proline, sugars, and amino acids to retain water within its cells and maintain turgor pressure (Sanders & Arndt, 2012). This adjustment helps protect cellular structures from dehydration. The plant also increases wax deposition on its leaves and thickens the cuticle to reduce water evaporation and protect against desiccation. While drought reduces photosynthetic activity, Indian mustard can maintain minimal energy production by adjusting its water and carbon dioxide utilization, enabling it to continue growing, albeit at a slower rate (Jankowsk *et al.*, 2020). In response to prolonged drought stress, Indian mustard may adopt an early maturation strategy, where it accelerates flowering and seed set to complete its reproductive cycle before the water deficit becomes too severe. This drought escape mechanism ensures that the plant produces seeds, even if the yield or seed size is reduced. Additionally, the plant relies on hormonal regulation, particularly the hormone abscisic acid (ABA), which triggers stomatal closure, promotes root growth, and enhances osmotic adjustment during drought conditions (Shah *et al.*, 2023). Together, these mechanisms allow Indian mustard to adapt to and tolerate drought, making it a resilient crop in water-scarce regions.

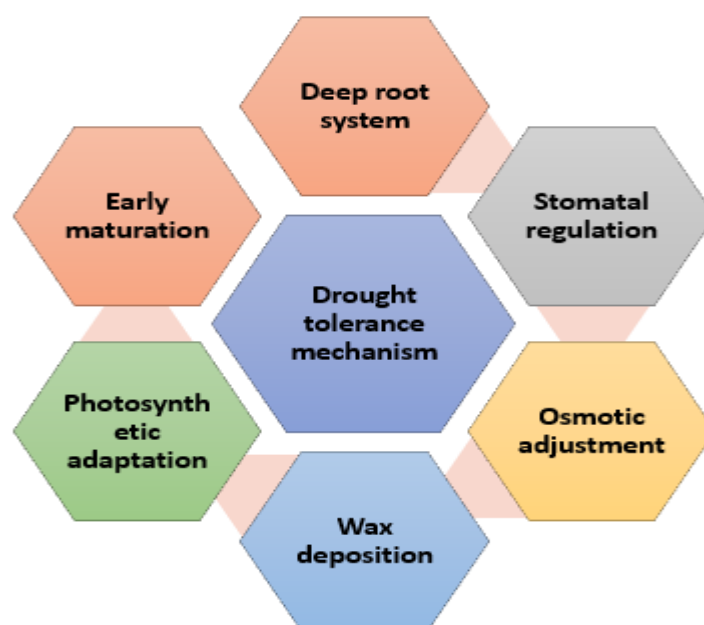


Figure 2.10 Drought tolerance mechanism of Indian mustard

2.13 Mustard Yield Responses to Variable Water Availability

Mustard yield is highly responsive to water availability, with the crop's growth, seed formation, and oil content significantly affected by both water abundance and water stress. Adequate water supply during critical growth stages, such as germination, vegetative growth, flowering, and seed filling, is essential for maximizing yield potential (Hake *et al.*, 2010). When mustard plants receive consistent and sufficient water, they produce a higher number of siliquae (seed pods), more seeds per pod, and larger seed sizes, all of which contribute to greater overall yield and improved oil content. During the early stages of growth, particularly germination and seedling development, adequate moisture is crucial for uniform plant emergence and strong root establishment. A lack of water during these stages can result in poor stand density and stunted growth, which negatively affects subsequent yield (Prasad *et al.*, 2008). As the plant enters the vegetative phase, moderate water is required to support rapid leaf development, photosynthesis, and biomass accumulation. Insufficient water during this period reduces leaf area and limits photosynthesis, ultimately lowering the plant's growth potential and yield (Ge *et al.*, 2012).

Water availability is particularly critical during the flowering and seed filling stages. Water stress during flowering can lead to increased flower abortion, reduced pollination, and fewer siliquae, all of which reduce seed yield. Similarly, water deficits during seed filling restrict nutrient and photosynthate translocation to the seeds, resulting in smaller, underdeveloped seeds with lower oil content (Ahmad *et al.*, 2022). On the other hand, excess water, particularly in poorly drained soils, can lead to waterlogging, which reduces root oxygenation, hampers nutrient uptake, and causes root rot, negatively affecting yield and seed quality. Under drought conditions, mustard plants may employ drought tolerance mechanisms such as deeper rooting, osmotic adjustment, and stomatal regulation to conserve water. However, prolonged water stress significantly reduces yield by limiting seed development and oil production (Pramanick *et al.*, 2023). Conversely, when water is adequately available, especially during flowering and seed filling, mustard plants can produce optimal yields with higher seed weight and better oil quality. In summary, mustard yield is highly dependent on water availability, with optimal water supply during key growth stages resulting in higher yields, better seed quality, and improved oil content (Ahmadi, & Bahrani, 2009). Water stress, particularly during flowering and seed filling, leads to

reduced seed formation, lower oil content, and decreased economic returns, underscoring the importance of effective water management for maximizing mustard productivity.

2.14 Impact of Humic Acid on Indian Mustard under Variable Water Regimes

Humic acid potentially improves the soil structure and boosts nutrient accessibility, all of which are critical for plant growth under varying water conditions (Tahoun *et al.*, 2022). These properties make humic acid particularly beneficial in semi-arid regions or areas prone to drought, where water scarcity is a limiting factor for mustard cultivation. Under water-stressed conditions, humic acid helps mitigate the negative effects of drought by improving the plant's water use efficiency. It enhances root growth and development, allowing the plant to access deeper soil moisture reserves. The increased root surface area also improves the uptake of essential nutrients which are crucial for sustaining plant growth under limited water availability (Li *et al.*, 2009).

Furthermore, humic acid supports osmotic adjustment in plants by promoting the accumulation of solutes like proline, which helps maintain cell turgor and prevents wilting during drought. This leads to better physiological performance, allowing the plant to continue growing despite reduced water availability. In well-watered conditions, humic acid further boosts growth by enhancing nutrient uptake and improving photosynthetic activity (Feng *et al.*, 2024). By chelating nutrients and improving soil cation exchange capacity, humic acid ensures that essential minerals are readily available to the plant. This leads to increased biomass production, more efficient chlorophyll synthesis, and greater energy capture through photosynthesis. Additionally, humic acid enhances root development, leading to better water and nutrient absorption even in optimal water conditions. Humic acid's ability to improve water retention in the soil is another key benefit under variable water regimes. It helps create a more stable soil environment, reducing water loss through evaporation and improving moisture availability to the plant over time (Haider *et al.*, 2015). This is particularly beneficial in soils that tend to dry out quickly or those with poor water-holding capacity. The increased water retention capacity allows Indian mustard to sustain growth during periods of irregular rainfall or extended dry spells. Research has shown that Indian mustard treated with humic acid under both drought and normal irrigation conditions

produces higher seed yields, better oil content, and improved seed quality compared to untreated plants (Walia *et al.*, 2024).

2.15 Effect of Sulphur on Indian Mustard under Variable Water Regimes

Sulphur plays a vital role in enhancing the growth, yield, and quality of Indian mustard, particularly under variable water regimes. This essential nutrient supports key physiological methods such as protein synthesis, enzyme activation, and chlorophyll formation, all of which are critical for plant development. Under water-stressed conditions, sulphur helps mitigate the negative effects of drought by improving nitrogen use efficiency, supporting protein synthesis, and enhancing the ability of plant to maintain cell turgor through osmotic adjustment (Sachan *et al.*, 2024). Additionally, sulphur promotes the production of glutathione, an antioxidant that protects plants from oxidative stress caused by water scarcity, enabling Indian mustard to better withstand periods of drought. In well-watered conditions, sulphur continues to play a critical role in optimizing growth and yield (Zenda *et al.*, 2021). It enhances chlorophyll production, improving photosynthesis and leading to increased vegetative growth and biomass accumulation. Sulphur also contributes to the efficient use of nitrogen, facilitating protein synthesis and promoting overall plant health. One of the most significant impacts of sulphur in well-watered conditions is its effect on seed quality and oil content. Sulphur is essential for fatty acid synthesis, which directly influences the oil production in mustard seeds, resulting in higher oil content and improved fatty acid composition. Seeds from sulphur-treated plants also tend to have a higher protein content, further enhancing their nutritional and market value (Singh *et al.*, 2021).

2.16 Combined Application of Humic Acid and Sulphur under Variable Water Regimes

The growth, production, and quality of Indian mustard are greatly improved by applying humic acid and sulphur together under varying water regimes. Both sulphur and humic acid have distinct roles in enhancing soil structure, nutrient availability, and plant stress tolerance. When combined, they have a positive synergistic effect that helps the plant, especially in situations when water availability is irregular (Sible *et al.*, 2021). Humic acid improves soil structure by enhancing aggregation and water retention, which is critical for nutrient absorption, especially under drought conditions. It also promotes root growth, allowing mustard plants to access deeper soil moisture and nutrients. Sulphur, on the other hand, plays an essential role in protein synthesis,

enzyme activation, and nutrient metabolism. By combining the two, the plants develop stronger roots and more efficient nutrient uptake, which supports growth even in challenging water conditions. Under water-limited conditions, humic acid helps retain soil moisture, reducing water stress on the plant, while sulphur aids in osmotic adjustment, enabling the plant to maintain cell turgor and prevent wilting (Chen *et al.*, 2022).

This combination enhances drought tolerance, reducing yield losses caused by water stress. Additionally, humic acid and sulphur together improve chlorophyll production and photosynthesis. Humic acid increases the availability of micronutrients required for chlorophyll synthesis, while sulphur plays a direct role in chlorophyll formation and nitrogen metabolism. The result is increased photosynthetic activity, leading to greater biomass production and healthier plants. In terms of seed quality, sulphur is crucial for fatty acid synthesis, directly influencing oil content in mustard seeds (Osman, & Rady, 2012). When combined with humic acid, which supports nutrient uptake and overall plant health, the result is seeds with higher oil content and improved quality. The combined application of humic acid and sulphur also enhances the plant's resilience to varying water conditions.

2.17 Effect of humic acid and sulphur on mustard seed oil quality

The impact of humic acid and sulfur on mustard seed oil quality, particularly under varying water regimes, has attained significant research attraction due to their role in improving plant development, nutrient uptake, and stress acceptance. Humic acid, a natural organic compound, enhances soil fertility by improving its structure and increasing the availability of essential nutrients, while sulfur plays a key role in oil synthesis, particularly in sulfur-containing amino acids that are crucial for oil quality. Studies examined the effects of humic acid on mustard under different water regimes suggest that it helps mitigate the impact of water stress by improving root growth and nutrient absorption. This leads to better seed development and oil accumulation, even under limited water conditions. Humic acid enhances the bioavailability of nutrients, including sulfur, which supports the synthesis of key compounds necessary for oil production. In addition, humic substances have been reported to improve the plant's water-holding capacity, thus reducing the negative effects of drought. The application of humic acid in mustard fields has been shown to increase seed yield, oil content, and

improve the fatty acid profile by enhancing the proportion of unsaturated fatty acids, particularly under water-limited environments (Sharif *et al.*, 2002; Shah *et al.*, 2018).

Sulphur, on the other hand, is critical for mustard plants, especially for oilseed crops where sulfur deficiencies are known to reduce oil content and degrade oil quality. Sulfur plays a pivotal role in the formation of sulfur-containing amino acids such as cysteine and methionine, which are integral to protein and oil biosynthesis. Research has shown that sulfur application under varying water regimes helps improve oil content and the nutritional quality of mustard seed oil by increasing the levels of polyunsaturated fatty acids (Ahmad *et al.*, 2007; Rathore *et al.*, 2015). Under water stress, sulfur aids in maintaining the metabolic processes related to oil synthesis, thus enhancing oil yield and quality. Sulfur also contributes to improving the oxidative stability of mustard oil, which is crucial for the shelf life and overall quality of the product (Zhao *et al.*, 2012).

Combined application of humic acid and sulfur under different water regimes has been found to synergistically enhance oil yield and quality in mustard crops. In water-deficit conditions, this combination improves stress tolerance by enhancing nutrient uptake and optimizing water use efficiency. Studies have demonstrated that the dual application leads to an improvement in oil content, a better balance of fatty acids, and increased proportions of beneficial unsaturated fatty acids (Kumar *et al.*, 2018; Singh *et al.*, 2020). This combination also positively influences the oxidative stability and overall nutritional quality of mustard seed oil, even under suboptimal water conditions. The application of humic acid and sulfur together enhances the plant's physiological processes, such as photosynthesis and nutrient assimilation, which are crucial for oil synthesis under water stress (Sharma *et al.*, 2019). Both humic acid and sulfur have demonstrated significant benefits for mustard seed oil quality under varying water regimes. Their combined application not only improves oil yield but also enhances the fatty acid composition, making the oil nutritionally superior. This approach has shown promise in mitigating the negative impacts of water stress on oil quality, suggesting that integrating humic acid and sulfur into mustard cultivation under different water conditions can be an effective strategy for maintaining oil productivity and quality.

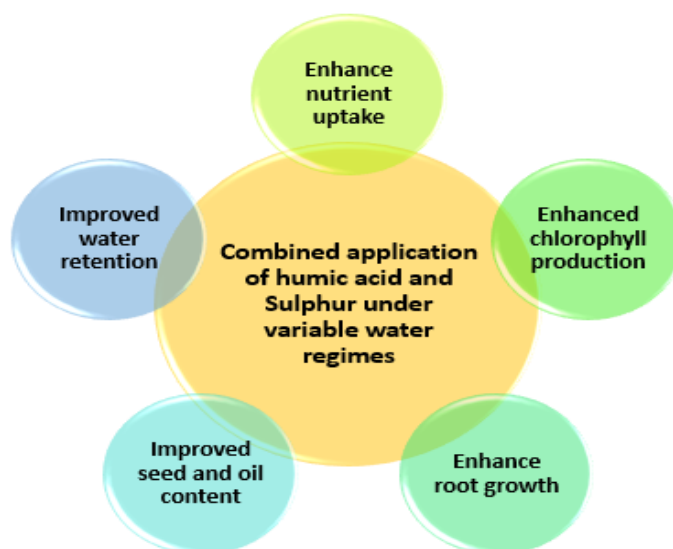


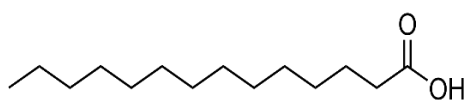
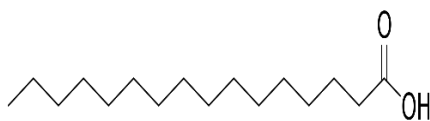
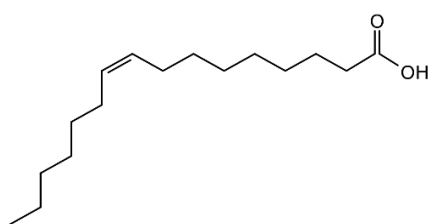
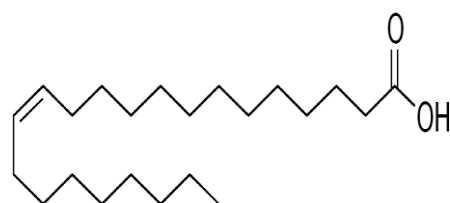
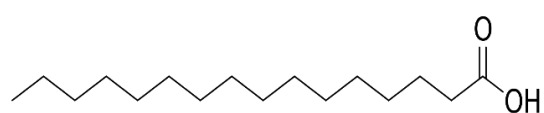
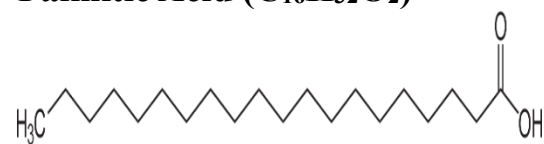
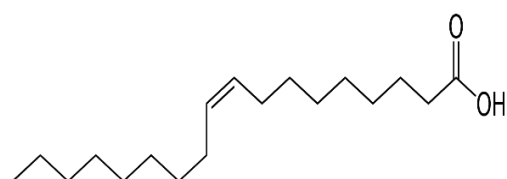
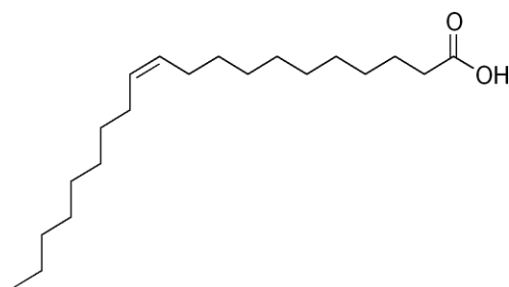
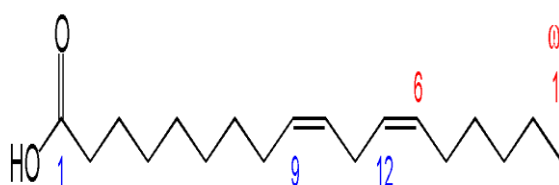
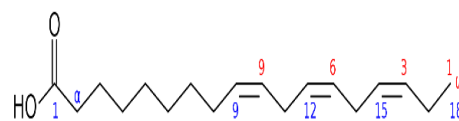
Figure 2.11 Combined synergistic effect of humic acid and Sulphur under variable water regimes

2.17.1 Effect of humic acid and sulphur on fatty acid profiling of mustard oil

The application of humic acid and sulfur has been shown to significantly influence the fatty acid composition of mustard seed oil. Both compounds play critical roles in improving oil yield and its quality by affecting the synthesis of essential fatty acids. MUFA, including oleic acid and erucic acid, are products of a linear reaction. Oleic acid, resulting from the first reaction, serves as the precursor for erucic acid synthesis. Consequently, when oleic acid levels increase, erucic acid levels decrease, and vice versa (Singh et al., 2023). By adding double bonds to the same 18-carbon chain, oleic acid is converted into PUFA, such as linoleic and linolenic acids. Accordingly, the relative amounts of these fatty acids show which steps in the fatty acid production pathway are dominant (Singh et al., 2023). Öz, M. et al., (2022) explored the sources, extraction methods, bioavailability, stability, safety, toxicology, and health benefits of polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs). PUFAs, with multiple double bonds, and MUFAs, with a single double bond, play crucial roles in human health by reducing the risk of cardiovascular diseases, cancer, Alzheimer's disease, and diabetes. They also alleviate symptoms in various skin and allergic conditions, asthma, and rheumatoid arthritis. Understanding these fatty acids' properties and benefits is essential for promoting human health and development. In a 3-month trial by Wu et al. 2022, 90 high-risk Chinese women compared the effects

of n-6 polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) on body weight and cardiometabolic health. Participants consumed diets rich in soybean oil (n-6 PUFA), olive oil, or camellia seed oil (both MUFA-rich). While no significant weight change occurred, MUFA-rich diets, particularly olive oil, improved cardiometabolic profiles: olive oil increased HDL cholesterol, and camellia seed oil reduced aspartate aminotransferase levels. These results suggest that MUFA-rich oils may offer greater cardiovascular health benefits for high-risk Chinese women. Palmitic acid is the first fatty acid produced during lipogenesis, constituting 20% to 30% of total fatty acids in phospholipid membranes and adipocyte triacylglycerols, is extensively studied for its association with cancer and obesity due to lipotoxicity - ‘hallmark for metabolic diseases’ (Yuan *et al.*, 2024).

It crucially modifies proteins through S-palmitoylation, wherein it binds to proteins via a thioester bond, facilitated by palmitoyl S-acyltransferases. Elevated intracellular PA levels contribute to metabolic disorders, neuronal disorders, and cancer (Yuan *et al.*, 2024). Mustard oils with high linoleic acid content are considered premium due to their ability to lower blood cholesterol levels and contribute significantly to preventing atherosclerosis (Poddar *et al.*, 2022).

**Myristic Acid (C₁₄H₂₈O₂)****Stearic Acid (C₁₈H₃₆O₂)****Palmitoleic Acid (C₁₆H₃₀O₂)****Erucic Acid (C₂₂H₄₂O₂)****Palmitic Acid (C₁₆H₃₂O₂)****Arachidic Acid (C₂₀H₄₀O₂)****Oleic Acid (C₁₈H₃₄O₂)****Cis-11 Eicosenoic Acid (C₂₀H₃₈O₂)****Linoleic acid (C₁₈H₃₂O₂)****Alpha-Linolenic Acid (C₁₈H₃₀O₂)****Figure 2.12** Fatty acids present in Indian mustard

A field experiment entitled “*Response of Indian mustard to humic acid and sulphur under variable water regimes*” was conducted at the Agronomy Research Farm of Lovely Professional University, Punjab, during *rabi* season of 2022-23 and 2023-24. This chapter provides a narrative of the materials and the experimental methodologies used, and the analytical techniques utilized to evaluate the recorded observations throughout the course of the investigations.

3.1. General details and information

3.1.1. Experimental site description

A consecutive two-year field experiment was conducted during *rabi* season 2022-2023 and 2023-2024 at the Lovely Professional University Farm (Tubewell-9), School of Agriculture, Lovely Professional University, Punjab, situated at 31°24'N latitude 75°71' E longitude, and an altitude of 235.11±3.00m above mean sea level.

3.1.2. The soil characteristics within the designated experimental field

In both years, composite representative soil samples were collected before initiating the experiment from a depth of 0-30 cm and kept in zip-lock bags at 2- 4 °C to examine the physicochemical aspects. The analysed soil samples provided information on the initial availability of key nutrients such as N, P, K, and S. The results of the soil analysis are presented in Table 3.1.

The soil of the experimental site had a sandy loam texture with good porosity (49.12%). The overall fertility status of the soil was categorized as low to medium in terms of nutrient availability. The organic carbon content (0.48%) was in the low range, indicating limited microbial activity and organic matter, which may impact soil fertility and structure. The available nitrogen (265.10 kg ha⁻¹) was also in the low range, suggesting the need for nitrogen fertilization to support healthy plant growth. The available phosphorus (18.35 kg ha⁻¹) falls within the medium range, which is adequate for moderate crop growth. Similarly, the available potassium (181.85 kg ha⁻¹) is categorized as medium, generally sufficient for crops. The available sulphur (9.55 ppm) was in the low range, indicating a potential deficiency that could affect sulphur-loving crops such as oilseeds. The soil pH (7.01) was neutral, ideal for most crops as it allows optimal nutrient availability. Furthermore, the electrical conductivity (EC) of 0.42 dS m⁻¹ was low, which suggested salinity is not a concern for this soil.

Table 3.1 Physico-chemical attributes of the soil in the experimental field

Particulars	Values	Methodology used
a. Physical Properties		
Sand (%)	62.89	International Pipette method (Piper, 1966)
Silt (%)	19.10	
Clay (%)	12.60	
Textural class		Sandy loam as per the USDA classification system and Piper (1966) guidelines
Bulk density (g.cm ⁻³)	1.37	Black, C.A. (1965)
Particle density (g.cm ⁻³)	2.61	
EC (dSm ⁻¹)	0.42	Digital Conductivity Meter
Pore space (%)	49.12	Graduated measuring cylinder
b. Chemical Properties		
Organic carbon (%)	0.48	Walkley and Black (1934)
Available nitrogen (kg ha ⁻¹)	265.10	Subbiah and Asija (1956)
Available P ₂ O ₅ (kg ha ⁻¹)	18.35	Olsen <i>et al.</i> (1954)
Available K ₂ O (kg ha ⁻¹)	181.85	Jackson (1967)
Available S (ppm)	9.55	Williams and Steinberg, (1959)
pH (1:2.5 soil: water ratio)	7.01	Digital pH meter

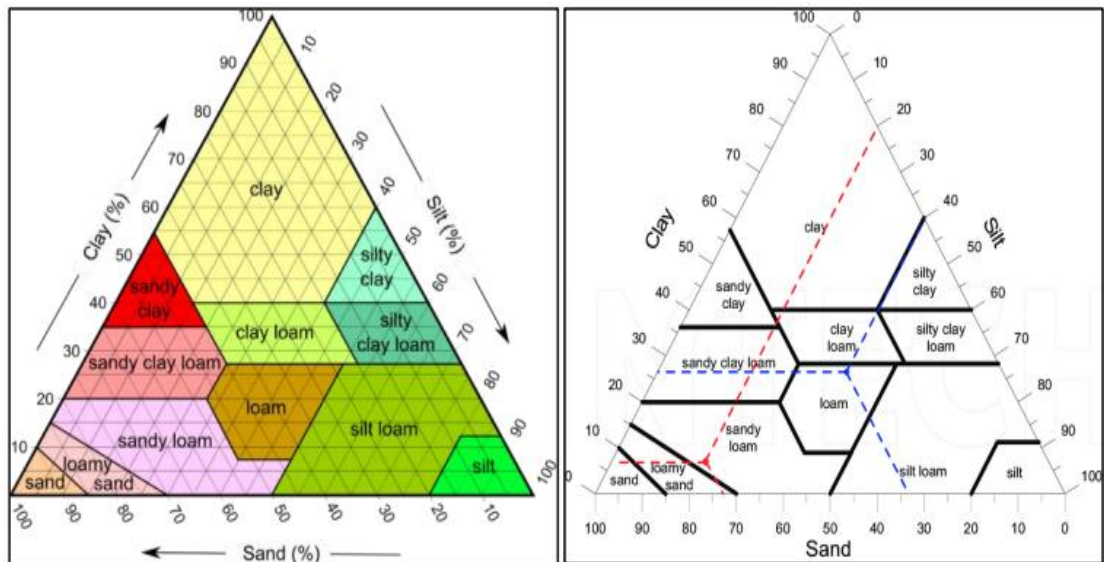


Figure 3.1 The USDA soil texture triangle (Source: Dream Civil,2023)

3.1.3 Meteorological and atmospheric conditions

The meteorological data were obtained from the meteorological department of Lovely Professional University, Phagwara, Punjab, for October to March (2022-23 & 2023-24) as Indian mustard was sown and harvested during this period.

In general, Lovely Professional University (LPU) as located in Phagwara, Punjab, India, at approximately 31.2°N latitude and 75.7°E longitude experiences a subtropical monsoon climate, characterized by distinct seasonal variations with hot summers (25°C–45°C), humid monsoons (25°C–35°C, 70–90% RH), and cool winters (as low as 4°C). Annual rainfall (816 mm) mainly occurs during the Southwest Monsoon (July–September), while Western Disturbances bring occasional winter rain. Winds shift seasonally, from hot 'Loo' winds in summer to moist monsoonal and cold north-westerly winter winds.

But, the meteorological data of October to March (2022-23 & 2023-24) reflected the seasonal variations in temperature, humidity, rainfall, and evaporation of the specific crop growth period. The maximum temperature ranged from 30.16°C to 11.76°C (2022-23) and 33.57°C to 12.50°C (2023-24), with the highest temperatures in October and the lowest in December and January. The relative humidity varied between 45.17% to 85.86% (2022-23) and 27.17% to 96.29% (2023-24), with higher moisture levels in December and January, especially in 2023-24, where relative humidity peaked at 96.29%.

Rainfall was minimal and sporadic, with higher precipitation in 2023-24, particularly in November (8.80 mm), January (6.09 mm), and March (2.68 mm), compared to lower rainfall in 2022-23, where the highest was in February (9.14 mm). Evaporation rates were higher in 2023-24, especially in November (17.01 mm), suggesting increased atmospheric dryness compared to 2022-23, where it remained between 0.44 mm to 3.20 mm.

Overall, 2023-24 experienced higher temperatures, humidity, and rainfall, along with greater evaporation, indicating fluctuating atmospheric conditions. These variations might have influenced soil moisture retention and overall crop growth and development in the specific region.

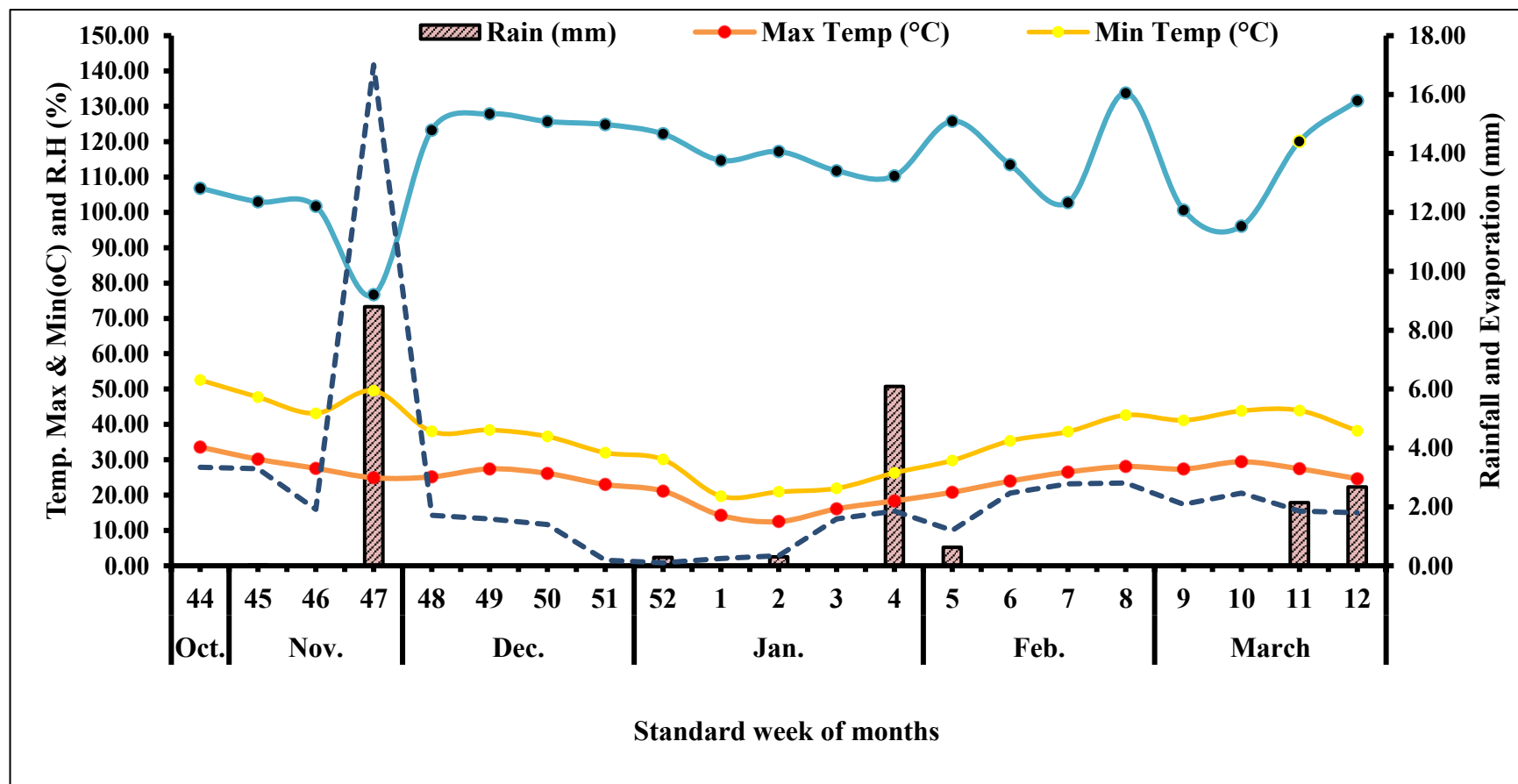


Fig.3.2 Weekly Agro-meteorological data during the crop growing *rabi* season (2022-23)

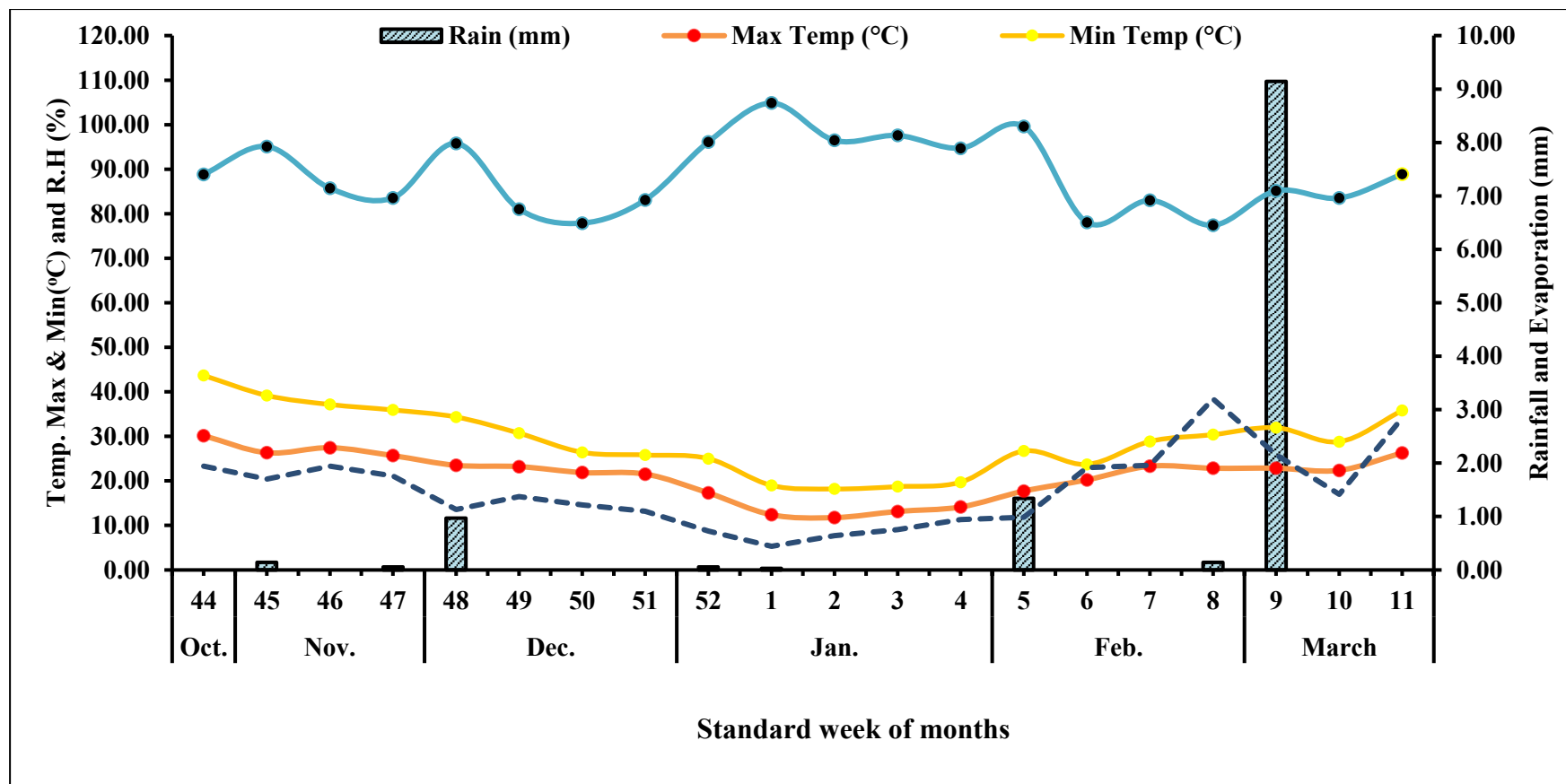


Fig.3.3 Weekly Agro-meteorological data during the crop growing *rabi* season (2023-24)

3.2 Experimental details pertaining to research work

The experiment on Indian mustard (RLC3) was carried out under a different set of treatments using a split-plot design replicated four times. The details of the treatments are outlined below.

Table 3.2 Experimental and treatment details

Location	Research Farm of Lovely Professional University, Jalandhar, Punjab (India)
Crop	Indian Mustard (<i>Brassica juncea</i> L. Czern and Coss)
Variety	RLC 3
Spacing	30 × 15cm
Experimental design	Split Plot Design (SPD)
Main plot [Irrigation regimes at critical Stage]	1. I ₀ = No post sowing irrigation 2. I ₁ = One post sowing irrigation (vegetative stage) 3. I ₂ = Two post sowing irrigation (I ₁ + flowering stage) 4. I ₃ = Three Post Sowing Irrigation (I ₁ + I ₂ + siliqua filling stage]
Subplot [Nutrient treatments]	1. S ₀ = Control 2. S ₁ = Humic acid [0.247 kg ha ⁻¹] 3. S ₂ = Sulphur [60 kg ha ⁻¹] 4. S ₃ = Humic acid + Sulphur
No. of total treatments	4 levels of irrigation regimes (main plot) × 4 levels of nutrient treatments (sub plot) =16
No. of replications	4
Total number of plots	4 Levels (main plot) ×4 levels (sub plot) × 4 (replication) = 64 Plots
Plot size	4.80 × 4.95 = 23.76 m ²
Total number of plots	16 × 4 = 64
Bund size	30 cm
Buffer Zone [Irrigation channel]	1.0 m
Net area	1520.64 m ²
Gross area	2135.64 m ²
Year of conductance	2022-2023 and 2023-2024

3.2.1. General features of Indian mustard var: “RLC 3” used for the experiment

Rai is thought to have originated in China. But RLC 3 (2015) is marked as the first '00' canola quality variety of mustard in India, this variety is characterized by yellow seeds and a medium-tall stature. It is developed by Punjab Agricultural University in Ludhiana with low erucic acid (0.5%) and high oleic acid levels and a glucosinolate content of 15 µ mole/g in its defatted meal (Banga *et al.*, 1983). This

variant was endorsed for widespread cultivation in Punjab state in 2024 (Anonymous, 2024) under irrigated conditions if timely sown. Demonstrating resistance to white rust, it achieves an average seed yield of 7.3 quintals per acre, accompanied by a 41.5 percent oil content. This variety reaches maturity in 145 days. 'Canola' is a globally accepted term for Brassica varieties or hybrids characterized by an oil containing erucic acid less than 2% erucic acid and fewer than 30 micro moles of glucosinolates per gram of defatted meal. The removal of long-chain erucic acid from canola varieties' oil results in an enhanced proportion of desirable MUFA (oleic acid), increasing from 18-20% to 60-67%. The oil derived from canola varieties is deemed a healthy choice for human consumption. Additionally, the defatted meal produced from such varieties is particularly well-suited for animal feed. Breeding programs in India have prioritized developing Canola-quality rapeseed-mustard varieties like RLC3 (Priyamedha, *et al.*, 2015). Additionally, the introduction of Canola-quality white mustard, featuring no erucic acid and minimal glucosinolate content, marks a notable advancement (Jankowski, *et al.*, 2020).

3.3. Land preparation and layout of the experimental field

The experimental field underwent comprehensive land preparation, involving thorough ploughing with a disc plough, subsequent cultivation with a cultivator, and levelling using a plank. Prior to applying treatments, the field experimental layout was established, creating bunds with a bund maker and setting plots i.e., per sub-plot size was $5 \times 5 = 25 \text{ m}^2$, per main-plot size was $20.9 \times 5 = 104.5 \text{ m}^2$, gross area was 2215 m^2 , net area was 1520.64 m^2 , bund size between each sub-plot was 30cm, and buffer zone of 1m each between the replications and main plot respectively. Canals were strategically incorporated between the plots to facilitate irrigation.

Soil application of different nutrient treatments, namely humic acid (0.247 kg ha^{-1}) and Sulphur (60 kg ha^{-1}) through gypsum in specific plots randomly arranged and replicated four times, was done during sowing of seed. Different irrigation regimes were applied to the experimental field based on the critical growth stage of the Indian mustard crop sourced from tubewell 9 of Lovely Professional University farm run by hybrid of solar panel and electricity. The treatment allocation and layout plan for Indian mustard is outlined in Fig. 3.4.

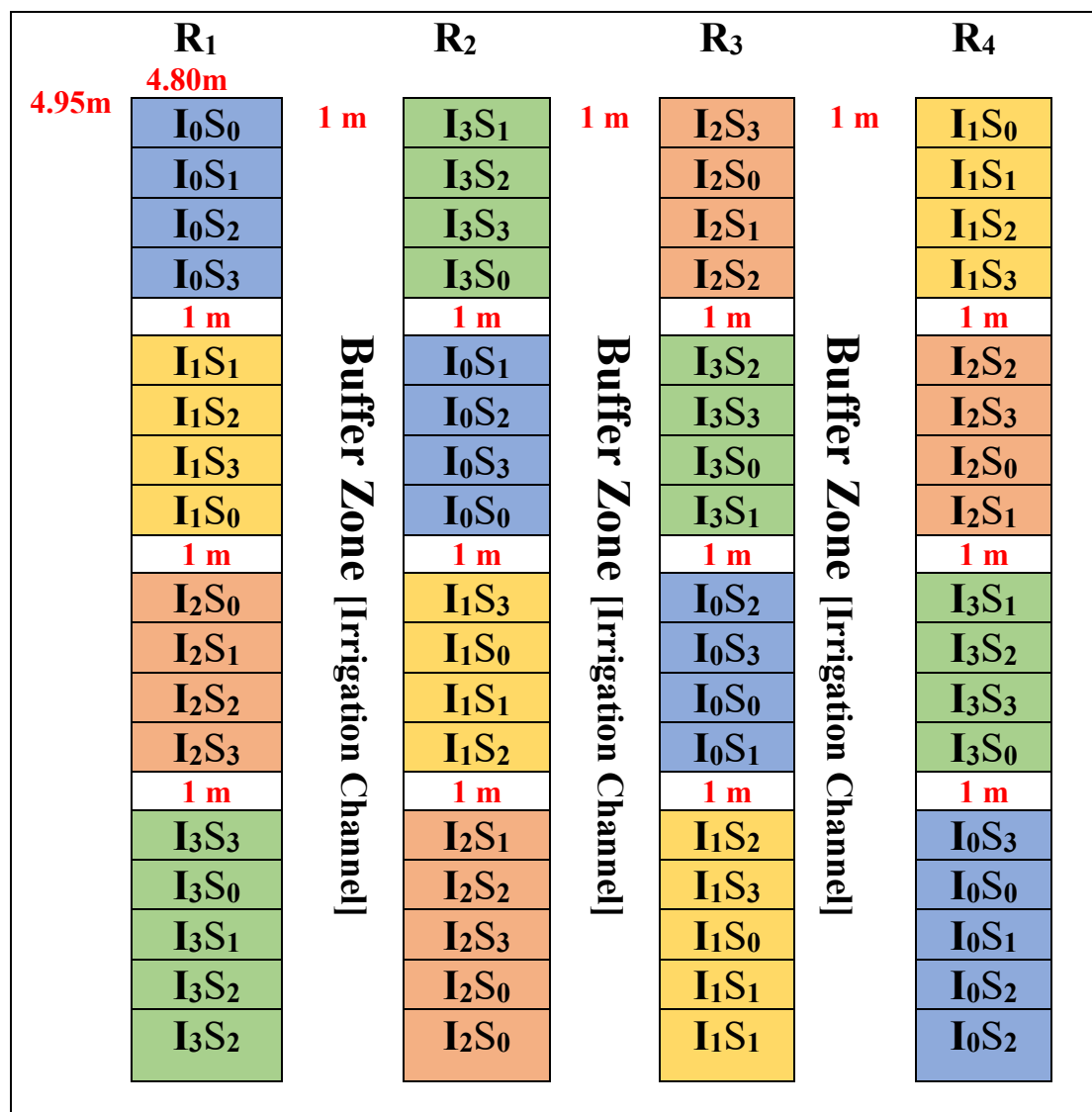


Fig.3.4 Layout of field experiment and treatment allocation in split plot design (SPD) *I₀=No Post Sowing Irrigation, I₁=One Post Sowing Irrigation (Vegetative stage), I₂=Two Post Sowing Irrigation (I₁+Flowering Stage), I₃=Three Post Sowing Irrigation (I₂+ Siliqua filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur

3.4. Details of agronomic practices and field operations

3.4.1 Calendar of field operations

No.	Name of operation	Date of Operation	
		2022-23	2023-24
1	Pre-sowing irrigation (Paleo)	2-11-2022	1-11-2023
2	Land and layout Preparation	26-10-2022	28-10-2023
3	Sowing of the crop	04-11-2022	04-11-2023
4	Thinning and Gap filling by transplanting	18-11-2022	20-11-2023
5	Plant Protection (Sawfly caterpillar)	NIL	12-12-2023
6	First Irrigation 1 st Irrigation at pre-flowering/vegetative stage at 30DAS (35- 45DAS)	8/12/2022 (35 DAS)	8/12/2023 (35 DAS)
7	Second Irrigation 1 st Irrigation at vegetative stage and 2 nd at flowering stage at 50DAS (45-70DAS) last till (15-25days)	02/01/2023 (60 DAS)	02/01/2024 (60 DAS)
8	Third Irrigation 1 st Irrigation at vegetative stage +2 nd at flowering stage+3 rd at siliqua filling stage at 90 days (90-110 DAS) lasting 30- 40 days.	11/02/2023 (100 DAS)	12/02/2024 (100 DAS)
9	Hand weeding	23-12-2022	23-12-2023
10	Harvesting	24-03-2023	17-03-2024
11	Thrashing	31-03-2023	27-03-2024

3.4.2 Nutrient and Fertilizer Application

In mustard, out of recommended doses under irrigated condition as per package of practices for crops of Punjab *Rabi* 2022-23 and 2023-24, 86.42 kg urea ha⁻¹, 197.68 kg gypsum ha⁻¹ and 64.17 kg DAP ha⁻¹ and 43.21 kg urea ha⁻¹ were applied at *rauni* (just before pre sowing irrigation) and the remaining 49.42kg N ha⁻¹ was applied as top dressing 45 post-sowing days. Humic acid (0.247 kg ha⁻¹) and Sulphur (60 kg ha⁻¹) through gypsum were applied at sowing at the specific plot as per layout in a uniform manner.

3.4.3 Irrigation Regimes

The Indian mustard was given paleo and then, after as per different irrigation regimes scheduled as per the research plan. Irrigation was scheduled at a physiologically critical water stage up to the field capacity. I_0 =No post sowing irrigation. I_1 = one post sowing irrigation *i.e.* first-time irrigation given at vegetative stages (30 DAS), I_2 = two post sowing irrigation *i.e.* second time at vegetative and flowering stage (50 DAS) and I_3 =three post sowing irrigation third time given at vegetative, flowering stage and seedfilling (90 DAS) stage by check basin method to field capacity.

3.4.4 Sowing details

Indian mustard *var.* RLC 3 seeds procured from seed store of Punjab Agriculture University (PAU), Ludhiana, Punjab, with genetic purity of 97% and germination of 85%. It was sown as sole crop following the line sowing method with spacing of 30*15cm, seed rate at 3.71kg ha⁻¹ and at 4-5cm depth. The seeds were mixed with moist soil the night before sowing to increase the bulk for uniform distribution while sowing.

3.4.5 Agronomic Cultural Operations

3.4.5.1 Gap filling and thinning

Thinning and gap filling were done in 15 post-sowing days, sowing wherever needed to uphold optimum plant populations.

3.4.5.2 Intercultural activity

One-time hand weeding was done in 4 weeks after sowing, which was adequate for keeping the plots free from weed infestation.

3.4.6 Plant protection

A single application of 250 ml of Ekalux 25 EC (quinalphos) in 60-80 L of water per acre was used to manage mustard sawfly (*Athalia lugens proxima*). The spray was applied when the initial signs of the pest appeared.

3.5 Biometrical Observations recorded

To assess growth parameters, 5 plants were randomly chosen and marked within the net plot area. Biometric data were collected exclusively from these tagged plants, except for dry weight measurements, which involved carefully uprooting five plants from the sample border rows. The average dry weight per plant was then calculated.

For biochemical analysis of leaves, five plants from the border area were randomly selected for destructive sampling. Observations of growth characteristics were systematically recorded every 30 days, beginning 30 post-sowing days (30, 60, 90 DAS) and concluding at harvest.

3.5.1 Moisture content of soil profile up to 30 cm depth at sowing and harvest (mm)

The moisture content of the soil profile up to 30 cm depth at sowing and harvest was determined using the gravimetric method, followed by volumetric method, and calculation of soil water moisture (mm). The soil up to 30 cm depth was collected using soil auger. In the gravimetric method, soil moisture content was calculated by measuring the weight difference between the fresh soil sample and the oven-dried soil sample using the formula as per Black *et al.*, 1965

$$\text{Soil Moisture Content (\%)} = (\text{FW} - \text{DW}) / \text{DW} \times 100$$

Wherein, FW = fresh weight and DW = dry weight after drying at 105°C for 24 hours.

Next, by using a volumetric method, converted the gravimetric moisture into a volumetric basis using the formula according to Hillel (1998).

$$\text{VMC (\%)} = \text{GM (\%)} \times \text{Bulk Density (BD)},$$

where the given bulk density is 1.37 g/cm³.

Finally, the total soil water moisture (mm) at 30 cm depth was determined using the formula as per Jury and Horton (2004).

$$\text{Soil Moisture (mm)} = \text{VMC (\%)} \times \text{Soil Layer Depth (mm)},$$

where the soil layer depth is 30 cm (300 mm).

3.5.2 Growth parameters

3.5.2.1 Initial and final Plant population plot¹:

A systematic agronomic methodology was employed to determine the initial and final plant population per plot of mustard with a spacing of 30 cm x 15 cm and a plot size of 25 m². The mustard seeds were first sown at a spacing of 30 cm between rows and 15 cm between plants within each row, ensuring optimal plant density for growth. After germination, typically 7-10 days after sowing, the initial plant population was determined by counting the number of mustard plants in a representative sample area, *i.e.*, 16 rows × 33 plants per row had 528 plants per plot. As the crop grew, regular monitoring was done to assess plant survival and to account for any losses due to environmental stress, diseases, or competition. At the time of physiological matured

stage, the final plant population was determined by counting the surviving plants in the same sample area.

3.5.2.2 Plant height (cm):

Randomly selected labelled plants from each experimental plot had their plant height measured in centimeters (cm) on different days and times. Using a meter scale, measurements were made from the base of the plant to the tip of its shoots. The average height was then calculated to illustrate the plant's overall growth.

3.5.2.3 Number of leaves plant⁻¹ (30, 60, and 90 DAS):

Five representative plant samples were selected randomly and tagged from each plot to assess the leaf development at 30, 60, and 90 DAS. Leaves were counted. Fully developed leaves, with more than 50% of the leaf blade visible and unfolded, were counted manually on the selected plants, while damaged or partially developed leaves were excluded. Tracking leaf development over time offers insights into plant health and growth.

3.5.2.4 Leaf area (cm² plant⁻¹) (30, 60, and 90 DAS):

Leaf area measurements were taken at three critical growth stages: 30, 60, and 90 DAS. Individual leaves from the sampled mustard plants were detached and placed in a leaf area meter. It scanned the leaf and provided an accurate digital reading of the leaf area in square centimetres (cm²). Recorded the leaf area of all individual leaves per plant and then summed them to get the total leaf area per plant. Measuring leaf area helps in understanding the growth pattern and the assessment of growth dynamics resulted by the effects of various agronomic treatments on leaf development.

3.5.2.5 Fresh weight and dry matter accumulation (g plant⁻¹)

After being cut down from the base, the five randomly chosen plants were first weighed and sun-dried before being oven-dried for about 48 hr. at 65°C to achieve a consistent weight. The electronic top pan balance was employed to weigh the samples. The average weight per plant was calculated by dividing the observed weight by five.

3.5.3 Growth analysis parameters (Computations)

3.5.3.1 Leaf area index (LAI)

It is the ratio of leaf area to ground area. All leaves were detached from the chosen plant and surface area was measured using a leaf area meter. (1/2-MDL-1000 LICOR

Ltd., USA) at 30, 60, 90 DAS and at harvest. Leaf area Index was calculated using the formulagiven by Watson (1947):

$$LAI = \text{Total leaf area (cm}^2\text{)} / \text{Total ground area (cm}^2\text{)}$$

3.5.3.2 Crop growth rate (CGR) (g m⁻² day⁻¹)

Crop growth rate indicates dry matter produced in a unit area per day. It was calculatedby using the formula by Hunt, (2012).

$$CGR = (W_2 - W_1) / (T_2 - T_1) * (1/\text{Sample area}) \text{ (g m}^{-2} \text{ day}^{-1}\text{)}$$

Where, T₁ and T₂ are time interval, W₁ and W₂ are dry weight at T₁ and T₂, respectively.

The CGR was computed for the duration between 30-60, 60-90 and 90-145 DAS

3.5.3.3 Relative growth rate (RGR) (g g⁻¹ day⁻¹)

RGR is a measure that signifies dry matter produced by one gram of existing drymatter per day. It was calculated by using the formula given by Hoffmann and Poorter, (2002)

$$RGR = (\ln W_2 - \ln W_1) / (T_2 - T_1) \text{ (g g}^{-1} \text{ day}^{-1}\text{)}$$

Where, W₁ and W₂ are dry weights at t₁ and t₂, respectively. The RGR was computed for the duration between 30-60, 60-90 and 90-145 DAS.

3.5.3.4 Net assimilation rate (NAR) (g m⁻² day⁻¹)

NAR is an increase in dry weight of plant per unit leaf area per unit time. It states Total photosynthates lost through respiration. It was computed by below formula formulated by Radford (1967)

$$NAR = (W_2 - W_1) / (T_2 - T_1) * (\ln A_2 - \ln A_1) / (A_2 - A_1) \text{ (g m}^{-2} \text{ day}^{-1}\text{)}$$

Wherein A₁ and A₂=Leaf Area, T₁ and T₂=time period, W₁and W₂=dry weight at T₁ andT₂. The NAR was computed for the duration between 30-60, 60-90 and 90-140 DAS

3.5.4 Crop phenological traits

3.5.4.1 Days taken for emergence

Days taken for emergence refer to the number of days taken by seeds to germinate and sprout after planting, depending on soil moisture and temperature. It aids in predicting growth onset, guiding farming schedules as it helps in understanding germination rate and early growth stages.

3.5.4.2 Days taken for branching:

The days taken for branching in Indian mustard were determined through systematic observation and measurement of growth stages under varying water regimes

and nutrient management. The vegetative growth period was tracked, with regular recordings of the post-sowing days (DAS) when the first branch appeared on the main stem for five randomly tagged plants till commencement of flowers. The average branching days were calculated from these observations. Data from field trials and experiments were compiled to assess the impact of different conditions on the branching pattern and timing.

3.5.4.3 Days taken for 50% flowering:

Accurate and reliable determination of the days taken for 50% flowering in Indian mustard involved comprehensive agronomic approach. A Field observation by documenting the exact date of sowing to conducting regular field checks to observe plant growth stages was done. Noted the date when the first flowers appeared. Monitored the field until 50% of the plants in the field had reached the flowering stage. This is when half of the plants had open flowers.

3.5.4.4 Days taken for maturity:

Determining the maturity of Indian mustard involves both agronomic and scientific methods. Field observations provide practical insights, starting with recording the sowing date, noting seedling emergence, and documenting key growth stages like vegetative growth, flowering, and pod formation. Visual indicators such as pod and seed colour changes, leaf senescence, and plant dryness are critical. As the plant matures, pods turn from green to yellow or brown, and seeds shift from green to yellowish, becoming hard and firm. Leaves dry, crumble, and fall off, while stems become brittle. The shattering test ensures timely harvest, and mature seeds should have 8-10% moisture to avoid spoilage. The entire plant appears dry with reduced green parts. Observing these indicators helps to optimize harvest timing for maximum yield and quality.

3.5.5 Physiological parameters (at 30, 60, and 90 DAS)

3.5.5.1 Relative leaf water content (RWC %)

Relative leaf water content (RWC) was calculated using following equation suggested by Wetherly (1962).

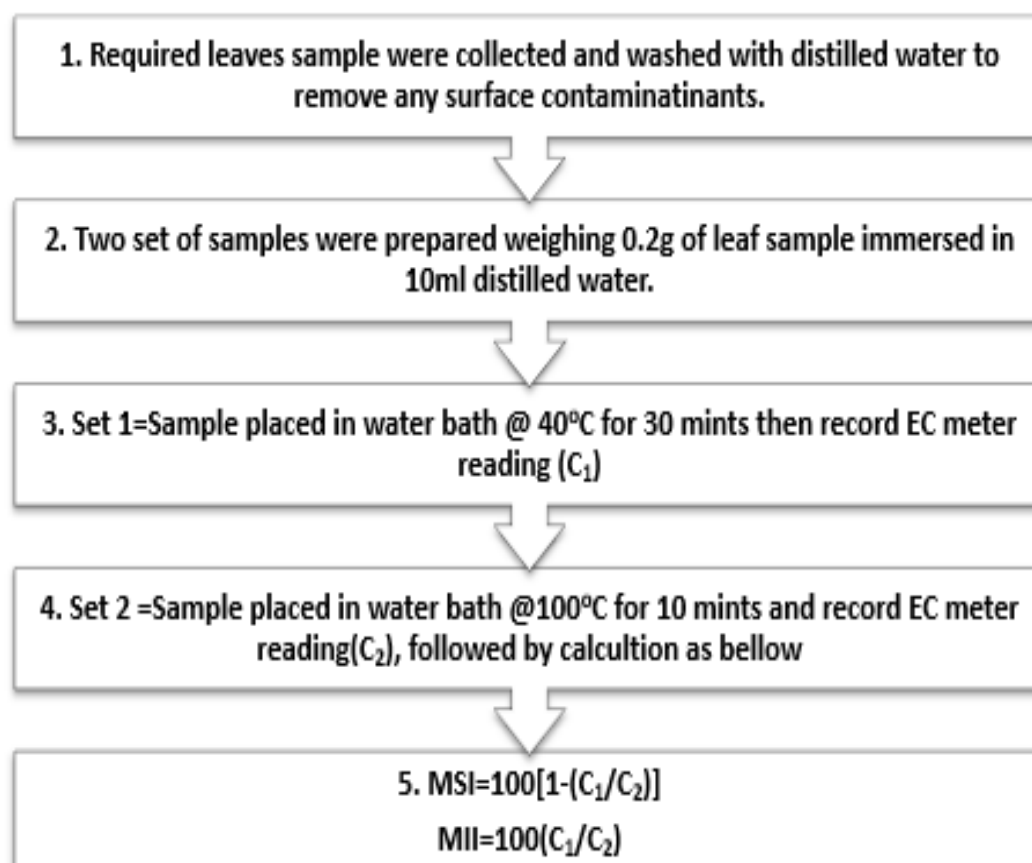
$$\text{RWC (\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

Wherein FW=Fresh weight of the leaf, DW= Dry weight of the leaf, TW=Turgid weight of the leaf

3.5.5.2 Membrane Injury Index (MII %) and Membrane stability index (MSI %)

Membrane injury index (MII %) and Membrane stability index (MSI %) was calculated using the procedure of (Sullivan *et al.*, 1972).

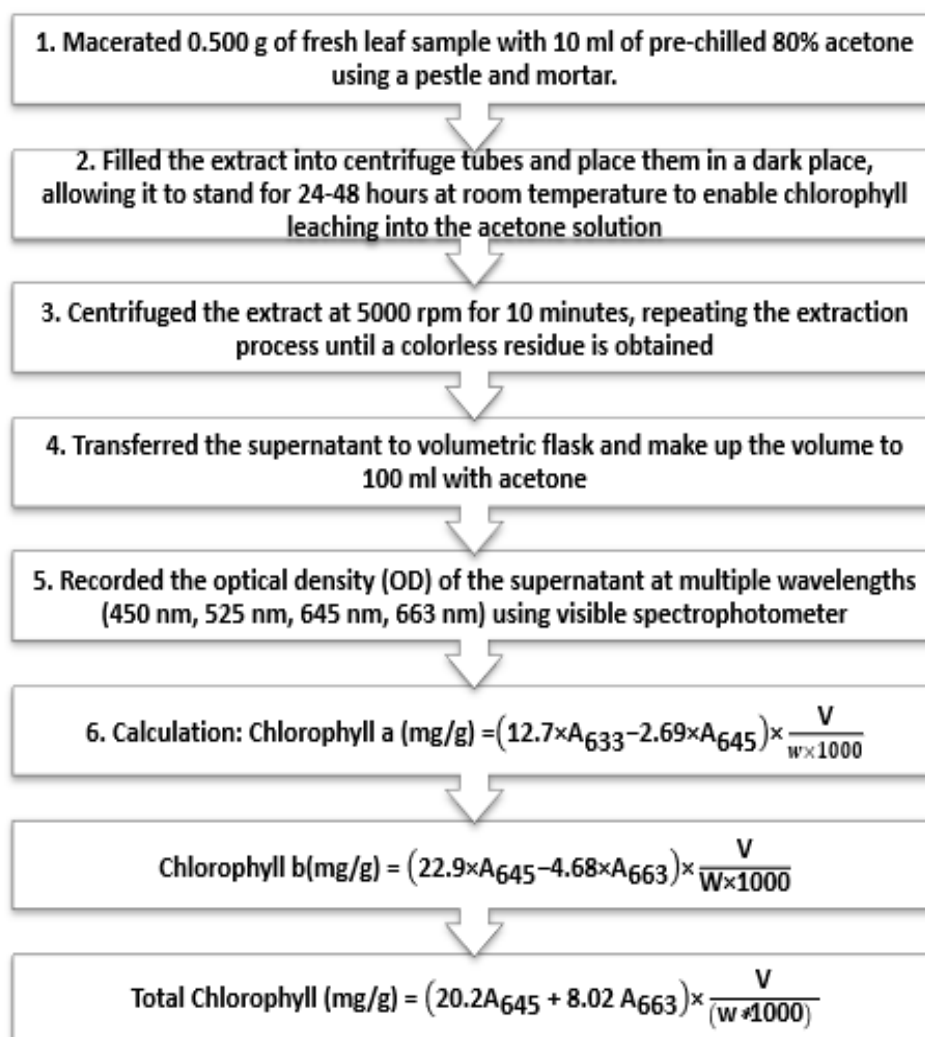
Procedure:



3.5.6 Biochemical Parameters at (60 and 90 DAS)

3.5.6.1 Chlorophyll a, Chlorophyll b, Total chlorophyll (mg g⁻¹ FW)

Chlorophyll a, Chlorophyll b, and Total chlorophyll (mg g⁻¹ FW) were determined following the method of Arnon, D.I., 1949 as below:

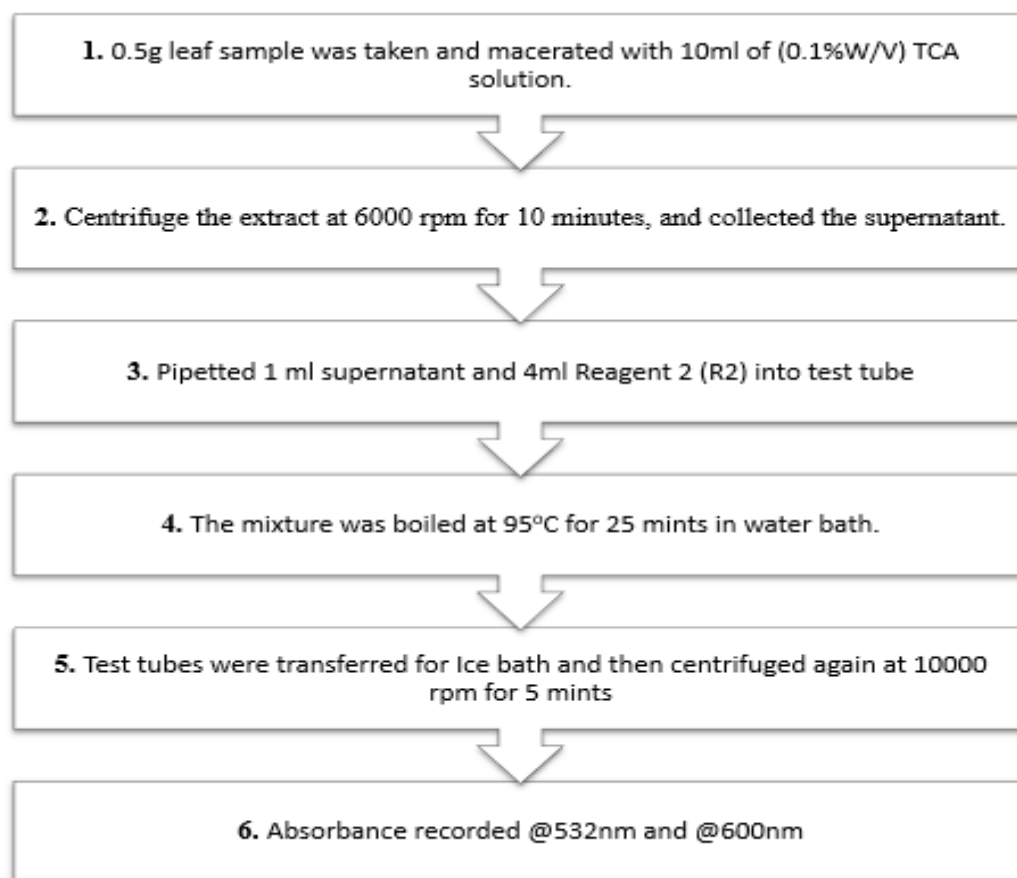
Extract preparation and Procedure:**3.5.6.2 Malondialdehyde (MDA) Content**

Malondialdehyde (MDA) content in terms of thiobarbituric acid reducing substances (TBARS) was determined using the method introduced by Heath and Packer (1968)

Reagents:

R₁= Trichloro Acetic Acid (TCA) (0.1%W/V) Solution, i.e., dissolving 0.100mg TCA in distilled water and then volume made up to 100 mL.

R₂= Thiobarbituric Acid (TBA) (0.50 %) in 20 % TCA solution, i.e., dissolved 20g TCA in 100 mL distilled water, and then 0.500mg TBA dissolved in a small volume of 20 % TCA, and final volume made up to 100 mL by 20 % TCA.

Procedure:**Calculation of MDA-TBA Complex:**

At 532, the MDA-TBA complex values were subtracted from the OD 600 readings. The Lambert-Beer law was used to determine the quantity of MDA-TBA Complex (red pigment), with an extinction coefficient of $\epsilon M = 155 \text{ mM}^{-1} \text{ cm}^{-1}$. MDA content values were extracted from measurements. The results are shown as fresh weight (FW) in $\mu\text{moles MDA g}^{-1}$.

3.5.6.3 Total soluble protein content ($\text{mg g}^{-1} \text{ FW}$)

Protein content was estimated by method described as per the Folin-Lowry method (1951).

Chemicals and reagents used:

1. BSA Stock solution (1 mg mL^{-1})
2. Distilled water (DW)
3. Freshly Prepared Sodium Phosphate Buffer (pH 7.4)

Solution A: 13.9g of 0.1M Monosodium phosphate/ Sodium dihydrogen phosphate (NaH_2PO_4) dissolved in dw and made-up volume to 1000 mL.

Solution B: To prepare a 0.1 M solution of disodium phosphate (Na_2HPO_4), 26.82 g of the compound was dissolved in distilled water, and the solution was diluted to a final volume of 1000 mL.

Solution A+ Solution B (39ml+61ml) and add 100 ml Dw. Final volume (200 mL)

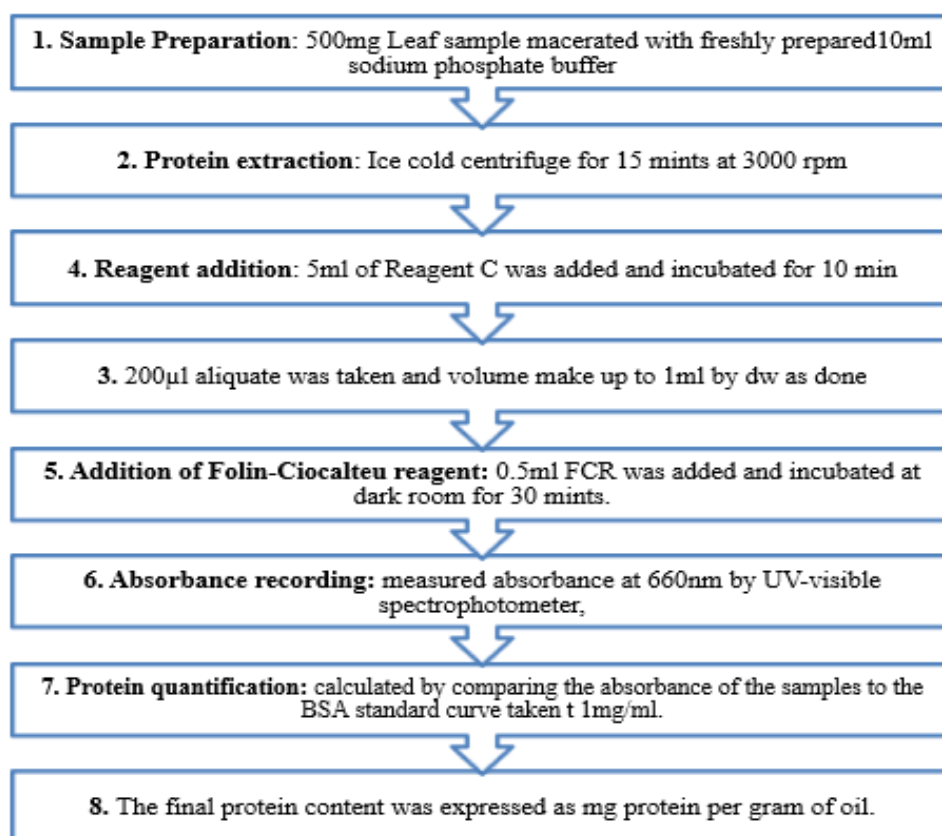
Reagents A: 2% Na_2CO_3 in 0.1% NaOH. [0.1gm NaOH dissolve in 100 mL dw+2gm Na_2CO_3

Reagent B: 0.5% CuSO_4 in 1% NaK Tartarate [1gm NaKT in 100ml dw+0.1gm CuSO_4]

Reagent C: 50ml A +1ml B

Reagent D: Folin-Phenol Reagent (FCR: Dw) in a 1:1 ratio

Procedure:



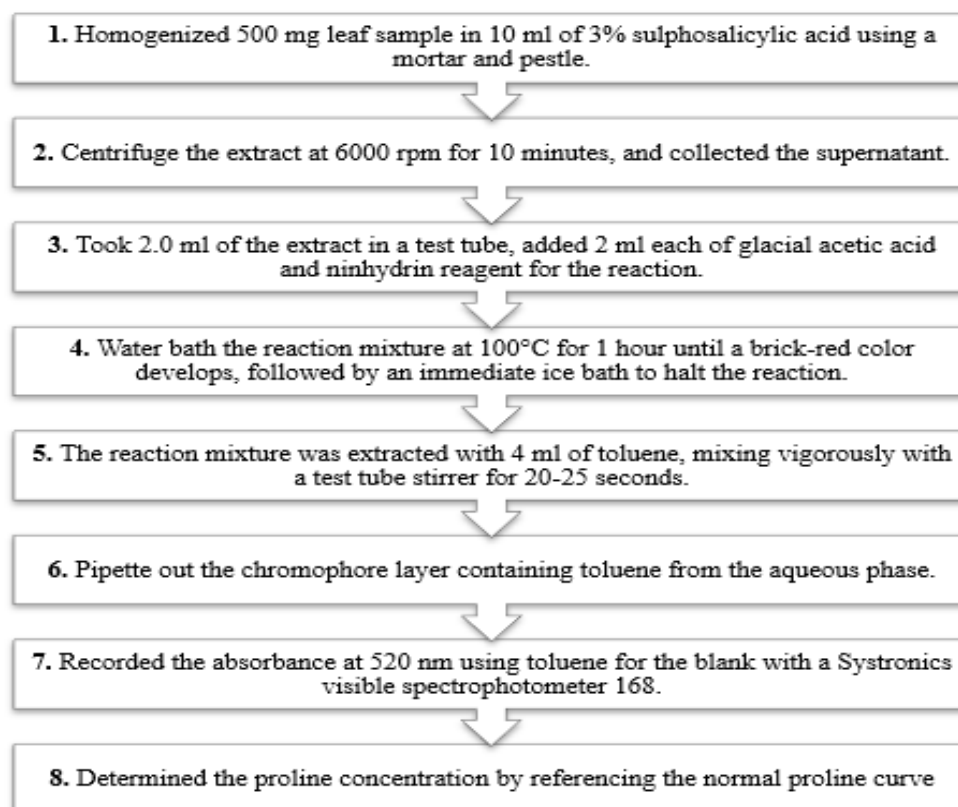
3.5.6.4 Proline content estimation ($\mu\text{g g}^{-1}$ FW)

Total Free Proline content estimation in leaves was conducted following the methodology outlined by Bates *et al.* (1973), utilizing the acid ninhydrin method and expressed in $\mu\text{g g}^{-1}$ fresh weight.

Chemicals and reagents used:

1. To make the Fresh Acid Ninhydrin reagent, 1.25 g of ninhydrin was dissolved with constant stirring in an ambient solution made up of 20 mL of 6 M phosphoric acid (pH 1.0) and 30 mL of glacial acetic acid. The reagent was used within 24 hours after being kept at 4°C in a dark amber bottle.
2. 3% Aqueous Sulphosalicylic acid solution prepared by dissolving 3 g of Sulphosalicylic acid in 100 ml distilled water.
3. Glacial Acetic Acid and organic solvent (Toluene)
4. Standard proline solution.

Procedure



Preparation of the standard curve for proline estimation:

10 mg of proline was liquefied in 3% aqueous sulphosalicylic acid, and the resulting mixture was then diluted with 100 mL of distilled water. Aliquots of 0.2, 0.4, 0.6, 0.8, and 1.0 mL were placed into different test tubes, and 3% aqueous sulphosalicylic acid solution was added to each tube to bring the volume down to 2 ml.

3.5.7 Yield and yield attributes at harvest***3.5.7.1 Number of primary and secondary branches plant⁻¹***

At harvest, the five randomly labelled plants' total number of branches was counted. The average number of branches per plant was calculated and displayed.

3.5.7.2 Number of siliquae plant⁻¹

By calculating the average number of siliquae from the five tagged plants utilized for growth observations, the number of siliquae per plant was determined. To calculate the quantity of siliqua per plant, the total number of siliquae was counted and then divided by the number of plants.

3.5.7.3 Average length of siliqua (cm) plant⁻¹

The average length of siliqua per mustard plant was determined using a vernier caliper. Five fully developed siliquae per plant from various position (upper, middle, and lower sections) were selected to get an average length. The Vernier caliper was calibrated and set to zero before taking measurements. It was used to measure the full length of the siliqua from the base (near the pedicel) to the tip, excluding the pedicel. Determination of the average siliqua length can help to assess yield potential and the overall health of the crop under different agronomic practices.

Average siliqua length = Sum of all siliqua length (cm) /Sum of number of siliqua plant⁻¹

3.5.7.4 Weight of siliqua plant⁻¹ (g)

Siliquae from five selected plants were divided, and weight of per plant was recorded and expressed in grams.

3.5.7.5 Test weight (g)

This was accomplished by manually counting 1000 seeds from a representative random sample of each treatment selected from clean and winnowed product. The total weight of the seeds was then calculated using an electronic balance and reported in grams.

3.5.7.6 Seed yield (kg ha^{-1})

The net plot area's harvest was used to calculate the yields of seeds and stover. The net plot area's plants were collected, individually tagged, and wrapped. Before being weighed, the gathered bundles were sun-dried to a specified moisture content. To separate the stover and seed, threshing was done. By deducting the seed yield from the overall bundle weight, the Stover yield was determined. The yields of seeds and stover, measured in kilograms per plot, were then translated to kilograms per hectare.

3.5.7.7 Biological yield (kg ha^{-1})

Each net plot's biological yield was calculated and expressed in kilograms per hectare. The stover and seed yields from the corresponding net plots were added to determine it. The following is the expression for the biological yield formula:

$$\text{Biological yield (kg ha}^{-1}\text{)} = \text{Seed yield} + \text{Stover yield.}$$

3.5.7.8 Harvest index (%)

The harvest index of mustard was determined as the ratio of the economic yield (seed yield) to the biological yield (total of seed and stover yield). The resulting value was expressed as a percentage, following the method outlined by Donald and Hamblin (1976):

$$\text{Harvest index (\%)} = \text{Economic Yield/ Biological Yield} \times 100$$

3.5.7.9 Oil content and oil yield (kg ha^{-1})

Quantification of oil content (%) was done as per Danlami, *et al.*, 2015 using Soxhlet extraction method.

Materials and apparatus used:

Soxhlet apparatus, extraction thimble, solvent (hexane), analytical balance, heating mantle, round-bottom flask, oilseed Sample (ground into fine particles), oven.

Procedure:

Dried the sample in an oven 65°C for 3-4 hours to remove moisture. Finely grounded oilseed sample (500g) was taken in a Soxhlet extraction thimble placed it in main chamber. Pre-weighed round-bottom flask to the Soxhlet apparatus and added the solvent (200 ml hexane) to the flask and heated the solvent using a heating mantle. Oil was extracted by allowing solvent to evaporate, condense in the condenser, and drip into the thimble containing the sample in the Soxhlet chamber for 6- 8 hours until no more oil is being extracted. The Soxhlet apparatus was allowed to cool. Carefully round

bottom flask was removed and left the flask in a fume hood to let solvent be evaporated, leaving behind the extracted oil in the flask followed by final weighing it. Oil content was calculated by below formula. The oil percentage was assessed using the Soxhlet's Apparatus, employing petroleum ether as the extractant, as described by Sankaran in 1966

Oil content (%) = Weight of extracted oil/ Weight of sample*100

The oil content was reported as a percentage of the dry weight of the sample

Oil yield was calculated by following formula:

Oil yield (kg ha^{-1}) = Oil content (%) * Seed yield (kg ha^{-1})

3.5.8 Oil quality parameters

Chemicals and Reagents: The chemicals required for assessment of oil qualities were procured from LPU lab 57-501. Chemicals and solvents used were of highest purity and of analytical grade.

Collection and preparation of grain and oil extract: The Indian mustard var. RLC 3 grains were collected from respective experimental plots and oil was extracted by Soxhlet apparatus. And oil so obtained was used for experiments.

3.5.8.1 Moisture content (%):

5g of fresh oil sample was taken levelled as W_s in petri plate and placed in a hot air oven to dry at 100-105°C until constant weight was recorded. The weight so obtained was labelled as W_o , reflecting oven-dried oil. Moisture content of Indian mustard oil was calculated as per AOAC. (2019), Method 925.40

Moisture content of Oil (%) = {(Weight of fresh oil- Weight of oven dry oil)/Weight of oven dry oil} ×100

3.5.8.2 Relative density (%)

The Relative density (specific gravity) of mustard oil was determined as per the standard procedure outlined in AOAC (2019) Method 920.212 using a pycnometer.

Materials and Equipment:

Pycnometer, thermometer, analytical Balance, water bath. At 25°C, a well cleaned and dried empty pycnometer was taken and weighed using an analytical balance (W_1). Filled the pycnometer with distilled water and weighed (W_2). Then after emptied and dried the pycnometer, and filled it with mustard oil and determined the

weight (W_3). The relative density (specific gravity) of mustard oil was computed as per AOAC. (2019) Method 920.212

Relative density (specific gravity) = Weight of oil ($W_3 - W_1$) / Weight of water ($W_2 - W_1$)

Wherein, W_1 = Weight of Empty pycnometer

W_2 = Water filled pycnometer weight

W_3 = Oil filled pycnometer weight

Relative density, also known as specific gravity, is a dimensionless quantity. Since it is a ratio of two densities, it has no units.

3.5.8.3 Total phenolic content (TPC) (mg GAE g⁻¹ oil)

The Folin-Ciocalteu reagent, as described by Teh and Birch (2013), was used in a spectrophotometric technique to quantify the total phenolic content (TPC).

Materials used:

Methanol (analytical grade for extraction), centrifuge tubes, mustard oil sample, folin-Ciocalteu reagent (1:10 diluted with distilled water), sodium carbonate (Na_2CO_3), 7.5% w/v solution (7.5g of Na_2CO_3 was dissolved in 10ml of distilled water and made up the volume to 100ml in a conical flask), gallic acid standard (for calibration curve), distilled water, spectrophotometer (Shimadzu UV-2700i, kyoto, Japan), Weighing balance (Sartorius India Pvt. Ltd. Mumbai India).

Procedure:

Preparation of standard calibration curve: A Stock solution of gallic acid was freshly prepared (1mg/ml) i.e, dissolved 10mg of GA in 10 ml methanol. Working solution was prepared by (C_1V_1 (stock solution) = C_2V_2 (Working solution): A Series of gallic acid standard solutions (e.g., 100, 200, 300, 400, 500, 700, and 900 $\mu\text{g/ml}$) was prepared by diluting a stock solution of gallic acid in distilled water. Added 1 mL of Folin-Ciocalteu reagent to each standard. After 3 minutes, added 10 mL of 7.5% sodium carbonate solution (dissolved 7.5 g in 100 mL doubled distilled water, to neutralize the solution). Mixed the solutions well and left for 30 minutes at room temperature in the dark. The absorbance was measured at 725 nm using Spectrophotometer (Shimadzu UV-2700i, kyoto, Japan). Plotted a calibration curve of absorbance vs. concentration of gallic acid to develop a standard calibration curve.

Oil Extraction and Analysis:

Methanol was used as a solvent to extract phenolic chemicals from mustard oil. 10 mL of methanol was mixed with 5 g of mustard oil, and the mixture was agitated violently for 10 minutes in order to carry out the extraction. The methanol layer containing the phenolic compounds was then separated from the mixture by centrifuging it for 10 minutes at 3000 rpm. The supernatant was carefully collected for additional examination. 5 mL of 10% Folin-Ciocalteu reagent was mixed with one mL of the methanolic extract to determine the phenolic content. 4 mL of a 7.5% sodium carbonate solution was added after three minutes, and the mixture was well mixed.

The TPC was calculated using the gallic acid calibration curve ($y = 0.0002x + 0.006$; $R^2 = 0.9983$) and expressed as mg GAE 100^{-1} mL of oil.

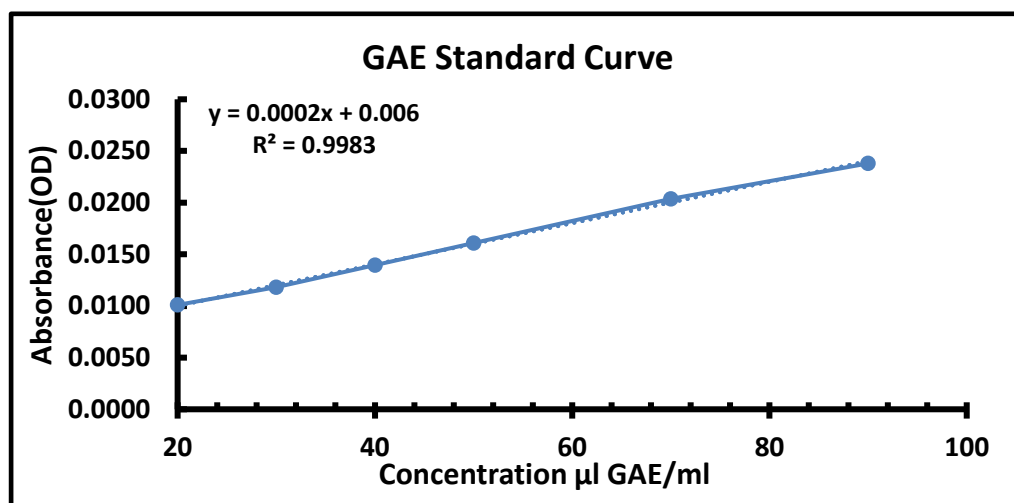


Figure 3.5 Standard curve of total phenolic content (TPC)

TPC (mg GAE 100^{-1} g sample) = (Dilution Factor Concentration from standard curve (mg mL^{-1}) \times Weight of sample (g) \times Volume of extract (mL) $\times 100$

3.5.8.4 Total antioxidant activity (%)

Total antioxidant activity (%) was evaluated by DPPH Assay using Ethyl acetate (Asha *et al.*, 2015).

Control=Ethyl acetate DPPH

Blank= Ethyl acetate (5mL)

Preparation of DPPH Solution (0.004%w/v):

1. 0.1mM DPPH ($6.09 \times 10^{-5} \text{ mol L}^{-1}$) solution Preparation: weigh 2.401 mg of DPPH on watch glass and dissolve in 100ml of Ethyl acetate.

2. Each time freshly prepared and stored in dark and cool place
3. For hydrophobic substances, ethyl acetate was employed as a superior solvent. After adding 0.2 mL of the Indian mustard oil sample to 3.8 mL of ethyl acetate to create 4 mL of the combination, 1 mL of DPPH (6.09×10^{-5} mol/L) solution was added to the ethyl acetate (for a total volume of 5 mL).
4. The absorbance was measured at a wavelength of 517 nm after 30 min.
5. One milliliter of DPPH solution and four milliliters of ethyl acetate served as the reference sample.
6. Antioxidant activity was expressed as percentage inhibition and was calculated using the following formula.
7. **% Inhibition** = $\{(\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of Control}\} \times 100$

The antioxidant activity in the DPPH assay is expressed as a percentage (%) because it quantifies the relative reduction of DPPH radicals by the sample, compared to a control (without the sample). So, it is typically expressed as a percentage (% inhibition).

3.5.8.5 Iodine value (g I_2 100 g^{-1} oil)

Iodine value/Iodine number/Iodine index was studied by A.O.A.C. (2000) Official method 920.159.

Materials, reagents, and equipment used:

Mustard oil sample, iodine monochloride solution (Wij's solution): Dissolve 13g of iodine trichloride (ICl_3) and 10g of iodine (I_2) in 1L of glacial acetic acid, potassium iodide (KI): 10% aqueous solution, sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$): 0.1 N standardized solution, starch indicator: freshly prepared 1% starch solution, carbon tetrachloride (CCl_4): anhydrous, glacial acetic acid: analytical grade, Erlenmeyer flasks: 250ml, burette with stand: 50 ml, pipettes, spatula, filler: 25 mL, 50 mL, glass stoppers: for flasks, analytical Balance: with a sensitivity of 0.0001 g, dark storage cabinet: to store the reaction mixture in the dark, measuring cylinder, funnel, beaker and hot plate.

Procedure:

Chemical Preparation:

1% Starch Solution: 50 mL of distilled water was measured into a 100 mL glass beaker and placed on a hot plate. The water was heated to a boil. Then, 0.5 g of soluble starch was transferred into the boiling water and stirred with a glass rod until it

dissolved. A clear and transparent solution indicated that the starch had completely dissolved. A filter paper was prepared and placed on a funnel, and the hot starch solution was filtered. After 30 minutes, the filtrate was collected and ready to be used as an indicator during titration.

0.1N Sodium Thiosulfate: 2.5 g of sodium thiosulfate crystals were weighed and dissolved in 80 mL of distilled water by heating the solution. After 10 minutes, the solution was allowed to cool to room temperature, and water was added to make the final volume 100 mL. The solution was standardized before use.

Sample and Blank Preparation: 0.3 g of mustard oil was weighed accurately into a clean, dry 250 mL Erlenmeyer flask, with the sample weight noted. For the blank, no sample was added.

Addition of Reagents: 10 mL of carbon tetrachloride (CCl_4) was added to dissolve the oil, and the flask was immediately closed. The same procedure was followed for the blank. Next, 25 mL of iodine monochloride (Wij's) solution was added to both flasks, and the flasks were gently swirled clockwise and counterclockwise to ensure thorough mixing.

Reaction: Immediately after adding the Wij's solution, the flask was stoppered and stored in a dark cabinet at room temperature (around 20-25°C) for 30 minutes.

Addition of Potassium Iodide: The flask was filled with 15 mL of potassium iodide (KI) solution after 30 minutes.

Titration: The reaction mixture was prepared by adding 100 mL of distilled water to the flask. While rotating the flask, the mixture was titrated with 0.1 N sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) solution until the yellow colour almost vanished (the initial burette reading was noted). The solution became blue when 1 mL of the 1% starch indicator solution was added. Titration proceeded until the solution turned milky white, signifying the terminus, and the blue colour vanished. If the blue colour returned after a vigorous shaking of the flask, the titration was carried out until it was finished. The burette's final reading was recorded.

Blank Determination: A blank determination was performed simultaneously by carrying out the same procedure, using the same quantities of reagents but without the oil sample. Both the initial and final burette readings were recorded.

Iodine Value (IV) was calculated using below formula:

Calculation:

$$\text{Iodine Value} = [12.69 \times (V_B - V_S) \times N] / W_s$$

Where: W_s = Sample weight

Normality of Sodium Thiosulphate (N) = 0.1N

Volume of Sodium Thiosulphate for blank (V_B) = (Final-initial burette reading).

Volume of Sodium Thiosulphate for Sample (V_S) = (Final-initial burette reading)

12.69 = Equivalent weight of iodine.

Iodine Value (IV) was expressed as grams of iodine absorbed per 100 grams of oil ($\text{g I} \cdot 100^{-1} \text{ g oil}$) representing the amount of iodine (in grams) that was absorbed by 100 grams of oil sample, that reflected the degree of unsaturation in the oil.

3.5.8.6 Saponification value ($\text{mg KOH g}^{-1} \text{ oil}$)

The saponification value of mustard oil was determined as per A.O.A.C. Official Method 920.160.

Preparation of the oil sample:

The mustard oil sample (2g) was transferred into a dry, clean, and appropriately labelled conical flask.

Addition of potassium hydroxide (KOH): 25 mL of 0.5 N alcoholic potassium hydroxide (KOH) solution was added to the flask containing the sample.

Boiling the Mixture: The flask was then attached to a reflux condenser, and the mixture was heated to a boil over a water bath for about 30 minutes. The heating allowed the oil to react with the KOH to complete saponification.

Cooling the Flask: After boiling, the flask was allowed to cool to room temperature before titration.

Titration: Once cooled, the contents of the flask were titrated against 0.5 N hydrochloric acid (HCl) using phenolphthalein as an indicator. The titration endpoint was identified when the pink colour of the solution disappeared, indicating neutralization of the KOH.

Blank Determination: A blank determination was also performed by carrying out the same procedure without the mustard oil sample to account for any excess KOH that did not react.

Calculation of Saponification Value as done using the following formula:

$$\text{Saponification Value} = [(B-S) \times N \times 56.1] / W$$

Where:

- B = mL of HCl used for blank determination
- S = mL of HCl used for sample titration
- N = Normality of HCl
- W = Weight of the mustard oil sample in grams
- 56.1 = Molecular weight of potassium hydroxide (KOH).

The result was expressed in mg KOH g⁻¹ of mustard oil.

3.5.8.7 Peroxide value (meq O₂ kg⁻¹ of oil)

Determination of the peroxide value in oils was done as per AOAC Official Method 965.33

Materials and apparatus used:

Burette with stand, measuring cylinder, Erlenmeyer flask (250 mL), pipettes, spatula, filler, test tubes with rack, funnel, beaker, hot plate, conical flask, balance machine.

Chemical Preparation: Glacial acetic acid: (Analytical grade), acetic acid, chloroform (Analytical grade), Potassium iodide (KI): Freshly ground, solid, Saturated potassium iodide solution (Made by dissolving KI in water), Starch indicator solution: Freshly prepared (0.5%).

Saturated Potassium Iodine Solution: Labelled test tube with KI, took 2ml distilled water into the test tube. Added KI into the test tube until the undissolved KI is left at the bottom.

Procedure:

1. **Sample Preparation:** An Oil sample (5.0g) was taken in a clean, dry 250 mL Erlenmeyer flask.
2. **Solvent Addition:** Added 30 mL of a mixture of glacial acetic acid and chloroform (3:2 v/v) to the flask containing the oil sample. Mixed by shaking and rotating. This solvent mixture dissolves the oil and facilitates the peroxide formation reaction.

Addition of Potassium Iodide: Added 0.5 mL of freshly prepared saturated potassium iodide solution to the flask. Swirl the flask gently to mix the contents thoroughly.

Reaction Time: Allow the reaction to proceed for exactly 1 minute, keeping the flask in a dark place (or covering it) to avoid any light-induced reactions. During this time, any peroxides present in the sample oxidized the iodide ion to iodine (I_2). After 1 minute, add 30 mL of distilled water to the flask. Shake to mix for 1 minute.

3. **Titration:** The liberated iodine was then titrated with a standard sodium thiosulfate solution (0.01 N). The titration continued until the yellow color of iodine faded, indicating the endpoint. Took initial n final burette reading.
4. **Starch Indicator:** Added 0.5 mL of freshly prepared starch indicator solution and continued titrating until the dark blue/ black color disappeared completely and turned white. Noted the final burette reading
5. **Calculation:** The peroxide value (in milliequivalents of peroxide per kilogram of oil) was calculated using the formula:

$$\text{Peroxide Value (meq/kg)} = V \times N \times 1000 / W_s \text{ (g)}$$

Where:

- Sample Weight (W_s)=5.00g
- V = Volume of Sodium thiosulfate used= final burette reading- Initial burette reading.
- N = Normality of the sodium thiosulfate solution =0.01N

The peroxide value of mustard oil was stated in milliequivalents of active oxygen per kilogram of oil ($\text{meq } O_2 \text{ kg}^{-1} \text{ oil}$) that quantifies the extent of oxidation in oils by measuring the amount of peroxide, a primary oxidation product, present in the oil sample.

3.5.9 Water used by the crop

3.5.9.1 Crop water use efficiency ($\text{kg ha}^{-1} \text{ mm}^{-1}$)

Evapotranspiration that is consumptive use of water was measured by field experimental plot method using water budget equation, wherein calculated irrigation was applied to meet the satisfactory growth of crop with no runoff and no deep percolation i.e $ET = P + Ir - R - Gw$ (Rodell, *et al.*,2011). Wherein, P =Precipitation, Ir =Irrigation, R =run off and Gw =ground water. Following equation was used to calculate the water use efficiency or water use by the crop.

Crop water use efficiency ($\text{kg ha}^{-1}\text{mm}^{-1}$) = Economic yield (kg ha^{-1})/Total water consumed (mm)

3.5.9.2 Water balance:

The evapo-transpiration 'ET' was estimated using the water balance equation according to Hati (2001) with slight modification. The water balance in the field during the cultivation of Indian mustard on sandy loam soil was determined using the equation given by Wang *et al.* (2024):

$$\Delta S = P + I - ET - R - D$$

In this equation, ΔS is water budget, P represents the amount of precipitation (mm) received during the growing season, while I denote irrigation applied (mm). Evapotranspiration (ET) was calculated as the sum of water loss due to soil evaporation and crop transpiration. Runoff (R) was estimated as the portion of precipitation or irrigation water that did not infiltrate the soil surface and instead flowed away from the plot. Deep percolation (D) accounted for water loss moving below the root zone, which could not be accessed by the crop.

3.6 Economic appraisal of treatments

The economic analysis of various treatments was conducted based on the mean yield values. The cost of cultivating Indian mustard was determined by considering the expenses associated with inputs utilized. This included calculating the power and labour requirements for different operations like ploughing, intercultural activities, plant protection measures, harvesting, and threshing, all calculated per hectare using the prevailing rates at the research farm, Lovely Professional University, Punjab. The costs of seeds, fertilizers, and pesticides were considered based on market prices at respective year's minimum support price (MSP)

3.6.1 Gross returns

Considering the current market values, the total output of Indian mustard, which includes both seeds and straw, was translated into gross returns in Rupees per hectare. The following formula is used to determine gross returns:

$$\text{Price of Stover} + \text{Price of Seed} = \text{Gross Returns}$$

3.6.2 Net returns

The entire cost of cultivation was subtracted from the gross return to determine the net profit per hectare. The following is how this calculation was expressed:

$$\text{Net returns} = \text{Gross returns} - \text{Total cost of cultivation}$$

3.6.3 Benefit: cost ratio (B: C ratio)

The following formula was used to calculate the benefit-to-cost ratio, which was then expressed as Rupees per Rupee invested.

$$\text{B: C ratio} = \text{Net returns} / \text{Total cost of production}$$

3.6 Statistical Analysis

A split-plot design was used to assess the significance of treatments across different parameters following the methodology outlined by Cochran and Cox (1967). The experimental data collected during the research study were subjected to statistical analysis through analysis of variance (ANOVA) at a significance level of $p \leq 0.05$, as described by Gomez and Gomez (1984), using the STAR (Statistical Tools for Agriculture Research) and RStudio version 2024.12.1 software packages.



Research field location and seeds of Indian mustard-RLC 3



Source of irrigation-Solar panel



Humic acid ($C_{106}H_{115}O_{59}N_9S$)



Gypsum ($CaSO_4 \cdot 2H_2O$)

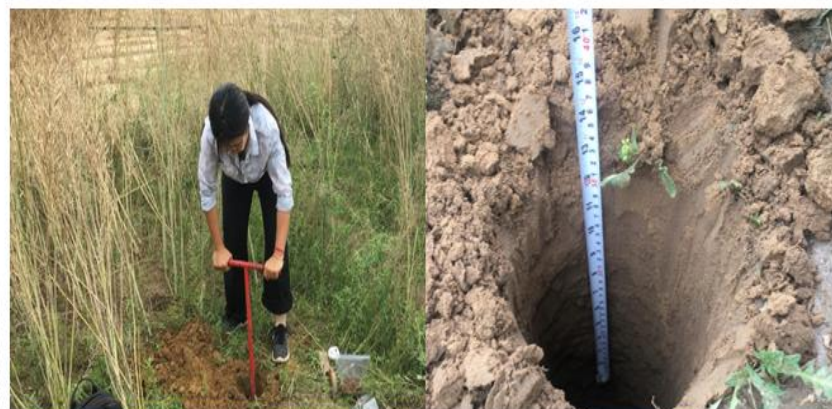


Determination of discharge rate

Picture 1. Location of experimental site and resource inputs



Collection of soil up to 30 cm. depth at sowing



Collection of soil up to 30 cm. depth at harvest



Weighing and cooling of soil samples



Initial and final Plant count done while tagging and at harvest

Picture 2. Moisture content of soil profile up to 30 cm. depth and plant population of mustard crop



Tagging plants for observation



Measurement of plant height



Samples collection



Measurement of leaf area (cm^2)



Weighing of sample

Picture 3. Random tagging of five plants plot^{-1} and recording plant height (cm), leaf area (cm^2) and dry weight (g)



Regular visual inspection of the experimental field



Visual of experimental field at branching and flowering stage plot



Visual of experimental field at physical maturity stage

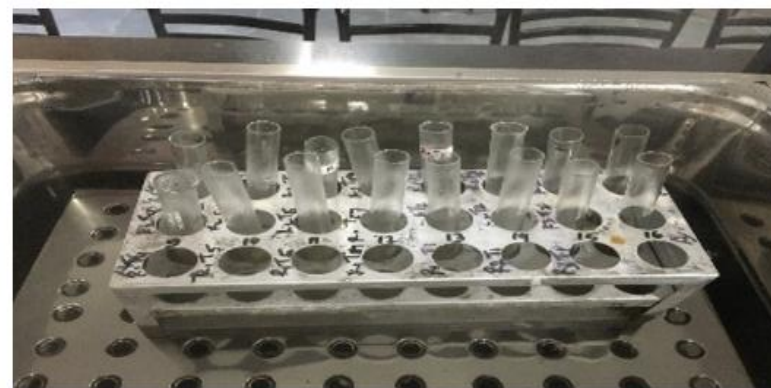
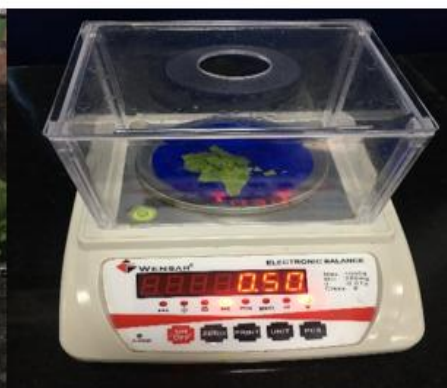


Drone view of experimental field at physical maturity stage

Picture 4. View of experimental field at different phenological stage



Fresh and turgid weighing of leaf samples



Water-bathing of leaf samples

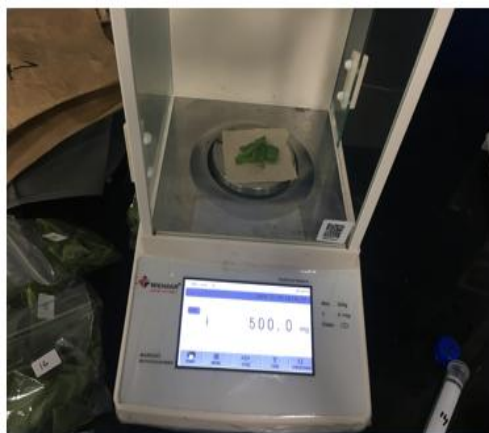


Cooling down of leaf samples



Reading of electrolytes leached out by EC meter

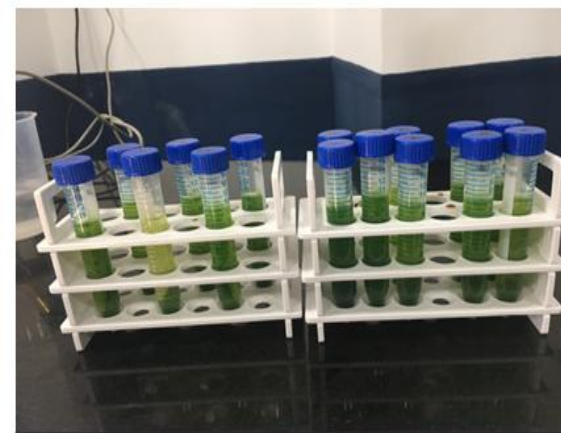
Picture 5. Determination of Relative water content , membrane stability index and membrane injury index of Indian mustard (RLC 3) leaves by Wetherly, 1962 and Sullivan *et al.*,1972 respectively.



Weighing of sample



Maceration of sample



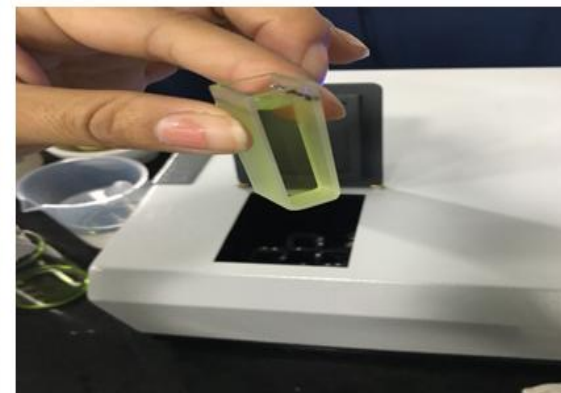
Volume make up of the sample



Centrifugation of sample

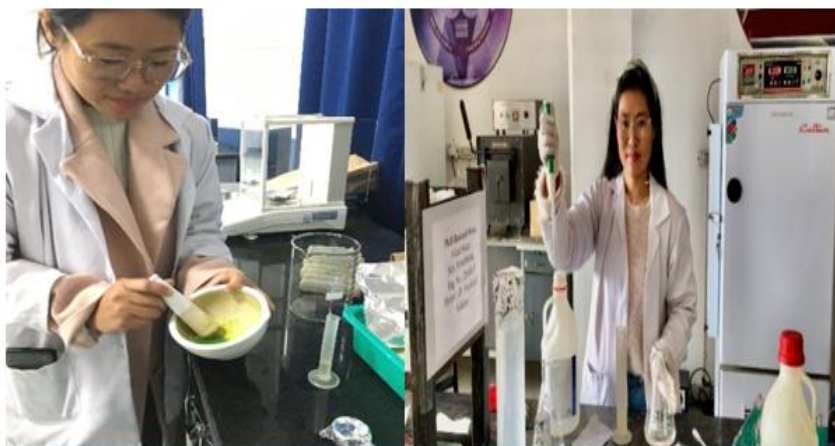


Dilution of sample



Optical density recording

Picture 6. Estimation of total chlorophyll, chlorophyll a, and chlorophyll b of Indian mustard leaf by Arnon, D.I., 1949



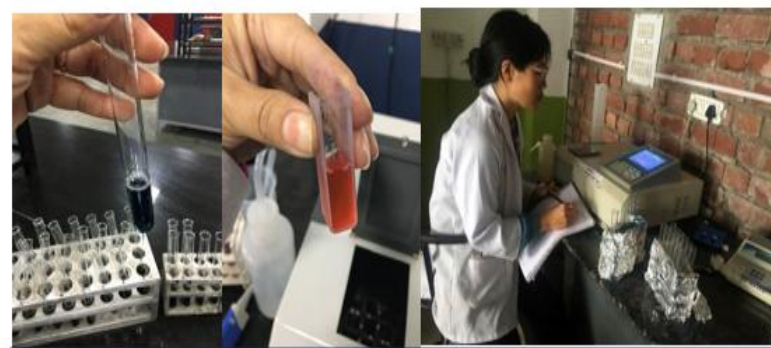
Sample and reagent preparation



Collection of supernatant after centrifugation



Water-bathing, cooling and incubation of samples



Optical density recording by visible spectrophotometer

Picture 7. Estimation of protein and proline in mustard leaves by Folin-Lowry *method* 1951, and Bates *et al.* 1973



Harvesting and threshing of 1m² per plot



Sieving out impurities from samples



Sun drying of sample



Weighing of Indian mustard seeds

Picture 8. Harvesting, Thrashing, Sieving and sun drying of Indian mustard



Counting number of branches per plant



Counting number of siliqua per plant



Measuring length of siliqua by vernier caliper



Weighing of siliqua

Picture 9. Determination of number of primary and secondary branches , siliqua per plant ,avg. length of siliqua, and wt. of siliqua of harvested India mustard (RLC 3)



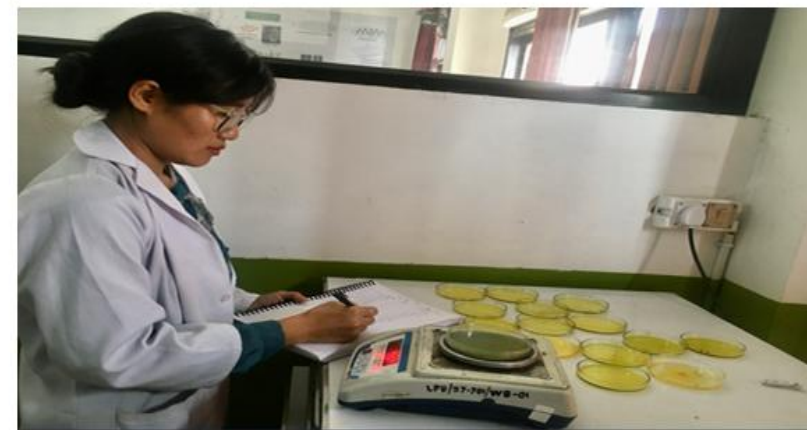
Sample preparation



Extraction of oil by soxhlet apparatus



Extracted oil samples



Moisture content of the oil

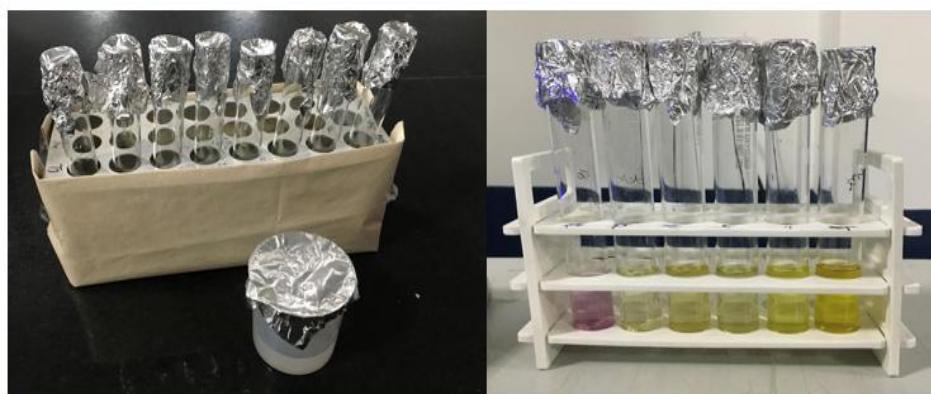
Picture 10. Determination of oil content, oil yield and moisture content of mustard oil



A, B, and C represents Pycnometer that is empty ,water and oil filled respectively for determining relative density of mustard oil



Sample and reagents preparation



Incubation of samples

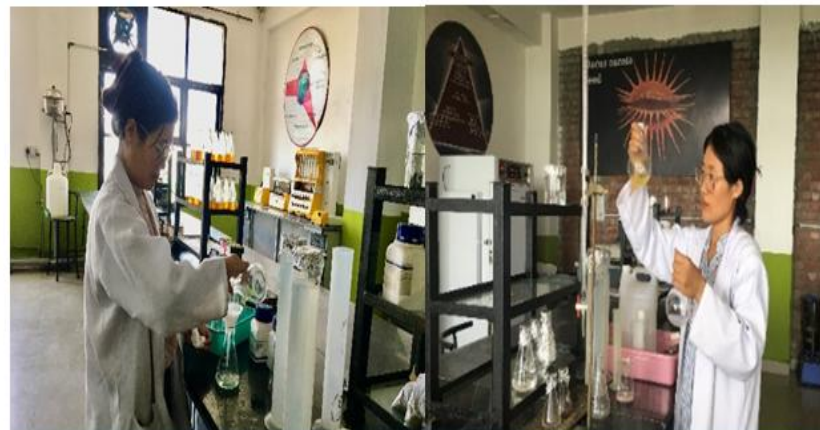


Recording optical density by spectrophotometer

Picture 11. Determination of relative density ,total phenolic content and antioxidant activity of mustard oil



Weighing of oil samples



Reagent preparation



Titration of blanks



Titration of oil samples

Picture 12. Determination of Iodine value, Saponification value and Peroxide value of mustard oil

4. Results and Discussion

4.1 Influence of humic acid and sulphur on the moisture content of the soil profile up to 30 cm depth at sowing and harvesting of Indian mustard (RLC 3) under different water regimes

The soil moisture content at sowing showed minimal variation across different irrigation and nutrient management treatments, with values ranging between 62.35 mm (I₂) and 66.90 mm (I₃) in 2022-23 and 63.19 mm (I₂) and 69.64 mm (I₃) in 2023-24 under irrigation regimes as shown in Table 4.1. Similarly, among nutrient management treatments, moisture content varied from 52.99 mm (S₀) to 79.82 mm (S₃) in 2022-23 and 53.08 mm (S₀) to 82.24 mm (S₃) in 2023-24. The non-significant differences at the sowing stage indicated that initial soil moisture availability was primarily governed by pre-sowing irrigation and inherent soil characteristics rather than the treatments applied after sowing (Evangelista *et al.*, 2014).

At harvest, soil moisture content was significantly affected by irrigation and nutrient management. The moisture content at harvest ranged from 42.54 mm (I₀) to 73.86 mm (I₃) in 2022-23 and 43.25 mm (I₀) to 69.99 mm (I₃) in 2023-24, showing that increasing irrigation frequency improved soil moisture conservation. The highest moisture retention was recorded under I₃, followed by I₂, which retained 70.26 mm in 2022-23 and 65.27 mm in 2023-24. The lowest soil moisture content was observed under I₀ (no irrigation), recording only 42.54 mm in 2022-23 and 43.25 mm in 2023-24, indicating severe depletion of soil moisture under rainfed conditions.

Among nutrient management treatments, S₃ retained the highest soil moisture at harvest, recording 81.31 mm in 2022-23 and 76.07 mm in 2023-24, followed by S₁ with 66.69 mm in 2022-23 and 65.71 mm in 2023-24. Conversely, the lowest moisture content was recorded under S₀ (control), with only 36.76 mm in 2022-23 and 37.30 mm in 2023-24, followed by S₂ with 53.53 mm in 2022-23 and 52.43 mm in 2023-24. The increased moisture retention in humic acid-treated plots can be attributed to its hydrophilic nature, which enhances soil aggregation, increases organic matter content, and improves water-holding capacity, while sulphur contributes to better soil structure and reduced moisture loss through evaporation (Kumar, *et al.*, 2024; Abbas, *et al.*, 2023).

A significant interaction in 2023-24 at harvest was observed between irrigation and nutrient management, indicating a synergistic effect on soil moisture retention. The

highest soil moisture content was recorded under I₃S₃ (three irrigations + humic acid and sulphur) with 84.99 mm, demonstrating that the combination of frequent irrigation and organic amendments significantly improved soil water availability. This suggested that humic acid helps retain irrigation water by reducing percolation losses and enhancing soil moisture-holding capacity, leading to sustained water availability during the crop growth cycle (Jat *et al.*, 2012). Meanwhile, the lowest soil moisture content was recorded under I₀S₀ (no irrigation + no nutrient application), confirming that the absence of both irrigation and organic amendments led to severe moisture depletion and poor soil moisture conservation (Rodriguez-Ramos *et al.*, 2022).

4.2 Growth parameters

4.2.1 Impact of different irrigation regimes and different nutrient management on initial plant population

The influence of different irrigation regimes on initial plant population of mustard was observed over two growing seasons, 2022–23 and 2023–24, along with an analysis of mean data across both years. In the 2022–23 season. Under the I₀ treatment, the initial plant population was recorded at 510.66 plants per hectare. The I₁ treatment led to a marginally higher initial population of 515.93 plants per hectare. The highest initial plant population for this season was observed under the I₃ treatment, with a recorded population of 517.64 plants per hectare. In the following season, 2023–24, a similar trend was observed with slightly higher initial plant populations across all treatments. The initial plant population under I₀ was 513.70 plants, and I₃ was highest with 518.89 plants per hectare. This trend aligns with established research showing that early irrigation creates favourable conditions for germination and seedling establishment, particularly in sandy loam soils with lower water retention capacities (Lamichhane and Soltani, 2020). By improving soil moisture levels during critical early growth stages, even minimal irrigation enhanced seed germination, as reflected in the slight population increase observed from I₀ to I₃. These findings are consistent with a recent study that indicated that targeted irrigation improves crop stand establishment, even when subsequent water availability is limited (El-Sanatawy *et al.*, 2021; Sushma *et al.*, 2023; Rai *et al.*, 2021).

Table 4.1. Soil moisture (0–30 cm) at sowing and harvesting as affected by irrigation, humic acid, and sulphur in Indian mustard

Treatments	Moisture content of soil profile up to 30 cm depth at sowing (mm)		Moisture content of soil profile up to 30 cm depth at harvest (mm)	
	2022-23	2023-24	2022-23	2023-24
Main plot (Irrigation regimes) (I)				
I ₀	64.99	67.80	42.54	43.25
I ₁	65.27	66.66	51.62	53.01
I ₂	62.35	63.19	70.26	65.27
I ₃	66.90	69.64	73.86	69.99
SEm±	0.56	1.27	1.71	1.73
C.D at 5%	NS	NS	5.56	5.56
Sub plot (Nutrient management) (S)				
S ₀	52.99	53.08	36.76	37.30
S ₁	68.75	71.53	66.69	65.71
S ₂	61.84	64.39	53.53	52.43
S ₃	79.82	82.24	81.31	76.07
SEm±	0.55	0.87	1.35	1.40
C.D at 5%	1.59	2.50	3.89	4.03
Interaction (S*I)	NS	NS	8.113	8.413

*I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation, I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage) S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, CD=Critical Difference, NS= non-significant. Cm=centimeter, mm=millimeter.

Table 4.1A. Interaction table of moisture content of soil profile up to 30 cm depth at harvest (mm)

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		28.53	48.80	34.41	58.44	42.54	27.42	50.15	35.36	60.06	43.25
I ₁		33.40	55.04	40.39	77.66	51.62	34.31	56.56	41.50	79.67	53.01
I ₂		41.29	79.63	65.71	94.43	70.26	42.43	81.83	57.25	79.58	65.27
I ₃		43.83	83.29	73.60	94.72	73.86	45.04	74.28	75.63	84.99	69.99
Mean (S)		36.76	66.69	53.53	81.31		37.30	65.71	52.43	76.07	
SEm±	S* I	3.425					3.459				
	I* S	2.897					2.980				
C.D at 5%	S* I	8.113					8.413				
	I* S	8.711					8.947				

*I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation, I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage) S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, CD=Critical Difference, NS= non-significant. DAS=Days after sowing, mm=millimeter.

The study assessed the influence of different nutrient management p on the initial plant population of mustard across two consecutive growing seasons (2022–23 and 2023–24), with mean data compiled across both years. The S₀ treatment recorded the lowest initial plant population at 508.55 plants per hectare, followed by S₂ with 511.97 plants per hectare. The S₁ treatment resulted in an improved population of 518.32 plants per hectare, while the highest initial population was observed under the S₃ treatment with 521.35 plants per hectare. In the 2023–24 season, a similar pattern emerged. The initial plant population in the S₀ treatment remained lower at 510.98 plants per hectare, while S₂ recorded 513.97 plants. The S₁ treatment further improved the population to 510.98 plants per hectare, and S₃ consistently achieved the highest population at 522.54 plants per hectare. Overall, humic acid was found to enhance water retention, augment soil structure, and stimulate microbial activity, making a conducive environment for early root establishment and seedling growth (Jindo *et al.*, 2022; Naik *et al.*, 2020). Similarly, sulphur contributes to protein synthesis, chlorophyll formation, and enzymatic functions, all critical for early vegetative growth and overall plant vigor (Singh *et al.*, 2023).

4.2.2 Influence of different irrigation regimes and nutrient management on final plant population

Throughout two growing seasons, 2022–2023 and 2023–2024, the final plant population of mustard under various irrigation regimes was studied. In the 2022–23 season, the final plant population gradually increased with additional irrigation applications. The I₀ treatment recorded the lowest final population at 479.10 plants per hectare. This was followed by I₁, which yielded a slightly higher population of 482.37 plants per hectare. The I₂ treatment resulted in a further increase to 485.95 plants per hectare, while I₃ showed the highest final plant population, with 487.64 plants per hectare. During the 2023–24 season, a similar pattern was observed. The I₀ treatment had a final plant population of 498.51 plants per hectare. The I₁ treatment saw a slight increase to 501.19 plants per hectare, and the I₂ treatment further improved the population to 503.35 plants per hectare. The most final plant population in 2023–24 was noted under I₃ treatment, reaching 503.89 plants per hectare. The mean data over both years reflects a consistent trend, with the final plant population increasing in line with irrigation frequency. This effect is particularly relevant in sandy loam soils, which have lower water-holding capacity. Intermittent irrigation prevents soil drying and

supports a healthier crop stand (Srinivasarao *et al.*, 2023). Studies have demonstrated that periodic irrigations enhance survival rates in water-sensitive crops by stabilizing root zone moisture levels and ensuring continuous water access (Kumar and Patel, 2023; Sharma and Kumar, 2023). The minimal variation in final plant population between I_0 and I_3 underscores mustard's adaptability to lower moisture levels. Literature supports this adaptability, revealing mustard's capacity to establish and maintain plant density in semi-arid conditions with limited soil moisture (Rashid *et al.*, 2021; Shukla and Chaudhary, 2020).

In the 2022–23 season, the final plant population varied significantly across the nutrient treatments. The S_0 treatment had the lowest final plant population at 477.55 plants per hectare, followed by S_2 with 480.79 plants. The S_1 treatment showed a notable improvement with 486.75 plants per hectare, while S_3 achieved the highest final population of 489.98 plants per hectare. In the 2023–24 season, a similar pattern was observed. The most final plant population was noted under S_3 treatment, with 507.54 plants per hectare. The mean data over both seasons showed a clear trend, with the final plant population consistently increasing in response to the different nutrient treatments. The S_0 treatment averaged 495.17 plants per hectare, while the S_2 treatment increased this to 489.85 plants. The S_1 treatment further improved the population, reaching an average of 505.32 plants. The S_3 treatment produced the highest mean final plant population, with an average of 507.54 plants per hectare. These findings indicate that both humic acid and sulphur positively impact the final plant population, particularly when applied together. Research has shown that humic acid applications promote root growth and nutrient uptake efficiency, improving plant establishment and survival across various soil types, including those with moderate nutrient deficiencies (Rigobelo, 2024). Sulphur alone may lack the enhancements in soil structure and nutrient availability provided by humic acid, explaining why the S_2 treatment did not achieve the same final plant population as S_3 (Tayade *et al.*, 2024).

Table 4.2 Impact of humic acid and sulphur applications on initial and final plant population density (plot⁻¹) in Indian mustard under differential water regimes

Treatments	Initial plant population plot ⁻¹		Final plant population plot ⁻¹	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24
I ₀	511	514	479	499
I ₁	516	517	482	501
I ₂	516	518	486	503
I ₃	518	519	488	504
SEm±	3.29	3.93	2.97	3.74
C.D at 5%	NS	NS	NS	NS
Sub plot (Nutrient management) (S)				
S ₀	509	511	478	495
S ₁	518	520	487	505
S ₂	512	514	481	499
S ₃	521	523	490	508
SEm±	1.95	2.13	1.99	2.03
C.D at 5%	5.59	6.10	5.70	5.83
Interaction (S*I)	NS	NS	NS	NS

*I=irrigation regimes, S=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂= Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C.D=Critical difference, DAS=Days after sowing. plot⁻¹=Per plot

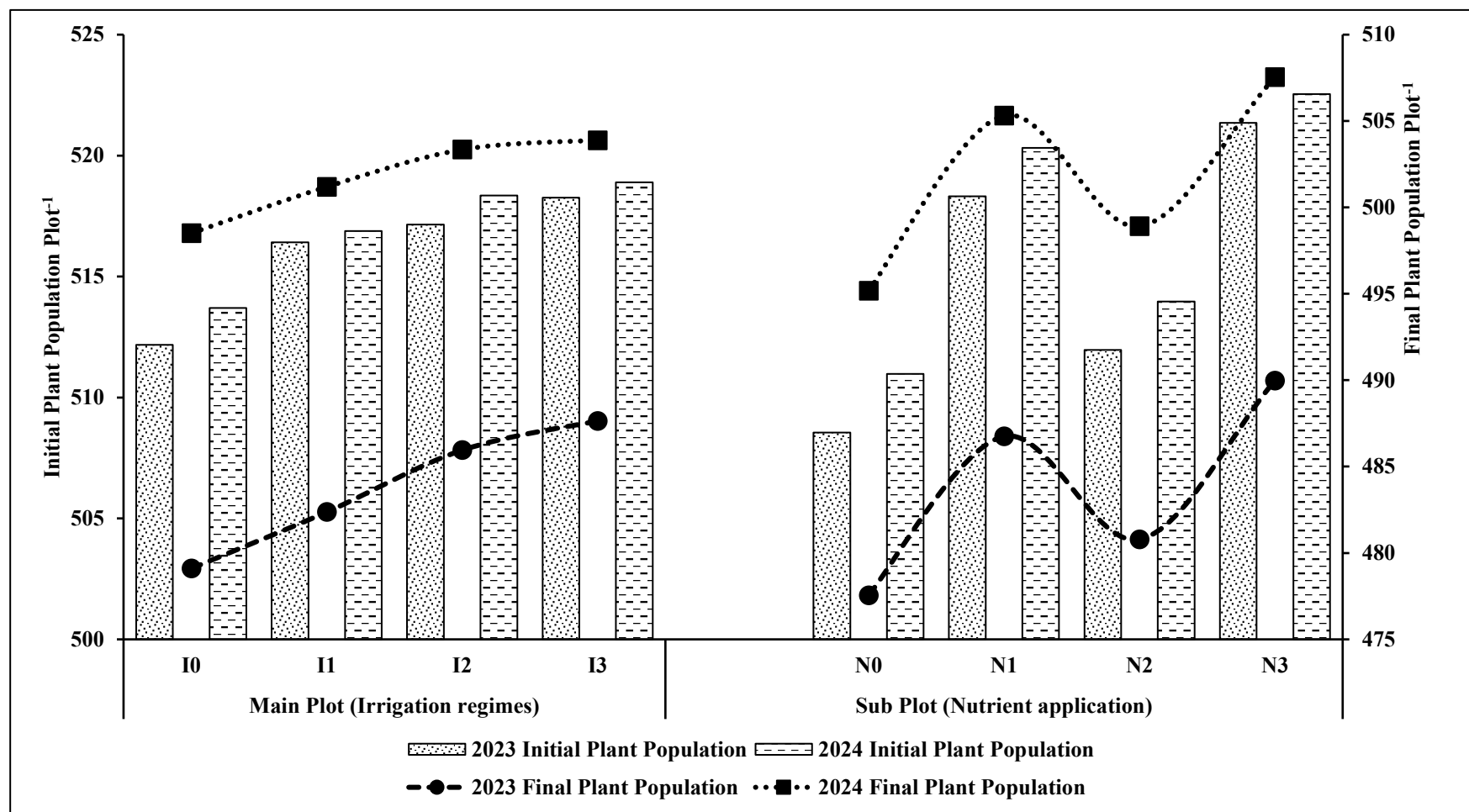


Figure 4.1 Effect of different irrigation regimes and different nutrient management on initial and final plant population

4.2.3 Impact of different irrigation regimes and different nutrient management on the plant height of the mustard crop

The study evaluated the impact of different irrigation regimes on the plant height of mustard across two growing seasons (2022–23 and 2023–24), with measurements taken at 30, 60, and 90 Days after sowing (DAS) and at harvest. In the 2022–23 season, plant heights were recorded as 11.98 cm, 108.73 cm, 154.58 cm, and 156.28 cm at 30 DAS, 60 DAS, 90 DAS, and harvest, respectively, for the I_0 treatment (no post-sowing irrigation). The I_1 treatment showed a slight increase, reaching 12.50 cm, 114.58 cm, 165.63 cm, and 165.45 cm at the same stages. With two post-sowing irrigations (I_2), plant heights rose further, reaching 12.42 cm, 120.33 cm, 178.98 cm, and 176.05 cm at each respective stage. This finding aligns with previous research indicating that increased soil moisture from frequent irrigation facilitates nutrient uptake and cellular expansion, processes essential for height growth (Bhattacharya and Bhattacharya, 2021). The tallest plants were observed in the I_3 treatment, with heights of 12.45 cm, 120.09 cm, 179.24 cm, and 187.02 cm. The 2023–24 season showed similar trends, with slightly higher heights across treatments. For I_0 , plant heights were 13.07 cm, 107.08 cm, 154.20 cm, and 155.95 cm across the four stages. The I_1 treatment recorded 13.22 cm, 117.12 cm, 168.41 cm, and 169.64 cm. The I_2 treatment further increased heights to 13.62 cm, 124.81 cm, 180.73 cm, and 185.53 cm, while the highest heights were again observed in I_3 , which measured 14.34 cm, 124.57 cm, 181.25 cm, and 188.78 cm at 30 DAS, 60 DAS, 90 DAS, and harvest.

In the 2022–23 season, at 30 DAS, plant heights ranged from 9.43 cm in the S_0 (control) treatment to 15.58 cm in the S_3 treatment. By 60 DAS, heights increased significantly, with S_0 reaching 107.28 cm and S_3 achieving the tallest plants at 122.32 cm. Similar trends were observed at 90 DAS, with heights ranging from 160.04 cm in S_0 to 177.51 cm in S_3 . At harvest, the final plant height was lowest in S_0 (162.23 cm) and highest in S_3 (179.38 cm). The 2023–24 season showed similar patterns with slightly taller plants. At 30 DAS, S_0 recorded 10.50 cm while S_3 reached 16.89 cm. By 60 DAS, plant heights ranged from 108.07 cm in S_0 to 125.42 cm in S_3 . At 90 DAS, S_0 produced plants of 161.60 cm, while S_3 again showed the tallest plants at 178.63 cm. At harvest, the final plant heights varied from 165.14 cm (S_0) to 181.86 cm (S_3), the maximum plant height was achieved only when both nutrients were applied together.

Table 4.3 Impact of different irrigation regimes and different nutrient management on the plant height plant⁻¹ (cm) of the Indian mustard

Treatments	Plant height (cm)							
	30 DAS		60 DAS		90 DAS		At harvest	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
I ₀	11.98	13.07	108.73	107.09	154.58	154.20	156.28	155.96
I ₁	12.50	13.23	114.58	117.12	165.63	168.41	165.45	169.64
I ₂	12.42	13.62	120.33	124.81	178.98	180.73	176.05	185.53
I ₃	12.45	14.34	120.09	124.57	179.24	181.25	187.02	188.78
SEm±	0.26	0.30	2.58	2.61	3.03	2.39	2.84	2.08
C.D at 5%	NS	NS	8.25	8.34	9.71	7.65	9.07	6.64
Sub plot (Nutrient management) (S)								
S ₀	9.43	10.50	107.28	108.07	160.04	161.60	162.22	165.14
S ₁	13.64	14.76	118.31	121.67	172.50	174.75	173.94	178.04
S ₂	10.71	12.10	115.82	118.42	168.39	169.63	169.26	174.86
S ₃	15.58	16.89	122.32	125.42	177.51	178.63	179.38	181.86
SEm±	0.17	0.21	1.03	1.14	1.07	1.35	1.048	1.272
C.D at 5%	0.50	0.60	2.95	3.28	3.07	3.88	3.007	3.647
Interaction (S*I)	NS	NS	NS	NS	NS	NS	NS	NS

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm± = Standard mean of error, C.D=Critical difference, DAS=Days after sowing, Cm=Centimeter

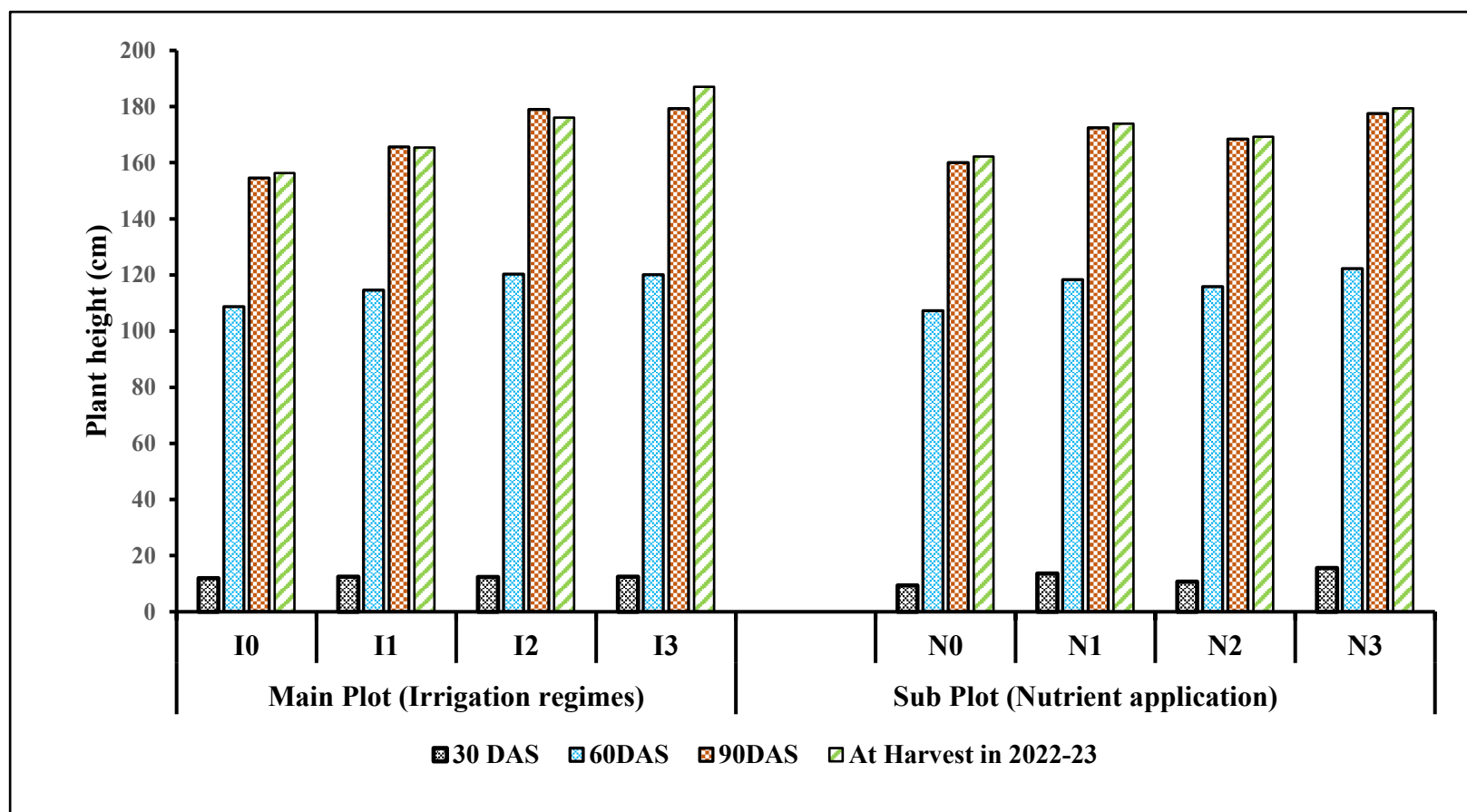


Figure 4.2A Effect of different irrigation regimes and different nutrient management on plant height (cm) of Indian mustard in 2022-23

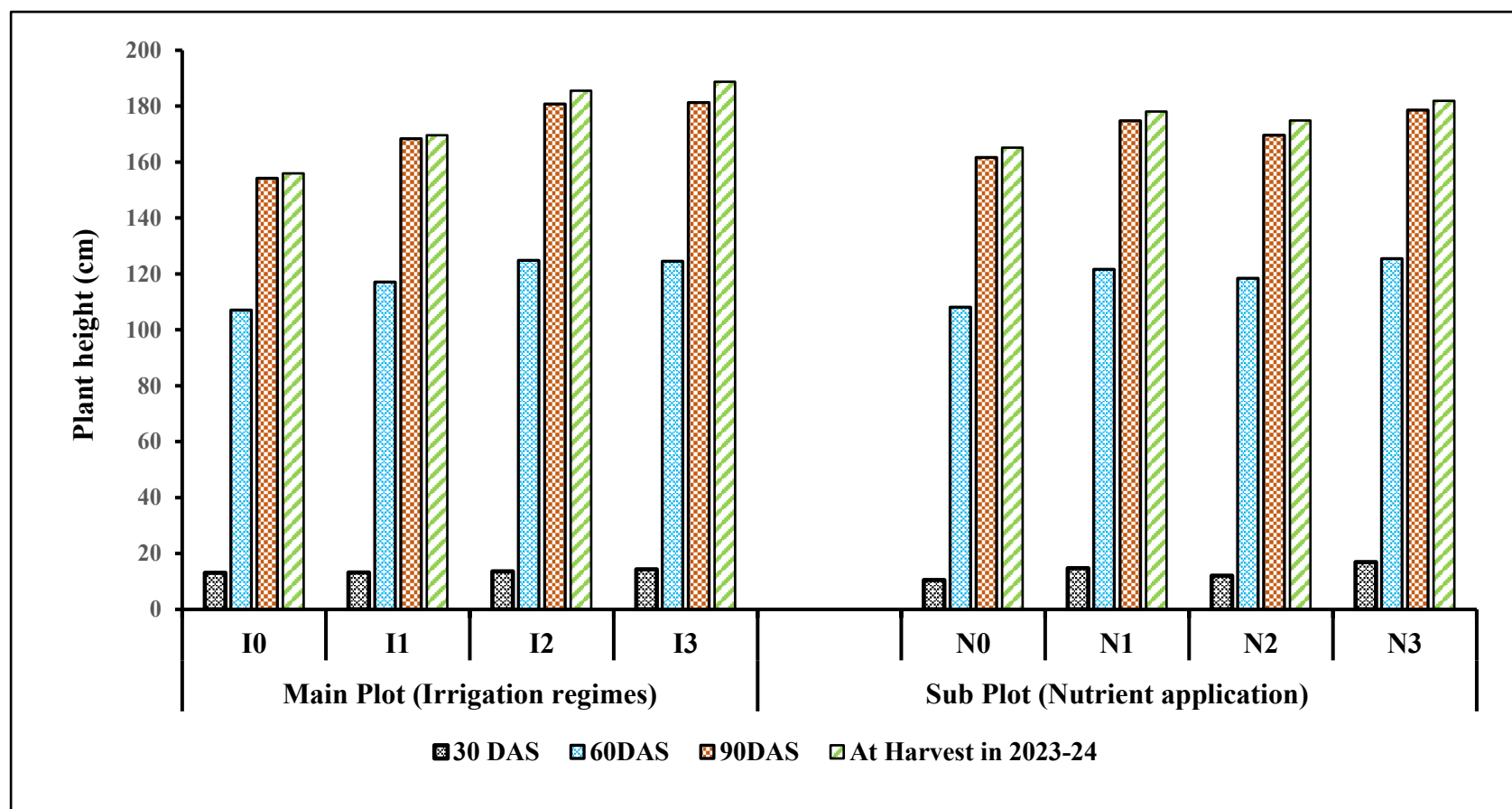


Figure 4.2B Effect of different irrigation regimes and different nutrient management on plant height (cm) of Indian mustard in 2023-24

4.2.4 Impact of different irrigation regimes and different nutrient management on the number of leaves

The impact of different irrigation regimes on the number of leaves per plant in mustard at various growth stages (30 DAS, 60 DAS, and 90 DAS) was consistent across both years (2022-23 and 2023-24), as well as in the combined mean data. In 2022-23, the I₃ treatment (three irrigations at vegetative, flowering, and siliqua filling stages) produced the highest number of leaves across all stages, with values of 4.06 leaves at 30 DAS, 28.82 leaves at 60 DAS, and 31.90 leaves at 90 DAS. In contrast, the I₀ treatment (no irrigation) had the lowest leaf count, recording 3.76 leaves at 30 DAS, 10.02 leaves at 60 DAS, and 11.05 leaves at 90 DAS. In 2023-24, the I₃ treatment again resulted in the highest number of leaves, with 4.22 leaves at 30 DAS, 29.03 leaves at 60 DAS, and 36.18 leaves at 90 DAS. I₀ again had the lowest leaf counts, with 3.91 leaves at 30 DAS, 9.98 leaves at 60 DAS, and 15.38 leaves at 90 DAS. Adequate soil moisture, as provided by I₃, maintained optimal metabolic and photosynthetic rates, promoting leaf growth and canopy expansion, particularly during the vegetative and reproductive stages (Ghadirnezhad Shiade *et al.*, 2023). The lower leaf counts observed in the I₀ treatment reflected the effects of water stress. Restricted moisture likely reduced nutrient uptake and limited photosynthetic efficiency, leading to fewer leaves. This trend aligned with findings from studies that indicated water stress could reduce leaf development as plants conserved resources under unfavorable conditions (Ullah, 2019).

The effect of nutrient management on the number of leaves per plant in mustard at various growth stages (30 DAS, 60 DAS, and 90 DAS) revealed significant differences across both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the S₃ treatment (humic acid + sulfur) produced the highest number of leaves per plant across all stages, with values of 5.05 leaves at 30 DAS, 21.68 leaves at 60 DAS, and 23.49 leaves at 90 DAS. In contrast, the S₀ treatment (control) had the lowest leaf count, recording 2.93 leaves at 30 DAS, 16.81 leaves at 60 DAS, and 16.78 leaves at 90 DAS. Humic acid improved soil structure, promoted root growth, and enhanced nutrient absorption, while sulfur played a crucial role in chlorophyll formation and protein synthesis, both essential for leaf development and overall vegetative growth (Tiwari *et al.*, 2023). In 2023-24, S₃ continued to result in the highest number of leaves, with 5.21 leaves at 30 DAS, 21.84 leaves at 60 DAS, and 27.77 leaves at 90 DAS. The

So treatment again had the lowest leaf counts, with 3.02 leaves at 30 DAS, 16.69 leaves at 60 DAS, and 22.19 leaves at 90 DAS.

4.2.5 Impact of different irrigation regimes and different nutrient management on leaf area (cm² plant⁻¹) of mustard crop

The effect of different irrigation regimes on the leaf area per plant (cm²) of mustard at various growth stages (30 DAS, 60 DAS, and 90 DAS) was significant across both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the I₃ treatment (three irrigations at vegetative, flowering, and siliqua filling stages) produced the largest leaf area at all stages, with values of 416.77 cm² at 30 DAS, 1898.22 cm² at 60 DAS, and 1305.52 cm² at 90 DAS. In contrast, the I₀ treatment (no irrigation) had the smallest leaf area, recording 379.08 cm² at 30 DAS, 1408.11 cm² at 60 DAS, and 821.17 cm² at 90 DAS. In 2023-24, the I₃ treatment again resulted in the largest leaf area, with 418.64 cm² at 30 DAS, 1961.78 cm² at 60 DAS, and 1344.83 cm² at 90 DAS. This effect was attributed to improved water availability, which enhanced cell turgor and expansion, resulting in larger leaf surfaces. Adequate soil moisture provided by the I₃ regime supported optimal photosynthetic rates and nutrient uptake, which are essential for maximizing leaf area, particularly during critical growth stages (Karnan *et al.*, 2023). I₀ once more had the smallest leaf area, with 384.14 cm² at 30 DAS, 1487.92 cm² at 60 DAS, and 908.90 cm² at 90 DAS. Studies have shown that water stress reduces leaf area as plants aim to conserve water, ultimately lowering photosynthetic potential and overall growth (Farooq *et al.*, 2019).

The effect of nutrient management on the leaf area per plant (cm²) of mustard at various growth stages (30 DAS, 60 DAS, and 90 DAS) was notable across both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the S₃ treatment (humic acid + sulfur) resulted in the largest leaf area across all stages, with values

Table 4.4 Impact of humic acid and sulphur on number of leaves plant⁻¹ of Indian mustard under variable water regimes

Treatments	Number of leaves plant ⁻¹					
	30 DAS		60 DAS		90 DAS	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
I ₀	3.76	3.91	10.02	9.98	11.05	15.38
I ₁	3.88	3.99	16.69	16.69	14.97	19.71
I ₂	4.00	4.16	21.56	21.72	24.60	29.50
I ₃	4.06	4.22	28.82	29.03	31.90	36.18
SEm±	0.09	0.11	0.61	0.67	0.49	0.57
C.D at 5%	NS	NS	1.94	2.13	1.57	1.84
Sub plot (Nutrient management) (S)						
S ₀	2.93	3.02	16.81	16.69	16.78	22.19
S ₁	4.42	4.59	20.23	20.38	22.10	26.38
S ₂	3.30	3.45	18.37	18.51	20.15	24.43
S ₃	5.05	5.21	21.68	21.84	23.49	27.77
SEm±	0.08	0.10	0.42	0.45	0.41	0.45
C.D at 5%	0.22	0.28	1.21	1.28	1.19	1.34
Interaction (S*I)	NS	NS	NS	NS	NS	NS

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm± = Standard mean of error, C.D=Critical difference, DAS=Days after sowing. Plant⁻¹=Per Plant.

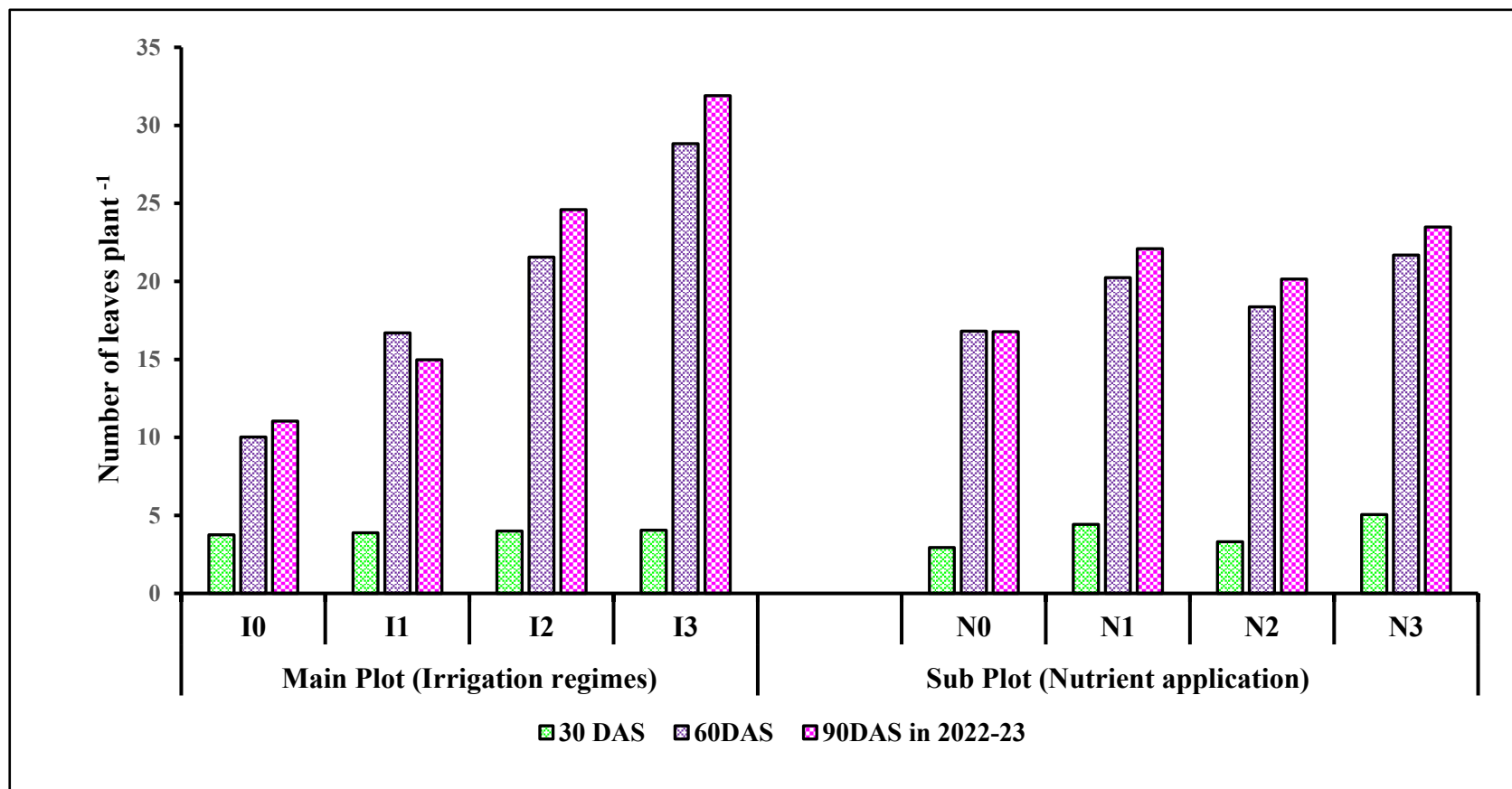


Figure 4.3A Effect of different irrigation regimes and different nutrient management on the number of leaves plant⁻¹ in 2022-23

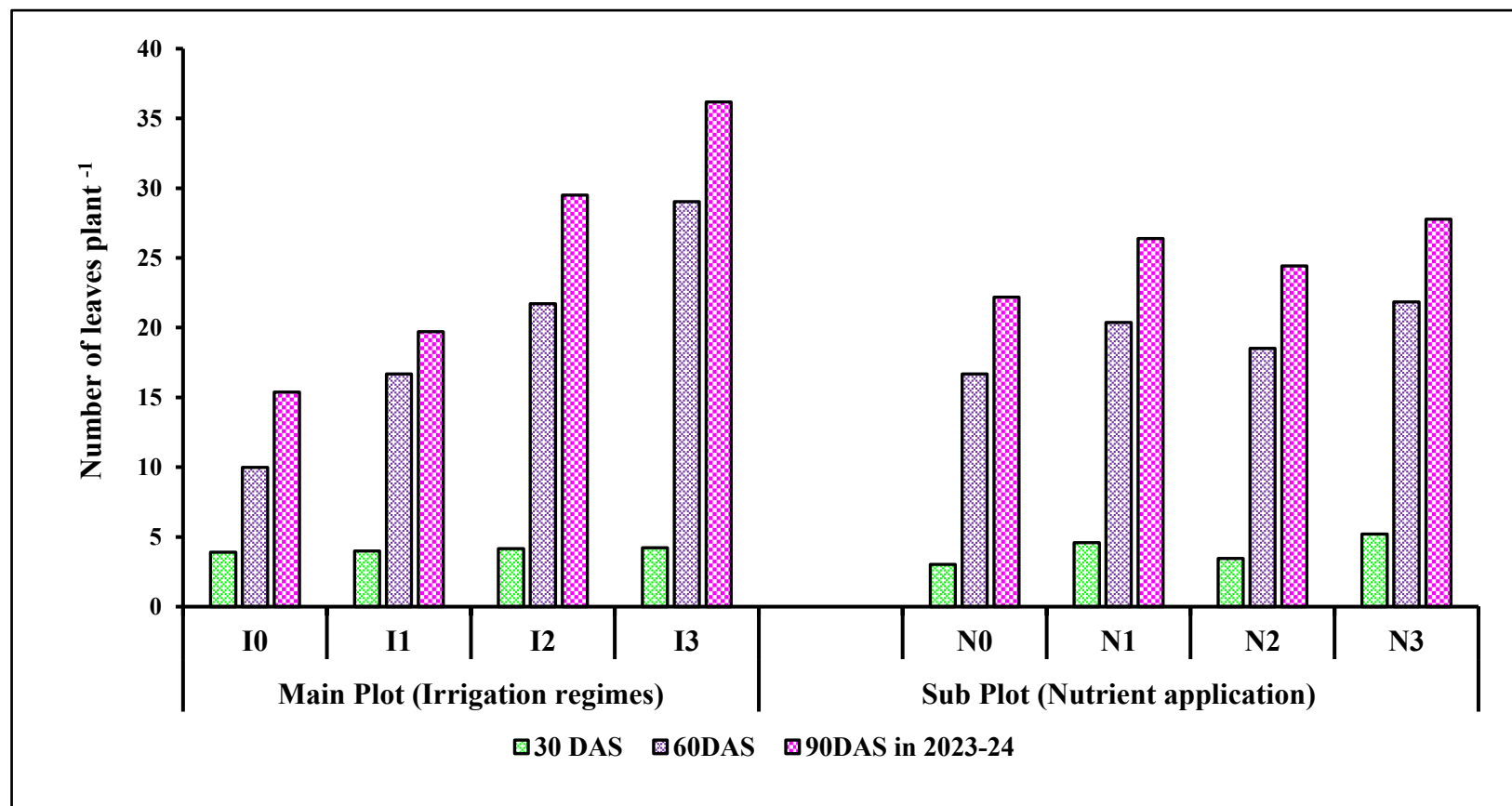


Figure 4.3B Effect of different irrigation regimes and different nutrient management on the number of leaves plant⁻¹ in 2023-24

of 493.47 cm² at 30 DAS, 1839.84 cm² at 60 DAS, and 1265.97 cm² at 90 DAS. The higher leaf area observed under S₃ was attributed to the synergistic effects of humic acid and sulfur, which together improved nutrient uptake, root growth, and overall plant health. Humic acid enhanced soil nutrient availability and retention, while sulfur played a vital role in protein synthesis and chlorophyll production, both of which contributed to greater leaf expansion (Ramadan *et al.*, 2024). In contrast, the S₀ treatment (control) had the smallest leaf area, recording 302.35 cm² at 30 DAS, 1583.36 cm² at 60 DAS, and 999.55 cm² at 90 DAS. In 2023-24, S₃ again produced the largest leaf area, with values of 490.66 cm² at 30 DAS, 1903.40 cm² at 60 DAS, and 1307.08 cm² at 90 DAS. The S₀ treatment remained the lowest, with 305.45 cm² at 30 DAS, 1687.05 cm² at 60 DAS, and 1084.18 cm² at 90 DAS. Plants likely experienced nutrient deficiencies that restricted cell division and elongation, curtailing leaf expansion and reducing overall photosynthetic capacity (Bhattacharya and Bhattacharya, 2021).

4.2.6 Impact of different irrigation regimes and different nutrient management on the fresh weight plant⁻¹(g)

The impact of different irrigation regimes on the fresh weight per plant (g) of mustard at various growth stages (30-60-90 DAS, and at harvest) was significant during both years (2022-23 and 2023-24), as well as in the mean data. In 2022-23, I₃ (three irrigations at vegetative, flowering, and siliqua filling stages) exhibited the highest fresh weight at all observation stages, with values of 13.59 g at 30 DAS, 117.78 g at 60 DAS, 175.44 g at 90 DAS, and 165.04 g at harvest. In contrast, I₀ (no irrigation) had the lowest fresh weight values across these stages, with 12.83 g at 30 DAS, 96.12 g at 60 DAS, 129.90 g at 90 DAS, and 118.11 g at harvest. The enhanced fresh weight observed under I₃ was attributed to the continuous availability of water during critical growth periods (vegetative, flowering, and siliqua filling), which supported cell turgor, nutrient uptake, and photosynthetic activity, ultimately leading to greater biomass (Oguz *et al.*, 2022). In 2023-24, a similar trend was observed, with I₃ consistently producing the highest fresh weight per plant across stages: 17.15 g at 30 DAS, 126.66 g at 60 DAS, 181.79 g at 90 DAS, and 170.02 g at harvest. Again, I₀ had the lowest fresh weight values, reaching only 15.71 g at 30 DAS, 104.30 g at 60 DAS, 130.72 g at 90 DAS, and 128.41 g at harvest. In contrast, the limited fresh weight observed under the I₀ treatment (no irrigation) was attributed to water stress, which likely hindered metabolic processes and

Table 4.5 Influence of humic acid and sulphur on number of leaves plant⁻¹ of Indian mustard under variable water regimes

Treatments	Leaf area (cm ²) plant ⁻¹					
	30 DAS		60 DAS		90 DAS	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
I ₀	379.08	384.14	1408.11	1487.92	821.17	908.90
I ₁	393.07	397.98	1777.24	1864.68	1186.02	1229.22
I ₂	406.43	409.02	1847.87	1911.43	1280.39	1323.87
I ₃	416.77	418.64	1898.22	1961.78	1305.52	1344.83
SEm±	9.19	7.82	34.27	39.73	35.25	26.90
C.D at 5%	NS	NS	109.63	127.08	112.75	86.04
Sub plot (Nutrient management) (S)						
S ₀	302.35	305.45	1583.36	1687.05	999.55	1084.18
S ₁	424.87	432.89	1772.99	1836.55	1190.56	1234.04
S ₂	374.65	380.78	1735.25	1798.81	1137.02	1181.51
S ₃	493.47	490.66	1839.84	1903.40	1265.97	1307.08
SEm±	18.00	4.59	15.27	24.48	18.40	16.47
C.D at 5%	6.29	13.15	43.78	70.19	52.78	47.23
Interaction (S*I)	NS	NS	NS	NS	NS	NS

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm± = Standard mean of error, C.D=Critical difference, DAS=Days after sowing, cm² = centimeter square ,Plant⁻¹=Per Plant.

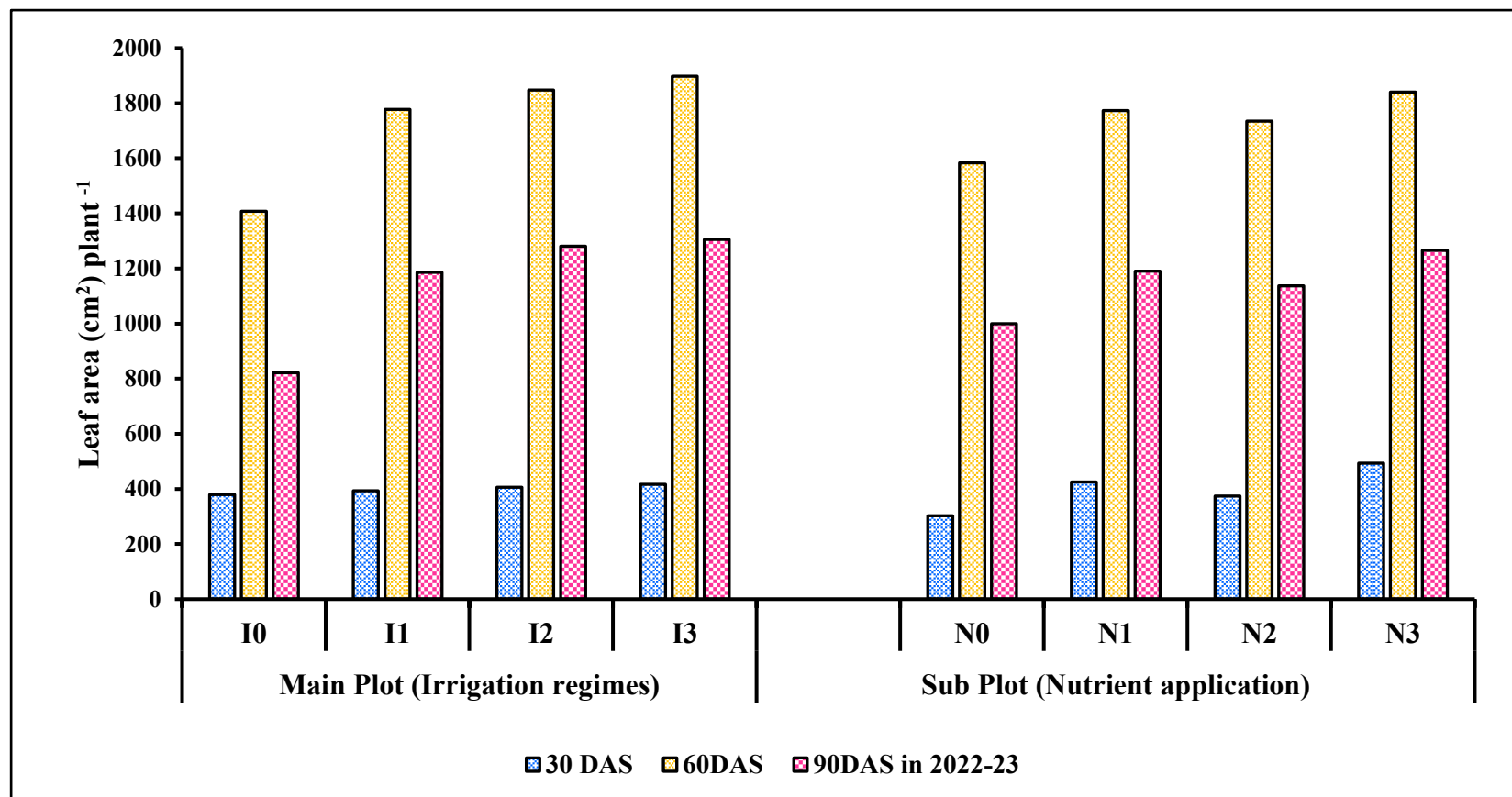


Figure 4.4A Effect of different irrigation regimes and different nutrient management on leaf area (cm² plant⁻¹) in 2022-23

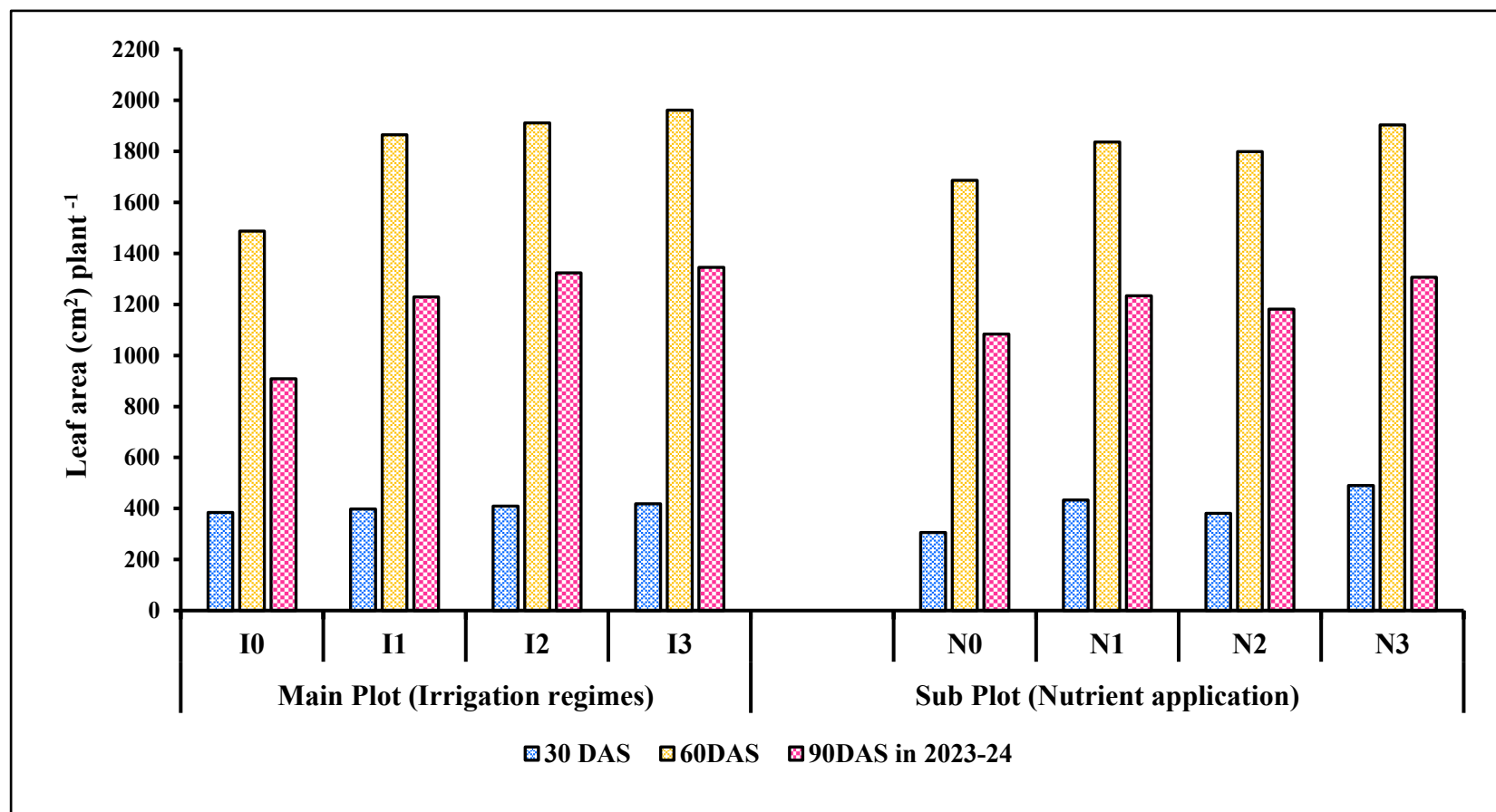


Figure 4.4B Effect of different irrigation regimes and different nutrient management on leaf area (cm² plant⁻¹) in 2023-24

restricted growth. When water availability was low, plants tended to conserve resources, resulting in reduced cell expansion and lower biomass accumulation (Ghadirnezhad Shiade, 2023). This observed trend aligned with studies showing that water stress during vegetative and reproductive stages significantly reduced yield and plant growth (Geremew *et al.*, 2019).

The effect of nutrient management on the fresh weight per plant (g) of mustard at different growth stages (30 DAS, 60 DAS, 90 DAS, and at harvest) was evident across both years (2022-23 and 2023-24) as well as in the mean data. In 2022-23, S₃ (humic acid + sulfur) treatment resulted in the highest fresh weight at all stages, with values of 16.13 g at 30 DAS, 117.50 g at 60 DAS, 168.44 g at 90 DAS, and 157.96 g at harvest. The lowest fresh weight values were observed under S₀ (control) with 10.30 g at 30 DAS, 99.50 g at 60 DAS, 144.05 g at 90 DAS, and 129.50 g at harvest. Humic acid improved soil structure, nutrient availability, and root growth, while sulfur contributed to essential processes such as protein synthesis, chlorophyll formation, and enzyme activity (Tiwari *et al.*, 2023). Together, these nutrients created favorable growth conditions, leading to larger plant size and higher fresh weight. The control treatment (S₀) consistently produced the lowest fresh weights, likely due to limited nutrient availability, which restricted physiological growth processes and limited biomass accumulation. Without additional nutrients, plants likely experienced nutrient stress, reducing cell expansion and overall growth potential (de Bang, 2021). Similarly, in 2023-24, the S₃ treatment produced the highest fresh weights: 19.13 g at 30 DAS, 124.41 g at 60 DAS, 172.94 g at 90 DAS, and 164.29 g at harvest. In comparison, the S₀ treatment had the lowest fresh weight values, measuring 11.87 g at 30 DAS, 110.27 g at 60 DAS, 148.34 g at 90 DAS, and 139.40 g at harvest. The consistently higher fresh weight observed under S₃ aligned with research indicating that combining organic and inorganic nutrients can enhance crop performance by collectively improving nutrient use efficiency, soil fertility, and plant metabolism (Bo *et al.*, 2024).

4.2.6a Interaction between different irrigation regimes and different nutrient management on the fresh weight plant⁻¹(g) of mustard crop at 60 DAS

The interaction between different irrigation regimes and nutrient management treatments had a notable impact on the fresh weight per plant (g) of mustard at 60 DAS during the years 2022-23, 2023-24, and in the mean data. In 2022-23, the I₃S₃ treatment

Table 4.6 Effect of humic acid and sulphur on fresh weight of Indian mustard under variable water regimes

Treatments	Fresh weight plant ⁻¹ (g)							
	30 DAS		60 DAS		90 DAS		At harvest	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
I ₀	12.83	15.71	96.12	104.30	129.90	130.72	118.11	128.41
I ₁	12.91	16.10	108.76	116.98	150.89	154.69	142.17	150.12
I ₂	13.62	16.55	115.22	123.26	167.65	172.31	151.88	157.32
I ₃	13.59	17.15	117.78	126.66	175.44	181.79	165.04	170.02
SEm±	0.326	0.523	2.107	2.300	3.845	3.796	3.17	4.00
C.D at 5%	NS	NS	6.739	7.357	12.299	12.143	10.13	12.81
Sub plot (Nutrient management) (S)								
S ₀	10.30	11.87	99.50	110.27	144.05	148.34	129.50	139.40
S ₁	13.73	17.73	111.56	120.36	160.04	164.47	149.55	155.82
S ₂	12.79	16.79	109.32	116.16	151.36	153.77	140.20	146.37
S ₃	16.13	19.13	117.50	124.41	168.44	172.94	157.96	164.29
SEm±	0.43	0.59	1.11	1.14	3.41	3.42	3.23	3.52
C.D at 5%	1.24	1.69	3.20	3.28	9.78	9.81	9.27	10.09
Interaction (S*I)	NS	NS	6.85	7.06	20.39	20.44	19.19	21.03

* I=irrigation regimes, N=nutrient management, I₀=No Post Sowing Irrigation], I₁=One Post Sowing Irrigation (Vegetative stage), I₂=Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃=Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀=Control, S₁=Humic acid, S₂=Sulphur, S₃=Humic acid + Sulphur, SEm±=Standard mean of error, C.D=Critical difference. NS= Nonsignificant, g plant⁻¹= gram per plant, DAS= Days after sowing.

Table 4.6A Interaction table of fresh weight plant⁻¹ (g) at 60 DAS

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		81.20	101.10	98.35	103.81	96.12	91.41	109.76	103.51	112.53	104.3
I ₁		96.41	111.61	110.52	116.49	108.76	112.41	120.41	114.05	121.06	116.98
I ₂		107.95	116.84	113.08	123.01	115.22	117.74	125.73	122.91	126.66	123.26
I ₃		112.44	116.69	115.31	126.69	117.78	119.52	125.56	124.16	137.41	126.66
Mean (S)		99.50	111.56	109.32	117.5		110.27	120.36	116.16	124.41	
SEm±	S * I	4.21					4.59				
	I * S	2.86					3.04				
C.D at 5%	S * I	6.85					7.06				
	I * S	8.79					9.38				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂= Sulphur, S₃ = Humic acid + Sulphur, SEm± = Standard mean of error, C. D=Critical difference. NS= Nonsignificant, g plant⁻¹= gram per plant, DAS= Days after sowing.

Table 4.6B: Interaction table of fresh weight plant⁻¹ (g) at 90 DAS

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
	I ₀	107.53	137.48	112.75	161.82	129.90	109.60	139.80	109.17	164.32	130.72
	I ₁	139.96	153.88	151.50	158.22	150.89	144.06	158.11	154.10	162.49	154.69
	I ₂	162.38	169.68	165.30	173.25	167.65	166.99	174.35	169.94	177.96	172.31
	I ₃	166.32	179.11	175.90	180.46	175.44	172.71	185.61	181.87	186.97	181.79
	Mean (S)	144.05	160.04	151.36	168.44		148.34	164.47	153.77	172.94	
SEm±	S * I	77.76					7.59				
	I * S	7.05					7.04				
C.D at 5%	S* I	20.39					20.44				
	I* S	21.07					21.02				

* I=irrigation regimes, N=nutrient management, I₀=No Post Sowing Irrigation], I₁=One Post Sowing Irrigation (Vegetative stage), I₂=Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃=Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀=Control, S₁=Humic acid, S₂=Sulphur, S₃=Humic acid + Sulphur, SEm±=Standard mean of error, C. D=Critical difference. NS= Nonsignificant, g plant⁻¹= gram per plant, DAS= Days after sowing.

Table 4.6C Interaction table of fresh weight plant⁻¹ (g) at harvest

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		88.81	128.76	101.79	153.10	118.11	106.42	136.64	109.44	161.15	128.41
I ₁		131.24	145.16	142.79	149.51	142.17	138.86	153.21	150.80	157.61	150.12
I ₂		146.61	153.91	149.53	157.48	151.88	151.42	159.56	155.13	163.18	157.32
I ₃		151.35	170.39	166.68	171.74	165.04	160.90	173.85	170.10	175.22	170.02
Mean (S)		129.50	149.55	140.20	157.96		139.40	155.82	146.37	164.29	
SEm±	S * I	6.33					8.01				
	I * S	6.43					7.29				
C.D at 5%	S* I	19.19					21.03				
	I* S	19.02					21.80				

* I=irrigation regimes, N=nutrient management, I₀=No Post Sowing Irrigation], I₁=One Post Sowing Irrigation (Vegetative stage), I₂=Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃=Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀=Control, S₁=Humic acid, S₂=Sulphur, S₃=Humic acid + Sulphur, SEm±=Standard mean of error, C.D=Critical difference. NS= Nonsignificant, g plant⁻¹= gram per plant, g=gram

(three irrigations with humic acid + sulphur) achieved the highest fresh weight per plant at 60 DAS, recording 126.69 g, while the I₀S₀ treatment (no irrigation and no added nutrients) had the lowest at 81.20 g. This combination provided optimal growth conditions, resulting in higher fresh weight under I₃S₃. Conversely, the lowest fresh weight observed in the I₀S₀ treatment highlighted the impact of water and nutrient deficiency, as both were crucial for cellular processes and energy transfer required for growth. Without irrigation or supplemental nutrients, plants likely experienced moisture and nutrient stress, limiting their growth potential and reducing fresh weight (Usman *et al.*, 2023). The findings aligned with previous studies demonstrating that well-managed irrigation and nutrient regimes enhance crop growth by supporting sustained photosynthesis, nutrient transport, and energy production (Ferreira *et al.*, 2024).

4.2.6b Interaction between different irrigation regimes and different nutrient management on the fresh weight plant⁻¹ (g) of mustard crop at 90 DAS

The interaction between different irrigation regimes and nutrient management treatments significantly affected the fresh weight per plant (g) of mustard at 90 DAS during the years 2022-23 and 2023-24, as well as in the mean data. In 2022-23, I₃S₃ (three irrigations with humic acid + sulfur) recorded the highest fresh weight per plant at 90 DAS, reaching 180.46 g, while I₀S₀ (no irrigation and no added nutrients) had the lowest at 107.53 g. The mean fresh weight for each irrigation regime ranged from 129.90 g under I₀ to 175.44 g under I₃, showing an increase in fresh weight with more frequent irrigation and nutrient supplementation. Consistent irrigation (I₃) maintained optimal soil moisture, facilitating efficient nutrient uptake and sustained growth, while humic acid and sulfur (S₃) improved soil fertility, nutrient availability, and physiological functions, leading to higher biomass (Wang *et al.*, 2022). In 2023-24, I₃S₃ again produced the highest fresh weight at 90 DAS, with a value of 186.97 g, whereas I₀S₀ had the lowest fresh weight of 109.60 g. In contrast, the lowest fresh weight under I₀S₀ highlighted the detrimental impact of both water and nutrient deficiencies, which limited plant growth potential and reduced biomass accumulation. Water scarcity and the absence of supplemental nutrients likely stressed the plants, restricting cell division and expansion processes vital for biomass production (Kumari *et al.*, 2022). This interaction effect aligned with research demonstrating that well-coordinated irrigation and nutrient management strategies are critical for optimizing crop growth, as water

and nutrients work synergistically to support higher fresh weights in mustard (Vikram *et al.*, 2022).

4.2.6c Interaction between different irrigation regimes and different nutrient management on the fresh weight plant⁻¹ (g) of mustard crop at harvest

The interaction between irrigation regimes and nutrient management significantly impacted the fresh weight per plant (g) of mustard at harvest in both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the I₃S₃ treatment produced the highest fresh weight at harvest at 171.74 g, while the I₀S₀ treatment yielded the lowest at 88.81 g. The mean fresh weight for each irrigation regime ranged from 118.11 g for I₀ to 165.04 g for I₃, showing a positive trend with increased irrigation frequency. The increased fresh weight under I₃S₃ was attributed to the combined influence of consistent water availability and enhanced nutrient uptake. Irrigation at key growth stages (I₃) maintained optimal soil moisture levels, facilitating nutrient absorption and improving physiological functions, while humic acid and sulfur (S₃) provided essential nutrients for growth and development (Wang *et al.*, 2022). In 2023-24, I₃S₃ again achieved the highest fresh weight at harvest with 175.22 g, whereas I₀S₀ was the lowest at 106.42 g. The mean fresh weight for each irrigation regime followed a similar trend, with values from 128.41 g for I₀ to 170.02 g for I₃. The combined mean data over the two years reaffirmed these patterns, with I₃S₃ consistently yielding the highest fresh weight at 173.48 g, and I₀S₀ the lowest at 97.61 g. Across irrigation regimes, the mean fresh weight ranged from 123.26 g for I₀ to 167.53 g for I₃. Humic acid enhanced soil structure and nutrient retention, boosting root growth and nutrient uptake, while sulfur played a critical role in protein synthesis and enzyme activation, leading to better biomass accumulation (Kaya *et al.*, 2020). Without supplemental water and nutrients, mustard plants likely experienced stress, limiting their growth and reducing fresh weight. Studies indicated that water stress and nutrient deficiency restricted cell division and elongation, leading to lower overall biomass (Kurepa and Smalle, 2022).

4.2.7 Impact of different irrigation regimes and different nutrient management on the Dry weight plant⁻¹ (g)

The impact of different irrigation regimes on the dry weight per plant (g) of mustard at various growth stages (30 DAS, 60 DAS, 90 DAS, and at harvest) was notable across both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, I₃ (three irrigations at vegetative, flowering, and siliqua filling stages) produced the highest dry weight per plant across all stages, with values of 1.39 g at 30 DAS, 21.74 g at 60 DAS, 45.28 g at 90 DAS, and 51.83 g at harvest. I₀ (no irrigation) had the lowest dry weight values, recording 1.36 g at 30 DAS, 14.27 g at 60 DAS, 28.11 g at 90 DAS, and 33.81 g at harvest. Sufficient moisture from three irrigations (I₃) allowed plants to maintain cell turgor and sustain photosynthetic rates, contributing to higher dry weight accumulation at each growth stage (Kang *et al.*, 2020). In 2023-24, a similar pattern was observed, with I₃ showing the highest dry weight values at all stages: 1.98 g at 30 DAS, 23.24 g at 60 DAS, 47.47 g at 90 DAS, and 51.96 g at harvest. I₀ recorded the lowest dry weight values again, with 1.91 g at 30 DAS, 15.52 g at 60 DAS, 30.12 g at 90 DAS, and 33.82 g at harvest. This finding aligned with research demonstrating that water stress could reduce dry matter production in crops by affecting nutrient transport and limiting photosynthetic efficiency (Seleiman, 2021).

The effect of nutrient management on the dry weight per plant (g) of mustard at various growth stages (30 DAS, 60 DAS, 90 DAS, and at harvest) showed notable differences across both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the S₃ treatment (humic acid + sulphur) resulted in the highest dry weight per plant at all stages, with values of 1.58 g at 30 DAS, 22.70 g at 60 DAS, 45.11 g at 90 DAS, and 48.67 g at harvest. Humic acid promoted soil structure, increased nutrient retention, and enhanced nutrient uptake efficiency, while sulfur played a critical role in amino acid and enzyme synthesis, contributing to improved biomass accumulation (Kaya *et al.*, 2020). In contrast, the S₀ treatment (control) had the lowest dry weight values across the stages, with 1.12 g at 30 DAS, 15.40 g at 60 DAS, 31.34 g at 90 DAS, and 40.23 g at harvest. In 2023-24, the S₃ treatment again showed the highest dry weight values, reaching 2.16 g at 30 DAS, 23.89 g at 60 DAS, 47.30 g at 90 DAS, and 48.86 g at harvest. Thus, without added nutrients, plants likely faced deficiencies that restricted cell division, elongation, and photosynthesis.

Table 4.7 Impact of humic acid and sulphur on dry matter of Indian mustard under variable water regimes

Treatments	Dry matter accumulation plant ⁻¹ (g)							
	30 DAS		60 DAS		90 DAS		At harvest	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
I ₀	1.36	1.91	14.27	15.52	28.11	30.12	33.81	33.82
I ₁	1.37	1.96	20.31	20.80	39.68	40.69	41.78	41.97
I ₂	1.38	1.97	20.99	21.68	41.86	44.14	48.98	48.98
I ₃	1.39	1.98	21.74	23.24	45.28	47.47	51.83	51.96
SEm±	0.01	0.02	0.09	0.31	0.16	0.35	0.90	0.68
C.D at 5%	NS	NS	0.28	1.01	0.50	1.12	2.88	2.19
Sub plot (Nutrient management) (S)								
S ₀	1.12	1.72	15.40	15.61	31.34	32.24	40.23	39.99
S ₁	1.46	2.04	20.78	21.97	41.87	44.06	44.96	45.15
S ₂	1.33	1.91	18.42	19.76	36.62	38.81	42.54	42.73
S ₃	1.58	2.16	22.70	23.89	45.11	47.30	48.67	48.86
SEm±	0.02	0.03	0.10	0.37	0.16	0.42	0.38	0.47
C.D at 5%	0.05	0.07	0.30	1.07	0.45	1.20	1.09	1.35
Interaction (S*I)	NS	NS	NS	NS	NS	NS	NS	NS

* I=irrigation regimes, N=nutrient management , I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm± = Standard mean of error, C.D=Critical difference, NS= Non-significant, DAS=Days after sowing, g plant⁻¹=gram per plant.

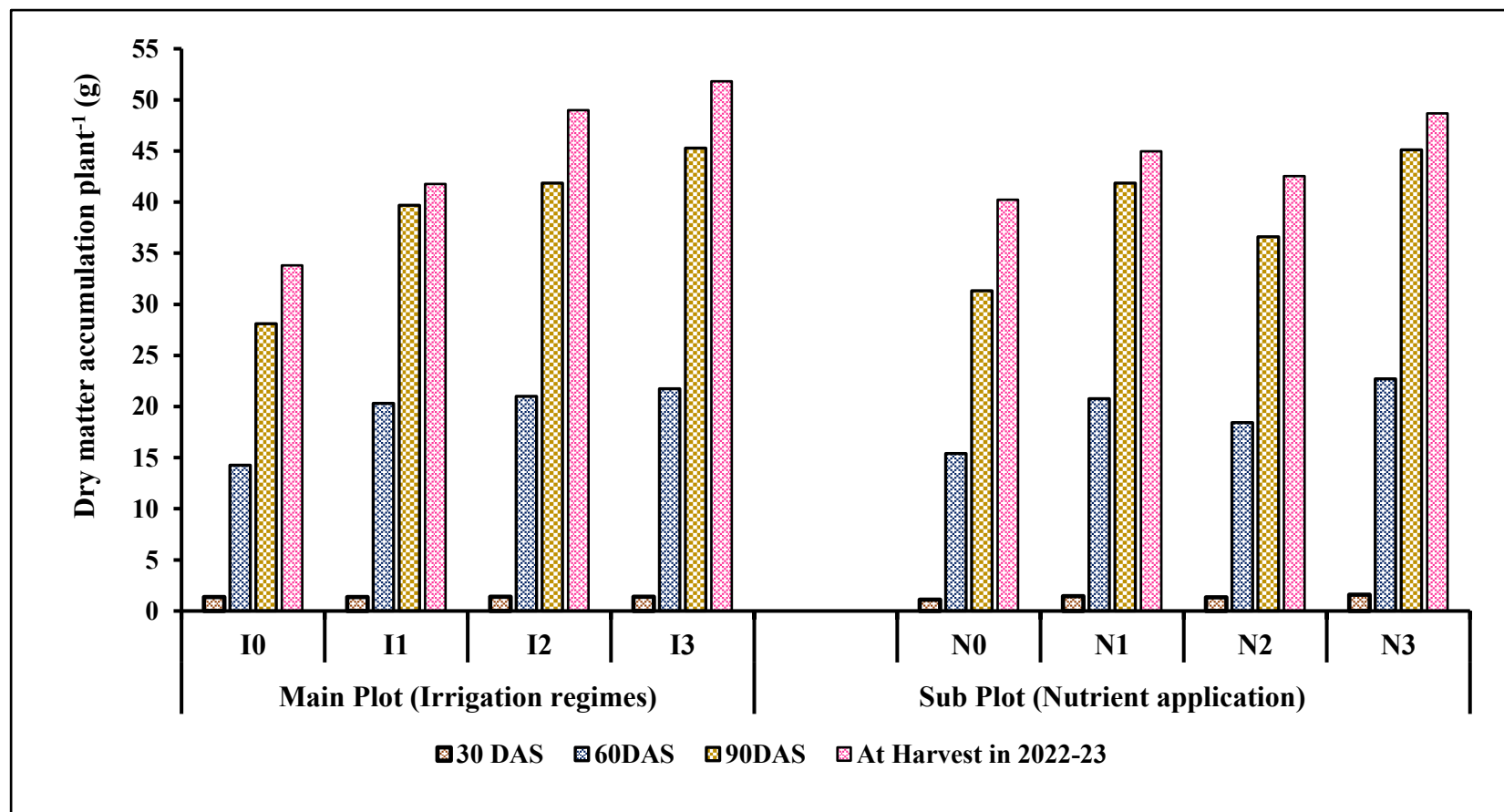


Figure 4.5A Effect of different irrigation regimes and nutrient management on dry weight plant⁻¹ (g) of Indian mustard in 2022-23

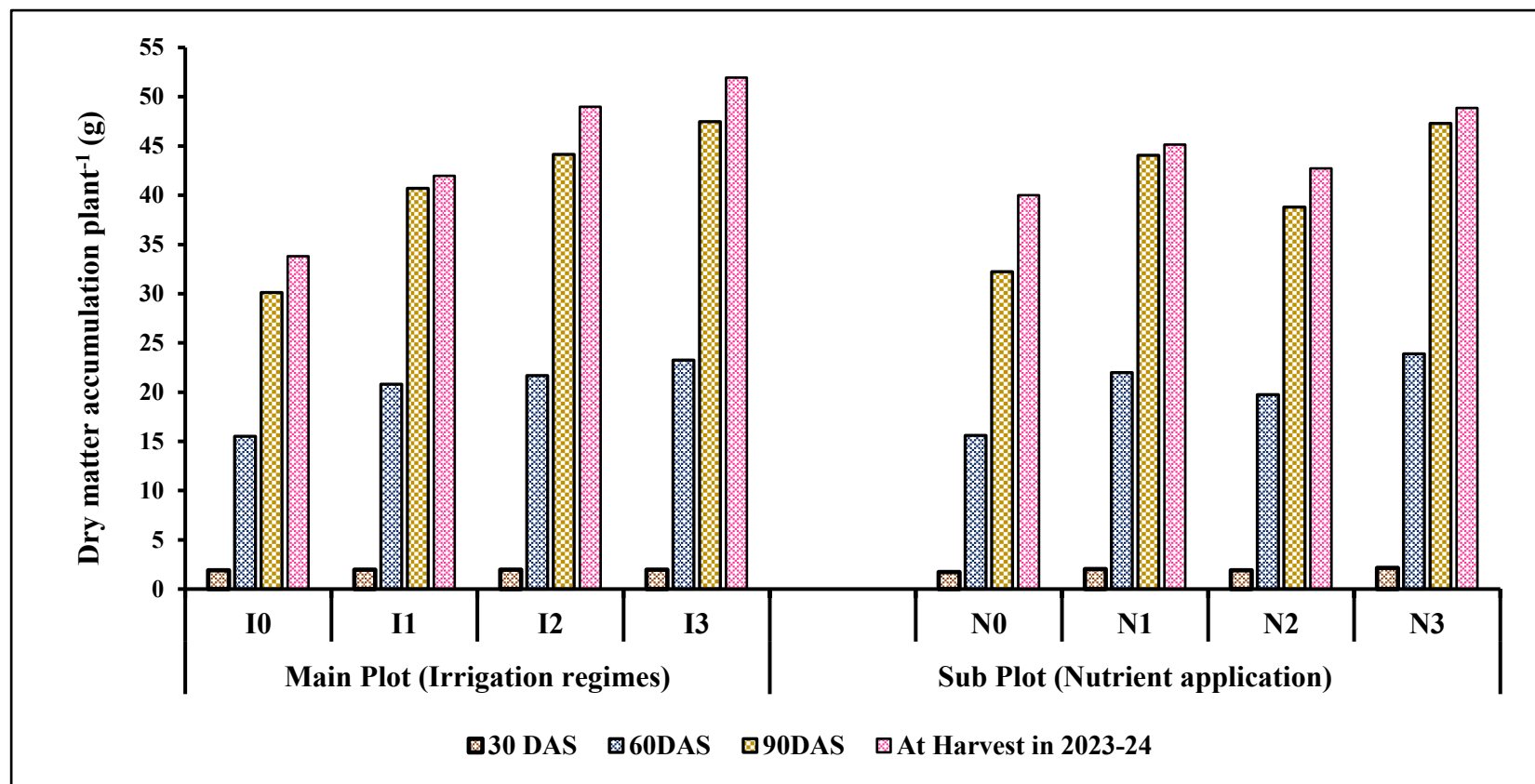


Figure 4.5B Effect of different irrigation regimes and nutrient management on dry weight plant⁻¹ (g) of Indian mustard in 2023-24

4.3 Growth analysis parameters (computations)

4.3.1 Impact of different irrigation regimes and different nutrient management on leaf area index (LAI)

The effect of different irrigation regimes on the leaf area index (LAI) of mustard at various growth stages (30 DAS, 60 DAS, and 90 DAS) was consistent across both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the I_3 treatment (three irrigations at vegetative, flowering, and siliqua filling stages) had the highest LAI at all stages, with values of 0.93 at 30 DAS, 4.14 at 60 DAS, and 2.99 at 90 DAS. In contrast, the I_0 treatment (no irrigation) had the lowest LAI values, recording 0.84 at 30 DAS, 3.12 at 60 DAS, and 1.96 at 90 DAS. In 2023-24, I_3 again produced the highest LAI values, with 0.93 at 30 DAS, 4.44 at 60 DAS, and 2.90 at 90 DAS. Adequate soil moisture enabled plants to maintain optimal metabolic functions, enhancing growth and contributing to a higher LAI, particularly during critical growth periods (Flack-Prain *et al.*, 2021). I_0 continued to show the lowest LAI, with 0.85 at 30 DAS, 3.32 at 60 DAS, and 1.89 at 90 DAS. This reduction in leaf area limited the plant's ability to capture sunlight, thereby affecting photosynthetic capacity and overall growth (Hussain *et al.*, 2017).

The effect of nutrient management on the leaf area index (LAI) of mustard at different growth stages (30 DAS, 60 DAS, and 90 DAS) showed notable differences across both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the S_3 treatment (humic acid + sulfur) resulted in the highest LAI values at all stages, with 1.10 at 30 DAS, 4.09 at 60 DAS, and 2.90 at 90 DAS. Together, these nutrients supported leaf expansion and contributed to an increased LAI, particularly during key growth stages (Liao *et al.*, 2022). In contrast, the S_0 treatment (control) had the lowest LAI, recording 0.67 at 30 DAS, 3.30 at 60 DAS, and 2.19 at 90 DAS. In 2023-24, the S_3 treatment again showed the highest LAI values with 1.09 at 30 DAS, 4.23 at 60 DAS, and 2.81 at 90 DAS. the S_0 treatment consistently showed the lowest LAI values, likely due to limited nutrient availability that restricted leaf growth and reduced canopy development. A lower LAI implied a diminished leaf area for sunlight capture, limiting photosynthesis and subsequently reducing biomass production (Parker, 2020).

Table 4.8 Influence of humic acid and sulphur on leaf area index of Indian mustard under variable water regimes

Treatments	Leaf area index (LAI)					
	30 DAS		60 DAS		90 DAS	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
I ₀	0.84	0.85	3.12	3.37	1.89	1.96
I ₁	0.87	0.88	3.80	4.30	2.59	2.78
I ₂	0.90	0.91	4.01	4.34	2.87	2.92
I ₃	0.93	0.93	4.14	4.44	2.99	2.90
SEm±	0.02	0.02	0.10	0.07	0.05	0.10
C.D at 5%	NS	NS	0.33	0.23	0.15	0.31
Sub plot (Nutrient management) (S)						
S ₀	0.67	0.68	3.30	3.97	2.19	2.44
S ₁	0.94	0.96	3.85	4.17	2.74	2.65
S ₂	0.83	0.85	3.83	4.02	2.57	2.58
S ₃	1.10	1.09	4.09	4.23	2.90	2.81
SEm±	0.01	0.01	0.06	0.07	0.05	0.07
C.D at 5%	0.04	0.03	0.18	0.19	0.14	0.21
Interaction (S*I)	NS	NS	NS	NS	NS	NS

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm± = Standard mean of error, C.D=Critical difference, NS= Non-significant ,DAS=Days after sowing.

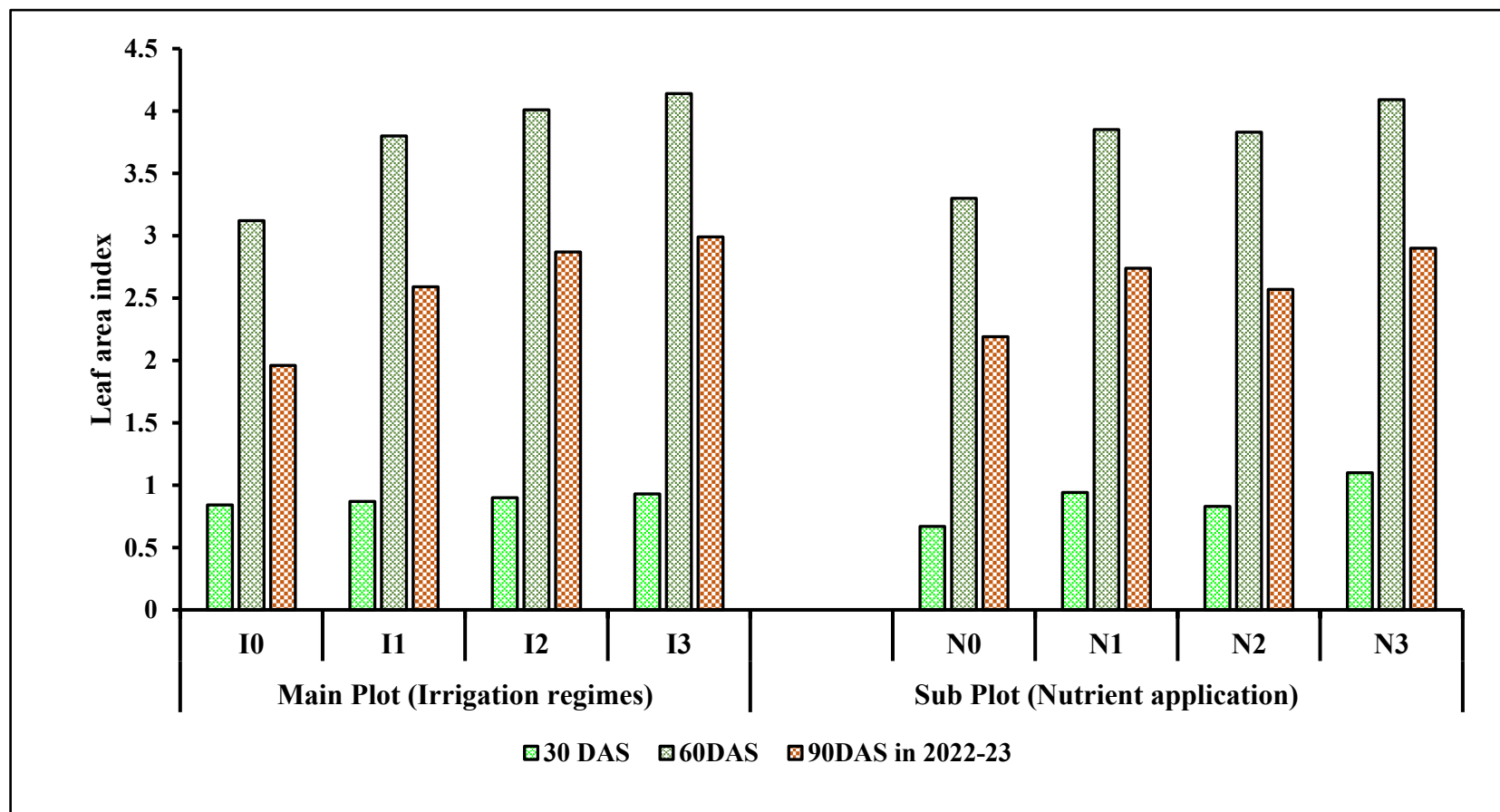


Figure 4.6A Effect of different irrigation regimes and different nutrient management on leaf area index of Indian mustard in 2022-23

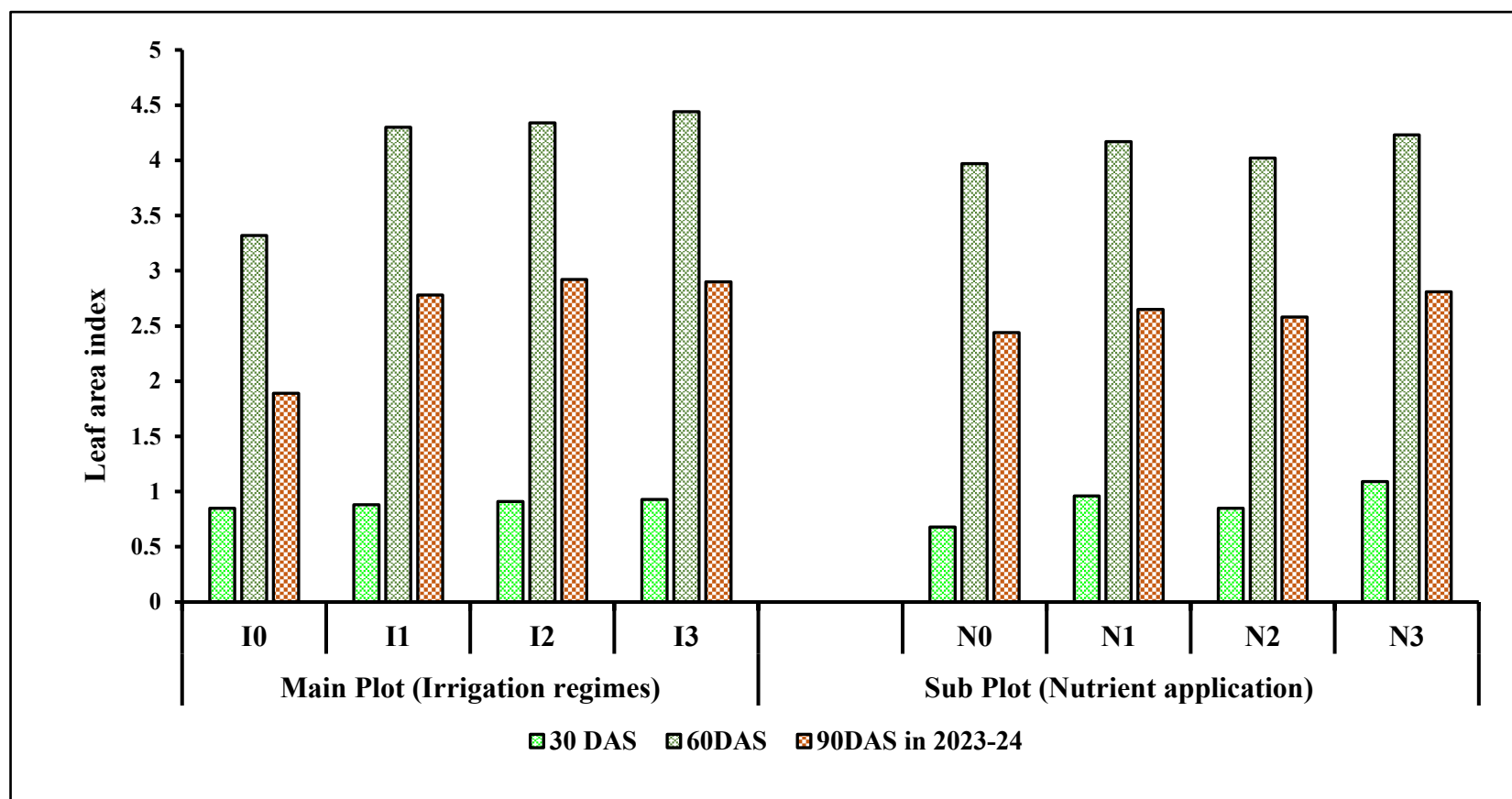


Figure 4.6B Effect of different irrigation regimes and different nutrient management on leaf area index of Indian mustard in 2023-24

4.3.2 Impact of different irrigation regimes and different nutrient management on the crop growth rate (CGR) ($\text{g m}^{-2} \text{ day}^{-1}$)

The effect of different irrigation regimes on the crop growth rate (CGR) of mustard in terms of $\text{g m}^{-2} \text{ day}^{-1}$ during the intervals of 30-60 DAS and 60-90 DAS showed distinct variations across both years (2022-23 and 2023-24) and in the combined mean data. In 2022-23, the I_3 treatment (three irrigations at vegetative, flowering, and siliqua filling stages) produced the highest CGR values, with $14.85 \text{ g m}^{-2} \text{ day}^{-1}$ for the 30-60 DAS interval and $16.75 \text{ g m}^{-2} \text{ day}^{-1}$ for the 60-90 DAS interval. The highest CGR observed under the I_3 treatment was attributed to the continuous water supply at key growth stages, which optimized nutrient uptake and promoted sustained photosynthesis. Adequate moisture provided by the I_3 regime supported active metabolic processes, enabling cell division and elongation, and ultimately leading to higher CGR during the vegetative and reproductive stages (Kumar *et al.*, 2022). Conversely, the I_0 treatment (no irrigation) had the lowest CGR values, recording $9.75 \text{ g m}^{-2} \text{ day}^{-1}$ for 30-60 DAS and $9.99 \text{ g m}^{-2} \text{ day}^{-1}$ for 60-90 DAS. In 2023-24, I_3 again resulted in the highest CGR, reaching $15.75 \text{ g m}^{-2} \text{ day}^{-1}$ for the 30-60 DAS interval and $18.87 \text{ g m}^{-2} \text{ day}^{-1}$ for the 60-90 DAS interval. I_0 continued to show the lowest CGR values, with $9.84 \text{ g m}^{-2} \text{ day}^{-1}$ for 30-60 DAS and $11.27 \text{ g m}^{-2} \text{ day}^{-1}$ for 60-90 DAS. The lowest CGR observed in the I_0 treatment highlighted the negative impact of water stress on mustard growth. Water limitations reduced nutrient transport and restricted photosynthetic efficiency, resulting in a lower CGR as plants struggled to maintain growth under moisture-deficient conditions (Saffari *et al.*, 2023).

In 2022-23, the S_3 treatment (humic acid + sulfur) produced the highest CGR values at both intervals, with $15.79 \text{ g m}^{-2} \text{ day}^{-1}$ for 30-60 DAS and $16.22 \text{ g m}^{-2} \text{ day}^{-1}$ for 60-90 DAS. The S_0 treatment (control) had the lowest CGR values, recording $9.93 \text{ g m}^{-2} \text{ day}^{-1}$ for 30-60 DAS and $11.79 \text{ g m}^{-2} \text{ day}^{-1}$ for 60-90 DAS. In 2023-24, S_3 again resulted in the highest CGR values, reaching $15.95 \text{ g m}^{-2} \text{ day}^{-1}$ for the 30-60 DAS interval and $17.72 \text{ g m}^{-2} \text{ day}^{-1}$ for the 60-90 DAS interval. Sulfur played a vital role in photosynthesis and protein synthesis, enabling more efficient metabolic activity and promoting robust growth. Together, these nutrients contributed to the higher CGR observed during the 30–60 DAS and 60–90 DAS intervals (Yang *et al.*, 2019). S_0 continued to show the lowest values, with $11.68 \text{ g m}^{-2} \text{ day}^{-1}$ for 30-60 DAS and $12.56 \text{ g m}^{-2} \text{ day}^{-1}$ for 60-90 DAS. The lowest CGR values in the S_0 treatment highlighted the

negative impact of nutrient limitations on growth. Without supplemental nutrients, mustard plants likely faced deficiencies that restricted cell division and elongation, reducing overall growth rates (Jalal *et al.*, 2023).

4.3.2a Interaction between different irrigation regimes and different nutrient management on the crop growth rate (CGR) ($\text{g m}^{-2} \text{ day}^{-1}$)

The interaction between different irrigation regimes and nutrient management treatments had a significant impact on the crop growth rate (CGR) of mustard ($\text{g m}^{-2} \text{ day}^{-1}$) during the years 2022-23, 2023-24, and in the combined mean data. In 2022-23, the I_3S_1 treatment (three irrigations with humic acid + sulphur) resulted in the highest CGR value at $18.90 \text{ g m}^{-2} \text{ day}^{-1}$, while the I_0S_0 treatment (no irrigation and no added nutrients) had the lowest at $6.23 \text{ g/m}^2/\text{day}$. This synergy between water and nutrients supported robust cell division and elongation, leading to an increased CGR, particularly during the vegetative and reproductive stages (Banik *et al.*, 2024). The I_0S_0 treatment consistently produced the lowest CGR values, highlighting the detrimental effects of water and nutrient limitations on mustard growth. Water stress and nutrient deficiencies restricted physiological processes, reducing photosynthetic efficiency and nutrient transport, which ultimately lowered CGR (Banerjee *et al.*, 2021).

4.3.3 Impact of different irrigation regimes and nutrient management on the relative growth rate (RGR) ($\text{g g}^{-1} \text{ day}^{-1}$)

The effect of different irrigation regimes on the relative growth rate (RGR) ($\text{g g}^{-1} \text{ day}^{-1}$) of mustard during the intervals of 30-60 DAS and 60-90 DAS revealed distinct patterns across both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the I_3 treatment (three irrigations at vegetative, flowering, and siliquea filling stages) showed the highest RGR values at both intervals, with $91.76 \text{ g g}^{-1} \text{ day}^{-1}$ for 30-60 DAS and $24.50 \text{ g g}^{-1} \text{ day}^{-1}$ for 60-90 DAS. This enhanced RGR under I_3 was attributed to consistent water availability, which supported metabolic processes, nutrient uptake, and overall growth. Adequate soil moisture enabled sustained photosynthesis and cellular expansion, leading to a higher RGR during critical growth stages (Lambers *et al.*, 2019). The I_0 treatment (no irrigation) recorded the lowest RGR values, with $78.17 \text{ g g}^{-1} \text{ day}^{-1}$ for 30-60 DAS and $22.59 \text{ g g}^{-1} \text{ day}^{-1}$ for 60-90 DAS. In 2023-24, I_3 again resulted in the highest RGR, reaching $81.76 \text{ g g}^{-1} \text{ day}^{-1}$ for 30-

Table 4.9 Effect of humic acid and sulphur on crop growth rate of Indian mustard under variable water regimes

Treatments	Crop growth rate ($\text{g m}^{-2} \text{ day}^{-1}$)			
	30-60 DAS		60-90 DAS	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24
I ₀	9.75	9.84	9.99	11.27
I ₁	13.41	15.11	15.04	14.40
I ₂	13.95	15.56	15.46	16.20
I ₃	14.85	15.75	16.75	18.87
SEm \pm	0.21	0.16	0.19	0.27
C.D at 5%	0.67	0.52	0.60	0.87
Sub plot (Nutrient management) (S)				
S ₀	9.93	11.68	11.79	12.56
S ₁	14.31	14.76	15.55	16.42
S ₂	11.92	13.85	13.66	14.03
S ₃	15.79	15.95	16.22	17.72
SEm \pm	0.17	0.20	0.26	0.23
C.D at 5%	0.48	0.57	0.75	0.65
Interaction (S*I)	NS	NS	1.54	1.36

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm \pm = Standard mean of error, C.D=Critical difference, NS= Non-significant , DAS=Days after sowing, gram plant⁻¹=gram per plant. $\text{g m}^{-2} \text{ day}^{-1}$ = Grams per square meter per day

Table 4.9A: Interaction table of crop growth rate ($\text{g m}^{-2} \text{ day}^{-1}$) at 60-90 DAS

Subplot (S) Main plot (I)	2022-23					2023-24				
	S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀	6.23	11.31	9.81	12.60	9.99	9.11	12.06	10.55	13.34	11.27
I ₁	11.45	15.51	14.88	18.32	15.04	13.16	16.26	13.13	15.06	14.40
I ₂	13.25	16.49	13.92	18.17	15.46	13.49	17.74	15.67	17.91	16.20
I ₃	16.25	18.90	16.04	19.80	16.75	14.49	19.65	16.79	24.55	18.87
Mean (S)	11.79	15.55	13.66	16.22		12.56	16.42	14.03	17.72	
SEm±	S * I	0.38				0.54				
	I * S	0.26				0.48				
C.D at 5%	S * I	1.54				1.36				
	I * S	1.44				1.43				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂= Sulphur, S₃ = Humic acid + Sulphur, SEm± = Standard mean of error, C.D=Critical difference, NS= Non-significant , DAS=Days after sowing, gram plant⁻¹=gram per plant. $\text{g m}^{-2} \text{ day}^{-1}$ = Grams per square meter per day

60 DAS and 24.30 g g⁻¹ day⁻¹ for 60-90 DAS, while I₀ maintained the lowest RGR values with 68.89 g g⁻¹ day⁻¹ for 30-60 DAS and 22.43 g g⁻¹ day⁻¹ for 60-90 DAS. Water limitations reduced photosynthesis and nutrient transport, restricting cell expansion and consequently lowering the growth rate. Under such conditions, mustard plants likely reduced their RGR as part of a strategy to conserve resources rather than prioritize growth (Valliere *et al.*, 2022).

The effect of different nutrient management treatments on the relative growth rate (RGR) (g g⁻¹ day⁻¹) of mustard during the intervals of 30-60 DAS and 60-90 DAS demonstrated distinct differences across both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the S₃ treatment (humic acid + sulfur) produced the highest RGR values, with 88.64 g g⁻¹ day⁻¹ for 30-60 DAS and 22.83 g g⁻¹ day⁻¹ for 60-90 DAS. The S₀ treatment (control) had the lowest RGR values, recording 86.94 g g⁻¹ day⁻¹ for 30-60 DAS and 23.48 g g⁻¹ day⁻¹ for 60-90 DAS. The higher RGR observed under S₃ was attributed to enhanced nutrient availability and improved metabolic processes facilitated by the synergistic effects of humic acid and sulfur. Humic acid improved soil health and nutrient uptake efficiency, while sulfur played a critical role in protein synthesis and chlorophyll production, both essential for promoting photosynthesis and growth during critical developmental stages (Ampong *et al.*, 2021). In 2023-24, S₃ again achieved the highest RGR values, reaching 79.92 g g⁻¹ day⁻¹ for 30-60 DAS and 22.72 g g⁻¹ day⁻¹ for 60-90 DAS, while S₀ showed the lowest RGR values with 75.50 g g⁻¹ day⁻¹ for 30-60 DAS and 23.28 g g⁻¹ day⁻¹ for 60-90 DAS. The lowest RGR values observed in the S₀ treatment highlighted the limitations of insufficient nutrient supplementation. Without additional nutrients, mustard plants likely experienced deficiencies that hindered metabolic activity and growth, particularly during the 60–90 DAS interval, a phase of heightened nutrient demand (SAREN, 2023).

4.3.3a Interaction between different irrigation regimes and different nutrient management on the relative growth rate (RGR) (g g⁻¹ day⁻¹) at 30-60 DAS

The interaction between different irrigation regimes and nutrient management treatments significantly affected the relative growth rate (RGR) (g g⁻¹ day⁻¹) of mustard across both years (2022-23 and 2023-24), as well as in the combined mean data. In 2022-23, the I₃S₃ treatment (three irrigations with humic acid + sulfur) achieved the highest RGR value at 92.10 g g⁻¹ day⁻¹, while the I₀S₀ treatment (no irrigation and no

added nutrients) recorded the lowest RGR at $75.37 \text{ g g}^{-1} \text{ day}^{-1}$. The synergy between adequate water and essential nutrients, such as sulfur, improved cell expansion and division, contributing to the increased RGR during critical growth phases (Kumar *et al.*, 2020). The I₀S₀ treatment consistently showed the lowest RGR values, illustrating the limitations imposed by the absence of both irrigation and nutrient supplementation. Under such conditions, plants experienced significant water and nutrient stress, reducing growth rates due to restricted metabolic functions and impaired biomass accumulation (Ullah, 2019).

4.3.3b Interaction between different irrigation regimes and different nutrient management on the relative growth rate (RGR) ($\text{g g}^{-1} \text{ day}^{-1}$) at 60-90 DAS

The interaction between different irrigation regimes and nutrient management treatments on the relative growth rate (RGR) ($\text{g g}^{-1} \text{ day}^{-1}$) of mustard at 60-90 DAS showed clear variations across 2022-23, 2023-24, and the combined mean data. In 2022-23, the I₃S₀ treatment (three irrigations with no added nutrients) achieved the highest RGR value of $25.49 \text{ g g}^{-1} \text{ day}^{-1}$, while I₀S₀ (no irrigation and no nutrients) had the lowest at $22.01 \text{ g g}^{-1} \text{ day}^{-1}$. This outcome was attributed to the reproductive phase benefiting from enhanced water availability, which promoted cellular expansion and metabolism even in the absence of supplemental nutrients (Zia *et al.*, 2021). The I₀S₃ treatment (no irrigation with humic acid + sulfur) consistently recorded the lowest RGR, underscoring the minimal impact of nutrient additions when water was restricted. Water stress hindered the plant's ability to efficiently utilize available nutrients, resulting in reduced growth rates (Fahad *et al.*, 2017).

4.3.4 Impact of different irrigation regimes and different nutrient management on the net assimilation ratio (NAR) ($\text{g m}^{-2} \text{ day}^{-1}$)

The impact of different irrigation regimes on the Net Assimilation Ratio (NAR) of mustard in $\text{g m}^{-2} \text{ day}^{-1}$ during the intervals of 30-60 DAS and 60-90 DAS showed consistent differences across both years (2022-23 and 2023-24) and in the combined mean data. In 2022-23, the I₃ treatment (three irrigations at vegetative, flowering, and siliqua filling stages) achieved the highest NAR at both intervals, with values of $0.0163 \text{ g m}^{-2} \text{ day}^{-1}$ for 30-60 DAS and $0.110 \text{ g m}^{-2} \text{ day}^{-1}$ for 60-90 DAS. In contrast, the I₀ treatment (no irrigation) had the lowest NAR values, recording $0.125 \text{ g m}^{-2} \text{ day}^{-1}$ for 30-60 DAS and $0.094 \text{ g m}^{-2} \text{ day}^{-1}$ for 60-90 DAS. Adequate soil moisture allowed plants to optimize their metabolic activity, enhancing photosynthetic efficiency and the

conversion of light energy into biomass (Huang *et al.*, 2021). In 2023-24, I₃ continued to show the highest NAR values, reaching 0.161 g m⁻² day⁻¹ for 30-60 DAS and 0.111 g m⁻² day⁻¹ for 60-90 DAS, while I₀ again recorded the lowest NAR with 0.124 g m⁻² day⁻¹ for 30-60 DAS and 0.096 g m⁻² day⁻¹ for 60-90 DAS. Under water-limited conditions, plants often close their stomata to conserve water, which restricts CO₂ intake and reduces photosynthetic assimilation (Mukhtiar *et al.*, 2023).

The effect of different nutrient management treatments on the Net Assimilation Ratio (NAR) of mustard (g m⁻² day⁻¹) during the intervals of 30-60 DAS and 60-90 DAS showed clear differences across both years (2022-23 and 2023-24), as well as in the combined mean data. In 2022-23, the S₃ treatment (humic acid + sulfur) produced the highest NAR values at both intervals, with 0.160 g m⁻² day⁻¹ for 30-60 DAS and 0.108 g m⁻² day⁻¹ for 60-90 DAS. The S₀ treatment (control) recorded the lowest NAR values, with 0.140 g m⁻² day⁻¹ for 30-60 DAS and 0.093 g m⁻² day⁻¹ for 60-90 DAS. In 2023-24, S₃ again achieved the highest NAR values, reaching 0.158 g m⁻² day⁻¹ for 30-60 DAS and 0.109 g m⁻² day⁻¹ for 60-90 DAS, while S₀ continued to have the lowest NAR values at 0.138 g m⁻² day⁻¹ for 30-60 DAS and 0.095 g m⁻² day⁻¹ for 60-90 DAS. Humic acid enhanced nutrient absorption and soil health, while sulfur played a key role in chlorophyll formation and protein synthesis, boosting carbon assimilation and NAR during critical growth phases (Dong *et al.*, 2024). S₀ treatment consistently produced the lowest NAR values, likely due to limited nutrient availability. Without supplemental nutrients, mustard plants may have experienced deficiencies that restricted photosynthetic capacity, ultimately resulting in lower NAR values (Ahmad *et al.*, 2022).

Table 4.10 Influence of humic acid and sulphur on relative growth rate of Indian mustard under variable water regimes

Treatments	Relative growth rate (g g ⁻¹ day ⁻¹)			
	30-60 DAS		60-90 DAS	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24
I ₀	75.17	75.89	21.59	22.43
I ₁	90.00	79.97	22.36	22.26
I ₂	85.52	76.09	21.68	21.55
I ₃	91.76	93.76	24.50	24.93
SEm±	0.22	0.17	0.10	0.09
C.D at 5%	0.70	0.56	0.31	0.29
Sub plot (Nutrient management) (S)				
S ₀	73.94	75.50	20.14	22.28
S ₁	76.15	78.91	23.05	24.10
S ₂	76.06	77.14	21.93	22.78
S ₃	78.64	79.92	26.83	26.99
SEm±	0.41	0.28	0.13	0.13
C.D at 5%	1.17	0.80	0.39	0.36
Interaction (S*I)	2.37	1.63	0.75	0.79

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm± = Standard mean of error ,C.D=Critical difference, DAS=Days after sowing, gram plant⁻¹=gram per plant. g g⁻¹ day⁻¹ = Grams per gram dry matter per day.

Table 4.10A: Interaction table of relative growth rate ($\text{g g}^{-1} \text{day}^{-1}$) at 30-60 DAS

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
	I ₀	75.37	80.04	76.26	80.99	78.17	64.78	71.19	67.00	72.60	68.89
	I ₁	89.62	90.03	89.93	90.42	90.00	77.90	80.64	79.77	81.56	79.97
	I ₂	91.07	90.86	90.47	91.07	85.52	79.33	81.49	80.34	82.23	76.09
	I ₃	91.71	91.66	91.58	92.10	91.76	79.99	82.31	81.46	83.27	81.76
	Mean (S)	86.94	88.15	87.06	88.64		75.50	78.91	77.14	79.92	
SEm±	S * I	1.15					0.35				
	I * S	1.04					0.51				
C.D at 5%	S* I	2.37					1.63				
	I * S	2.15					1.50				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm± = Standard mean of error ,C.D=Critical difference, DAS=Days after sowing, gram plant⁻¹=gram per plant. $\text{g g}^{-1} \text{day}^{-1}$ = Grams per gram dry matter per day.

Table 4.10B: Interaction table of relative growth rate ($\text{g g}^{-1} \text{day}^{-1}$) at 60-90 DAS

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		22.01	22.40	23.49	22.47	22.59	21.85	22.27	23.25	22.35	22.43
I ₁		22.92	22.54	22.04	21.96	22.36	22.76	22.43	21.95	21.89	22.26
I ₂		23.49	23.25	22.56	22.82	21.68	23.30	23.12	22.44	22.71	21.55
I ₃		25.49	24.79	23.64	24.09	24.50	25.20	24.59	23.48	23.93	24.30
Mean (S)		23.48	23.25	22.93	22.83		23.28	23.10	22.78	22.72	
SEm±	S * I	0.20					0.18				
	I * S	0.25					0.24				
C.D at 5%	S* I	0.79					0.75				
	I* S	0.74					0.69				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm± = Standard mean of error ,C.D=Critical difference, DAS=Days after sowing, gram plant^{-1} =gram per plant. $\text{g g}^{-1} \text{day}^{-1}$ = Grams per gram dry matter per day.

Table 4.11 Impact of humic acid and sulphur on net assimilation rate of Indian mustard under variable water regimes

Treatments	Net assimilation rate ($\text{g m}^{-2} \text{ day}^{-1}$)			
	30-60 DAS		60-90 DAS	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24
I ₀	0.123	0.124	0.094	0.096
I ₁	0.158	0.156	0.098	0.099
I ₂	0.151	0.149	0.094	0.095
I ₃	0.163	0.161	0.110	0.113
SEm \pm	0.002	0.002	0.003	0.003
C.D at 5%	0.006	0.006	0.009	0.009
Sub plot (Nutrient management) (S)				
S ₀	0.138	0.141	0.093	0.095
S ₁	0.157	0.154	0.106	0.103
S ₂	0.150	0.148	0.095	0.096
S ₃	0.160	0.166	0.108	0.114
SEm \pm	0.002	0.002	0.002	0.002
C.D at 5%	0.005	0.005	0.005	0.004
Interaction (S*I)	NS	NS	NS	NS

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm \pm = Standard mean of error, C.D=Critical difference, NS= Non-significant, DAS=Days after sowing, gram plant⁻¹=gram per plant. $\text{g m}^{-2} \text{ day}^{-1}$ = gram per metre square per day.

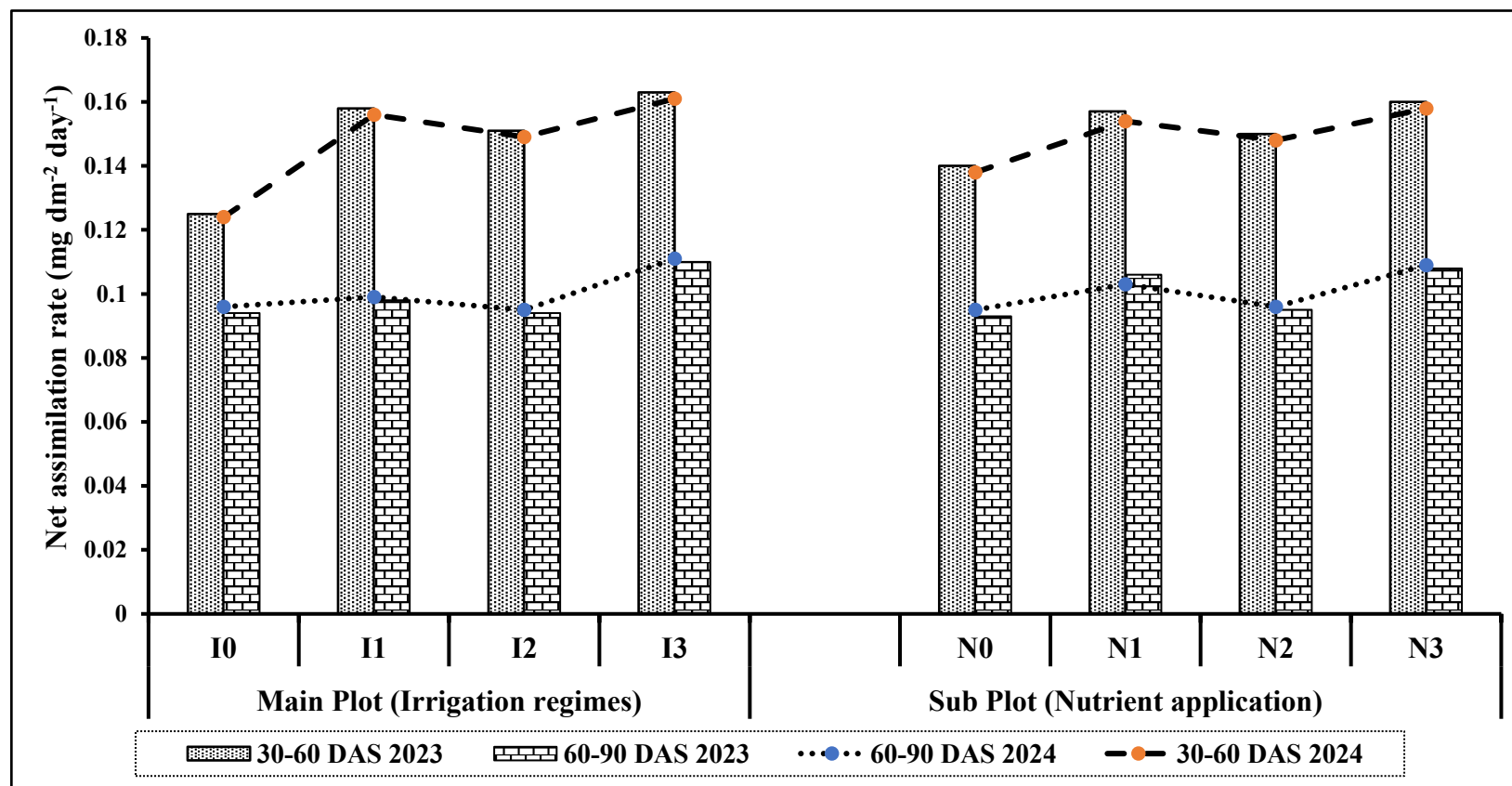


Figure 4.7 Effect of different irrigation regimes and different nutrient management on net assimilation of Indian mustard in 2022-23 and 2023-24

4.4 Crop phenological traits

4.4.1 Effect of different irrigation regimes and different nutrient management on the days taken for emergence (DTE), days taken for branching (DTB), days taken for 50% flowering (DTF 50 %), days taken to maturity (DTM)

In the 2022-23 season, irrigation treatments significantly affected the days taken for various growth stages of the mustard crop. The days taken for emergence (DTE) ranged from 3.44 to 4.55 days, with the I₃ treatment (three post-sowing irrigations) showing the fastest emergence (3.44 days) compared to I₀ (no irrigation), which showed the slowest emergence (4.55 days). For days taken to branching (DTB), I₃ again demonstrated faster progression with 39.17 days, whereas I₀ required 49.85 days. The days taken for 50% flowering (DTF 50 %) followed a similar trend, with I₃ reaching this stage in 37.90 days and I₀ taking significantly longer at 64.03 days. Days to maturity (DTM) were shortest in I₃ at 129.19 days and longest in I₀ at 95.82 days. In 2023-24, similar patterns were observed. Adequate water during vegetative growth improves cell expansion and division, which are vital for rapid seedling establishment and progression through subsequent growth stages (Oguz *et al.*, 2022). DTB for I₃ was 35.07 days, while I₀ had a prolonged branching period of 40.27 days. DTF 50 % ranged from 37.90 days in I₃ to 64.03 days in I₀. Days to maturity (DTM) also showed the shortest duration with I₃ at 109.28 days, compared to the longest duration with I₀ at 114.25 days. Research demonstrates that water availability enhances nutrient mobility in the soil, improving the absorption of essential nutrients like nitrogen and phosphorus, which are critical for flowering and fruiting (Bhattacharya and Bhattacharya, 2021). The I₃ treatment consistently resulted in shorter DTM, reflecting the benefits of strategic irrigation during critical growth phases. This finding aligns with studies showing that timely irrigation accelerates crop maturation and enhances yield potential (Maurya *et al.*, 2023).

Nutrient management had a notable effect on the days taken for mustard crop growth stages, including days taken for emergence (DTE), branching (DTB), 50% flowering (DTF50%), and maturity (DTM), across the treatments S₀ (control), S₁ (humic acid), S₂ (sulphur), and S₃ (humic acid + sulphur). In terms of days taken for emergence (DTE), S₃ exhibited the fastest emergence with a mean of 3.25 days across both years, followed closely by S₁ with 3.56 days. S₀ took the longest time to emerge at 4.25 days, suggesting that the addition of humic acid and sulfur accelerates the

emergence stage. This was attributed to the synergistic effects of humic acid and sulfur on root development and nutrient uptake efficiency. Humic acid enhanced root penetration and nutrient absorption, while sulfur was essential for synthesizing key amino acids and enzymes involved in growth (Sarlaki *et al.*, 2024; Narayan *et al.*, 2023). The delayed progression to these stages in the control treatment (S₀) implied that the lack of supplemental nutrients limited physiological growth rates, possibly due to restricted nitrogen and sulfur availability, which were vital for protein synthesis and metabolic activities (O'Hearn, *et al.*, 2023). The quicker transition to flowering and maturity observed in the S₃ treatment aligned with studies that indicated humic acid improved soil nutrient availability and plant photosynthesis, while sulfur boosted chlorophyll formation and reduced flowering time by supporting efficient metabolic pathways (Shah *et al.*, 2023).

4.4.1a Interaction between different irrigation regimes and different nutrient management on the days taken to maturity of Indian mustard crop at harvest

The interaction between different irrigation regimes and nutrient management treatments significantly influenced the days taken to maturity (DTM) of the mustard crop at harvest across the two years (2022-23 and 2023-24) and in the mean data. In 2022-23, I₃S₃ (three irrigations with humic acid + sulphur) had the longest DTM of 133.52 days, while I₀S₀ (no irrigation and no added nutrients) had the shortest DTM of 86.68 days. The DTM increased with more frequent irrigation and enhanced nutrient management. The observed trend of delayed maturity with increased irrigation and nutrient inputs was consistent with studies indicating that crops grown with optimal water and nutrient availability tend to exhibit prolonged vegetative and reproductive phases, allowing higher yield potential but extending the crop cycle (Varshney *et al.*, 2021). In 2023-24, the trend was similar, with I₃S₃ again showed the longest DTM (138.49 days) and I₀S₀ the shortest (91.65 days).

Table 4.12 Influence of humic acid and sulphur on days taken for emergence and days taken for branching in Indian mustard under variable water regimes

Treatments	Days taken for emergence		Days taken for branching	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24
I ₀	4.00	4.55	49.85	40.27
I ₁	3.75	4.41	44.96	37.96
I ₂	3.69	4.20	40.82	36.99
I ₃	3.44	4.15	39.17	35.07
SEm±	0.10	0.18	0.68	0.77
C.D at 5%	NS	NS	2.16	2.47
Sub plot (Nutrient management) (S)				
S ₀	4.25	5.74	48.84	42.23
S ₁	3.56	3.74	40.46	37.94
S ₂	3.81	4.60	41.69	33.96
S ₃	3.25	3.24	36.83	30.16
SEm±	0.14	0.21	0.42	0.60
C.D at 5%	0.40	0.61	1.20	1.72
Interaction (S*I)	NS	NS	NS	NS

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂= Sulphur, S₃ = Humic acid + Sulphur, SEm± = Standard mean of error, C. D=Critical difference, NS= non-significant

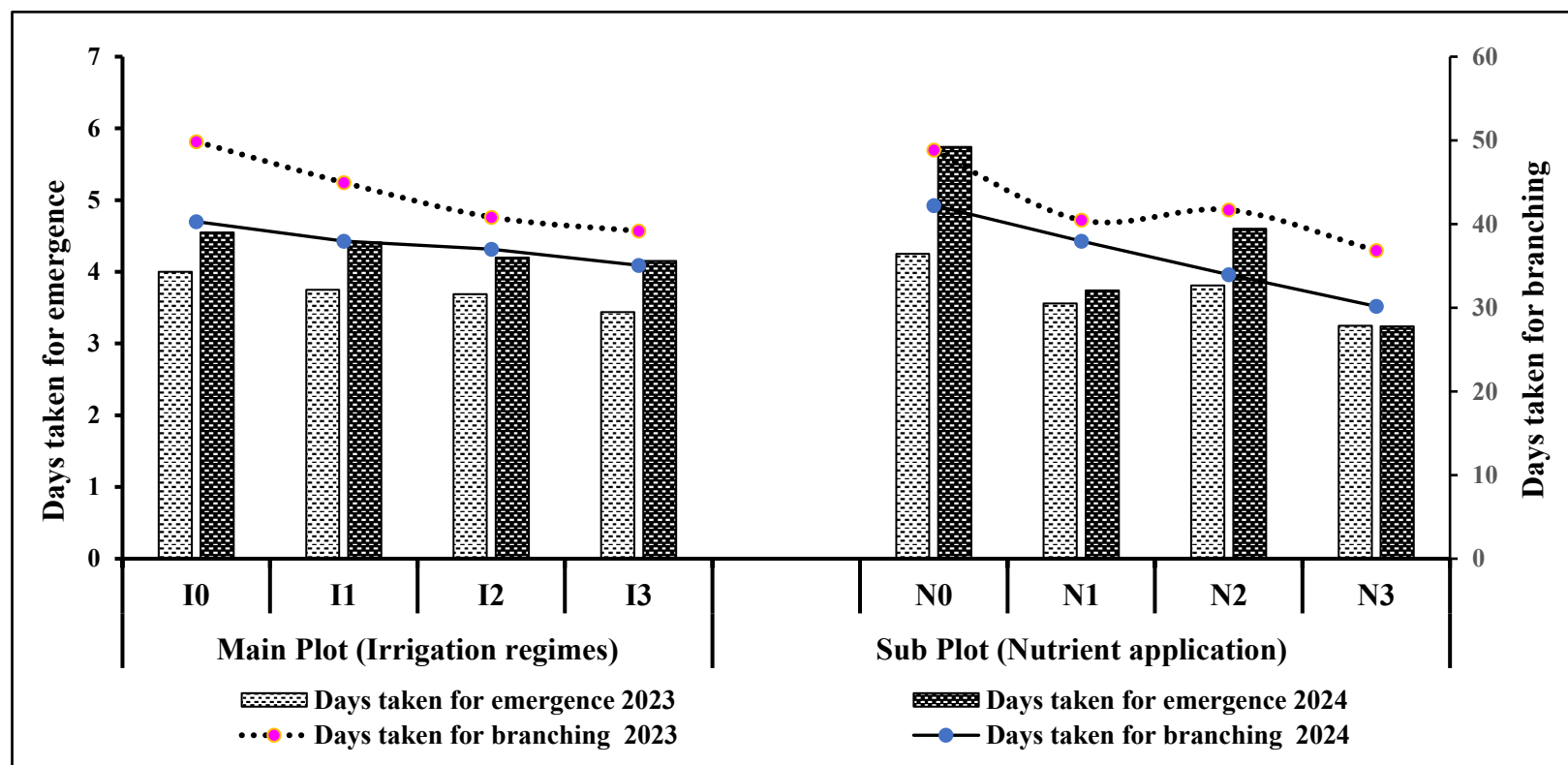


Figure 4.8 Effect of different irrigation regimes and different nutrient management on days taken for emergence and days taken for branching in Indian mustard in 2022-23 and 2023-24

Table 4.13 Effect of humic acid and sulphur on days taken for 50% flowering and days taken for maturity in Indian mustard under variable water regimes

Treatments	Days taken for 50% flowering		Days taken for maturity	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24
I ₀	64.03	51.74	95.82	100.70
I ₁	49.25	45.34	109.28	114.25
I ₂	39.34	38.08	122.60	127.57
I ₃	37.90	37.01	129.19	133.16
SEm±	0.69	0.69	1.49	1.30
C.D at 5%	2.21	2.21	4.75	4.17
Sub plot (Nutrient management) (S)				
S ₀	55.97	47.13	104.02	105.99
S ₁	46.12	44.53	117.40	122.18
S ₂	50.64	45.26	112.80	117.15
S ₃	43.81	42.21	129.67	132.35
SEm±	0.45	0.45	0.72	0.67
C.D at 5%	1.28	1.28	2.07	1.93
Interaction (S*I)	NS	NS	4.46	4.14

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C. D=Critical difference. NS= non-significant.

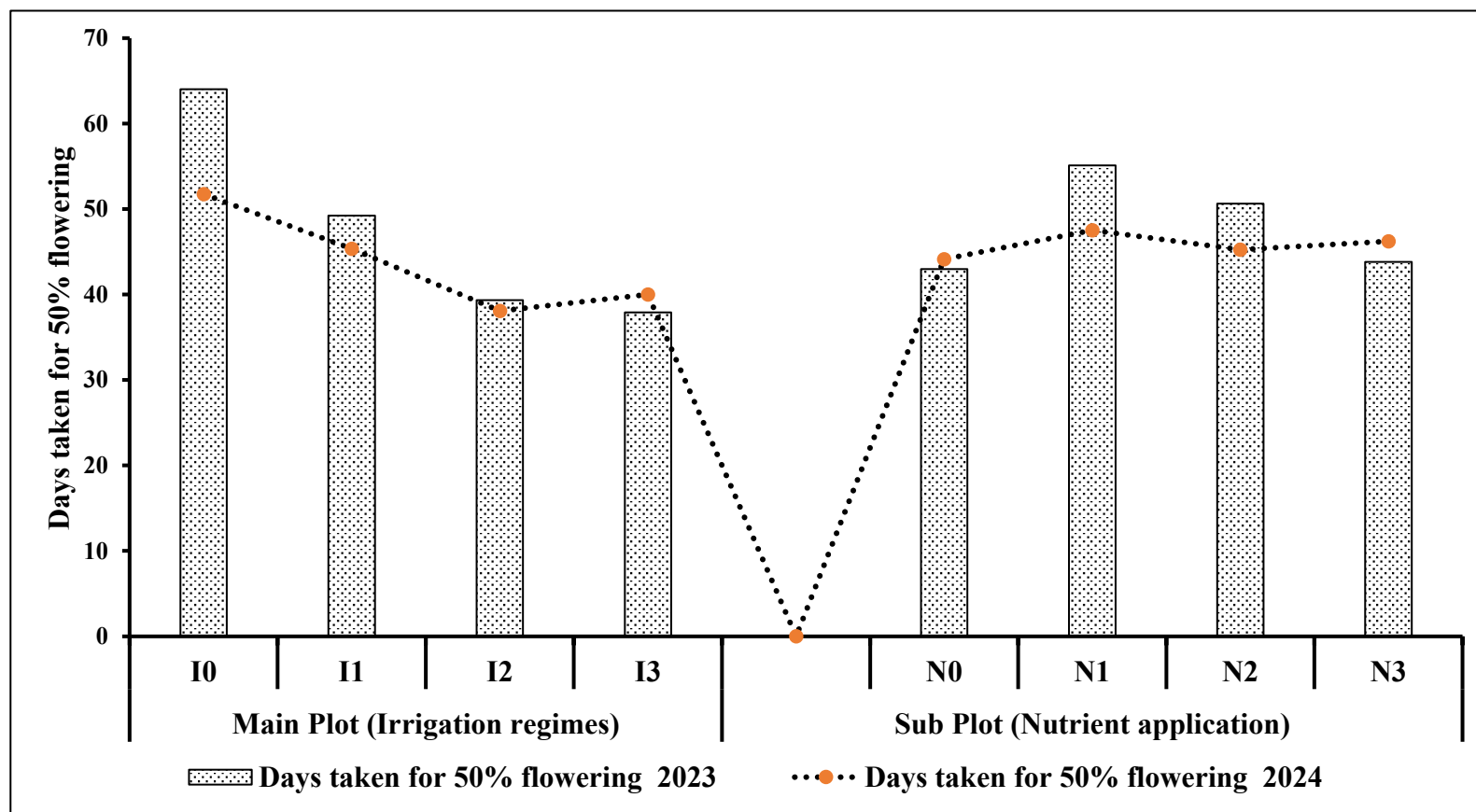


Figure 4.9 Effect of different irrigation regimes and different nutrient management on days taken for 50% flowering in Indian mustard

Table 4.13A: Interaction table of days taken for maturity (days)

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
	I ₀	86.68	97.81	92.79	106.01	95.82	91.65	102.03	95.30	113.81	100.70
	I ₁	102.63	109.52	106.79	118.16	109.28	107.60	114.49	111.76	123.13	114.25
	I ₂	121.66	130.28	125.46	133.01	127.60	126.63	135.25	130.43	137.98	132.57
	I ₃	125.12	131.98	126.15	133.52	129.19	130.09	136.95	131.126	138.49	134.16
	Mean (S)	109.02	117.40	112.80	122.67		113.99	122.18	117.15	128.35	
SEm±	S * I	2.98					2.61				
	I* S	1.94					1.75				
C.D at 5%	S* I	4.46					4.14				
	I* S	6.01					5.38				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C. D=Critical difference. NS= non-significant.

4.5 Physiological parameters (at 30, 60, and 90 DAS)

4.5.1 Impact of different irrigation regimes and different nutrient management on relative water content (RWC) of Indian mustard leaves

The effect of different irrigation regimes on the relative water content (RWC) (%) of mustard at various growth stages (30 DAS, 60 DAS, and 90 DAS) showed consistent patterns across both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the I₃ treatment (three irrigations at vegetative, flowering, and siliqua filling stages) resulted in the highest RWC values at all stages, with 65.89% at 30 DAS, 79.54% at 60 DAS, and 77.29% at 90 DAS. This enhancement in RWC was attributed to consistent water availability, which helped maintain cell turgor and supported optimal physiological functions during critical growth stages. Higher RWC values reflected improved hydration and cell expansion, both of which were vital for growth and metabolic activities (Bandurska *et al.*, 2022). In contrast, the I₀ treatment (no irrigation) recorded the lowest RWC values, with 60.32% at 30 DAS, 64.90% at 60 DAS, and 51.04% at 90 DAS. In 2023-24, I₃ again produced the highest RWC values, reaching 69.22% at 30 DAS, 87.70% at 60 DAS, and 86.13% at 90 DAS, while I₀ maintained the lowest RWC values with 66.88% at 30 DAS, 73.88% at 60 DAS, and 59.98% at 90 DAS. The lower RWC under I₀ highlighted increased vulnerability to drought stress, which ultimately resulted in reduced growth and yield potential (Arab *et al.*, 2023).

The effect of different nutrient management treatments on the relative water content (RWC) (%) of mustard at various growth stages (30 DAS, 60 DAS, and 90 DAS) showed clear trends across both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the S₃ treatment (humic acid + sulphur) produced the highest RWC values at each stage, with 66.10% at 30 DAS, 75.63% at 60 DAS, and 64.21% at 90 DAS. The S₀ treatment (control) had the lowest RWC values, recording 60.78% at 30 DAS, 69.90% at 60 DAS, and 57.40% at 90 DAS. In 2023-24, S₃ continued to show the highest RWC, reaching 91.15% at 30 DAS, 82.60% at 60 DAS, and 72.98% at 90 DAS, while S₀ again recorded the lowest values with 88.44% at 30 DAS, 78.15% at 60 DAS, and 67.14% at 90 DAS. Humic acid improved soil moisture retention and nutrient absorption, while sulfur supported essential metabolic processes that enhanced water retention within cells. This combination helped maintain higher hydration levels, which were crucial for cell turgor and physiological functioning (Chen *et al.*, 2022). In

contrast, the S₀ treatment consistently showed the lowest RWC values, underscoring the limitations imposed by a lack of nutrient supplementation. Without additional nutrients, mustard plants experienced nutrient stress, reducing cell water retention and leading to lower RWC, especially under drier conditions (Singh *et al.*, 2022).

4.5.1a Interaction between different irrigation regimes and different nutrient management on relative water content (RWC) of Indian mustard crop at 60 DAS

The interaction between different irrigation regimes and nutrient management treatments had a significant effect on the relative water content (RWC) (%) of mustard at 60 DAS during both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the I₃S₃ treatment (three irrigations with humic acid + sulfur) produced the highest RWC value at 81.97%, while the I₀S₀ treatment (no irrigation and no nutrients) recorded the lowest RWC at 61.11%. Humic acid improved soil structure and water-holding capacity, while sulfur supported metabolic functions essential for water retention within cells (Bello *et al.*, 2021). The mean RWC values across irrigation regimes showed that I₃ had the highest average RWC at 79.54%, followed by I₂ at 76.01%, I₁ at 70.75%, and I₀ at 64.90%. In 2023-24, I₃S₃ again resulted in the highest RWC, reaching 90.15%, while I₀S₀ remained the lowest at 72.68%. Across irrigation regimes, I₃ had the highest mean RWC at 83.62%, followed by I₂ at 77.82%, I₁ at 74.94%, and I₀ at 69.39%. The I₀S₀ treatment consistently produced the lowest RWC, highlighting the adverse effects of combined water and nutrient deficiencies. Without sufficient moisture or nutrients, mustard plants experienced water stress, leading to reduced cell turgor and impaired physiological functions, ultimately resulting in lower RWC (Khan *et al.*, 2024).

4.5.1b Interaction between different irrigation regimes and different nutrient management on relative water content (RWC) of mustard crop at 90 DAS

The interaction between different irrigation regimes and nutrient management treatments significantly influenced the relative water content (RWC) (%) of mustard at 90 DAS across both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the I₃S₃ treatment (three irrigations with humic acid + sulphur) achieved the highest RWC at 79.70%, while the I₀S₀ treatment (no irrigation and no nutrients) recorded the lowest RWC at 46.83%. The combination of three irrigations (I₃) and nutrient addition (S₃: humic acid + sulphur) maintained high cell hydration by enhancing soil water availability and improving nutrient uptake. Humic acid

contributed to soil moisture retention and enhanced water absorption, while sulfur supported metabolic processes that retained water within plant cells, resulting in higher RWC values (Kaya *et al.*, 2020). Across irrigation regimes, I₃ had the highest mean RWC at 81.71%, followed by I₂ at 65.21%, I₁ at 57.77%, and I₀ at 55.51%. When both resources were restricted, mustard plants experienced increased water stress, leading to reduced turgor pressure and overall physiological stress, which ultimately lowered RWC (Ahmad *et al.*, 2022). The two-year mean data emphasized that the integrated approach of combining irrigation and nutrient management, as in the I₃S₃ treatment, was essential for sustaining higher RWC at the 90 DAS stage. This integration proved critical for ensuring optimal plant hydration, supporting physiological processes, and enhancing crop resilience.

4.5.2 Impact of different irrigation regimes and different nutrient management on membrane stability index (MSI)

The impact of different irrigation regimes on the membrane stability index (MSI) in mustard at 30, 60, and 90 DAS varied distinctly across the years 2022-23 and 2023-24, with a trend of increased MSI associated with higher irrigation frequency. In 2022-23, the I₃ treatment (three irrigations) recorded the highest MSI values, reaching 38.65% at 30 DAS, 62.83% at 60 DAS, and 69.53% at 90 DAS. This pattern continued in 2023-24, where MSI under I₃ peaked at 71.15% at 60 DAS and 77.46% at 90 DAS. Conversely, the I₀ treatment (no irrigation) showed the lowest MSI values, with 39.88%, 54.92%, and 57.39% at 30, 60, and 90 DAS, respectively. Adequate water availability supported active nutrient transport and metabolic processes, which were beneficial for growth but appeared to heighten the vulnerability of cell membranes to injury, especially when exposed to environmental stresses. The greater membrane fluidity and permeability in adequately hydrated cells likely explained the higher MSI observed in I₃ (Bhattacharya, 2022) in 2022-23. The trend was consistent in 2023-24, where I₀ yielded MSI values of 43.34% at 30 DAS and 57.39% at 90 DAS. These results consistently indicate that increased irrigation levels are associated with a rise in MSI values. The impact of different irrigation regimes on the MSI in mustard at 30, 60, and 90 DAS varied distinctly across the years 2022-23 and 2023-24, with a trend of

Table 4.14 Impact of humic acid and sulphur on the relative water content in leaf of Indian mustard under variable water regimes

Treatments	Relative water content (%) of leaf					
	30 DAS		60 DAS		90 DAS	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
I ₀	60.32	66.88	64.90	73.88	51.04	59.98
I ₁	64.11	67.74	70.75	79.12	53.41	62.13
I ₂	64.98	68.52	76.01	85.62	60.46	69.97
I ₃	65.89	69.22	79.54	87.70	77.29	86.13
SEm±	0.31	0.21	0.46	0.41	0.38	0.32
C.D at 5%	0.99	0.68	1.45	1.31	1.22	1.04
Sub plot (Nutrient management) (S)						
S ₀	60.78	64.91	69.90	78.15	57.40	67.14
S ₁	64.18	68.26	73.89	80.85	60.95	69.71
S ₂	63.24	67.57	71.78	78.72	59.63	68.38
S ₃	66.10	69.62	75.63	82.60	64.21	72.98
SEm±	0.30	0.35	0.22	0.27	0.39	0.38
C.D at 5%	0.85	1.02	0.62	0.78	1.13	1.10
Interaction (S*I)	NS	NS	1.35	2.07	2.18	1.93

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁ = Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C.D=Critical difference, NS= non-significant., DAS=Days after sowing, %=Percent

Table 4.14A: Interaction table of relative water content (%) at 60 DAS

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		61.11	66.84	63.70	67.97	64.90	72.68	74.95	71.79	76.08	73.88
I ₁		67.15	71.56	69.95	74.36	70.75	76.25	79.68	78.06	82.50	79.12
I ₂		74.35	76.62	74.83	78.24	77.01	78.52	80.05	78.25	83.67	84.62
I ₃		77.00	80.53	78.64	81.97	79.54	85.15	88.71	86.80	88.15	87.70
Mean (S)		69.90	73.89	71.78	75.63		78.15	80.85	78.72	82.60	
SEm±	S * I	0.91					0.82				
	I * S	0.59					0.63				
C.D at 5%	S* I	1.35					2.07				
	I* S	1.83					1.49				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁ = Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C.D=Critical difference, NS= non-significant., DAS=Days after sowing, %=Percent

Table 4.14B: Interaction table of the relative water content of leaves (%) at 90 DAS

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		46.83	50.51	50.11	56.70	51.04	56.48	59.20	58.82	65.43	59.98
I ₁		51.56	53.96	52.37	55.77	53.41	60.26	62.68	61.07	64.49	62.13
I ₂		56.10	61.47	59.60	64.67	60.46	67.85	70.23	68.35	73.44	69.97
I ₃		75.12	77.88	76.45	79.70	77.29	83.97	86.72	85.27	87.01	86.13
Mean (S)		57.40	60.95	59.63	64.21		67.14	69.71	68.38	72.98	
SEm±	S * I	0.69					0.65				
	I * S	0.73					0.74				
C.D at 5%	S * I	2.18					1.93				
	I * S	2.15					1.86				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁ = Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C.D=Critical difference, NS= non-significant., DAS=Days after sowing, %=Percent

increased MSI associated with higher irrigation frequency. The I₀ treatment, characterized by the absence of post-sowing irrigation, consistently exhibited the lowest MSI values, suggesting that limited water availability reduced membrane permeability and vulnerability to injury. Water stress conditions generally led to reduced metabolic rates and created more rigid, less permeable membranes, which inherently limited susceptibility to injury. However, this benefit occurred at the expense of reduced cellular function and overall growth (Ahluwalia *et al.*, 2021).

The impact of different nutrient management treatments on the membrane stability index (MSI) in mustard at 30, 60, and 90 DAS demonstrated distinct patterns across both years, 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the S₃ treatment (humic acid + sulphur) consistently showed the highest MSI values across all stages, with 66.07% at 60 DAS and 70.00% at 90 DAS, suggesting an increase in membrane injury with this nutrient combination. Humic acid contributed to improved soil health and nutrient absorption, while sulfur played a vital role in cellular metabolic functions, supporting growth and expansion. These processes may have heightened membrane vulnerability to environmental stress, particularly in the later growth stages (Bhadwal *et al.*, 2024). Conversely, the S₀ treatment (control) recorded the lowest MSI values, with 29.69% at 30 DAS and 57.52% at 90 DAS. This pattern continued in 2023-24, where S₃ reached the highest MSI values, peaking at 74.02% at 60 DAS and 77.41% at 90 DAS, while S₀ exhibited lower MSI values of 61.48% at 60 DAS and 64.59% at 90 DAS. The S₀ treatment (control) consistently exhibited the lowest MSI values, indicating that nutrient-limited conditions reduced membrane permeability and susceptibility to injury. Limited nutrient availability likely constrained cellular expansion and metabolic activity, resulting in a more stable but less dynamic cellular environment. This stability lowered MSI but also restricted the crop's growth potential (Waqas *et al.*, 2019).

4.5.2a Interaction between different irrigation regimes and different nutrient management on membrane stability index (MSI) of mustard crop at 60 DAS

The interaction between different irrigation regimes and nutrient management treatments had a notable effect on the membrane stability index (MSI) of mustard at 60 DAS in both years, 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the I₃S₃ treatment (three irrigations with humic acid + sulphur) exhibited the

highest MSI at 71.43%, while the I₀S₀ treatment (no irrigation and no nutrients) recorded the lowest MSI at 46.98%. In 2023-24, the I₃S₃ treatment again resulted in the highest MSI value at 75.93%, while the lowest MSI value was observed in I₀S₀ at 52.11%. For this year, I₃ maintained the highest average MSI at 71.43%, compared to the lowest in I₀ at 46.98%. The highest MSI values, observed under the I₃S₃ treatment, suggested that ample irrigation combined with nutrient-rich conditions enhanced cellular activity and membrane permeability, potentially increasing susceptibility to injury under stress. Humic acid improved soil health and nutrient uptake, while sulphur played a crucial role in metabolic processes that supported cell expansion and membrane fluidity. These factors likely contributed to heightened MSI, reflecting increased cellular vulnerability to environmental stresses (Ciriello *et al.*, 2024).

4.5.2b Interaction between different irrigation regimes and different nutrient management on membrane stability index (MSI) of mustard crop at 90 DAS

The interaction between different irrigation regimes and nutrient management treatments significantly influenced the membrane stability index (MSI) of mustard at 90 DAS across both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the I₃S₃ treatment (three irrigations with humic acid + sulphur) recorded the highest MSI at 74.28%, while the I₀S₀ treatment (no irrigation and no added nutrients) showed the lowest MSI at 49.41%. In 2023-24, I₃S₃ continued to show the highest MSI at 80.24%, while I₀S₀ had the lowest MSI value at 54.21%. Frequent irrigations maintained cellular hydration and reduced oxidative stress, while humic acid improved soil moisture retention and nutrient absorption. Sulfur played a pivotal role in strengthening antioxidant defenses, protecting cellular membranes from oxidative damage. These findings aligned with those of Abd El-Mageed *et al.* (2020), who reported improved membrane integrity under integrated irrigation and nutrient management. In contrast, the I₀S₀ treatment (no irrigation and no nutrient supplementation) consistently recorded the highest MSI values, reflecting severe membrane damage. The absence of irrigation exacerbated dehydration and oxidative stress, while nutrient deficiencies limited the plants' ability to counteract these stresses. Similar trends were observed by Rajabi *et al.* (2024), who demonstrated increased membrane damage under water-deficient conditions.

Table 4.15 Influence of humic acid and sulphur on membrane injury index of leaves of Indian mustard under variable water regimes

Treatments	Membrane stability index (%)					
	30 DAS		60 DAS		90 DAS	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
I ₀	39.88	43.34	54.92	60.06	57.39	63.04
I ₁	38.59	44.80	63.25	58.90	64.48	72.90
I ₂	39.49	45.52	61.33	69.75	66.52	74.75
I ₃	38.65	44.55	62.83	71.15	69.53	77.46
SEm±	0.48	0.49	0.46	0.58	0.56	0.67
C.D at 5%	NS	NS	1.49	1.85	1.79	2.15
Sub plot (Nutrient management) (S)						
S ₀	29.69	35.41	53.71	61.48	57.52	64.59
S ₁	43.81	49.36	62.52	70.41	66.45	73.80
S ₂	37.28	42.98	58.03	65.95	61.95	69.34
S ₃	45.82	50.46	66.07	74.02	70.00	77.41
SEm±	0.45	0.44	0.41	0.47	0.45	0.56
C.D at 5%	1.30	1.27	1.17	1.35	1.29	1.61
Interaction (S*I)	NS	NS	2.44	2.83	2.69	3.37

* I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁ = Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C. D=Critical difference, NS= Non-significant, DAS=Days after sowing. % = per cent

Table 4.15A: Interaction table of membrane stability index (%) of leaves at 60 DAS

Subplot (S) Main plot (I)	2022-23					2023-24				
	S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀	46.98	58.82	51.61	62.29	54.92	52.11	63.97	56.75	67.43	60.06
I ₁	54.74	62.28	58.96	67.03	61.25	64.19	72.01	68.69	71.74	70.90
I ₂	56.06	63.49	60.28	69.49	61.33	64.34	71.96	68.73	78.96	71.75
I ₃	57.06	65.49	61.281	71.43	62.83	65.30	73.71	69.648	75.91	71.15
Mean (S)	53.71	62.52	58.03	66.07		61.48	70.41	65.95	74.02	
SEm±	S * I	0.93				1.16				
	I * S	0.84				1.01				
C.D at 5%	S * I	2.44				2.83				
	I * S	2.53				3.01				

* I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C. D=Critical, Difference, NS= Non-significant, DAS=Days after sowing.

Table 4.15B: Interaction table of membra stability index (%) of leaves at 90 DAS

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
	I ₀	49.41	61.28	54.08	64.77	57.39	54.21	67.23	60.01	70.69	63.04
	I ₁	57.96	65.51	62.18	70.26	64.48	66.19	74.01	70.69	75.04	72.90
	I ₂	61.25	68.69	65.46	72.69	66.52	69.34	76.96	73.73	78.96	74.75
	I ₃	61.42	70.33	66.100	74.28	67.53	68.61	77.02	72.958	80.24	74.46
	Mean (S)	57.52	66.45	61.95	70.00		64.59	73.80	69.34	77.41	
SEm±	S * I	1.15					1.35				
	I * S	0.96					1.19				
C.D at 5%	S* I	2.69					3.37				
	I * S	2.87					3.55				

* I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C. D=Critical difference, NS= Non-significant, DAS=Days after sowing.

4.5.3 Impact of different irrigation regimes and different nutrient management on membrane injury index (MII)

The effect of different irrigation regimes on the membrane injury index (MII) (%) of mustard at various growth stages (30 DAS, 60 DAS, and 90 DAS) showed notable differences across both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the I₃ treatment (three irrigations at vegetative, flowering, and silique filling stages) resulted in the highest MII values across most stages, with 61.35% at 30 DAS, 37.17% at 60 DAS, and 18.47% at 90 DAS. The I₀ treatment (no irrigation) recorded the lowest MII values, with 60.12% at 30 DAS, 45.08% at 60 DAS, and 43.61% at 90 DAS. In 2023-24, I₃ again showed the highest MII values at 55.45% at 30 DAS, 28.85% at 60 DAS, and 25.54% at 90 DAS. The improved MII in I₃ was attributed to adequate water availability, which supported cell membrane integrity by maintaining cellular turgor and reducing oxidative stress. Higher MII reflected stronger and more resilient cellular membranes, essential for sustained growth and productivity (Semida *et al.*, 2020). The lowest MII values were observed in the I₀ treatment, which recorded 56.66% at 30 DAS, 39.94% at 60 DAS, and 36.96% at 90 DAS. Reduced MII suggested that plants under I₀ experienced elevated cellular stress, compromising membrane integrity and overall plant health (Rajabi *et al.*, 2024).

The effect of different nutrient management treatments on the membrane injury index (MII) (%) of mustard at various growth stages (30 DAS, 60 DAS, and 90 DAS) showed clear trends across both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the S₃ treatment (humic acid + sulphur) resulted in the highest MSI values across all stages, with 45.18% at 30 DAS, 33.93% at 60 DAS, and 30.00% at 90 DAS. The S₀ treatment (control) had the lowest MII values, recording 70.31% at 30 DAS, 46.29% at 60 DAS, and 42.48% at 90 DAS. In 2023-24, S₃ again produced the highest MII values with 49.54% at 30 DAS, 25.98% at 60 DAS, and 22.59% at 90 DAS. The lowest MII values were observed in S₀, with 70.31% at 30 DAS, 46.29% at 60 DAS, and 42.48% at 90 DAS. Humic acid improved soil properties and enhanced nutrient absorption, while sulfur played a critical role in boosting antioxidant defenses that protected cellular membranes from oxidative damage. This combination supported improved membrane stability, essential for maintaining cellular health and resilience to stress (Liu *et al.*, 2019; Beigi *et al.*, 2019). The S₀ treatment consistently exhibited the lowest MSI values, highlighting the negative effects of nutrient deficiencies on

membrane stability. Without supplemental nutrients, mustard plants experienced heightened oxidative stress, compromising cell membrane integrity and reducing MSI, particularly under challenging environmental conditions (Sharma, 2020).

4.5.3a Interaction between different irrigation regimes and different nutrient management on membrane stability index (MII) at 60 DAS

The interaction between different irrigation regimes and nutrient management treatments had a significant effect on the membrane injury index (MII) (%) of mustard at 60 DAS across both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the I₃S₃ treatment (three irrigations with humic acid + sulphur) achieved the highest MII value of 32.53%, while the I₀S₀ treatment (no irrigation and no added nutrients) recorded the lowest MII at 53.02%. Humic acid improved soil moisture retention and nutrient uptake, while sulphur played a key role in antioxidative defence mechanisms, protecting cell membranes from oxidative stress (Ennab *et al.*, 2023). In contrast, the I₀S₀ treatment consistently showed the lowest MSI values, highlighting the detrimental effects of water and nutrient deficiencies on cell membrane integrity. Without irrigation and nutrients, mustard plants faced increased oxidative stress, leading to reduced membrane stability (Khan *et al.*, 2024). The two-year mean data underscored the importance of an integrated approach combining irrigation and nutrient management, as observed in the I₃S₃ treatment, for sustaining higher MII. This strategy proved critical in maintaining cell membrane stability and ensuring better stress resilience in mustard plants.

4.5.3b Interaction between different irrigation regimes and different nutrient management on membrane stability index (MSI) at 90 DAS

The interaction between different irrigation regimes and nutrient management treatments significantly influenced the membrane injury index (MII) (%) of mustard at 90 DAS during both 2022-23 and 2023-24, as well as in the combined mean data. In 2022-23, the I₃S₃ treatment (three irrigations with humic acid + sulfur) achieved the highest MSI at 27.72%, while the I₀S₀ treatment (no irrigation and no nutrients) recorded the lowest MII at 50.56%. Across irrigation regimes. The combination of three irrigations (I₃) and nutrient additions, specifically humic acid and sulfur (S₃), enhanced membrane stability by ensuring optimal cellular hydration and supplying essential nutrients that protected cellular membranes from oxidative stress. Humic acid improved soil moisture retention and nutrient uptake, while sulfur played a vital role in cellular

defenses against oxidative damage, resulting in higher MII (Abd El-Mageed *et al.*, 2020). The I₀S₀ treatment consistently showed the lowest MII values, highlighting the negative effects of water and nutrient deficiencies on membrane stability. Without adequate water or nutrients, mustard plants were more susceptible to stress, which compromised membrane integrity and reduced MII (Dey *et al.*, 2024).

4.6 Biochemical Parameters at (60 and 90 DAS)

4.6.1 Impact of different irrigation regimes and different nutrient management on total chlorophyll of Indian mustard (RLC3)

The impact of various irrigation regimes on the total chlorophyll content of mustard plants was assessed at 60 and 90 Days after sowing (DAS) over two growing seasons, 2022-23 and 2023-24. The total chlorophyll content, measured in mg g⁻¹ fresh weight (FW), showed a clear trend in response to the different irrigation treatments. The treatment with three post-sowing irrigations (I₃) consistently recorded the highest chlorophyll levels, with values of 2.261 and 2.075 mg g⁻¹ FW at 60 and 90 DAS, respectively, in 2022-23. Similarly, in 2023-24, I₃ reached 2.283 and 2.096 mg g⁻¹ FW at 60 and 90 DAS, respectively. In contrast, the treatment with no post-sowing irrigation (I₀) consistently recorded the lowest chlorophyll content, with values of 2.015 and 1.769 mg g⁻¹ FW at 60 and 90 DAS, respectively, in 2022-23, and 2.036 and 1.784 mg g⁻¹ FW in 2023-24. This trend indicated that greater soil moisture availability in frequently irrigated treatments enhanced photosynthetic activity and chlorophyll synthesis, likely due to improved nutrient uptake, maintained leaf turgidity, and overall plant vigor. These results align with findings from Bhattacharya and Bhattacharya (2021), which highlighted the pivotal role of adequate water supply in promoting chlorophyll accumulation and sustaining photosynthetic capacity in crop plants.

The effect of different nutrient management treatments on the total chlorophyll content of mustard was evaluated at 60 and 90 Days after sowing (DAS) across two cropping seasons, 2022-23 and 2023-24. Chlorophyll content (mg g⁻¹ FW) varied

Table 4.16 Influence of humic acid and sulphur on membrane injury index of leaves of Indian mustard under variable water regimes

Treatments	Membrane injury index (%)					
	30 DAS		60 DAS		90 DAS	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
I ₀	60.12	56.66	45.08	39.94	43.61	36.96
I ₁	61.41	55.20	38.75	29.10	35.52	27.10
I ₂	60.51	54.48	36.39	28.47	31.51	25.25
I ₃	61.35	55.45	37.17	28.85	18.47	25.54
SEm±	0.48	0.49	0.46	0.58	0.56	0.67
C.D at 5%	NS	NS	1.49	1.85	1.79	2.19
Sub plot (Nutrient management) (S)						
S ₀	70.31	64.59	46.29	38.52	42.48	35.41
S ₁	56.19	50.64	37.48	29.59	33.55	26.20
S ₂	62.72	57.02	41.97	34.05	38.05	30.66
S ₃	54.18	49.54	33.93	25.98	30.00	22.59
SEm±	0.45	0.44	0.41	0.47	0.45	0.56
C.D at 5%	1.30	1.27	1.17	1.35	1.29	1.61
Interaction (S*I)	NS	NS	2.44	2.83	2.70	3.37

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, NS= Non-significant, DAS=Days after sowing. %=Percent

Table 4.16A: Interaction table of membrane injury index (%) of leaves at 60 DAS

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		53.02	41.18	48.39	37.71	45.08	47.89	36.03	43.25	32.57	39.94
I ₁		45.27	37.72	41.04	30.97	38.75	35.81	27.99	31.31	21.26	29.10
I ₂		43.94	36.51	39.72	34.51	36.39	35.66	28.04	31.27	26.04	28.47
I ₃		42.94	34.51	38.719	32.53	37.17	34.70	26.29	30.352	24.07	28.85
Mean (S)		46.29	37.48	41.97	33.93		38.52	29.59	34.05	25.98	
SEm±	S * I	0.93					1.16				
	I* S	0.85					1.00				
C.D at 5%	S* I	2.44					2.83				
	I* S	2.53					3.01				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, NS= Non-significant, DAS=Days after sowing. %=Percent

Table 4.16B: Interaction table of membrane injury index (%) of leaves at 90 DAS

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
	I ₀	50.56	38.72	45.92	35.23	42.61	45.79	32.77	39.99	29.31	36.96
	I ₁	42.04	34.49	37.82	27.74	35.52	33.81	25.99	29.31	19.26	27.10
	I ₂	38.75	31.31	34.54	29.31	31.51	30.66	23.04	26.27	21.04	23.77
	I ₃	38.58	29.67	33.900	27.72	32.47	31.39	22.98	27.042	20.76	25.54
	Mean (S)	42.48	33.55	38.05	30.01		35.41	26.20	30.66	22.59	
SEm±	S * I	1.18					1.35				
	I* S	0.96					1.18				
C.D at 5%	S* I	2.70					3.37				
	I* S	2.88					3.55				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂= Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, NS= Non-significant, DAS=Days after sowing. %=Percent

significantly with nutrient management practices, with the highest chlorophyll levels observed in the treatment with combined humic acid and sulphur application (S_3). At 60 DAS, chlorophyll content for S_3 was $2.280 \text{ mg g}^{-1} \text{ FW}$ in 2022-23 and $2.304 \text{ mg g}^{-1} \text{ FW}$ in 2023-24. The lowest values were recorded in the control treatment (S_0), with $1.991 \text{ mg g}^{-1} \text{ FW}$ in 2022-23 and $2.009 \text{ mg g}^{-1} \text{ FW}$ in 2023-24. A similar trend was observed at 90 DAS, where S_3 exhibited the highest chlorophyll content ($2.215 \text{ mg g}^{-1} \text{ FW}$ in 2022-23 and $2.123 \text{ mg g}^{-1} \text{ FW}$ in 2023-24), while S_0 had the lowest values, averaging $1.775 \text{ mg g}^{-1} \text{ FW}$ across the two years. The superior performance of S_3 was attributed to the synergistic effects of humic acid and sulphur in promoting chlorophyll synthesis. Humic acid enhanced nutrient uptake and improved soil health, while sulphur supported chloroplast function and the synthesis of essential amino acids, which contributed to increased chlorophyll content. These findings aligned with those of Beigi *et al.* (2019), who reported improved chlorophyll levels in crops treated with humic acid and sulphur. In contrast, the S_0 treatment (control) consistently showed the lowest chlorophyll levels, highlighting the adverse effects of nutrient deficiencies. Without adequate supplementation, plants lacked the resources necessary for efficient chlorophyll synthesis, resulting in suboptimal photosynthetic performance. Similar trends were observed by Kaya *et al.* (2020), who reported reduced chlorophyll content in mustard plants under nutrient-deficient conditions. The two-year mean data demonstrated that treatments such as S_1 (humic acid) and S_2 (sulphur) independently improved chlorophyll content compared to the control, although their combined application (S_3) resulted in the most significant enhancement.

4.6.1a Interaction between different irrigation regimes and different nutrient management on total chlorophyll at 60 DAS

The interaction between different irrigation regimes and nutrient management treatments had a notable impact on the total chlorophyll content of mustard at 60 Days after sowing (DAS) over two cropping seasons, 2022-23 and 2023-24. In both years, the combination of the highest irrigation regime (I_3) with the combined nutrient treatment of humic acid and sulphur (S_3) yielded the highest chlorophyll levels. Specifically, in 2022-23, the I_3S_3 treatment combination achieved a chlorophyll content of $2.38 \text{ mg g}^{-1} \text{ FW}$, which increased slightly to $2.30 \text{ mg g}^{-1} \text{ FW}$ in 2023-24. In contrast, the lowest chlorophyll values were recorded in the control treatments with no irrigation (I_0) and no nutrient application (S_0), with values of $1.851 \text{ mg g}^{-1} \text{ FW}$ in 2022-23 and $1.867 \text{ mg g}^{-1} \text{ FW}$ in 2023-24.

g⁻¹ FW in 2023-24. The positive impact of humic acid and sulphur was likely due to their roles in improving soil structure, nutrient absorption, and chlorophyll biosynthesis (Wang *et al.*, 2024). In contrast, the lowest chlorophyll levels were recorded under the I₀S₀ treatment, where limited water and nutrient availability likely restricted chlorophyll production due to water stress and nutrient deficiencies. These results underscored the detrimental effects of inadequate resources on physiological processes critical to crop growth and productivity. These findings emphasized the importance of integrating sufficient irrigation with balanced nutrient management to maximize chlorophyll content and support optimal mustard crop growth (Rana and Parihar, 2019).

4.6.1b Interaction between different irrigation regimes and different nutrient management on total chlorophyll at 90 DAS

The interaction between different irrigation regimes and nutrient management practices influenced the total chlorophyll content of mustard at 90 Days after sowing (DAS) across two cropping seasons, 2022-23 and 2023-24. Chlorophyll content, measured in mg g⁻¹ fresh weight (FW), varied significantly based on the irrigation and nutrient combinations. In both years, the highest chlorophyll content was observed in the treatment combination of I₃ (three post-sowing irrigations) and S₃ (combined humic acid and sulphur), with values of 2.59 mg g⁻¹

FW in 2022-23 and 2.35 mg g⁻¹ FW in 2023-24. The lowest chlorophyll levels were recorded in the I₀S₀ treatment (no irrigation and no additional nutrients), with values of 1.60 mg g⁻¹ FW in 2022-23 and 1.63 mg g⁻¹ FW in 2023-24. The highest chlorophyll content was achieved in the treatment with three irrigations (I₃) combined with the S₃ nutrient management, indicating that adequate irrigation coupled with essential nutrients benefited photosynthetic activity and plant vigor. This combination likely enhanced nutrient availability, improved soil health, and promoted chlorophyll biosynthesis, which was essential for optimal plant growth and productivity (Kaya *et al.*, 2020). The lowest chlorophyll content recorded in the I₀S₀ treatment further underscored the negative impact of water stress and nutrient deficiency on chlorophyll production, likely due to restricted metabolic activity and impaired photosynthetic efficiency under limited resource conditions (Ahluwalia *et al.*, 2021).

Table 4.17 Effect of humic acid and sulphur on total chlorophyll of Indian mustard under variable water regimes

Treatments	Total chlorophyll (mg g ⁻¹ FW)			
	60 DAS		90 DAS	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24
I ₀	2.015	2.036	1.769	1.784
I ₁	2.096	2.122	1.993	1.897
I ₂	2.248	2.271	2.032	2.055
I ₃	2.261	2.283	2.075	2.096
SEm±	0.0109	0.0118	0.0108	0.012
C.D at 5%	0.035	0.038	0.035	0.039
Sub plot (Nutrient management) (S)				
S ₀	1.991	2.009	1.764	1.786
S ₁	2.219	2.251	1.992	2.011
S ₂	2.130	2.149	1.899	1.911
S ₃	2.280	2.304	2.215	2.123
SEm±	0.004	0.005	0.013	0.016
C.D at 5%	0.015	0.015	0.043	0.046
Interaction (S*I)	0.029	0.320	0.089	0.095

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, DAS=Days after sowing, mg g⁻¹ FW= milligram per gram fresh weight

Table 4.17A: Interaction table of total chlorophyll (mg g⁻¹ FW) of leaves at 60 DAS

Subplot (S) Main plot (I)	2022-23					2023-24				
	S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀	1.85	2.09	1.98	2.15	2.02	1.87	2.12	1.99	2.17	2.04
I ₁	1.95	2.13	2.07	2.24	2.10	1.96	2.17	2.09	2.27	2.12
I ₂	2.08	2.35	2.21	2.36	2.25	2.10	2.37	2.23	2.38	2.27
I ₃	2.09	2.31	2.26	2.38	2.26	2.11	2.33	2.29	2.40	2.28
Mean (S)	1.99	2.22	2.13	2.28		2.01	2.25	2.15	2.30	
SEm±	S * I	0.021				0.024				
	I * S	0.014				0.015				
C.D at 5%	S* I	0.032				0.029				
	I* S	0.043				0.046				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, DAS=Days after sowing, mg g⁻¹ FW= milligram per gram fresh weight

Table 4.17B: Interaction table of total chlorophyll (mg g^{-1} FW) of leaves at 90 DAS

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		1.60	1.85	1.74	1.89	1.77	1.63	1.86	1.75	1.90	1.78
I ₁		1.75	1.87	1.82	2.53	1.99	1.77	1.89	1.82	2.11	1.90
I ₂		1.82	2.13	1.93	2.25	2.03	1.84	2.15	1.96	2.27	2.05
I ₃		1.89	2.13	2.10	2.59	2.08	1.90	2.15	2.12	2.35	2.10
Mean (S)		1.76	1.99	1.90	2.22		1.79	2.01	1.91	2.12	
SEm±	S * I	0.022					0.025				
	I * S	0.028					0.031				
C.D at 5%	S * I	0.089					0.095				
	I * S	0.083					0.090				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂= Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, DAS=Days after sowing, mg g^{-1} FW= milligram per gram fresh weight

4.6.2 Impact of different irrigation regimes and different nutrient management on chlorophyll a

The impact of different irrigation regimes on chlorophyll a content in mustard plants was evaluated at 60 and 90 DAS over the 2022-23 and 2023-24 growing seasons. Chlorophyll a content, measured in mg g^{-1} fresh weight (FW), varied across irrigation treatments, with higher irrigation levels generally resulting in increased chlorophyll a levels. This suggested that optimal water availability supported chlorophyll synthesis, enhancing photosynthetic capacity and promoting better plant growth (Farouk *et al.*, 2021). At 60 DAS, the highest chlorophyll a content was recorded in the treatment with three post-sowing irrigations (I_3), with values of 1.866 mg g^{-1} FW in 2022-23 and 1.956 mg g^{-1} FW in 2023-24. Higher irrigation levels likely improved nutrient absorption and cellular hydration, factors essential for chlorophyll a production and photosynthetic efficiency (Muhammad *et al.*, 2022). Conversely, the lowest chlorophyll a content was found in the no irrigation treatment (I_0), with values of 1.661 mg g^{-1} FW in 2022-23 and 1.759 mg g^{-1} FW in 2023-24. At 90 DAS, a similar trend was observed, with I_3 recording the highest chlorophyll a content (1.203 mg g^{-1} FW in 2022-23 and 1.729 mg g^{-1} FW in 2023-24), while I_0 had the lowest chlorophyll a content (1.018 mg g^{-1} FW in 2022-23 and 1.441 mg g^{-1} FW in 2023-24). These results aligned with previous research showing that water availability was critical for chlorophyll production and overall plant health (Nemeskér *et al.*, 2019). The observed trends underscored the importance of adequate irrigation in mustard cultivation, particularly in water-limited regions. By implementing irrigation strategies that maintained soil moisture at optimal levels, farmers enhanced chlorophyll a content, leading to improved photosynthetic efficiency, higher crop vigor, and potentially greater yield.

The effect of different nutrient management treatments on chlorophyll a content in mustard plants was analysed at 60 and 90 Days after sowing (DAS) across the 2022-23 and 2023-24 seasons. The chlorophyll a content (mg g^{-1} FW) varied according to the nutrient treatments, with the combined humic acid and sulphur application (S_3) resulting in the highest levels of chlorophyll a. At 60 DAS, the S_3 treatment recorded the highest chlorophyll a content with 1.876 mg g^{-1} FW in 2022-23 and 1.983 mg g^{-1} FW in 2023-24. The lowest chlorophyll a content was found in the control treatment (S_0), with values of 1.617 mg g^{-1} FW in 2022-23 and 1.725 mg g^{-1} FW in 2023-24. At 90 DAS, the trend persisted, with S_3 displaying the highest chlorophyll a values of 1.278 mg g^{-1}

FW in 2022-23 and 1.724 mg g⁻¹ FW in 2023-24, resulting in a mean of 1.501 mg g⁻¹ FW. Humic acid likely improved soil structure and nutrient mobility, while sulphur played a vital role in chlorophyll biosynthesis, both contributing to the higher chlorophyll levels observed (Bano *et al.*, 2022). The control treatment (S₀) again showed the lowest chlorophyll a content, with values of 1.018 mg g⁻¹ FW in 2022-23 and 1.452 mg g⁻¹ FW in 2023-24, averaging 1.235 mg g⁻¹ FW. In contrast, the lowest chlorophyll a level in the control treatment (S₀) indicated that nutrient supplementation was essential for optimal chlorophyll production and overall plant health (Aye and Masih, 2023). These findings aligned with existing research showing that balanced nutrient management significantly impacted chlorophyll content and photosynthetic efficiency (Shankar *et al.*, 2021).

4.6.2a Interaction between different irrigation regimes and different nutrient management on chlorophyll a at 60 DAS

The interaction between different irrigation regimes and nutrient management treatments had a notable impact on chlorophyll a content in mustard plants at 60 Days after sowing (DAS) across the 2022-23 and 2023-24 cropping seasons. Chlorophyll a content, measured in mg g⁻¹ fresh weight (FW), increased with higher irrigation and nutrient levels. The highest chlorophyll a content was observed in the combination of three post-sowing irrigations (I₃) with the application of humic acid and sulphur (S₃), with values of 1.978 mg g⁻¹ FW in 2022-23 and 2.060 mg g⁻¹ FW in 2023-24. Conversely, the lowest chlorophyll a content was recorded in the combination of no irrigation (I₀) with no nutrient application (S₀), with values of 1.510 mg g⁻¹ FW in 2022-23 and 1.620 mg g⁻¹ FW in 2023-24, resulting in a mean of 1.565 mg g⁻¹ FW. The combined application of humic acid and sulphur, alongside adequate irrigation, improved chlorophyll a content in plants by enhancing nutrient availability, soil structure, and chlorophyll biosynthesis, each critical for efficient photosynthesis and growth. Humic acid improved soil structure and increased nutrient availability by promoting root growth and enhancing the uptake of key nutrients like iron, which was essential for chlorophyll formation (Tiwari *et al.*, 2023). Meanwhile, sulphur supported chlorophyll synthesis by being integral to amino acid and enzyme production, which were crucial for photosynthetic pathways (Kharwar *et al.*, 2021). When supplied with adequate water, which aided in nutrient transport within the plant, the combined effects of humic acid and sulphur resulted in higher chlorophyll levels, which translated to

enhanced photosynthetic efficiency and stronger plant growth (Bela *et al.*, 2019). On the other hand, the lowest chlorophyll a level recorded in the I₀S₀ treatment highlighted the negative effects of limited water and nutrient availability, likely due to restricted nutrient mobility and reduced chlorophyll biosynthesis under stress conditions (Kumari *et al.*, 2022).

4.6.2b Interaction between different irrigation regimes and different nutrient management on chlorophyll a at 90 DAS

The interaction between different irrigation regimes and nutrient management treatments influenced chlorophyll a content in mustard plants at 90 Days after sowing (DAS) over the 2022-23 and 2023-24 seasons. Chlorophyll a content, measured in mg/g fresh weight (FW), varied significantly across irrigation and nutrient combinations, with the highest values observed under the three-irrigation regime (I₃) combined with the application of humic acid and sulphur (S₃). Specifically, at 90 DAS, the I₃S₃ combination resulted in chlorophyll a content values of 1.203 mg g⁻¹ FW in 2022-23 and 1.842 mg g⁻¹ FW in 2023-24. The lowest chlorophyll a content was recorded in the no irrigation and no nutrient treatment (I₀S₀), with values of 0.919 mg g⁻¹ FW in 2022-23 and 1.316 mg g⁻¹ FW in 2023-24. For nutrient management, the mean values across irrigation regimes revealed that S₃ exhibited the highest chlorophyll a level, while S₀ recorded the lowest. These findings underscore the critical importance of integrating irrigation and nutrient management strategies in mustard cultivation to maximize chlorophyll a content and improve crop productivity (Zulfiqar *et al.*, 2023). This approach was particularly beneficial in semi-arid regions, where efficient management of water and nutrients significantly improved crop health and productivity (Hayati *et al.*, 2022).

4.6.3 Impact of different irrigation regimes and different nutrient management on chlorophyll b

The impact of different irrigation regimes on chlorophyll b content in mustard plants was assessed at 60 and 90 DAS over the 2022-23 and 2023-24 growing seasons. Chlorophyll b content, measured in mg g⁻¹ fresh weight (FW), showed a positive response to increased irrigation levels. At 60 DAS, the highest chlorophyll b content was recorded in the treatment with two post-sowing irrigations (I₂), with values of 0.423 mg g⁻¹ FW in 2022-23 and 0.461 mg g⁻¹ FW in 2023-24.

Table 4.18 Impact of humic acid and sulphur on chlorophyll a of Indian mustard under variable water regimes

Treatments	Chlorophyll a (mg g ⁻¹ FW)			
	60 DAS		90 DAS	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24
I ₀	1.661	1.759	1.018	1.441
I ₁	1.708	1.847	1.148	1.532
I ₂	1.826	1.935	1.171	1.659
I ₃	1.866	1.956	1.203	1.729
SEm±	0.007	0.008	0.006	0.010
C.D at 5%	0.025	0.027	0.020	0.032
Sub plot (Nutrient management) (S)				
S ₀	1.617	1.725	1.018	1.452
S ₁	1.824	1.941	1.149	1.633
S ₂	1.744	1.848	1.095	1.553
S ₃	1.876	1.983	1.278	1.724
SEm±	0.004	0.004	0.009	0.013
C.D at 5%	0.010	0.011	0.025	0.037
Interaction (S*I)	0.023	0.024	0.051	0.013

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, DAS=Days after sowing, mg g⁻¹ FW= milligram per gram fresh weight.

Table 4.18A: Interaction table of chlorophyll a (mg g^{-1} FW) of leaves at 60 DAS

Subplot (S) Main plot (I)	2022-23					2023-24				
	S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀	1.510	1.720	1.625	1.790	1.661	1.620	1.845	1.713	1.860	1.759
I ₁	1.545	1.758	1.698	1.833	1.708	1.690	1.898	1.830	1.970	1.847
I ₂	1.698	1.913	1.790	1.903	1.826	1.785	2.020	1.895	2.040	1.935
I ₃	1.715	1.905	1.865	1.978	1.866	1.805	2.003	1.955	2.060	1.956
Mean (S)	1.617	1.824	1.744	1.876		1.725	1.941	1.848	1.983	
SEm±	S * I	0.015				0.016				
	I * S	0.010				0.012				
C.D at 5%	S* I	0.023				0.024				
	I* S	0.030				0.32				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, DAS=Days after sowing, mg g^{-1} FW= milligram per gram fresh weight.

Table 4.18B: Interaction table of chlorophyll a (mg g^{-1} FW) of leaves at 90 DAS

Subplot (S) Main plot (I)	2022-23					2023-24				
	S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀	0.919	1.065	1.002	1.088	1.018	1.316	1.503	1.411	1.534	1.441
I ₁	1.009	1.074	1.051	1.459	1.148	1.430	1.521	1.473	1.701	1.532
I ₂	1.050	1.226	1.114	1.295	1.171	1.488	1.735	1.579	1.835	1.659
I ₃	1.093	1.232	1.215	1.298	1.203	1.572	1.772	1.748	1.842	1.729
Mean (S)	1.018	1.149	1.095	1.278		1.452	1.633	1.553	1.724	
SEm±	S * I	0.013				0.010				
	I * S	0.016				0.033				
C.D at 5%	S* I	0.051				0.013				
	I* S	0.048				0.018				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, DAS=Days after sowing, mg g^{-1} FW= milligram per gram fresh weight.

The lowest chlorophyll b levels were observed in the no irrigation treatment (I_0), with values of $0.354 \text{ mg g}^{-1} \text{ FW}$ in 2022-23 and $0.403 \text{ mg g}^{-1} \text{ FW}$ in 2023-24. At 90 DAS, a similar trend was observed, with I_3 and I_2 showing higher chlorophyll b levels, while I_0 consistently exhibited the lowest content. This increase suggested that higher soil moisture facilitated chlorophyll synthesis, improving photosynthetic capacity and overall plant health (Li *et al.*, 2019). Chlorophyll b, critical for capturing light energy for photosynthesis, showed a marked increase with frequent irrigation, likely due to enhanced hydration and nutrient transport within the plant (Miao *et al.*, 2020). In contrast, the reduced chlorophyll b content in the no irrigation treatment (I_0) highlighted the negative effects of water stress, which restricted photosynthetic activity and diminished chlorophyll production under drought conditions (Batool *et al.*, 2020). These results underscore the vital role of adequate irrigation in increasing chlorophyll b content and maintaining photosynthetic performance in mustard cultivation. Especially in semi-arid regions, irrigation practices that ensured optimal soil moisture were shown to enhance chlorophyll levels, improve crop vigor, and boost yields, supporting sustainable crop production in water-limited environments (Kang *et al.*, 2021).

The effect of different nutrient management treatments on chlorophyll b content in mustard plants was analysed at 60 and 90 Days after sowing (DAS) across the 2022-23 and 2023-24 seasons. Chlorophyll b content, measured in mg g^{-1} fresh weight (FW), varied with nutrient treatments, showing higher levels under the combined application of humic acid and sulphur (S_3). At 60 DAS, the S_3 treatment recorded the highest chlorophyll b content, with values of $0.405 \text{ mg g}^{-1} \text{ FW}$ in 2022-23 and $0.457 \text{ mg g}^{-1} \text{ FW}$ in 2023-24. The lowest values were observed in the control treatment (S_0), with $0.375 \text{ mg g}^{-1} \text{ FW}$ in 2022-23 and $0.413 \text{ mg g}^{-1} \text{ FW}$ in 2023-24. At 90 DAS, a similar trend was observed, with S_3 exhibiting the highest chlorophyll b content ($0.299 \text{ mg g}^{-1} \text{ FW}$ in 2022-23 and $0.530 \text{ mg g}^{-1} \text{ FW}$ in 2023-24, with a mean of $0.414 \text{ mg g}^{-1} \text{ FW}$). Humic acid enhanced soil porosity and nutrient retention, promoting efficient nutrient uptake, while sulphur directly contributed to chlorophyll synthesis by supporting essential metabolic processes (Tahoun *et al.*, 2022; Majumdar *et al.*, 2023). The marked increase in chlorophyll b content under the S_3 treatment reflected improved photosynthetic efficiency and underscored the potential for increased crop productivity through nutrient optimization. This improvement illustrated the synergistic effects of humic

acid and sulphur in facilitating better photosynthetic performance by ensuring optimal chlorophyll production. These findings were consistent with existing studies, which have emphasized the role of balanced nutrient management in promoting chlorophyll synthesis and enhancing photosynthetic activity. For example, Tiwari *et al.* (2020) demonstrated that integrating nutrient management practices improved crop vigor and sustainability, particularly in nutrient-deficient soils. The results reinforced the importance of adopting such nutrient management strategies to improve plant health and productivity, highlighting their relevance for sustainable agriculture, especially in areas where soil fertility is a limiting factor.

4.6.3a Interaction between different irrigation regimes and different nutrient management on chlorophyll b at 60 DAS

The interaction between different irrigation regimes and nutrient management treatments had a significant impact on chlorophyll b content in mustard plants at 60 Days after sowing (DAS) across the 2022-23 and 2023-24 cropping seasons. Chlorophyll b content, measured in mg g^{-1} fresh weight (FW), showed variations across irrigation and nutrient combinations. The highest chlorophyll b levels were observed under the combination of two irrigations (I_2) with humic acid and sulphur application (S_3), with values of 0.454 mg g^{-1} FW in 2022-23 and 0.483 mg g^{-1} FW in 2023-24. Conversely, the lowest chlorophyll b content was recorded in the no irrigation (I_0) and no nutrient (S_0) treatment, with values of 0.340 mg g^{-1} FW in 2022-23 and 0.380 mg g^{-1} FW in 2023-24. This indicated that a balanced supply of water and essential nutrients fostered chlorophyll synthesis, enhancing photosynthetic capacity and supporting plant growth. Humic acid likely aided nutrient absorption and soil structure improvement, while sulphur, crucial for chlorophyll formation, contributed to the increased chlorophyll b levels under these conditions (Wang *et al.*, 2024).

4.6.3b Interaction between different irrigation regimes and different nutrient management on chlorophyll b at 90 DAS

The interaction between different irrigation regimes and nutrient management treatments influenced chlorophyll b content in mustard plants at 90 Days after sowing (DAS) across the 2022-23 and 2023-24 seasons. Chlorophyll b content, measured in mg g^{-1} fresh weight (FW), showed notable variations across combinations of irrigation and nutrient treatments. The highest chlorophyll b content was recorded under the two-irrigation regime (I_2) combined with humic acid and sulphur (S_3), with values of 0.302

mg g⁻¹ FW in 2022-23 and 0.569 mg g⁻¹ FW in 2023-24. Conversely, the lowest chlorophyll b content was observed in the no irrigation and no nutrient application treatment (I₀S₀), with values of 0.216 mg g⁻¹ FW in 2022-23 and 0.410 mg g⁻¹ FW in 2023-24. These findings underscored the importance of an integrated approach to water and nutrient management for maximizing chlorophyll b content in mustard, leading to greater photosynthetic efficiency and potential yield improvements (Meena *et al.*, 2024).

4.6.4 Impact of different irrigation regimes and different nutrient management on malondialdehyde (MDA) content

Malondialdehyde (MDA) content, measured in µmol g⁻¹ FW, varied significantly with irrigation levels, showing higher values under water-limited conditions, indicating oxidative stress. At 60 DAS, the highest MDA content was recorded in the no irrigation treatment (I₀), with values of 25.78 µmol g⁻¹ FW in 2022-23 and 28.70 µmole/g in 2023-24. Conversely, the lowest MDA content was observed in the three-irrigation treatment (I₃), with values of 13.04 µmol g⁻¹ FW in 2022-23 and 15.91 µmol g⁻¹ FW in 2023-24. A similar trend was observed at 90 DAS, where I₀ exhibited the highest MDA content (32.47 µmol g⁻¹ FW in 2022-23 and 36.66 µmol g⁻¹ FW in 2023-24), while I₃ showed the lowest values (10.61 µmol g⁻¹ FW in 2022-23 and 13.47 µmol g⁻¹ FW in 2023-24). MDA, a byproduct of lipid peroxidation, served as a reliable biomarker for oxidative damage in plants (Heath & Packer, 1968). The elevated MDA levels under the no-irrigation (I₀) treatment reflected heightened oxidative stress caused by water deficiency, which disrupted cellular homeostasis, damaged membranes, and accelerated lipid peroxidation (El-Sanatawy *et al.*, 2021). Water scarcity reduced turgor pressure and impaired the plant's antioxidative defense mechanisms, leading to the accumulation of reactive oxygen species (ROS) and increased lipid peroxidation.

Table 4.19 Effect of humic acid and sulphur on chlorophyll b of Indian mustard under variable water regimes

Treatments	Chlorophyll b (mg g ⁻¹ FW)			
	60 DAS		90 DA	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24
I ₀	0.354	0.403	0.238	0.448
I ₁	0.388	0.430	0.269	0.476
I ₂	0.423	0.461	0.274	0.515
I ₃	0.397	0.461	0.281	0.517
SEm±	0.004	0.001	0.001	0.003
C.D at 5%	0.013	0.004	0.004	0.009
Sub plot (Nutrient management) (S)				
S ₀	0.375	0.413	0.238	0.447
S ₁	0.396	0.454	0.269	0.502
S ₂	0.386	0.431	0.256	0.478
S ₃	0.405	0.457	0.299	0.530
SEm±	0.003	0.001	0.002	0.004
C.D at 5%	0.009	0.003	0.006	0.012
Interaction (S*I)	0.018	0.006	0.012	0.024

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, DAS=Days after sowing, mg g⁻¹ FW= milligram per gram fresh weight.

Table 4.19A: Interaction table of chlorophyll b (mg g⁻¹ FW) of leaves at 60 DAS

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		0.340	0.367	0.353	0.356	0.354	0.380	0.428	0.393	0.410	0.403
I ₁		0.401	0.369	0.371	0.409	0.388	0.400	0.440	0.428	0.453	0.430
I ₂		0.381	0.439	0.419	0.454	0.423	0.433	0.480	0.448	0.483	0.461
I ₃		0.378	0.409	0.400	0.400	0.397	0.438	0.468	0.458	0.483	0.461
Mean (S)		0.375	0.396	0.386	0.405		0.413	0.454	0.431	0.457	
SEm±	S * I	0.008					0.003				
	I * S	0.010					0.004				
C.D at 5%	S* I	0.019					0.006				
	I* S	0.020					0.012				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, DAS=Days after sowing, mg g⁻¹ FW= milligram per gram fresh weight.

Table 4.19B: Interaction table of chlorophyll b (mg g^{-1} FW) of leaves at 90 DAS

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		0.216	0.249	0.234	0.255	0.238	0.410	0.468	0.439	0.477	0.448
I ₁		0.236	0.251	0.246	0.341	0.269	0.445	0.472	0.458	0.528	0.476
I ₂		0.245	0.288	0.260	0.302	0.274	0.462	0.538	0.490	0.569	0.515
I ₃		0.255	0.288	0.284	0.313	0.281	0.471	0.530	0.523	0.576	0.517
Mean (S)		0.238	0.269	0.256	0.299		0.447	0.502	0.478	0.530	
SEm±	S * I	0.003					0.006				
	I * S	0.001					0.010				
C.D at 5%	S* I	0.012					0.024				
	I* S	0.010					0.021				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, DAS=Days after sowing, mg g^{-1} FW= milligram per gram fresh weight.

Conversely, the lower MDA levels observed in the three-irrigation treatment (I₃) suggested that sufficient water supply helped mitigate oxidative stress by maintaining cellular integrity and reducing ROS generation. Adequate hydration enhanced the plant's physiological processes, including nutrient transport and enzymatic activity, thereby limiting oxidative damage to membranes (Mukarram *et al.*, 2021).

The effect of different nutrient management treatments on malondialdehyde (MDA) content in mustard plants was assessed at 60 and 90 Days after sowing (DAS) across the 2022-23 and 2023-24 growing seasons. MDA content, measured in $\mu\text{mol g}^{-1}$ FW, varied across nutrient treatments, with the highest levels observed in the control treatment (S₀), indicating increased oxidative stress. At 60 DAS, the highest MDA content was recorded in the S₀ treatment, with values of 23.75 $\mu\text{mol g}^{-1}$ FW in 2022-23 and 26.49 $\mu\text{mol g}^{-1}$ FW in 2023-24. The lowest MDA levels were observed in the S₃, with values of 13.58 $\mu\text{mol g}^{-1}$ FW in 2022-23 and 18.46 $\mu\text{mol g}^{-1}$ FW in 2023-24. A similar trend was observed at 90 DAS, where S₀ consistently showed the highest MDA content (27.31 $\mu\text{mol g}^{-1}$ FW in 2022-23 and 29.81 $\mu\text{mol g}^{-1}$ FW in 2023-24). Malondialdehyde content in mustard crops, indicating decreased oxidative stress and improved cellular integrity. MDA, a byproduct of lipid peroxidation, served as a key indicator of cellular damage caused by oxidative stress (Heath & Packer, 1968). The pronounced reduction in MDA levels under the S₃ treatment suggested that humic acid and sulphur contributed to stress mitigation by enhancing nutrient uptake, improving water retention, and supporting the plant's antioxidative mechanisms. Humic acid increased root growth, soil nutrient availability, and enzymatic activities associated with oxidative stress defense (de *et al.*, 2021). Similarly, sulphur, as a critical component of glutathione and other sulphur-containing compounds, played a pivotal role in detoxifying reactive oxygen species (ROS) and maintaining redox homeostasis (Alvi *et al.*, 2023). These combined effects likely fortified the plant's resilience to oxidative stress. The observed trend aligned with findings from similar studies, which highlighted the role of humic substances and sulphur in reducing oxidative damage and improving crop stress tolerance (Kaya *et al.*, 2023; Belal *et al.*, 2019).

Table 4.20 Influence of humic acid and sulphur on malondialdehyde content of Indian mustard under variable water regimes

Treatments	Malondialdehyde content ($\mu\text{mol g}^{-1}$ FW)			
	60 DAS		90 DAS	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24
I ₀	25.78	28.70	32.47	36.66
I ₁	22.31	25.87	27.11	29.63
I ₂	13.44	18.00	22.66	21.16
I ₃	13.04	15.91	10.61	13.47
SEm \pm	0.20	0.22	0.27	0.32
C.D at 5%	0.63	0.71	0.87	1.01
Sub plot (Nutrient management) (S)				
S ₀	23.75	26.49	27.31	29.81
S ₁	17.50	20.22	21.46	22.96
S ₂	20.76	23.32	25.57	27.38
S ₃	13.58	18.46	18.52	20.77
SEm \pm	0.15	0.22	0.19	0.34
C.D at 5%	0.42	0.64	0.55	0.97
Interaction (S*I)	0.84	1.28	1.16	2.01

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm \pm = Standard error of mean , C.D=Critical difference, DAS=Days after sowing, $\mu\text{mol g}^{-1}$ FW= micromoles per gram fresh weight

Table 4.20A: Interaction table of malondialdehyde content ($\mu\text{mol g}^{-1}$ FW) of leaves at 60 DAS

Subplot (S) Main plot (I)	2022-23					2023-24				
	S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀	30.51	24.42	28.15	20.04	25.78	33.67	24.48	29.32	27.34	28.70
I ₁	28.10	21.16	25.10	14.90	22.31	30.29	24.03	27.70	21.47	25.87
I ₂	19.23	12.63	15.80	10.12	14.44	22.01	17.08	19.12	13.79	18.00
I ₃	17.15	11.79	13.976	9.26	13.04	20.01	15.27	17.121	11.24	15.91
Mean (S)	23.75	17.50	20.76	13.58		26.49	20.22	23.32	18.46	
SEm±	S * I	0.397				0.445				
	I * S	0.321				0.447				
C.D at 5%	S* I	0.882				1.329				
	I* S	0.970				1.327				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, DAS=Days after sowing, $\mu\text{mol g}^{-1}$ FW= micromoles per gram fresh weight

Table 4.20B: Interaction table of malondialdehyde content ($\mu\text{mol g}^{-1}$ FW) of leaves at 90 DAS

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		36.77	30.35	35.73	27.04	32.47	42.26	33.35	39.98	31.04	36.66
I ₁		31.10	25.31	29.16	22.90	27.11	33.61	27.83	31.67	25.42	29.63
I ₂		27.23	20.63	25.67	17.12	22.66	25.23	18.63	23.67	17.13	21.16
I ₃		14.16	9.54	11.734	7.02	10.61	18.14	12.03	14.218	9.50	13.47
Mean (S)		27.31	21.46	25.57	18.52		29.81	22.96	27.38	20.77	
SEm±	S * I	0.547					0.632				
	I * S	0.431					0.665				
C.D at 5%	S* I	1.163					2.01				
	I* S	1.304					1.97				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, DAS=Days after sowing, $\mu\text{mol g}^{-1}$ FW= micromoles per gram fresh weight

4.6.4a Interaction between different irrigation regimes and different nutrient management on malondialdehyde (MDA) content at 60 DAS

MDA content, measured in $\mu\text{mol g}^{-1}$ FW, varied significantly depending on irrigation and nutrient combinations, with higher levels observed under reduced irrigation and in control treatments. The highest MDA content was recorded in the no irrigation (I_0) treatment with no nutrient application (S_0), with values of $30.51 \mu\text{mol g}^{-1}$ FW in 2022-23 and $33.67 \mu\text{mol g}^{-1}$ FW in 2023-24, averaging $32.09 \mu\text{mol g}^{-1}$ FW. In contrast, the lowest MDA content was observed in the three-irrigation (I_3) regime with the combined humic acid and sulphur treatment (S_3), showing values of $9.26 \mu\text{mol g}^{-1}$ FW in 2022-23 and $11.24 \mu\text{mol g}^{-1}$ FW in 2023-24, resulting in a mean of $10.25 \mu\text{mol g}^{-1}$ FW. Across all treatments, the mean MDA content was highest in I_0 and lowest in I_3 , while among nutrient treatments, S_3 consistently resulted in the lowest MDA content. MDA, a byproduct of lipid peroxidation, served as a biomarker for oxidative stress in plants (Heath & Packer, 1968). Higher MDA levels under limited irrigation (I_0 and I_1) indicated increased stress due to water scarcity, which impaired cellular homeostasis and accelerated lipid peroxidation (Gholami *et al.*, 2022). Nutrient treatments significantly influenced MDA levels, with the combined application of humic acid and sulphur (S_3) showing the most pronounced reduction. Humic acid enhanced water retention, nutrient uptake, and antioxidant activity (Abbas *et al.*, 2022), while sulphur contributed to synthesising sulphur-containing amino acids and glutathione, essential components of the plant's antioxidant defence system (Majumdar *et al.*, 2023).

4.6.4b Interaction between different irrigation regimes and different nutrient management on MDA content at 90 DAS

MDA content, measured in $\mu\text{mole/g}$, varied significantly depending on irrigation and nutrient combinations, with higher levels observed under reduced irrigation and in control treatments. The highest MDA content was recorded in the no irrigation (I_0) treatment with no nutrient application (S_0), with values of $30.51 \mu\text{mol g}^{-1}$ FW in 2022-23 and $33.67 \mu\text{mol g}^{-1}$ FW in 2023-24, averaging $32.09 \mu\text{mol g}^{-1}$ FW. In contrast, the lowest MDA content was observed in the three-irrigation (I_3) regime with the combined humic acid and sulphur treatment (S_3), showing values of $9.26 \mu\text{mol g}^{-1}$ FW in 2022-23 and $11.24 \mu\text{mol g}^{-1}$ FW in 2023-24, resulting in a mean of $10.25 \mu\text{mol g}^{-1}$ FW. Across all treatments, the mean MDA content was highest in I_0 and lowest in I_3 , while among nutrient treatments, S_3 consistently resulted in the lowest MDA content.

Their synergistic effects likely improved the plants' resilience to oxidative stress across various irrigation regimes. These results demonstrated the importance of integrated nutrient management and appropriate irrigation scheduling in reducing oxidative damage and improving the physiological health of mustard crops.

4.6.5 Impact of different irrigation regimes and different nutrient management on total soluble protein content of Indian mustard (RLC3)

The impact of different irrigation regimes on the protein content of mustard plants was evaluated at 60 and 90 Days after sowing (DAS) across the 2022-23 and 2023-24 growing seasons. Protein content, measured in mg g^{-1} fresh weight (FW), varied significantly with irrigation levels, showing higher values with increased irrigation. At 60 DAS, the highest protein content was observed in the treatment with three post-sowing irrigations (I_3), recording values of 39.22 mg g^{-1} FW in 2022-23 and 41.90 mg g^{-1} FW in 2023-24. Conversely, the lowest protein content was found in the no irrigation treatment (I_0), with values of 26.95 mg g^{-1} FW in 2022-23 and 30.21 mg g^{-1} FW in 2023-24, averaging 28.58 mg g^{-1} FW. A similar trend was observed at 90 DAS, where I_3 consistently exhibited the highest protein content (41.74 mg g^{-1} FW in 2022-23 and 46.87 mg g^{-1} FW in 2023-24), while I_0 had the lowest values (27.48 mg g^{-1} FW in 2022-23 and 33.32 mg g^{-1} FW in 2023-24). This trend suggested that adequate water availability supported protein synthesis by enhancing nutrient absorption and stimulating metabolic processes essential for protein formation. Adequate irrigation promoted cellular activities involved in amino acid and protein synthesis, ultimately improving plant health and nutritional quality (Saleem *et al.*, 2023). Protein content serves as a critical marker of plant vitality and nutritional value, with higher values indicating optimal physiological function under well-hydrated conditions. In contrast, the low protein content observed in the no-irrigation treatment (I_0) reflected the detrimental effects of water stress, likely due to reduced nutrient uptake and suppressed protein synthesis caused by limited metabolic activity. This aligned with previous findings emphasising the role of sufficient water in facilitating nutrient assimilation and metabolic pathways, leading to greater protein accumulation in crops (Farooq *et al.*, 2019).

The effect of different nutrient management treatments on the protein content of mustard plants was assessed at 60 and 90 Days after sowing (DAS) over the 2022-23 and 2023-24 growing seasons. Protein content, measured in mg g^{-1} fresh weight

(FW), varied with nutrient applications, showing higher levels in treatments with enhanced nutrient inputs. At 60 DAS, the highest protein content was recorded in the combined humic acid and sulphur treatment (S_3), with values of 36.35 mg g⁻¹ FW in 2022-23 and 40.08 mg g⁻¹ FW in 2023-24. The lowest protein content was observed in the control treatment (S_0), with values of 29.23 mg g⁻¹ FW in 2022-23 and 31.59 mg g⁻¹ FW in 2023-24. At 90 DAS, a similar trend was observed, with S_3 consistently showing the highest protein content (39.94 mg g⁻¹ FW in 2022-23 and 45.54 mg g⁻¹ FW in 2023-24, while S_0 recorded the lowest protein content (28.85 mg g⁻¹ FW in 2022-23 and 35.59 mg g⁻¹ FW in 2023-24). This increase suggested that humic acid and sulphur synergistically improved nutrient uptake and activated metabolic pathways vital for protein synthesis. Humic acid enhanced protein synthesis by improving nutrient availability, especially nitrogen and sulphur, which are essential for amino acid and protein formation, and by promoting enzyme production stimulated by microbial activity in the soil (Nardi and Francioso, 2021). Meanwhile, sulphur directly contributed to amino acid synthesis as a key component of sulphur-containing amino acids like cysteine and methionine, essential precursors for protein formation (Papet *et al.*, 2019).

4.6.5a Interaction between different irrigation regimes and different nutrient management on total soluble protein content at 60 DAS

The interaction between different irrigation regimes and nutrient management treatments on protein content in mustard plants was examined at 60 Days after sowing (DAS) across the 2022-23 and 2023-24 growing seasons. Protein content, measured in mg g⁻¹ fresh weight (FW), showed significant variation based on the combination of irrigation and nutrient management. The highest protein levels were observed in the treatment combining three irrigations (I_3) with humic acid and sulphur application (S_3), with values of 42.74 mg g⁻¹ FW in 2022-23 and 45.68 mg g⁻¹ FW in 2023-24. In contrast, the lowest protein content was recorded in the no irrigation and no nutrient treatment (I_0S_0), with values of 24.51 mg g⁻¹ FW in 2022-23 and 27.59 mg g⁻¹ FW in 2023-24. The highest protein content observed in the I_3S_3 treatment indicated that adequate soil moisture and essential nutrients collectively optimized metabolic functions, including protein synthesis. Humic acid and sulphur played a crucial role by promoting nutrient uptake and assimilation, creating favourable conditions for protein formation under well-irrigated settings (Ancín *et al.*, 2024).

4.6.5b Interaction between different irrigation regimes and different nutrient management on total soluble protein content at 90 DAS

The interaction between different irrigation regimes and nutrient management treatments on protein content in mustard plants was examined at 90 Days after sowing (DAS) across the 2022-23 and 2023-24 growing seasons. Protein content, measured in mg g⁻¹ fresh weight (FW), showed significant variation depending on the combination of irrigation and nutrient treatments. The highest protein levels were recorded in the treatment with three irrigations (I₃) combined with the application of humic acid and sulphur (S₃), yielding values of 47.48 mg g⁻¹ FW in 2022-23 and 50.72 mg g⁻¹ FW in 2023-24. Conversely, the lowest protein content was observed in the no irrigation and no nutrient treatment (I₀S₀), with values of 23.59 mg g⁻¹ FW in 2022-23 and 27.95 mg g⁻¹ FW in 2023-24. In contrast, the minimal protein levels found in the I₀S₀ treatment demonstrated the detrimental effects of water and nutrient deficiencies on protein synthesis, likely resulting from restricted nutrient transport and inhibited metabolic activity under stress conditions. Integrating practices that ensure sufficient water supply and balanced nutrient application, particularly of humic acid and sulphur, can enhance plant health, potentially leading to higher yields and resilience (Rathor *et al.*, 2023).

4.6.6 Impact of different irrigation regimes and different nutrient management on proline content of mustard crop

Proline content, measured in µg g⁻¹ FW, varied significantly across irrigation treatments, with higher proline levels observed under reduced irrigation. At 60 DAS, the highest proline content was recorded in the no irrigation treatment (I₀), with values of 104.73 µg g⁻¹ FW in 2022-23 and 103.40 µg g⁻¹ FW in 2023-24. Conversely, the lowest proline content was observed in the three-irrigation treatment (I₃), with values of 86.41 µg g⁻¹ FW in 2022-23 and 85.31 µg g⁻¹ FW in 2023-24. A similar trend was observed at 90 DAS, where I₀ exhibited the highest proline levels (149.08 µg g⁻¹ FW in 2022-23 and 146.37 µg g⁻¹ FW in 2023-24), while I₃ consistently showed the lowest values (125.91 µg g⁻¹ FW in 2022-23 and 123.63 µg g⁻¹ FW in 2023-24).

Table 4.21 Effect of humic acid and sulphur total soluble protein content of Indian mustard under variable water regimes

Treatments	Total soluble protein content (mg g ⁻¹ FW)			
	60 DAS		90 DAS	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24
I ₀	26.95	30.21	27.48	33.32
I ₁	31.71	34.45	32.85	39.49
I ₂	39.04	41.10	37.86	44.03
I ₃	39.22	41.90	41.74	47.87
SEm±	0.39	0.33	0.25	0.36
C.D at 5%	1.23	1.06	0.81	1.16
Sub plot (Nutrient management) (S)				
S ₀	29.23	31.59	28.85	35.59
S ₁	33.11	35.54	33.65	39.77
S ₂	36.68	39.94	37.49	42.80
S ₃	36.35	40.08	39.94	45.54
SEm±	0.28	0.30	0.33	0.28
C.D at 5%	0.79	0.86	0.93	0.81
Interaction (S*I)	1.66	1.80	1.914	1.693

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, DAS=Days after sowing, mg g⁻¹ FW = milligrams of protein per gram of fresh weight

Table 4.21A: Interaction table of total soluble protein content (mg g⁻¹ FW) of leaves at 60 DAS

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		24.51	26.41	27.64	29.25	26.95	27.59	29.24	30.22	33.78	30.21
I ₁		27.54	32.72	32.92	33.65	31.71	28.53	34.61	36.54	38.14	34.45
I ₂		29.97	35.45	36.78	39.77	37.49	32.41	37.54	39.72	42.71	38.10
I ₃		34.91	37.85	41.381	42.74	39.22	37.85	40.79	43.264	45.68	41.90
Mean (S)		29.23	33.11	34.68	36.35		31.59	35.54	37.44	40.08	
SEm±	S * I	0.770					0.660				
	I * S	0.612					0.617				
C.D at 5%	S* I	1.66					1.795				
	I* S	1.85					1.840				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, DAS=Days after sowing, mg g⁻¹ FW = milligrams of protein per gram of fresh weight

Table 4.21B: Interaction table of total soluble protein content (mg g^{-1} FW) of leaves at 90 DAS

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		145.86	149.41	151.20	149.86	149.08	143.19	146.69	148.44	147.14	146.37
I ₁		141.19	142.32	144.34	143.18	142.76	138.62	139.73	141.72	140.58	140.16
I ₂		130.63	134.32	137.21	135.20	134.34	128.26	131.88	134.72	132.74	131.90
I ₃		119.95	126.07	130.68 0	126.94	125.91	117.78	123.78	128.325	124.63	123.63
Mean (S)		134.41	138.03	140.86	138.79		131.96	135.52	138.30	136.27	
SEm±	S * I	1.457					1.385				
	I * S	1.050					1.019				
C.D at 5%	S* I	2.668					2.628				
	I* S	3.208					3.105				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, DAS=Days after sowing, mg g^{-1} FW = milligrams of protein per gram of fresh weight

Proline functioned as an osmo-protectant, stabilising cellular structures and maintaining osmotic balance under water stress, suggesting its critical role in the plant's adaptive response to drought (Hosseinifard *et al.*, 2022). Elevated proline levels in water-stressed plants indicated the activation of protective mechanisms, whereas lower proline levels in adequately irrigated plants (I_3) reflected reduced stress. These results highlighted proline as a reliable biochemical marker of water stress in mustard and underscored the plant's inherent adaptation to drought (Soni *et al.*, 2024). Understanding these responses can guide irrigation practices aimed at optimising water use and enhancing crop resilience, particularly in drought-prone regions (Sikka *et al.*, 2022).

Proline content, measured in $\mu\text{mole/g}$, varied with nutrient treatments, showing slightly higher levels in treatments with reduced or no additional nutrients. At 60 DAS, the highest proline content was observed in the control treatment (S_0), with values of $91.98 \mu\text{g g}^{-1}$ FW in 2022-23 and $90.81 \mu\text{g g}^{-1}$ FW in 2023-24. The lowest proline content at 60 DAS was recorded in the S_2 treatment, with values of $96.71 \mu\text{g g}^{-1}$ FW in 2022-23 and $95.47 \mu\text{g g}^{-1}$ FW in 2023-24. A similar trend was observed at 90 DAS, with S_0 again showing the highest proline levels ($134.41 \mu\text{g g}^{-1}$ FW in 2022-23 and $131.96 \mu\text{g g}^{-1}$ FW in 2023-24, while S_2 exhibited the lowest values ($140.86 \mu\text{g g}^{-1}$ FW in 2022-23 and $138.30 \mu\text{g g}^{-1}$ FW in 2023-24). Proline accumulation is often associated with stress responses, and the higher levels observed in the control treatment (S_0) likely indicated that nutrient deficiency prompted a mild stress response in the plants, increasing proline synthesis (Trovato *et al.*, 2019). Proline acts as an osmo-protectant, stabilising cell structures and maintaining osmotic balance under stress. In contrast, treatments with added nutrients (such as S_2) displayed lower proline levels, potentially reflecting reduced stress due to improved nutrient availability, which supports normal metabolic functioning and lessens the need for proline as a stress marker. These findings highlighted the role of proline as an indicator of plant stress, particularly in nutrient-deficient conditions (Dhaliwal *et al.*, 2022).

4.6.6a Interaction between different irrigation regimes and different nutrient management on proline content at 60 DAS

Proline content, measured in $\mu\text{g g}^{-1}$ FW, showed significant variations depending on the irrigation and nutrient combination. The highest proline content was observed in the no irrigation (I_0) combined with the S_2 nutrient treatment, with values

of 108.19 $\mu\text{g g}^{-1}$ FW in 2022-23 and 106.80 $\mu\text{g g}^{-1}$ FW in 2023-24. In contrast, the lowest proline levels were recorded under the three-irrigation regime (I_3) with the S_0 nutrient treatment, with values of 85.38 $\mu\text{g g}^{-1}$ FW in 2022-23 and 84.29 $\mu\text{g g}^{-1}$ FW in 2023-24. while I_3 exhibited the lowest across both years. Among nutrient treatments, S_2 showed the highest mean proline levels across irrigation regimes, while S_0 showed the lowest. Proline, a key osmo-protectant, accumulates under stress to support plants by maintaining osmotic balance and stabilising cellular components during adverse conditions (Ghosh, 2022). The elevated proline levels in the I_0S_2 treatment suggested that, under water-deficient conditions, supplementary nutrients enhanced stress-related metabolic pathways, stimulating proline synthesis as a protective response. Conversely, lower proline levels observed in the well-irrigated treatment (I_3) reflected reduced stress, as adequate water diminished the need for proline accumulation as a biochemical marker.

4.6.6b Interaction between different irrigation regimes and different nutrient management on proline content at 90 DAS

Proline content, measured in $\mu\text{g g}^{-1}$ FW, showed significant variation based on irrigation and nutrient combinations, with higher proline levels observed in treatments with less irrigation. The highest proline content was recorded in the no irrigation treatment (I_0) with the S_2 nutrient management, reaching 151.20 $\mu\text{g g}^{-1}$ FW in 2022-23 and 148.44 $\mu\text{g g}^{-1}$ FW in 2023-24. In contrast, the lowest proline content was observed in the three-irrigation (I_3) and no nutrient (S_0) combination, with values of 119.95 $\mu\text{g g}^{-1}$ FW in 2022-23 and 117.78 $\mu\text{g g}^{-1}$ FW in 2023-24. Among nutrient treatments, S_2 resulted in the highest mean proline levels across irrigation regimes, while S_0 showed the lowest. These findings highlighted proline's role as an indicator of both water and nutrient stress in mustard crops. The interaction between irrigation and nutrient inputs influencing proline levels underscored the potential for targeted water and nutrient management strategies to optimize plant stress adaptation (Ben *et al.*, 2024).

Table 4.22 Effect of humic acid and sulphur on proline content of Indian mustard under variable water regimes

Treatments	Proline content ($\mu\text{g g}^{-1}$ FW)			
	60 DAS		90 DAS	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24
I ₀	104.73	103.40	149.08	146.37
I ₁	97.01	95.77	142.76	140.16
I ₂	87.74	84.59	134.34	131.90
I ₃	86.41	85.31	125.91	123.63
SEm \pm	0.48	0.48	0.73	0.69
C.D at 5%	1.55	1.53	2.33	2.22
Sub plot (Nutrient management) (S)				
S ₀	94.98	97.81	140.01	141.96
S ₁	93.94	92.75	138.03	135.52
S ₂	96.71	95.47	140.86	138.30
S ₃	95.26	94.04	134.79	129.27
SEm \pm	0.39	0.38	0.44	0.43
C.D at 5%	1.11	1.09	1.25	1.24
Interaction (S*I)	2.319	2.288	2.668	2.628

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm \pm = Standard error of mean ,C.D=Critical difference, DAS=Days after sowing, $\mu\text{g g}^{-1}$ FW =microgram of proline per gram of fresh weight.

Table 4.22A: Interaction table of proline content ($\mu\text{g g}^{-1}$ FW) of leaves at 60 DAS

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		104.37	103.58	101.19	99.79	102.23	102.10	99.27	101.80	98.43	100.40
I ₁		94.08	96.50	99.89	97.56	97.01	92.88	95.27	98.61	96.32	95.77
I ₂		88.10	89.72	91.17	89.96	89.74	86.97	88.58	90.01	88.81	88.59
I ₃		85.38	85.98	87.583	86.72	86.41	84.29	84.88	86.464	85.62	85.31
Mean (S)		91.98	93.94	96.71	95.26		90.81	92.75	95.47	94.04	
SEm±	S * I	0.971					0.955				
	I * S	0.826					0.814				
C.D at 5%	S * I	2.319					2.288				
	I * S	2.483					2.446				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean ,C.D=Critical difference, DAS=Days after sowing, $\mu\text{g g}^{-1}$ FW =microgram of proline per gram of fresh weight.

Table 4.22B: Interaction table of proline content ($\mu\text{g g}^{-1}$ FW) of leaves at 90 DAS

Subplot (S) Main plot (I)	2022-23					2023-24				
	S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀	149.86	147.41	147.20	145.86	147.08	147.19	146.69	144.44	142.14	145.17
I ₁	141.19	142.32	144.34	143.18	142.76	138.62	139.73	141.72	140.58	140.16
I ₂	130.63	134.32	137.21	135.20	134.34	128.26	131.88	134.72	132.74	131.90
I ₃	119.95	126.07	130.68 0	126.94	125.91	117.78	123.78	128.325	124.63	123.63
Mean (S)	134.41	138.03	140.86	138.79		131.96	135.52	138.30	136.27	
SEm±	S * I	1.457				1.385				
	I * S	1.050				1.019				
C.D at 5%	S* I	2.668				2.628				
	I* S	3.208				3.105				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean ,C.D=Critical difference, DAS=Days after sowing, $\mu\text{g g}^{-1}$ FW = microgram of proline per gram of fresh weight.

4.7 Yield and yield attributes at harvest

4.7.1 Effect of different irrigation regimes and different nutrient management on number of primary branches, number of secondary branches, number of siliqua and average length of siliqua plant⁻¹ of Indian mustard (RLC3)

The effect of different irrigation regimes on mustard plant characteristics, including the number of primary and secondary branches, number of siliquae, and average siliqua length per plant, was assessed across the 2022-23 and 2023-24 growing seasons. Increased irrigation positively influenced all four parameters, with the highest values recorded in the three-irrigation treatment (I₃). In the number of primary branches, the I₃ treatment showed the highest count (8.44 in 2022-23 and 15.65 in 2023-24), while the no irrigation treatment (I₀) had the lowest average at 6.71 branches. For the number of secondary branches (23.87 in 2022-23 and 22.73 in 2023-24), and I₀ recorded the lowest at 9.93 branches. The number of siliquae per plant was highest in I₃, whereas I₀ showed the lowest value of 250.68 siliqua and highest value was found for 442.36 in 2023-24. For average siliqua length, I₃ again had the longest siliqua (5.86 cm in 2022-23 and 6.35 cm in 2023-24), while the shortest siliqua length was observed in I₀, 2.77 cm. Adequate water availability facilitated enhanced nutrient uptake, photosynthesis, and cell expansion, which collectively contributed to improved vegetative and reproductive development. These processes are critical during key growth stages, and their optimization under sufficient irrigation resulted in superior plant performance. In contrast, the absence of irrigation (I₀) imposed water stress, which restricted physiological processes such as nutrient assimilation and photosynthesis, ultimately reducing growth and yield potential. Water deficits during crucial growth phases inhibited cell division and expansion, resulting in lower branch numbers and diminished siliqua production and size. These findings were consistent with those of Islam (2021) and Kumar (2019), who reported that irrigation alleviates the adverse effects of water stress and supports higher crop productivity.

The effect of different nutrient management treatments on mustard plant characteristics, including the number of primary and secondary branches, number of siliquae, and average siliqua length per plant, was assessed across the 2022-23 and 2023-24 growing seasons. Higher nutrient input, especially with humic acid and sulphur (S₃), positively influenced all four parameters. In the number of primary branches, the S₃ treatment had the highest count (5.09 in 2022-23 and 5.11 in 2023-24),

while the lowest average was observed in the control (S_0) with 3.51 branches. For the number of secondary branches. The number of siliquae per plant was highest in the S_3 treatment, with 319.83 in 2022-23 and 444.95 in 2023-24, whereas S_0 showed the lowest average of 266.70 siliquae. For average siliqua length, S_3 again had the longest siliqua (5.09 cm in 2022-23 and 5.11 cm in 2023-24), while the shortest siliqua length was observed in S_0 . This improvement was attributed to enhanced nutrient uptake and metabolic activity, which supported vegetative growth and reproductive success. In contrast, the lower values observed in the control treatment (S_0) highlighted the adverse effects of nutrient deficiencies, which restricted growth and pod formation due to impaired metabolic and physiological processes (Kumar *et al.*, 2021). Similar studies demonstrated that humic acid improved root growth and nutrient availability, while sulphur played a critical role in protein synthesis and antioxidative defense, collectively supporting pod development (Rathor *et al.*, 2023; Singh *et al.*, 2024).

4.7.1a Interaction between different irrigation regimes and different nutrient management on number of primary branches plant⁻¹

Increased irrigation and nutrient input positively affected the number of primary branches, with the highest values observed under the three-irrigation regime (I_3) combined with humic acid and sulphur application (S_3). In 2022-23, the I_3S_3 combination resulted in an average of 10.75 primary branches, while in 2023-24, this combination increased to 17.06 branches. The lowest count was observed in the no irrigation (I_0) and no nutrient (S_0) treatment, with an average of 4.71 branches in 2022-23 and 6.13 branches in 2023-24. Irrigation facilitated optimal cell expansion, nutrient transport, and photosynthesis, while humic acid and sulphur improved root development, nutrient uptake, and enzymatic activities essential for shoot growth (Rana *et al.*, 2019; Ahmad *et al.*, 2023). Conversely, the lowest branch count in the I_0S_0 treatment underscored the detrimental effects of water and nutrient deficiencies, which limited cellular processes and vegetative development (Longnecker, 2021). Similar studies emphasised that integrated water and nutrient management strategies significantly improved vegetative traits in crops by addressing both water stress and nutrient limitations (Kang *et al.*, 2021; Ullah *et al.*, 2019). These findings highlighted the critical role of combining irrigation and nutrient management in enhancing branching, a key determinant of canopy structure and yield potential in mustard cultivation.

4.7.1b Interaction between different irrigation regimes and different nutrient management on number of secondary branches plant⁻¹

The interaction between different irrigation regimes and nutrient management treatments on the number of secondary branches per mustard plant was examined across the 2022-23 and 2023-24 growing seasons. Both increased irrigation and nutrient input positively impacted the number of secondary branches, with the highest counts observed in the three-irrigation regime (I₃) combined with humic acid and sulphur application (S₃). In 2022-23, the I₃S₃ treatment resulted in an average of 29.65 secondary branches, which slightly decreased to 29.13 branches in 2023-24. The lowest count was recorded in the no irrigation (I₀) and no nutrient (S₀) treatment, with an average of 6.68 branches in 2022-23 and 7.86 branches in 2023-24. Sufficient irrigation facilitated nutrient transport, photosynthesis, and hormonal regulation, creating optimal conditions for silique formation (Gupta *et al.*, 2024). Furthermore, humic acid and sulphur enhanced nutrient uptake, metabolic activity, and antioxidative defences, all of which were critical for reproductive success (de Moura *et al.*, 2023; Radi *et al.*, 2023). In contrast, the lowest silique count observed in the I₀S₀ treatment highlighted the adverse impact of water and nutrient deficiencies, which restricted resource allocation to reproductive organs (Manna and Siddique, 2009). Similar studies corroborated these findings, with Riar and McDonald (2020) reporting increased pod numbers under adequate water and nutrient regimes and Sekaran *et al.* (2021) emphasising the role of integrated management in improving reproductive traits. These results underscore the importance of holistic resource management, combining optimised irrigation and nutrient strategies, to maximise silique production and overall yield potential in mustard cultivation.

4.7.1c Interaction between different irrigation regimes and different nutrient management on average length of silique

The interaction between different irrigation regimes and nutrient management treatments on the average length of silique per mustard plant was analysed across the 2022-23 and 2023-24 growing seasons. Increased irrigation and nutrient application had a positive impact on silique length, with the longest silique observed under the three-irrigation regime (I₃) combined with humic acid and sulphur application (S₃). In 2022-23, the I₃S₃ treatment resulted in an average silique length of 6.56 cm, which increased to 7.42 cm in 2023-24. The shortest silique length was recorded in the no

irrigation (I₀) and no nutrient (S₀) treatment, with an average of 2.42 cm in 2022-23 and 2.78 cm in 2023-24. These physiological improvements directly contributed to higher seed count and yield potential. In contrast, the shortest siliqua length observed under the I₀S₀ treatment underscored the detrimental effects of resource limitations. Water stress hindered cell expansion and nutrient transport, impairing pod elongation, while nutrient deficiencies restricted protein synthesis and essential metabolic processes. This constrained physiological activity diminished reproductive development, leading to stunted siliqua formation (Nadeem *et al.*, 2009).

4.7.2 Effect of different irrigation regimes and different nutrient management on test weight (g), weight of siliqua plant⁻¹, seed yield (kg ha⁻¹) and oil content (%)

The effect of different irrigation regimes on test weight, weight of siliqua per plant, seed yield, and oil content of mustard was evaluated across the 2022-23 and 2023-24 growing seasons. Increased irrigation positively influenced all four parameters, with the highest values observed under the three-irrigation regime (I₃). For test weight, the I₃ treatment produced the highest values, with an average of 4.38 g in 2022-23 and 4.44 g in 2023-24. In contrast, the no irrigation treatment (I₀) showed the lowest test weight. The weight of siliqua per plant was also highest in the I₃ treatment, averaging 45.48 g in 2022-23 and 45.55 g in 2023-24. The lowest siliqua weight was found in the I₀ treatment. In terms of seed yield, the I₃ treatment again achieved the highest results, with 2752.45 kg ha⁻¹ in 2022-23 and 2969.13 kg ha⁻¹ in 2023-24. The lowest seed yield was recorded in the I₀ treatment. For oil content, the I₃ treatment exhibited the highest oil percentages, with 41.21% in 2022-23 and 46.89% in 2023-24. The I₀ treatment had the lowest oil content across both years. The substantial increase in seed yield and oil content under the I₃ treatment highlighted the pivotal role of irrigation in promoting reproductive success and oil biosynthesis in mustard, as supported by studies like Sharma *et al.* (2024) and Ghadirnezhad Shiade *et al.* (2023). Adequate water availability supported photosynthesis and other metabolic functions necessary for seed formation and oil accumulation (Ebrahimian *et al.*, 2019).

Table 4.23 Effect of humic acid and sulphur on the number of primary and secondary branches and number of siliquae of Indian mustard under variable water regimes

Treatment	No. of pri. branches plant ⁻¹		No. of second. branches plant ⁻¹		No. of siliqua plant ⁻¹	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
I ₀	6.71	9.43	9.60	9.93	250.68	396.05
I ₁	7.06	11.41	14.86	14.81	273.70	416.19
I ₂	7.92	12.89	16.96	18.84	311.79	426.03
I ₃	8.44	15.65	21.87	24.73	335.55	442.36
SEm±	0.15	0.19	0.52	0.40	4.80	7.19
C.D at 5%	0.49	0.61	1.65	1.29	15.37	23.00
Sub plot (Nutrient management) (S)						
S ₀	5.45	9.66	11.44	12.02	266.70	398.65
S ₁	8.40	13.17	17.00	17.15	286.33	415.22
S ₂	6.29	11.84	15.57	16.10	298.86	421.81
S ₃	10.00	14.71	19.08	21.95	319.83	444.95
SEm±	0.08	0.17	0.35	0.39	2.23	7.23
C.D at 5%	0.23	0.48	1.02	1.12	6.39	20.73
Interaction (S*I)	0.501	1.005	2.150	2.316	NS	NS

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (I₁+ Flowering Stage), I₃ =Three Post Sowing Irrigation (I₁+I₂Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, NS= Non-significant, DAS=Days after sowing, No. of 1^o Branches plant⁻¹= Number of primary branches per plant, No. of 2^o Branches Plant⁻¹= Number of secondary branches per plant.

Table 4.23A: Interaction table of number of primary branches plant⁻¹

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
	I ₀	4.71	7.13	5.67	9.31	6.71	6.13	10.29	8.83	12.47	9.43
	I ₁	5.14	7.53	6.12	9.47	7.06	9.30	11.94	10.52	13.88	11.41
	I ₂	5.50	9.21	6.52	10.47	7.92	10.47	14.18	11.49	15.44	12.89
	I ₃	6.43	9.71	6.86	10.75	8.44	12.74	16.27	16.53	17.06	15.65
	Mean (S)	5.45	8.40	6.29	10.00		9.66	13.17	11.84	14.71	
SEm±	S * I	0.305					0.381				
	I * S	0.208					0.348				
C.D at 5%	S* I	0.501					1.005				
	I* S	0.639					1.040				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, NS= Non-significant, DAS=Days after sowing, No. of 1° Branches Plant⁻¹= Number of primary branches per plant.

Table 23B: Interaction table of number of secondary branches plant⁻¹

Subplot (S) Main plot (I)	2022-23					2023-24				
	S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀	6.68	8.95	8.53	14.24	9.60	7.86	10.59	9.86	11.42	9.93
I ₁	9.87	15.62	14.86	19.08	14.86	10.59	14.89	13.97	19.80	14.81
I ₂	12.03	18.15	15.50	22.15	16.96	13.30	19.36	18.84	23.84	18.84
I ₃	17.15	25.28	23.38	29.65	23.87	16.31	23.75	21.71	29.13	22.73
Mean (S)	11.44	17.00	15.57	21.28		12.02	17.15	16.10	21.05	
SEm±	S * I	1.031				0.804				
	I* S	0.802				0.785				
C.D at 5%	S* I	2.150				2.316				
	I* S	2.430				2.336				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, NS= Non-significant, DAS=Days after sowing, No. of 2° Branches Plant⁻¹= Number of secondary branches per plant.

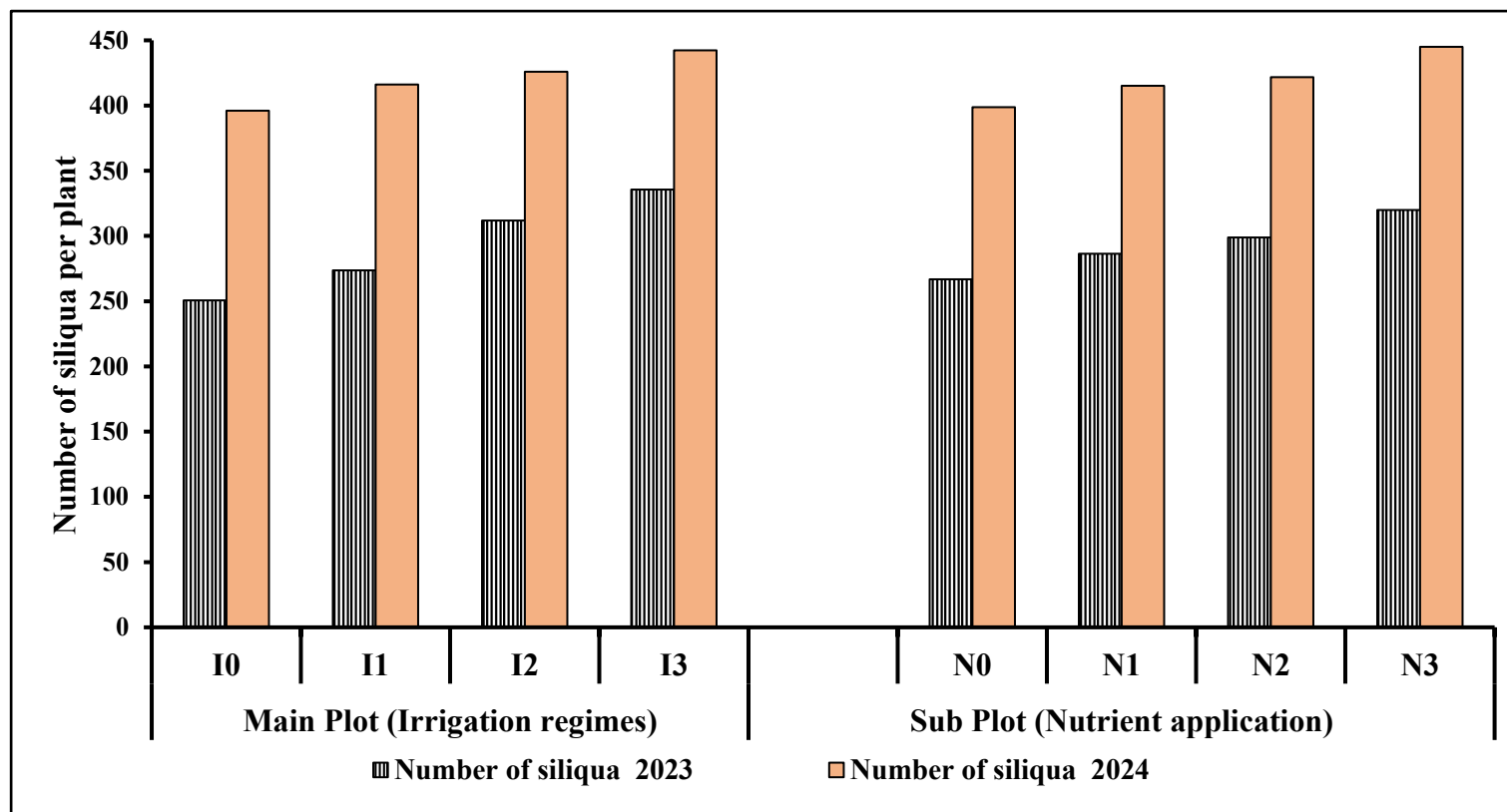


Figure 4.10 Effect of different irrigation regimes and different nutrient management on the number of siliqua plant⁻¹ in Indian mustard in 2022-23 and 2023-24

The higher oil content observed in the I₃ treatment was particularly beneficial, adding economic value to the crop and enhancing the commercial appeal of mustard oil. In contrast, the lowest values across all parameters in the no irrigation (I₀) treatment illustrated the restrictive effects of water stress on crop performance. Water deficits likely limited nutrient absorption and cellular expansion, leading to reduced siliqua formation, lower seed yield, and diminished oil content (Sujat *et al.*, 2023). These findings underscore the importance of effective irrigation strategies, particularly during critical growth stages, to maximise yield and economic value in semi-arid regions (Hussain *et al.*, 2020).

The effect of different nutrient management treatments on test weight, weight of siliqua per plant, seed yield, and oil content of mustard was evaluated across the 2022-23 and 2023-24 growing seasons. Higher nutrient input, especially with the combined application of humic acid and sulphur (S₃), positively influenced all four parameters. For test weight, the S₃ treatment produced the highest values, with an average of 4.80 g in 2022-23 and 4.02 g in 2023-24. The lowest test weight was observed in the control treatment (S₀) in both years. The weight of siliqua per plant was also highest in the S₃ treatment, 39.60 g in 2022-23 and 45.37 g in 2023-24. In terms of seed yield, the S₃ treatment achieved the highest results, with 2482.17 kg ha⁻¹ in 2022-23 and 2680.31 kg ha⁻¹ in 2023-24. The lowest seed yield was recorded in the S₀ treatment, with 1216.07 kg ha⁻¹ in 2022-23 and 1328.83 kg ha⁻¹ in 2023-24. For oil content, the S₃ treatment exhibited the highest percentages, with 44.33% in 2022-23 and 47.02% in 2023-24. The S₀ treatment had the lowest oil content, with 33.60% in 2022-23 and 37.18% in 2023-24. These improvements aligned with findings by Mahmud *et al.* (2023), who reported that humic acid enhances soil structure, improves nutrient uptake, and fosters plant vigour, thereby directly contributing to better seed filling and siliqua development. The higher test weight and siliqua weight observed under S₃ reflected improved reproductive success, consistent with Ranjan *et al.* (2023), who highlighted the benefits of organic amendments and sulphur in boosting crop productivity. Sulphur played a pivotal role in amino acid synthesis and protein formation, both essential for seed development and oil biosynthesis, as demonstrated by Pramanik *et al.* (2022). The notable increase in oil content under the S₃ treatment further supported this, aligning with the findings of Joshi *et al.* (2021), who linked sulphur nutrition to enhanced oil accumulation in oilseed crops. These combined effects

not only improved yield and quality but also increased the economic viability of mustard cultivation. The enhanced productivity and oil content under S₃ made mustard a more profitable and attractive crop for farmers, emphasizing the importance of balanced nutrient management in sustainable agriculture.

4.7.2a Interaction between different irrigation regimes and different nutrient management on test weight (g) of mustard crop

The interaction between different irrigation regimes and nutrient management treatments on test weight of mustard seeds was analysed across the 2022-23 and 2023-24 growing seasons. Both increased irrigation and enhanced nutrient application positively impacted test weight, with the highest values observed under the three-irrigation regime (I₃) combined with humic acid and sulphur application (S₃). In 2022-23, the I₃S₃ treatment produced a test weight of 4.99 g, which was consistent at 4.95 g in 2023-24. In contrast, the lowest test weight was recorded in the no irrigation (I₀) and no nutrient (S₀) treatment, with values of 2.52 g in 2022-23 and 2.31 g in 2023-24, giving a mean of 2.42 g. Additionally, humic acid and sulphur application improved soil fertility and nutrient uptake efficiency, directly influencing protein synthesis and seed development (Belal *et al.*, 2019). Humic substances enhanced root growth and nutrient absorption by improving soil structure and stimulating microbial activity (Nardi *et al.*, 2021). Sulphur, an essential macronutrient, played a vital role in synthesizing sulphur-containing amino acids and proteins, contributing to seed density and weight (Nagesh *et al.*, 2024). In contrast, the lowest test weight was recorded in the I₀S₀ treatment, characterized by the absence of irrigation and nutrient supplementation. Water scarcity likely restricted the translocation of nutrients and carbohydrates to developing seeds, as corroborated by Martínez-Ballesta *et al.* (2020), who emphasized the role of water availability in determining reproductive success and seed weight. Similarly, nutrient limitations impeded cellular processes, reducing the accumulation of storage reserves in seeds (Bakhtavar and Afzal, 2020).

4.7.2b Interaction between different irrigation regimes and different nutrient management on weight of silique plant⁻¹ of mustard crop

Both increased irrigation and nutrient application positively impacted silique weight, with the highest values recorded under the three-irrigation regime (I₃) combined with humic acid and sulphur application (S₃). In 2022-23, the I₃S₃ treatment resulted in a silique weight of 43.69 g, which increased further in 51.16 g in 2023-24. Conversely,

the lowest silique weight was observed in the no irrigation (I₀) and no nutrient (S₀) treatment, with values of 16.37 g in 2022-23 and 19.79 g in 2023-24. Adequate irrigation improved nutrient transport and cell expansion, consistent with findings by Rana *et al.* (2019), who reported that water availability during critical growth stages enhanced reproductive success. The role of humic acid in improving soil structure and root development, alongside Sulphur's contribution to nutrient uptake and amino acid synthesis, further supported silique formation and weight, as observed by Feng and Zhang (2021) and Shah and Mohammad (2022). In contrast, the lowest silique weight under the I₀S₀ treatment highlighted the impact of water and nutrient scarcity on reproductive performance, as restricted translocation of nutrients limited silique development. These findings aligned with Kumar *et al.* (2015), who emphasized the negative effects of water and nutrient deficits on crop productivity.

4.7.2c Interaction between different irrigation regimes and different nutrient management on seed yield (kg ha⁻¹)

Both increased irrigation and nutrient input had a positive effect on seed yield, with the highest yields observed under the three-irrigation regime (I₃) combined with humic acid and sulphur application (S₃). In 2022-23, the I₃S₃ treatment yielded 2752.45 kg/ha, which increased to 3797.60 kg ha⁻¹ in 2023-24. In contrast, the lowest seed yield was recorded in the no irrigation (I₀) and no nutrient (S₀) treatment, with values of 633.75 kg ha⁻¹ in 2022-23 and 706.79 kg ha⁻¹ in 2023-24. The I₃S₃ treatment, combining optimal irrigation with humic acid and sulphur, significantly improved productivity by supporting growth and seed development. Adequate irrigation facilitated nutrient transport and photosynthesis, as noted by Ullah *et al.* (2019) and Meena *et al.* (2024), boosting biomass and yield. Humic acid enhanced soil health and nutrient uptake (Zhou *et al.*, 2019), while sulphur supported amino acid synthesis and photosynthesis, improving seed yield (Shah *et al.*, 2022). In contrast, the lowest yields in I₀S₀ highlighted the detrimental effects of water and nutrient scarcity, which inhibited physiological functions essential for growth, as observed by Ostmeier *et al.* (2020). These findings underscored the importance of integrated strategies in irrigation and nutrient management, ensuring higher yields and sustainable mustard cultivation. Hayati *et al.* (2022) reported that such approaches increased oilseed productivity by 20–30%.

4.7.2d Interaction between different irrigation regimes and different nutrient management on oil content (%)

The interaction between different irrigation regimes and nutrient management treatments on oil content in mustard seeds was evaluated across the 2022-23 and 2023-24 growing seasons. Both increased irrigation and enhanced nutrient input positively influenced oil content, with the highest values recorded under the three-irrigation regime (I_3) combined with humic acid and sulphur application (S_3). In 2022-23, the I_3S_3 treatment produced an oil content of 47.43%, which further increased to 51.13% in 2023-24. The lowest oil content was observed in the no irrigation (I_0) and no nutrient (S_0) treatment, with values of 28.32% in 2022-23 and 28.89% in 2023-24. Adequate irrigation enhanced nutrient uptake, photosynthesis, and metabolic pathways essential for lipid synthesis. Similar findings by Sajid *et al.* (2023) reported that proper irrigation during flowering and seed development stages improved photosynthate translocation, leading to higher oil content. Furthermore, Ebrahimian *et al.* (2019) emphasized that irrigation facilitated lipid metabolism, boosting oil yields in oilseed crops. Humic acid contributed to this improvement by enhancing soil structure, root health, and nutrient availability, which collectively increased enzymatic efficiency critical for lipid synthesis (Vikram *et al.*, 2022). Sulphur played a particularly vital role in mustard oil production by aiding fatty acid synthesis and improving oil quality. As noted by Karmakar *et al.* (2024), sulphur fertilization enhanced enzyme activity linked to photosynthesis and glucosinolate production, both of which are essential for high-quality oil. In contrast, the lowest oil content observed in the I_0S_0 treatment highlighted the negative effects of water and nutrient deficiencies, which severely restricted physiological processes required for lipid biosynthesis. Odukoya *et al.* (2019) similarly observed that water scarcity limited nutrient transport and fatty acid synthesis, reducing oil yield. These results underscored the critical role of integrating irrigation with nutrient management. The I_3S_3 treatment not only maximized oil content but also demonstrated its potential for economic and sustainable mustard cultivation, aligning with findings by Manna and Siddique (2024), who advocated for combined water and nutrient strategies to ensure optimal yield and profitability.

Table 4.24 Influence of humic acid and sulphur on average length and weight of siliqua plant per plant and test weight of Indian mustard under variable water regimes

Treatment	Average length of siliqua plant ⁻¹ (cm)		Weight of siliqua plant ⁻¹ (g)		Test weight (g)	
	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
Main plot (Irrigation regimes) (I)						
I ₀	2.77	3.16	21.27	27.94	3.74	2.61
I ₁	3.30	3.74	26.10	33.24	3.99	3.15
I ₂	4.57	4.20	33.47	41.13	4.17	3.91
I ₃	5.86	6.35	45.48	45.55	4.38	4.44
SEm±	0.09	0.12	0.41	0.62	0.03	0.08
C.D at 5%	0.29	0.37	1.31	2.00	0.09	0.26
Sub plot (Nutrient management) (S)						
S ₀	3.51	3.77	22.13	26.36	3.10	3.03
S ₁	4.01	4.38	30.19	35.08	3.85	3.58
S ₂	3.90	4.18	34.40	41.06	4.48	3.47
S ₃	5.09	5.11	39.60	45.37	4.64	4.81
SEm±	0.08	0.08	0.43	0.40	0.03	0.01
C.D at 5%	0.24	0.23	1.24	1.16	0.10	0.03
Interaction (S*I)	0.49	0.50	2.55	2.46	0.20	0.08

* I=irrigation regimes, N=nutrient management, I₀=No Post Sowing Irrigation], I₁=One Post Sowing Irrigation (Vegetative stage), I₂=Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃=Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, plant⁻¹= per plant ,Cm=Centimeter, g=gram

Table 4.24A: Interaction table of average length of siliqua plant⁻¹ (cm)

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		2.42	2.75	2.54	3.36	2.77	2.78	3.15	3.11	3.60	3.16
I ₁		2.80	3.27	3.40	3.74	3.30	3.09	3.48	3.44	4.93	3.74
I ₂		3.57	3.91	4.12	6.68	4.57	3.87	4.25	4.19	4.48	4.20
I ₃		5.26	6.11	5.527	6.56	5.86	5.35	6.65	5.965	7.42	6.35
Mean (S)		3.51	4.01	3.90	5.09		3.77	4.38	4.18	5.11	
SEm±	S * I	0.18					0.23				
	I * S	0.15					0.17				
C.D at 5%	S * I	0.49					0.47				
	I * S	0.50					0.54				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, plant⁻¹= per plant

Table 4.24B: Interaction table of weight of siliqua plant⁻¹ (g)

Subplot (S) Main plot (I)	2022-23					2023-24				
	S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀	16.37	19.65	23.98	25.09	21.27	19.79	26.34	31.49	34.14	27.94
I ₁	18.89	25.04	28.62	31.87	26.10	24.48	33.16	35.38	39.94	33.24
I ₂	22.85	30.62	36.73	43.69	33.47	28.95	39.17	45.24	51.16	41.13
I ₃	30.40	45.46	48.285	57.78	45.48	32.21	41.64	52.118	56.24	45.55
Mean (S)	22.13	30.19	34.40	39.60		26.36	35.08	41.06	45.37	
SEm±	S * I	0.82				1.24				
	I * S	0.85				0.93				
C.D at 5%	S* I	2.55				2.46				
	I * S	2.52				2.84				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, plant⁻¹= per plant ,g=grams

Table 4.24C: Interaction table of test weight (g)

Subplot (S) Main plot (I)	2022-23					2023-24				
	S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀	2.52	3.58	4.19	4.55	3.74	2.31	2.64	2.43	3.06	2.61
I ₁	3.03	3.77	4.33	4.78	3.99	2.53	3.15	3.29	3.63	3.15
I ₂	3.20	3.98	4.63	4.87	4.17	3.38	3.80	4.01	4.46	3.91
I ₃	3.64	4.05	4.795	4.99	4.38	3.90	4.75	4.167	4.95	4.44
Mean (S)	3.10	3.85	4.48	4.80		3.03	3.58	3.47	4.02	
SEm±	S * I	0.054				0.163				
	I * S	0.065				0.084				
C.D at 5%	S* I	0.200				0.076				
	I* S	0.191				0.271				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, DAS=Days after sowing, g=grams

4.7.3 Effect of different irrigation regimes and different nutrient management on oil yield (kg ha⁻¹), biological harvest (kg ha⁻¹), harvest index and moisture content (%) of mustard crop

During the 2022–2023 and 2023–2024 growing seasons, the impact of various irrigation schedules on mustard oil's moisture content, biological harvest, harvest index, and oil output was examined. All parameters were positively impacted by increased irrigation, with the three-irrigation regime (I₃) showing the highest values. With an average of 1151.92 kg ha⁻¹ in 2022–2023 and 1407.10 kg ha⁻¹ in 2023–2024, the I₃ treatment produced the most oil. I₀ produced the least amount of oil. With values of 6972.12 kg ha⁻¹ in 2022–2023 and 7438.74 kg ha⁻¹ in 2023–2024, the I₃ treatment had the highest biological harvest. On the other hand, with 1177.11 kg ha⁻¹, the I₀ treatment had the lowest biological harvest. The harvest index was highest with the I₃ treatment, 38.67% in 2022–2023 and 38.47% in 2023–2024. With values of 0.28% in 2022–2023 and 0.40% in 2023–2024, the I₃ treatment had the lowest moisture content in oil. The I₃ treatment, with optimal irrigation, recorded the highest oil yield, reflecting the positive effects of adequate water on seed development and oil biosynthesis. Irrigation facilitated nutrient transport, photosynthesis, and lipid metabolic processes, all essential for oil accumulation. Kaur *et al.* (2024) observed a 30% increase in oil yield in oilseed crops under sufficient irrigation compared to water-deficient conditions. Furthermore, reduced moisture content in oil from the I₃ treatment indicated better storage stability and quality, supported by Wijewardana *et al.* (2020), who found that optimal water availability during seed maturation enhanced oil properties.

The I₃ treatment also demonstrated superior biological harvest and harvest index, highlighting improved biomass production and resource-use efficiency. Adequate water availability enhanced root activity, nutrient uptake, and photosynthetic efficiency, leading to increased biomass. Wijewardana *et al.* (2019) reported that higher irrigation levels improved biological yield in oilseed crops by optimizing vegetative and reproductive growth. Increased irrigation in the I₃ treatment enhanced primary and secondary branch development, siliqua number, and siliqua length, which are critical for higher yields as they determine seed-setting capacity. Mamatha *et al.* (2022) noted that irrigation significantly improved siliqua size and count by reducing water stress during flowering and seed formation stages.

During the 2022–2023 and 2023–2024 growing seasons, the impact of various nutrient management techniques on mustard oil's moisture content, biological harvest, harvest index, and oil output was assessed. All metrics benefited from increased nutrient input, especially when sulphur (S_3) and humic acid were applied. With 1151.92 kg ha⁻¹ in 2022–2023 and 1407.10 kg ha⁻¹ in 2023–2024, the S_3 treatment produced the most oil, with 1117.73 kg ha⁻¹ in 2022-23 and 1274.04 kg ha⁻¹ in 2023–2024. The control treatment (S_0) produced the least amount of oil, over two years. With values of 6486.68 kg ha⁻¹ in 2022–2023 and 6941.34 kg ha⁻¹ in 2023–2024, the S_3 treatment had the largest biological harvest. On the other hand, the biological harvest from the S_0 treatment was the lowest. The S_3 treatment had the highest harvest index, with values of 39.85% in 2022–2023 and 39.69% in 2023–2024. S_0 treatment had the lowest harvest index. With values of 0.43% in 2022–2023 and 0.53% in 2023–2024, the S_3 treatment had the highest moisture content in oil. Humic acid enhanced root health and nutrient uptake, while sulphur played a vital role in lipid metabolism and enzyme activation, critical for oil biosynthesis. Studies by Izhar Shafi *et al.* (2020) and Rathore *et al.* (2022) confirmed that sulphur supplementation in mustard increased fatty acid synthesis and oil content. Moreover, the reduced moisture content in oil under S_3 indicated superior quality and storage potential, consistent with findings by Sharma *et al.* (2021). In contrast, the S_0 treatment recorded the lowest oil yield and biological harvest, highlighting the adverse effects of nutrient deficiency on plant metabolism and seed development. Li *et al.* (2022) observed similar outcomes, where insufficient nutrients limited biomass accumulation and oil biosynthesis in oilseed crops. The I_3S_3 treatment demonstrated the highest oil yield, illustrating the synergistic effects of adequate water and nutrients. Optimal irrigation enhanced nutrient transport, photosynthesis, and metabolic activities, while humic acid and sulphur further improved nutrient absorption and enzymatic functions. This interaction created an ideal environment for oil biosynthesis and seed filling, as supported by Shahrajabian and Sun (2024). Conversely, the lowest oil yield in the I_0S_0 treatment highlighted the compounded negative impact of water stress and nutrient deficiency, which inhibited physiological functions necessary for crop productivity. Singh *et al.* (2021) reported that water scarcity and poor nutrient availability significantly reduced seed quality and oil content in mustard crops.

4.7.3a Interaction between different irrigation regimes and different nutrient management on oil yield (kg ha⁻¹) of mustard crop

Both increased irrigation and nutrient application positively impacted oil yield, with the highest yields recorded under the three-irrigation regime (I₃) combined with humic acid and sulphur application (S₃). In 2022-23, the I₃S₃ treatment produced an oil yield of 1672.69 kg ha⁻¹, which increased to 1941.08 kg ha⁻¹ in 2023-24. Conversely, the lowest oil yield was observed in the no irrigation (I₀) and no nutrient (S₀) treatment, with values of 179.03 kg ha⁻¹ in 2022-23 and 203.93 kg ha⁻¹ in 2023-24. Studies such as Kumar *et al.* (2021) showed that optimized irrigation schedules in mustard substantially enhanced seed yield and oil content by ensuring water availability during critical growth phases. Similarly, Amirkhiz *et al.* (2021) highlighted that irrigation improved enzymatic activities and metabolic pathways involved in lipid biosynthesis, contributing to higher oil yields. The application of humic acid and sulphur further amplified oil yield by promoting root development, nutrient uptake, and enzymatic processes essential for plant metabolism. Humic acid increased microbial activity and enhanced the bioavailability of macro- and micronutrients, while sulphur played a vital role in synthesizing amino acids and enzymes critical for oil biosynthesis. Research by Shah *et al.* (2022) demonstrated that sulphur application enhanced chlorophyll content and improved oil quality in mustard crops. Song *et al.* (2022) also found that humic acid improved nutrient-use efficiency and plant growth, leading to better yield outcomes. In contrast, the lowest oil yield, observed under the I₀S₀ treatment, underscored the detrimental effects of water stress and nutrient deficiency. Limited water availability disrupted physiological processes such as cell turgor, enzymatic activity, and nutrient transport, inhibiting oil synthesis and seed development. These findings aligned with Qiao *et al.* (2024), who reported that water stress significantly reduced oil content by impairing photosynthesis and assimilate translocation. Nutrient deficiencies further exacerbated these effects, as insufficient levels of sulphur and humic acid constrained metabolic activity and oil formation.

4.7.3b Interaction between different irrigation regimes and different nutrient management on biological harvest (kg ha⁻¹) of mustard crop

The interaction between different irrigation regimes and nutrient management treatments on the biological harvest per hectare in mustard was analysed across the 2022-23 and 2023-24 growing seasons. Both increased irrigation and nutrient

application positively impacted biological harvest, with the highest values observed under the three-irrigation regime (I_3) combined with humic acid and sulphur application (S_3). In 2022-23, the I_3S_3 treatment produced a biological harvest of 8012.59 kg ha⁻¹, which increased to 8705.57 kg ha⁻¹ in 2023-24. In contrast, the lowest biological harvest was recorded in the no irrigation (I_0) and no nutrient (S_0) treatment, with values of 3236.64 kg ha⁻¹ in 2022-23 and 3511.37 kg ha⁻¹ in 2023-24. This finding aligned with established evidence that adequate irrigation promotes nutrient solubility and uptake, enabling efficient photosynthesis and plant metabolism (Farooq *et al.*, 2019). These physiological improvements drove enhanced vegetative growth and biomass accumulation.

The application of humic acid in the I_3S_3 treatment likely boosted nutrient efficiency and root architecture, fostering greater water and nutrient uptake. Humic substances are known to improve soil structure, enhance cation exchange capacity, and promote microbial activity, contributing to better nutrient availability (Guo *et al.*, 2019). Similarly, sulphur supplementation supported protein synthesis and enzymatic activities, essential for plant growth, particularly in sulphur-responsive crops like mustard (Zenda *et al.*, 2021). Conversely, the reduced biological harvest in the I_0S_0 treatment underscored the detrimental impact of water and nutrient scarcity. Limited irrigation likely inhibited cellular processes such as photosynthesis and turgor maintenance, restricting plant growth (Singh *et al.*, 2021). Nutrient deficiencies, particularly in nitrogen and sulphur, further exacerbated these limitations, as these nutrients are critical for chlorophyll synthesis and energy metabolism (Muneer *et al.*, 2024).

4.7.3c Interaction between different irrigation regimes and different nutrient management on number of harvest index

Increased irrigation and nutrient input positively influenced the harvest index, with the highest values recorded under the three-irrigation regime (I_3) combined with humic acid and sulphur application (S_3). In 2022-23, the I_3S_3 treatment achieved a harvest index of 46.97%, which was consistent at 46.65% in 2023-24. The lowest harvest index was observed in the no irrigation (I_0) and no nutrient (S_0) treatment, with values of 19.59% in 2022-23 and 20.15% in 2023-24. A higher harvest index indicated efficient allocation of resources toward reproductive growth and seed production relative to vegetative biomass, a crucial factor for maximizing yield efficiency (Hossain

et al., 2019). The I₃ treatment ensured adequate water availability, which enhanced photosynthesis, nutrient translocation, and assimilate partitioning to developing seeds, thereby boosting yield components such as seed weight and number (Farooq *et al.*, 2019). The synergistic effect of humic acid and sulphur in the S₃ treatment likely contributed to enhanced root growth and nutrient uptake efficiency. Humic acids improved soil structure, promoted root elongation, and facilitated nutrient absorption, while sulphur played a key role in protein synthesis and enzymatic activities essential for reproductive development (Chen *et al.*, 2022). Enhanced nutrient availability and utilization directly supported seed filling and maturation, further increasing the harvest index. Conversely, the I₀S₀ treatment exhibited the lowest harvest index due to water and nutrient limitations, which impaired photosynthesis and carbohydrate production, reducing the translocation of assimilates to reproductive organs. This aligned with findings by Harrison Day *et al.* (2022), who reported that water stress reduced reproductive success in plants by limiting resource availability during critical growth stages. Nutrient deficiencies, particularly nitrogen and sulphur, further compromised seed production by disrupting metabolic pathways essential for reproductive development (Raza, 2021).

4.8 Oil quality parameters

4.8.1 Effect of different irrigation regimes and different nutrient management on relative density, antioxidant activity, total phenolic content and saponification value of mustard oil

The results showed that increased irrigation reduced relative density, antioxidant activity, and total phenolic content while increasing the saponification value. The three-irrigation regime (I₃) produced the lowest RD, with values of 0.86% in 2022-23 and 2023-24. The highest RD was observed in the no irrigation treatment (I₀), with a mean of 0.95%. Antioxidant activity decreased with increased irrigation, with I₃ showing the lowest AA at 24.89% in 2022-23 and 25.78% in 2023-24. The highest AA was observed in the I₀ treatment 34.03% in 2022-23 and 35.73% in 2023-24. TPC also decreased with more irrigation.

Table 4.25 Impact of humic acid and sulphur on seed yield, biological yield, and harvest index of Indian mustard under variable water regimes

Treatment	Seed yield (kg ha ⁻¹)		Biological yield (kg ha ⁻¹)		Harvest index (%)	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
I ₀	1177.11	1287.13	4536.00	4901.82	24.33	24.57
I ₁	1436.40	1563.75	5181.75	5598.49	32.09	32.20
I ₂	2080.19	2251.61	5513.29	6007.59	37.21	37.13
I ₃	2752.45	2969.13	6972.12	7438.74	38.45	38.67
SEm±	32.87	36.46	108.36	118.43	0.94	0.95
C.D at 5%	106.65	118.29	351.58	384.24	2.82	2.86
Sub plot (Nutrient management) (S)						
S ₀	1216.07	1328.83	4620.34	4834.57	24.64	24.88
S ₁	1652.36	1794.85	5876.90	6401.60	28.91	28.96
S ₂	2095.55	2267.64	5219.24	5769.14	38.89	38.84
S ₃	2482.17	2680.31	6486.68	6941.34	39.65	39.81
SEm±	35.85	38.19	89.15	103.40	0.85	0.84
C.D at 5%	103.25	109.99	256.75	297.50	2.41	2.41
Interaction (S*I)	212.21	226.47	534.93	618.50	4.97	4.98

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C.D=Critical difference, DAS=Days after sowing, kg ha⁻¹=Kilograms of seed produced per hectare of land

Table 4.25A: Interaction table of seed yield (kg ha⁻¹)

Subplot (S) Main plot (I)	2022-23					2023-24				
	S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀	633.75	1102.9 2	1378.0 7	1032.3 4	1177.11	706.79	1207.98	1501.61	1732.1 5	1287.13
I ₁	880.30	1338.1 3	1619.5 4	1474.3 2	1436.40	970.14	1459.03	1759.20	2066.6 4	1563.75
I ₂	1366.59	1740.9 8	2314.7 8	2304.0 0	2080.19	1489.8 5	1890.07	2501.66	3124.8 7	2251.61
I ₃	1983.63	2427.4 3	3069.8 1	3528.9 3	2752.45	2148.5 5	2622.31	3308.07	3797.6 0	2969.13
Mean (S)	1216.07	1652.3 6	2095.5 5	2482.1 7		1328.8 3	1794.85	2267.64	2680.3 1	
SEm±	S * I	65.74				72.93				
	I * S	70.261				75.54				
C.D at 5%	S * I	212.21				226.47				
	I * S	207.95				223.95				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, DAS=Days after sowing, kg ha⁻¹=Kilograms of seed produced per hectare of land

Table 4.25B: Interaction table of biological yield (kg ha⁻¹)

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		3236.6 4	6370.5 5	4314.1 3	5334.8 1	4814.03	3511.3 7	5787.62	4681.30	6912.7 4	5223.2 6
I ₁		4505.1 0	4446.2 1	4472.3 1	4504.5 9	4482.05	4824.3 1	4886.94	4852.63	4885.4 1	4862.3 2
I ₂		4644.8 5	5653.6 0	5558.1 8	6196.5 5	5513.29	5038.6 4	6132.90	6030.87	6720.2 5	5980.6 7
I ₃		6673.5 1	7081.1 1	6725.3 6	8012.5 9	7123.14	7240.8 9	7683.24	7296.75	8705.5 7	7731.6 1
Mean (S)		4765.0 3	5887.8 7	5267.5 0	6012.1 3		5153.8 0	6122.68	5715.39	6805.9 9	
SEm±	S * I	227.19					260.957				
	I * S	191.67					214.62				
C.D at 5%	S* I	536.17					593.17				
	I* S	576.51					646.99				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, kg ha⁻¹=Kilograms of total biomass produced per hectare of land

Table 4.25C: Interaction table of harvest index (%)

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		19.59	20.75	31.97	25.01	24.33	20.15	20.95	32.12	25.06	24.57
I ₁		19.82	29.72	36.27	42.53	32.09	20.14	29.88	36.33	42.47	32.20
I ₂		29.41	30.86	41.68	44.90	37.21	29.55	30.87	41.54	44.58	37.13
I ₃		29.75	34.31	45.65	46.97	38.67	29.70	34.16	45.35	46.65	38.47
Mean (S)		24.64	28.91	38.89	39.85		24.88	28.96	38.84	39.69	
SEm±	S * I	1.75					1.76				
	I * S	1.69					1.68				
C.D at 5%	S* I	4.97					4.98				
	I* S	5.03					5.04				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C. D=Critical difference, %=Percent

I₃ had the lowest TPC, with values of 24.92 mg GAE g⁻¹ in 2022-23 and 26.80 mg GAE g⁻¹ in 2023-24. I₀ recorded the highest TPC, 46.99 mg GAE g⁻¹. Increased irrigation led to a higher SV, with I₃ recording the lowest values at 167.82 mg KOH g⁻¹ in 2022-23 and 163.71 mg KOH g⁻¹ in 2023-24. In contrast, the highest SV was observed in the I₀ treatment, 181.30 mg KOH g⁻¹. The lowest relative density and antioxidant activity in the I₃ treatment indicated that abundant water availability diluted the concentration of bioactive compounds in mustard seeds. This phenomenon aligned with findings by Yadav *et al.* (2021), which suggested that excessive irrigation disrupted secondary metabolite synthesis, leading to a decline in antioxidant compounds. Lower antioxidant activity and phenolic content reduced the oil's oxidative stability, as these compounds were essential for neutralizing free radicals and preserving oil quality over extended storage periods (Machado *et al.*, 2023). Phenolics played a critical role in determining oil quality, enhancing its health benefits and resistance to oxidative degradation. Conversely, the increased saponification value observed in the I₃ treatment suggested a higher presence of short-chain fatty acids. These changes, as noted by Aslam *et al.* (2020), enhanced the oil's suitability for industrial uses, such as soap production, but compromised nutritional quality if bioactive compounds were diminished. The higher relative density, antioxidant activity, and phenolic content in the no-irrigation treatment (I₀) suggested that water scarcity concentrated bioactive compounds, improving the oil's health-promoting properties and oxidative stability (Wang *et al.*, 2023).

Enhanced nutrient input, especially with humic acid and sulphur (S₃), affected these oil parameters. The S₃ treatment produced a relatively stable RD, with values of 0.84% in 2022-23 and 0.88% in 2023-24. The highest RD was recorded in the control treatment (S₀). Antioxidant activity decreased with higher nutrient input. The S₃ treatment showed the lowest AA, with values of 25.17% in 2022-23 and 26.83% in 2023-24. The highest AA was recorded in the S₀ treatment. TPC was lowest in the S₃ treatment, with values of 31.58 mg GAE g⁻¹ oil in 2022-23 and 30.06 mg GAE g⁻¹ oil in 2023-24. In contrast, the highest TPC was observed in the S₀ treatment 43.15 mg GAE g⁻¹ oil in 2022-23 and 47.88 mg GAE g⁻¹ oil in 2023-24. The S₂ treatment produced the highest SV, with values of 169.69 mg KOH g⁻¹ oil in 2022-23 and 166.07 mg KOH g⁻¹ oil in 2023-24. The highest SV was recorded in the S₀ treatment with 184.36 mg KOH g⁻¹ oil in 2022-23. The lower relative density, antioxidant activity, and TPC observed in the S₃ treatment suggested that enhanced nutrient availability favored seed

development at the expense of bioactive compound synthesis. This phenomenon aligned with findings by Savarese *et al.* (2022), which reported that humic acid improved nutrient uptake and metabolic efficiency but could dilute secondary metabolites under highly optimized growth conditions. Reduced antioxidant activity and TPC, critical indicators of oil quality, compromised oxidative stability, as these compounds were vital for neutralizing free radicals and preserving oil freshness during storage (Kurek *et al.*, 2024). A decrease in these bioactive compounds potentially lowered the oil's health benefits and shelf life. Conversely, the increase in saponification value with the S₃ treatment suggested a shift toward a higher proportion of short-chain fatty acids. While these enhanced functional properties for industrial applications, such as soap production, it indicated a reduction in long-chain fatty acids, which were crucial for the oil's nutritional profile (Chen and Liu, 2020). Interestingly, the higher antioxidant activity and TPC observed in the S₀ treatment suggested that nutrient limitations led to increased accumulation of bioactive compounds, possibly as a plant defense mechanism (Khan *et al.*, 2024). However, this advantage was counterbalanced by lower seed yield and oil production, demonstrating the trade-off between yield and oil quality.

4.8.1a Interaction between different irrigation regimes and different nutrient management on total phenolic content (mg GAE gm⁻¹ oil) of mustard oil

The interaction between different irrigation regimes and nutrient management treatments on total phenolic content (TPC) in mustard oil was evaluated across the 2022-23 and 2023-24 growing seasons. Increased irrigation and nutrient management influenced TPC, with the highest values observed in the no irrigation (I₀) and no nutrient (S₀) treatments. The TPC was highest in the I₀S₀ treatment, with values of 52.77 mg GAE g⁻¹ oil in 2022-23 and 58.88 mg GAE g⁻¹ oil in 2023-24. The lowest TPC was recorded in the I₃S₃ treatment, with values of 21.07 mg GAE g⁻¹ oil in 2022-23 and 17.82 mg GAE g⁻¹ oil in 2023-24. This outcome aligned with previous studies indicating that water and nutrient scarcity often induced plant stress responses, including enhanced biosynthesis of secondary metabolites like phenolic compounds (Jan *et al.*, 2021). These metabolites played a critical role in mitigating oxidative damage, serving as antioxidants that protected cellular structures during stress conditions. Consequently, the elevated TPC in the I₀S₀ treatment likely enhanced the oxidative stability, storage life, and health-promoting properties of the oil. Phenolic

compounds contributed significantly to the antioxidant activity of oils, as they neutralized free radicals and prevented oxidative degradation. The increased TPC in the resource-scarce treatments (I_0 and S_0) suggested that plants redirected metabolic resources toward secondary metabolite production when primary growth processes were constrained (Mehta *et al.*, 2024). This adaptation enhanced the antioxidant quality of mustard oil, potentially offering greater consumer and commercial value. In contrast, the lowest TPC observed in the high-input I_3S_3 treatment was attributed to reduced physiological stress. Ample water and nutrient availability likely prioritized primary growth processes over secondary metabolite production, diminishing phenolic synthesis (Hickman *et al.*, 2021). Although high irrigation and nutrient inputs improved seed yield and oil quantity, the corresponding reduction in TPC likely lowered the antioxidant potential and stability of the oil.

4.8.1b Interaction between different irrigation regimes and different nutrient management saponification value (mg KOH g⁻¹ oil) of mustard oil

Both increased irrigation and nutrient application influenced SV, with the highest values observed under the three-irrigation regime (I_3) combined with humic acid and sulphur application (S_2). The lowest SV was recorded in the I_0S_0 treatment, with values of 190.07 mg KOH g⁻¹ oil in 2022-23 and 194.23 mg KOH g⁻¹ oil in 2023-24. The SV was recorded in the I_3S_2 treatment, with values of 168.38 mg KOH g⁻¹ oil in 2022-23 and 162.22 mg KOH g⁻¹ oil in 2023-24. This aligned with studies showing that sufficient hydration and nutrient uptake enhanced enzymatic activities involved in lipid biosynthesis, favoring shorter-chain fatty acids (Pegg and Amarowicz *et al.*, 2023). Oils with higher SV were often valued in industrial applications such as soap production due to their superior saponification properties (Ofori *et al.*, 2023). However, while a higher SV enhanced the functional properties of mustard oil for industrial uses, it reflected a shift in fatty acid composition that could slightly alter the oil's nutritional profile. Shorter-chain fatty acids provided distinct benefits, but longer-chain fatty acids, typically associated with lower SV, were more desirable for cardiovascular and overall health (Islam *et al.*, 2024). Conversely, the lowest SV recorded in the I_0S_0 treatment reflected the impact of water and nutrient limitations, which hindered metabolic pathways critical for fatty acid diversity. This resulted in an increased proportion of longer-chain fatty acids, known for their superior nutritional qualities but reduced industrial utility. These results emphasized the need to balance irrigation and nutrient

management to optimize both the industrial and nutritional value of mustard oil, catering to diverse market demands.

4.8.2 Effect of different irrigation regimes and different nutrient management on iodine value and peroxide value of mustard oil

The effect of different irrigation regimes on the iodine value and peroxide value of mustard oil was evaluated across the 2022-23 and 2023-24 growing seasons. Increased irrigation decreased both the iodine value and peroxide value, with the lowest values observed under the three-irrigation regime (I_3). The highest iodine value was recorded in the no irrigation treatment (I_0), with values of 97.09 meq O_2 kg^{-1} of oil in 2022-23 and 95.18 meq O_2 kg^{-1} of oil in 2023-24. The lowest iodine value was observed in the I_3 treatment. The highest peroxide value was observed in the I_0 treatment, with values of 7.64 meq O_2 kg^{-1} of oil in 2022-23 and 8.09 meq O_2 kg^{-1} of oil in 2023-24. The lowest peroxide value was recorded in the I_3 treatment, with a mean of 4.12 meq O_2 kg^{-1} of oil. This aligned with findings by Machado *et al.* (2023), which indicated that water stress often led to increased synthesis of unsaturated fatty acids, enhancing nutritional properties but rendering the oil more susceptible to oxidation. Conversely, the lower IV in the I_3 treatment indicated a reduced proportion of unsaturated fatty acids, likely due to ample water availability supporting a shift toward saturated fatty acid synthesis. Saturated fatty acids improved the oil's oxidative stability, as they were less prone to degradation and rancidity (Arefin, 2023). The PV results further supported this trend, with the highest PV recorded in the I_0 treatment, suggesting that increased unsaturation and potential stress-induced lipid oxidation under limited irrigation exacerbated primary oxidation processes (Duhan *et al.*, 2019). In contrast, the lowest PV in the I_3 treatment reflected enhanced oxidative stability, likely due to a reduced unsaturation level and diminished oxidative stress. These findings underscored the critical role of irrigation management in mustard cultivation, as water availability not only affected yield but also modulated oil quality. While reduced irrigation enhanced unsaturation and improved nutritional quality, it raised oxidation risks, compromising storage stability.

Table 4.26 Influence of humic acid and sulphur on oil content, oil yield and moisture content in oil of Indian mustard under variable water regimes

Treatment	Oil content (%)		Oil yield (kg ha ⁻¹)		Moisture content in oil (%)	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
I ₀	35.95	35.61	437.07	474.40	0.25	0.47
I ₁	39.05	40.97	573.83	621.75	0.29	0.47
I ₂	39.95	45.08	847.89	1030.64	0.31	0.44
I ₃	41.21	46.89	1151.55	1407.10	0.28	0.40
SEm±	0.43	0.39	14.37	19.74	0.014	0.020
C.D at 5%	1.38	1.25	45.96	63.16	NS	NS
Sub plot (Nutrient management) (S)						
S ₀	33.60	37.18	423.36	520.62	0.18	0.34
S ₁	37.51	40.91	792.60	947.03	0.30	0.49
S ₂	40.71	43.45	677.01	792.20	0.23	0.42
S ₃	44.33	47.02	1117.73	1274.04	0.43	0.53
SEm±	0.36	0.36	15.58	18.34	0.02	0.02
C.D at 5%	1.04	1.03	44.67	52.59	0.06	0.06
Interaction (S*I)	2.17	2.14	92.23	109.34	NS	NS

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C.D=Critical difference, kg ha⁻¹=Kilograms per hectare, %=Percent

Table 4.26A: Interaction table of oil content (%)

Subplot (S) Main plot (I)	2022-23					2023-24				
	S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀	28.32	35.82	39.63	40.04	35.95	28.89	34.40	37.15	42.01	35.61
I ₁	33.81	37.30	40.18	44.90	39.05	35.42	39.76	42.88	45.83	40.97
I ₂	35.32	38.29	41.24	44.95	39.95	41.19	44.12	45.90	49.11	45.08
I ₃	36.97	38.64	41.80	47.43	41.21	43.21	45.35	47.86	51.13	46.89
Mean (S)	33.60	37.51	40.71	44.33		37.18	40.91	43.45	47.02	
SEm±	S * I	0.86				0.78				
	I * S	0.76				0.74				
C.D at 5%	S* I	2.17				2.14				
	I * S	2.28				2.19				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C.D=Critical difference, kg ha⁻¹=Kilograms per hectare, %=Percent

Table 4.26B: Interaction table of oil yield (kg ha⁻¹)

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		179.03	493.61	437.08	638.57	437.07	203.93	516.49	448.89	728.28	474.40
I ₁		297.89	603.81	537.53	856.09	573.83	333.66	666.68	595.69	890.98	621.75
I ₂		483.05	886.10	718.81	1303.58	847.89	614.27	1103.56	868.90	1535.8 1	1030.64
I ₃		733.47	1186.87	1014.64	1672.69	1151.92	930.63	1501.37	1255.30	1941.0 8	1407.10
Mean (S)		423.36	792.60	677.01	1117.73		520.62	947.03	792.20	1274.0 4	
SEm±	S * I	28.73					39.49				
	I* S	30.57					37.40				
C.D at 5%	S* I	92.23					109.34				
	I* S	90.49					111.50				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C.D=Critical difference, kg ha⁻¹=Kilograms per hectare, %=Percent

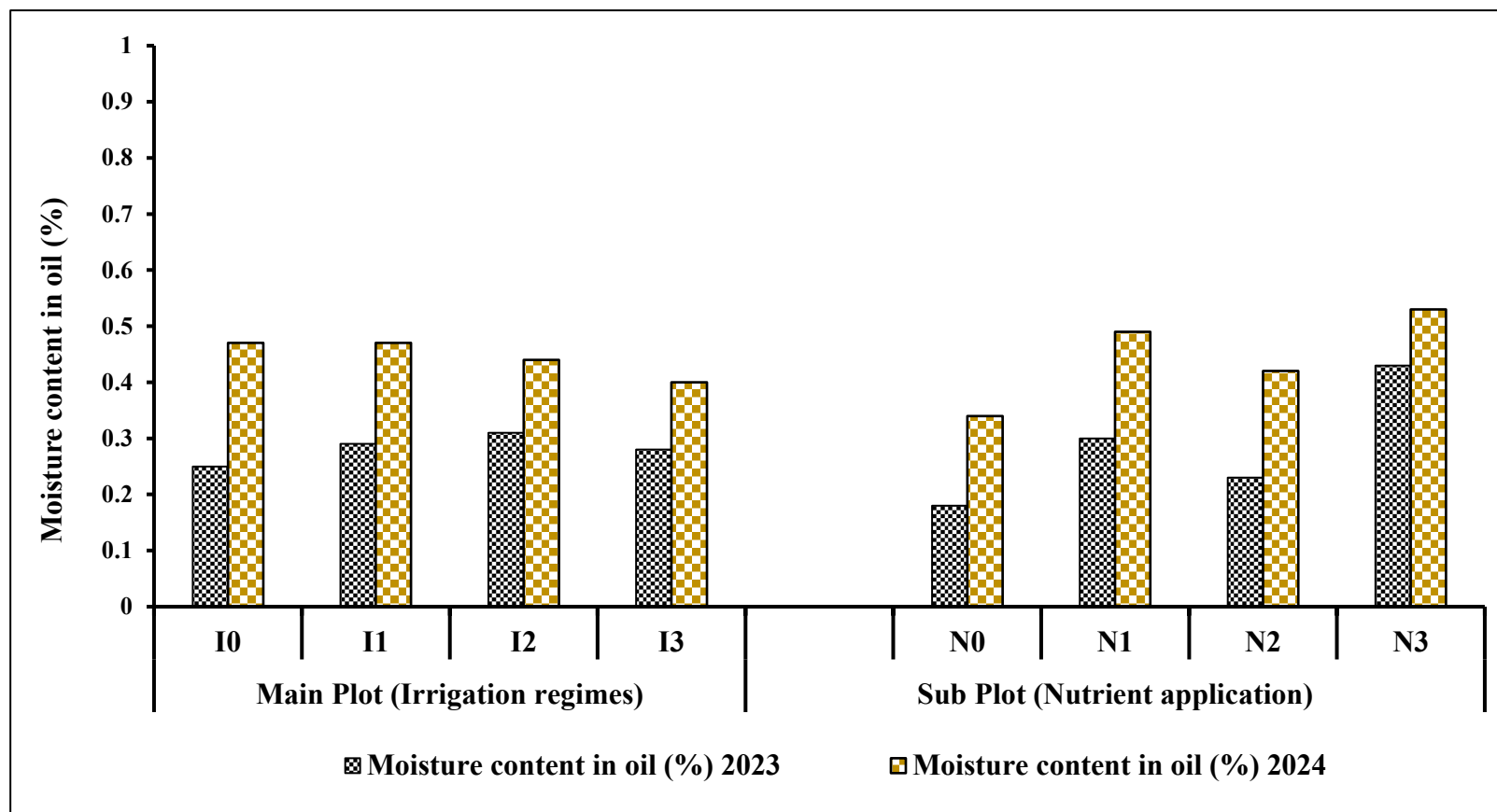


Figure 4.11 Effect of different irrigation regimes and different nutrient management on moisture content in Indian mustard oil

Table 4.27 Impact of humic acid and sulphur on the relative density of oil, total phenolic content, and total antioxidant oil quality of Indian mustard under variable water regimes

Treatment	Relative density of oil		TPC (mg GAE g ⁻¹ oil)		Total Antioxidant (%)	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
I ₀	0.95	0.95	46.99	50.18	34.03	35.73
I ₁	0.89	0.93	42.92	43.60	27.31	28.42
I ₂	0.86	0.90	30.70	32.85	25.36	26.09
I ₃	0.85	0.86	24.92	26.80	24.89	25.78
SEm±	0.01	0.01	0.39	0.46	0.33	0.32
C.D at 5%	0.04	0.03	1.24	1.48	1.05	1.01
Sub plot (Nutrient management) (S)						
S ₀	0.97	0.94	43.15	47.88	30.71	31.61
S ₁	0.90	0.92	34.31	34.83	26.92	27.95
S ₂	0.85	0.90	36.50	40.65	28.78	29.95
S ₃	0.84	0.88	31.58	30.06	25.17	26.83
SEm±	0.01	0.00	0.38	0.37	0.27	0.17
C.D at 5%	0.04	0.01	1.10	1.07	0.77	0.49
Interaction (S*I)	NS	NS	2.273	2.243	NS	NS

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, NS= Non-significant, mg GAE g⁻¹ oil=Milligrams of Gallic Acid Equivalents per gram of oil,%=Percent

Table 4.27A: Interaction table of total phenol content (mg GAE g⁻¹ oil) of oil

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀		52.77	44.74	47.47	43.00	46.99	58.88	45.52	51.09	45.22	50.18
I ₁		50.01	43.25	43.25	35.16	42.92	55.00	40.05	45.76	33.58	43.60
I ₂		36.83	28.14	30.73	27.11	30.70	41.01	29.88	36.89	23.62	32.85
I ₃		32.97	21.10	24.53	21.07	24.92	36.65	23.88	28.87	17.82	26.80
Mean (S)		43.15	34.31	36.50	31.58		47.88	34.83	40.65	30.06	
SEm±	S * I	0.75					0.92				
	I * S	0.77					0.79				
C.D at 5%	S* I	2.273					2.243				
	I* S	2.281					2.386				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, NS= Non-significant, mg GAE g⁻¹ oil=Milligrams of Gallic Acid Equivalents per gram of oil

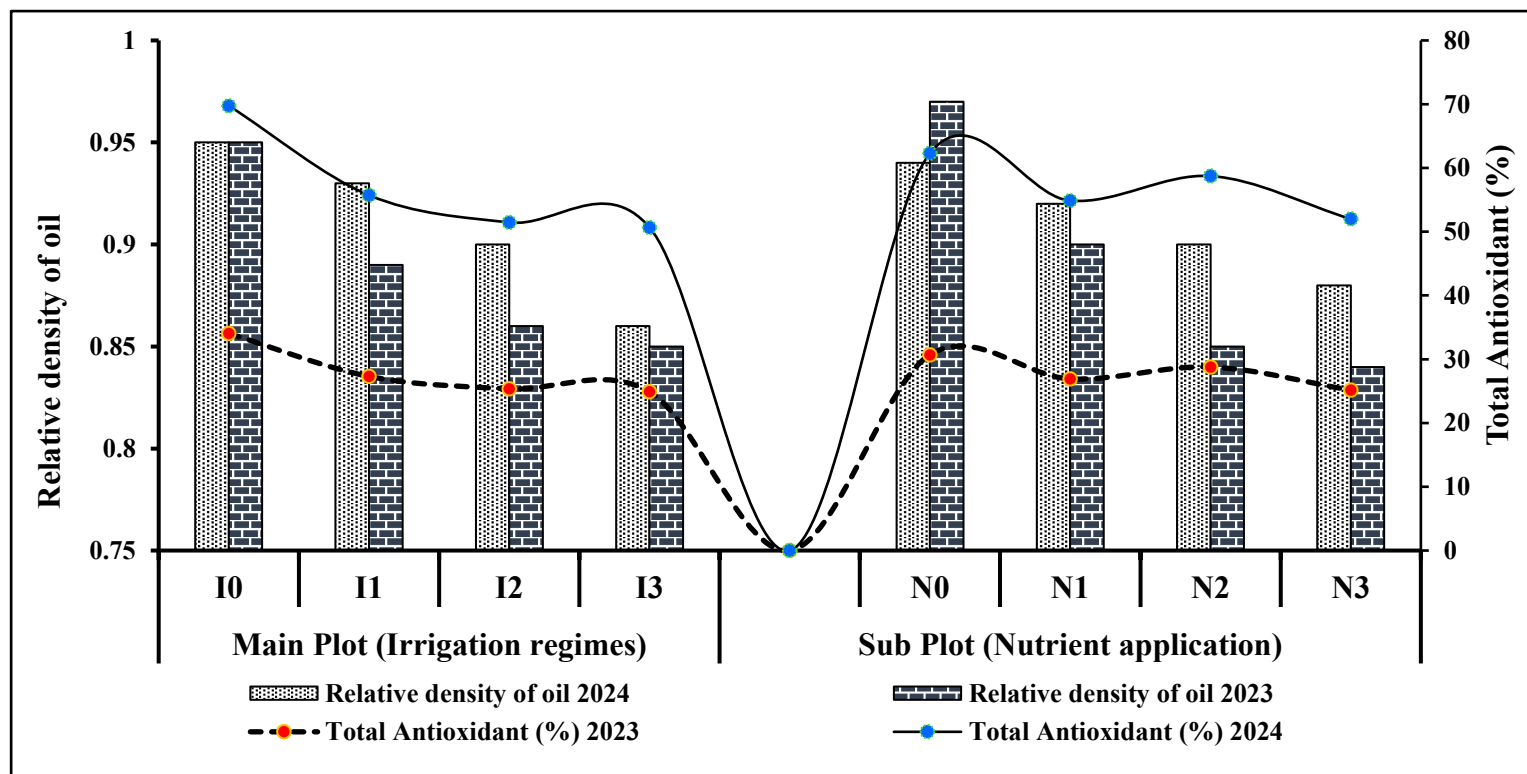


Figure 4.12 Effect of different irrigation regimes nutrient management on relative density and antioxidant activity in Indian mustard oil

During the 2022–2023 and 2023–2024 growing seasons, the impact of various nutrient management techniques on the iodine and peroxide values of mustard oil was examined. Both metrics were impacted by nutrient treatment the S₃ treatment, with sulphur and humic acid, had the greatest iodine value of 128.73 g I₂ 100 g⁻¹ oil in 2022–2023 and 121.77 g I₂ 100 g⁻¹ oil in 2023–2024. The S₀ treatment had the lowest iodine value, oil in two years with 92.77 g I₂ 100 g⁻¹ oil in 2022–2023 and 95.08 g I₂ 100 g⁻¹ oil in 2023–2024. The S₀ treatment had the greatest peroxide value, measuring 7.28 meq O₂ kg⁻¹ of oil in 2022–2023 and 9.26 meq O₂ kg⁻¹ of oil in 2023–24. Unsaturated fatty acids, particularly polyunsaturated fats, were known to offer health benefits, including improved cardiovascular health and anti-inflammatory properties (Sachan *et al.*, 2024). Despite the elevated IV, the low PV in the S₁ treatment indicated good oxidative stability, potentially due to inherent antioxidant compounds or reduced exposure to pro-oxidative conditions during seed development. In contrast, the highest PV recorded in the S₀ treatment, combined with a lower IV, indicated higher susceptibility to oxidation and reduced oxidative stability. This outcome suggested that the oil from the S₀ treatment had a lower proportion of unsaturated fatty acids, which were more prone to oxidation, yet paradoxically faced greater oxidative stress. This imbalance may have resulted from the lack of humic acid and sulphur, which were known to enhance plant stress tolerance and antioxidant defenses (Ennab *et al.*, 2023).

The S₃ treatment, which integrated humic acid and sulphur, demonstrated moderate IV and PV values, indicating a balanced fatty acid composition. This balance was crucial for producing oil with a desirable degree of unsaturation while maintaining stability. Humic acid improved nutrient uptake and soil health, while sulphur played a key role in lipid metabolism and antioxidant enzyme activity, supporting oxidative stability (Ahmad *et al.*, 2023).

4.8.2a Interaction between different irrigation regimes and different nutrient management on iodine value (g I₂/100 g oil) of mustard oil

Both irrigation and nutrient application influenced the iodine value, which is a measure of unsaturation in the oil. The highest iodine values were recorded in the I₃S₃ treatment, with values of 140.20 g I₂ 100 g⁻¹ oil in 2022-23 and 136.22 g I₂ 100 g⁻¹ oil in 2023-24. Among nutrient treatments, S₃ consistently showed the highest iodine values across all irrigation levels, while S₀ had the lowest values. Unsaturated fatty acids, especially polyunsaturated ones, were associated with several health benefits, including

improved cardiovascular health and reduced inflammation (Margină *et al.*, 2020). The elevated IV in the I₀S₁ treatment suggested that resource limitations, such as water and nutrient scarcity, induced a stress response in plants, triggering the accumulation of unsaturated fatty acids. This stress adaptation aligned with findings by Zahedi *et al.* (2021), which indicated that plants under water stress allocated metabolic resources toward the synthesis of compounds that enhanced adaptability and survival. Conversely, the lowest IV observed in the high-irrigation treatment (I₃) with no nutrient inputs (S₀) suggested a reduction in unsaturated fatty acids. Adequate water availability likely prioritized primary growth and biomass production over the synthesis of secondary metabolites, including unsaturated fatty acids, leading to oils with lower IV. While this reduction enhanced the oxidative stability of the oil, making it less prone to rancidity during storage, it may have compromised the oil's nutritional value (Pattnaik *et al.*, 2021).

4.8.2b Interaction between different irrigation regimes and different nutrient management on peroxide value (meq O₂ kg⁻¹ of oil) of mustard oil

The interaction between different irrigation regimes and nutrient management treatments on the peroxide value (PV) of mustard oil was analysed for the 2022-23 and 2023-24 growing seasons. Both irrigation and nutrient treatments affected the peroxide value, which indicates the level of primary oxidation and potential rancidity in the oil. The highest PV was observed in the I₀S₃ treatment, with values of 16.66 meq O₂ kg⁻¹ of oil in 2022-23 and 20.51 meq O₂ kg⁻¹ of oil in 2023-24. The I₃S₁ treatment's low PV suggested that ample water supply mitigated oxidative stress in plants, reducing the formation of primary oxidation products like hydroperoxides. Reduced PV was associated with improved oil quality and extended shelf life, making the oil less prone to rancidity during storage. This observation aligned with studies by Ahmad *et al.* (2021), which highlighted the role of adequate water availability in reducing plant oxidative stress, leading to more stable oil profiles. In contrast, the highest PV in the I₀S₂ treatment, observed under no irrigation and additional nutrient inputs, underscored the detrimental effects of water stress combined with nutrient loading. Under water-limited conditions, plants experienced increased lipid peroxidation as a stress response, leading to higher oxidative degradation of unsaturated fatty acids (Hassan *et al.*, 2024). Additional nutrient supply under these conditions may have exacerbated metabolic imbalances, further elevated oxidative stress and compromised oil quality. The results

also suggested that balanced irrigation and nutrient management were critical in modulating oil oxidative stability. While adequate water reduced oxidative stress, the absence of humic acid and sulphur in the S₁ treatment may have minimised pro-oxidative enzyme activity associated with sulphur metabolism (Dragoev *et al.*, 2024).

4.9 Effect of different irrigation regimes and different nutrient management on crop water use efficiency (CWUE) of mustard crop

CWUE measures the efficiency with which water is used in producing yield, and different irrigation levels significantly influenced these values. The highest CWUE was observed in the I₀ (no-irrigation treatment), with 70.60 kg ha⁻¹ mm⁻¹ across the two years. CWUE values were exceptionally high in this treatment due to the minimal water input, which maximized the efficiency of water use in yield production. The I₁ treatment had CWUE 50.56 kg ha⁻¹ mm⁻¹, which was significantly lower than I₀. Also, I₂ was low and I₃ was lowest in both years 27.79 kg ha⁻¹ mm⁻¹ in 2022-23, and 29.00 kg ha⁻¹ mm⁻¹ in 2023-24. Although two irrigations supported higher yield, the additional water input reduced the overall efficiency compared to I₀ and I₁. The lowest CWUE among irrigated treatments was observed in the three-irrigation regime. This finding concurred with the observations of Baghbani-Arani *et al.* (2020), who noted that intermediate irrigation regimes were effective in achieving higher productivity with moderate water use in oilseed crops. Conversely, treatments with two (I₂) and three (I₃) irrigations resulted in lower CWUE due to excessive water application, which, while increasing yields, diluted the efficiency metric. Such outcomes corroborated with the results of Pawar *et al.* (2020), who highlighted the diminishing returns of additional water on CWUE in mustard cultivation. These results emphasized the importance of fine-tuning irrigation schedules to achieve an optimal balance between yield and water resource sustainability, especially in arid and semi-arid regions where water was a limiting factor.

Table 4.28 Effect of humic acid and sulphur on iodine value, saponification value, and peroxide value of Indian mustard under variable water regimes

Treatment	Iodine value (g I ₂ 100 g ⁻¹ oil)		Saponification value (mg KOH g ⁻¹ oil)		Peroxide value (meq O ₂ kg ⁻¹ of oil)	
	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
Main plot (Irrigation regimes) (I)						
I ₀	97.04	95.18	178.01	181.30	7.64	8.09
I ₁	103.61	99.91	176.21	178.88	6.20	7.12
I ₂	110.38	110.73	171.13	169.87	4.73	5.83
I ₃	120.89	116.72	167.82	163.71	4.16	4.09
SEm±	1.56	1.44	1.77	0.96	0.04	0.04
C.D at 5%	4.98	4.61	5.66	3.08	0.14	0.12
Sub plot (Nutrient management) (S)						
S ₀	92.77	95.08	184.36	187.96	7.28	9.26
S ₁	101.40	102.46	176.99	179.70	6.45	8.62
S ₂	115.93	110.15	169.69	166.07	4.74	4.02
S ₃	128.73	121.77	162.13	160.03	4.26	3.24
SEm±	1.28	0.93	0.61	0.79	0.03	0.05
C.D at 5%	3.68	2.67	1.74	2.26	0.10	0.15
Interaction (S*I)	7.69	2.63	3.81	2.23	0.203	0.31

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C.D=Critical difference, DAS=Days after sowing. g I₂ 100 g⁻¹ oil = Grams of iodine absorbed by 100 grams of oil

Table 4.28A: Interaction table of iodine value (g I₂ 100 g⁻¹ oil)

Subplot (S) Main plot (I)	2022-23					2023-24				
	S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀	76.09	89.47	100.53	122.08	97.04	84.81	90.07	101.61	104.22	95.18
I ₁	88.85	92.99	104.91	127.68	103.61	87.45	93.41	104.26	114.52	99.91
I ₂	101.58	116.37	126.23	124.95	110.38	100.78	113.58	124.14	132.11	110.73
I ₃	104.54	106.77	132.05	140.20	120.89	107.26	112.78	110.61	136.22	116.72
Mean (S)	92.77	101.40	115.93	128.73		95.08	102.46	110.15	121.77	
SEm±	S * I	3.15				2.88				
	I * S	2.71				2.16				
C.D at 5%	S* I	7.69				2.63				
	I* S	8.14				3.06				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂= Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C.D=Critical difference, DAS=Days after sowing. g I₂ 100 g⁻¹ oil = Grams of iodine absorbed by 100 grams of oil

Table 4.28B: Interaction table of saponification value (mg KOH g⁻¹ oil)

Subplot (S) Main plot (I)	2022-23					2023-24				
	S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
I ₀	190.07	180.68	174.28	159.83	176.01	194.23	187.17	172.09	158.55	178.03
I ₁	181.43	182.75	173.72	154.77	173.21	191.84	185.18	171.2	153.51	175.43
I ₂	186.90	171.69	168.39	150.47	169.36	187.64	178.07	162.22	150.32	169.57
I ₃	179.04	172.86	162.38	147.00	165.82	178.12	168.37	158.77	149.59	163.71
Mean (S)	184.36	176.99	169.69	162.13		187.96	179.7	166.07	160.03	
SEm±	S * I	3.53				1.93				
	I * S	2.06				1.67				
C.D at 5%	S* I	3.81				2.23				
	I* S	6.48				2.36				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, mg KOH g⁻¹ oil = milligrams of potassium hydroxide (KOH) required to saponify one gram of oil

Table 4.28C: Interaction table of peroxide value (meq O₂ kg⁻¹ of oil)

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
	I ₀	9.14	9.28	6.31	6.69	7.84	11.39	11.79	5.02	9.51	9.75
	I ₁	8.30	6.76	5.13	5.19	6.35	10.31	9.26	4.67	7.93	7.12
	I ₂	6.45	4.70	4.08	2.10	4.73	8.96	5.47	3.93	6.28	5.83
	I ₃	5.22	5.06	3.45	2.89	4.16	6.38	6.22	2.45	2.32	4.34
	Mean (S)	7.28	6.45	4.74	4.26		9.26	8.62	4.02	3.24	
SEm±	S * I	0.089					0.073				
	I * S	0.073					0.098				
C.D at 5%	S * I	0.203					0.311				
	I * S	0.221					0.290				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean , C.D=Critical difference, meq O₂ kg⁻¹ of oil = Milliequivalents of reactive oxygen per kilogram of oil

The impact of different nutrient management treatments on crop water use efficiency (CWUE) of mustard oil was evaluated across the 2022-23 and 2023-24 growing seasons. The CWUE values reflect the efficiency of water use in achieving yield, with higher values indicating better efficiency. The CWUE values for S₀ were 53.02 kg ha⁻¹ mm⁻¹ in 2022-23 and decreased to 44.41 kg ha⁻¹ mm⁻¹ in 2023-24, indicating limited water use efficiency under minimal nutrient input. The S₁ treatment significantly lowered CWUE 43.54 kg ha⁻¹ mm⁻¹ in 2022-23 to 42.10 kg ha⁻¹ mm⁻¹ in 2023-24. This increase suggests that adequate nutrient availability can enhance water use efficiency by promoting plant growth and yield under similar water conditions. In the S₂ treatment, CWUE had a value of 48.33 kg ha⁻¹ mm⁻¹, lower efficiency than S₁ and S₃. The CWUE values were 53.02 kg ha⁻¹ mm⁻¹ in 2022-23 and 44.41 kg ha⁻¹ mm⁻¹ in 2023-24, The highest CWUE was recorded in the S₀ treatment, 53.02 kg ha⁻¹ mm⁻¹ in 2022-23 and 44.41 kg ha⁻¹ mm⁻¹ in 2023-24. The superior CWUE observed in the S₃ treatment, combining humic acid and sulphur, highlighted the importance of balanced nutrient availability in enhancing water utilization efficiency. This was consistent with findings by Samreen *et al.* (2022) and Mekdad *et al.* (2022), who reported that sulphur and humic acid supplementation improved nutrient uptake and enzymatic activity in oilseed crops, thereby optimizing plant growth and water use efficiency. The increased CWUE in S₃ was attributed to enhanced root development and metabolic activity, which facilitated efficient water and nutrient absorption, resulting in higher yields per unit of water applied. In contrast, the lowest CWUE in the control treatment (S₀), devoid of added nutrients, underscored the detrimental effects of nutrient deficiency on crop growth and resource efficiency. This observation aligned with findings by Yusuf *et al.* (2024), who demonstrated that insufficient nutrient availability limited biomass production and water utilization efficiency, leading to suboptimal economic returns, particularly in water-scarce regions. While the S₀ treatment minimized input costs, its lower yields diminished overall water productivity, emphasizing the need for effective nutrient management strategies. The results suggested that integrating humic acid and sulphur into nutrient regimes could enhance both yield and water use efficiency, particularly in areas with limited water resources. These findings underscored the potential of nutrient optimization as a sustainable practice for improving CWUE in mustard oil production while balancing economic and environmental considerations.

4.9a Interaction between different irrigation regimes and different nutrient management on crop water use efficiency (CWUE) of mustard oil

The interaction between different irrigation regimes and nutrient management treatments on Crop Water Use Efficiency (CWUE) of mustard oil was analyzed for the 2022-23 and 2023-24 growing seasons. CWUE reflects the efficiency of water utilization in yield production, and both irrigation frequency and nutrient application impacted these values. In the I_0 treatment, the highest CWUE was recorded with S_0 , with values of $83.07 \text{ kg ha}^{-1} \text{ mm}^{-1}$ in 2022-23 and $69.56 \text{ kg ha}^{-1} \text{ mm}^{-1}$ in 2023-24. The limited water input in I_0 led to high CWUE values, with S_0 optimizing yield per unit of water used. CWUE for I_3 $27.79 \text{ kg ha}^{-1} \text{ mm}^{-1}$, reflecting that increased irrigation volume diluted the water efficiency, despite producing high yields. Nutrient treatment S_3 , combining humic acid and sulphur, yielded the highest CWUE across all irrigation regimes, especially under I_0 . The high CWUE observed in I_0 with S_3 supplementation suggested that balanced nutrient availability enhanced physiological and metabolic processes, enabling plants to maximize yield per unit of water used. This aligned with the work of Kaya *et al.* (2020), who reported that sulphur and humic acid improved nutrient absorption and drought resilience in oilseed crops, thus optimizing water use efficiency

Table 4.29 Effect of humic acid and sulphur on crop water use efficiency of Indian mustard under variable water regimes

Treatment	Crop water use efficiency (kg ha ⁻¹ mm ⁻¹)	
	2022-23	2023-24
Main plot (Irrigation regimes) (I)		
I ₀	58.14	51.02
I ₁	50.56	35.81
I ₂	37.02	30.59
I ₃	31.79	29.94
SEm±	0.63	0.57
C.D at 5%	2.05	1.85
Sub plot (Nutrient management) (S)		
S ₀	53.02	44.32
S ₁	43.54	36.10
S ₂	48.33	42.79
S ₃	41.08	30.41
SEm±	0.58	0.53
C.D at 5%	1.67	1.53
Interaction (S*I)	3.17	3.44

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C.D=Critical difference, kg ha⁻¹ mm⁻¹= Kilograms per hectare per millimeter

Table 4.29A: Interaction table of crop water use efficiency ($\text{kg ha}^{-1} \text{mm}^{-1}$)

Subplot (S) Main plot (I)		2022-23					2023-24				
		S ₀	S ₁	S ₂	S ₃	Mean (I)	S ₀	S ₁	S ₂	S ₃	Mean (I)
	I ₀	83.07	61.94	76.11	61.26	70.60	69.56	56.27	67.63	42.21	58.92
	I ₁	56.96	48.09	51.01	46.17	50.56	41.27	34.79	38.16	33.94	37.04
	I ₂	41.19	36.95	37.58	32.37	37.02	32.22	30.72	30.74	30.23	30.98
	I ₃	30.88	27.17	28.61	24.52	27.79	29.95	24.69	28.45	23.66	26.69
	Mean (S)	53.02	43.54	48.33	41.08		43.25	36.62	41.24	32.51	
SEm±	S * I	1.06					1.26				
	I* S	1.12					1.18				
C.D at 5%	S* I	3.71					3.44				
	I* S	3.31					3.52				

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm±= Standard error of mean, C.D=Critical difference, $\text{kg ha}^{-1} \text{mm}^{-1}$ =Kilograms per hectare per millimeter

4.10 Effect of different irrigation regimes and different nutrient management on economic appraisal of Indian mustard RLC3

The economic analysis of mustard oil production under varying irrigation regimes during the 2022-23 and 2023-24 growing seasons reveals substantial impacts on gross returns, net returns, and the benefit-cost (B:C) ratio. A progressive increase in irrigation led to higher profitability metrics across all evaluated parameters. In the no irrigation treatment (I_0), the gross return was Rs 232124.69 per hectare in 2022-23 while Rs 267149.34 per hectare in 2022-24. This resulted in a net return of Rs 131978.738 per hectare in 2022-23 while Rs 162957.21 per hectare in 2022-24 and a B:C ratio of 1.31 in 2022-23 while 1.56 in 2022-24, indicating relatively lower profitability compared to treatments with more frequent irrigation. The economic returns were limited by the absence of additional water, which likely constrained plant growth and yield potential. Despite the lower input costs, the restricted moisture availability in I_0 reduced yield outputs, thereby limiting the overall economic returns. The one-irrigation treatment (I_1) showed improvement over I_0 , with a gross return of Rs 265421.60 per hectare in 2022-23 while Rs 305117.81 per hectare in 2022-24, which corresponded to a higher net return of Rs 16363.215 per hectare in 2022-23 while Rs 198762.76 per hectare in 2022-24 and an increased B:C ratio of 1.61 in 2022-23 while 1.87 in 2022-24. The increase in profitability in I_1 reflects the positive impact of a single irrigation event, which likely provided essential moisture at a critical growth stage, enhancing plant health and yield outcomes. The slight rise in cultivation costs was offset by the more substantial gains in returns, resulting in a notable boost in net profitability compared to I_0 . In the two-irrigation treatment (I_2), the economic returns were further elevated, with a gross return of Rs 278420.69 per hectare in 2022-23 while Rs 327413.79 per hectare in 2022-24 and a net return of Rs 177162.782 per hectare in 2022-23 while Rs 222109.69 per hectare in 2022-24. The B:C ratio also increased to 1.75 in 2022-23 and 2.11 in 2022-24, indicating that the benefits from additional irrigation inputs continued to exceed the costs. I_3 was best elevated, with a gross return of Rs 352079.17 per hectare in 2022-23 while Rs 405411.50 per hectare in 2022-24 and a net return of Rs 251346.744 per hectare in 2022-23 while Rs 300632.89 per hectare in 2022-24. The B:C ratio also increased to 2.49 in 2022-23 and 2.86 in 2022-24. The consistent water supply in I_2 likely optimized plant growth and yield formation, enhancing the economic returns per hectare. The additional cost of cultivation in I_2 was

outweighed by the improvement in gross and net returns, reflecting the value of regular irrigation for sustaining high productivity and profitability in mustard cultivation. The improved yield, coupled with superior oil characteristics, likely increased market value, translating into enhanced profitability. These findings aligned with studies such as Kaya *et al.* (2020) and Ahmad *et al.* (2023), which reported that humic substances and sulphur played critical roles in improving nutrient efficiency, photosynthetic activity, and crop resilience. Conversely, the S₀ treatment, characterized by minimal nutrient input, yielded the lowest economic returns and B ratio. Limited nutrient availability restricted plant biomass accumulation and oil yield, leading to suboptimal productivity despite reduced input costs. While this approach minimized expenditure, the insufficient returns highlighted the economic inefficiency of low-input strategies for mustard cultivation. The superior performance of the S₃ treatment demonstrated that strategic nutrient management incorporating humic acid and sulphur could substantially enhance the profitability of mustard farming.

The economic appraisal of mustard oil production under various nutrient management treatments across the 2022-23 and 2023-24 growing seasons demonstrates significant variations in gross returns, net returns, and the benefit-cost ratio. The data indicate that increasing nutrient input, especially with humic acid and sulphur (S₃), positively impacts profitability. The S₀ treatment, with minimal nutrient input, resulted in the lowest economic returns with gross return of Rs 236365.18 per hectare in 2022-23 while Rs 263484.19 per hectare in 2022-24, which corresponded to a higher net return of Rs 137471.22 per hectare in 2022-23 while Rs 160412.67 per hectare in 2022-24 and an increased B:C ratio of 1.39 in 2022-23 while 1.56 in 2022-24. Although this treatment incurred the lowest cultivation costs, the limited nutrient input restricted yield potential, resulting in reduced profitability compared to other nutrient treatments. The S₁ treatment significantly enhanced economic outcomes, with a mean gross return of Rs 296783.74 per hectare in 2022-23 while Rs 348886.94 per hectare in 2022-24, which corresponded to a higher net return of Rs 197252.65 per hectare in 2022-23 while Rs 245178.30 per hectare in 2022-24 and an increased B:C ratio of 1.98 in 2022-23 while 2.36 in 2022-24 demonstrating substantial profitability. The nutrient input in S₁ supported improved plant growth and yield, increasing returns while keeping cultivation costs manageable. The S₂ treatment showed moderate economic benefits, with a mean gross return of Rs 267339.98 per hectare in 2022-23 while Rs 314418.01

per hectare in 2022-24, which corresponded to a higher net return of Rs 164911.24 per hectare in 2022-23 while Rs 207811.71 per hectare in 2022-24 and B:C ratio of 1.61 in 2022-23 while 1.95 in 2022-24. S_2 provided higher returns than the control (S_0), it was less profitable than S_1 and S_3 , suggesting that the combined application of nutrients may optimize growth and yield potential more effectively than individual nutrient applications. The S_3 treatment yielded the highest economic returns, with a mean gross return of Rs 327557.25 per hectare in 2022-23 while Rs 378303.30 per hectare in 2022-24, which corresponded to a higher net return of Rs 224491.38 per hectare in 2022-23 while Rs 271059.87 per hectare in 2022-24 and B: C ratio of 2.18 in 2022-23 while 2.53 in 2022-24 the highest among all treatments, indicating the most profitable nutrient management strategy. The application of both humic acid and sulphur in S_3 optimized nutrient availability and plant health, leading to higher yields and oil quality, which in turn enhanced gross and net return

Table 4.30 Influence of humic acid and sulphur on the economics of Indian mustard under variable water regimes

Treatment	Gross return (Rs ha ⁻¹)		Net return (Rs ha ⁻¹)		B:C ratio	
Main plot (Irrigation regimes) (I)	2022-23	2023-24	2022-23	2023-24	2022-23	2023-24
I ₀	232124.69	267149.34	131978.738	162957.21	1.31	1.56
I ₁	265421.60	305117.81	163638.215	198762.76	1.61	1.87
I ₂	278420.69	327413.79	177162.782	222109.69	1.75	2.11
I ₃	352079.17	405411.50	251346.744	300632.89	2.49	2.86
SEm±	5736.22	6453.91	5735.59	6454.30	0.06	0.06
C.D at 5%	18609.85	20938.24	18346.38	20645.30	0.18	0.19
Sub plot (Nutrient management) (S)						
S ₀	236365.18	263484.19	137471.22	160412.67	1.39	1.56
S ₁	296783.74	348886.94	197252.65	245178.30	1.98	2.36
S ₂	267339.98	314418.01	164911.24	207811.71	1.61	1.95
S ₃	327557.25	378303.30	224491.38	271059.87	2.18	2.53
SEm±	4501.07	5635.47	4501.07	5635.47	0.04	0.05
C.D at 5%	12962.39	16229.28	12907.83	16160.99	0.13	0.15
Interaction (S*I)	NS	NS	NS	NS	NS	NS

* I=irrigation regimes, N=nutrient management, I₀ =No Post Sowing Irrigation], I₁ =One Post Sowing Irrigation (Vegetative stage), I₂ =Two Post Sowing Irrigation (Vegetative + Flowering Stage), I₃ =Three Post Sowing Irrigation (Vegetative + Flowering + Seed filling stage), S₀ = Control, S₁= Humic acid, S₂ = Sulphur, S₃ = Humic acid + Sulphur, SEm± = Standard mean of error, C.D = Critical difference, Rs. ha⁻¹ = Rupees per hectare, B:C ratio = Benefit cost ratio

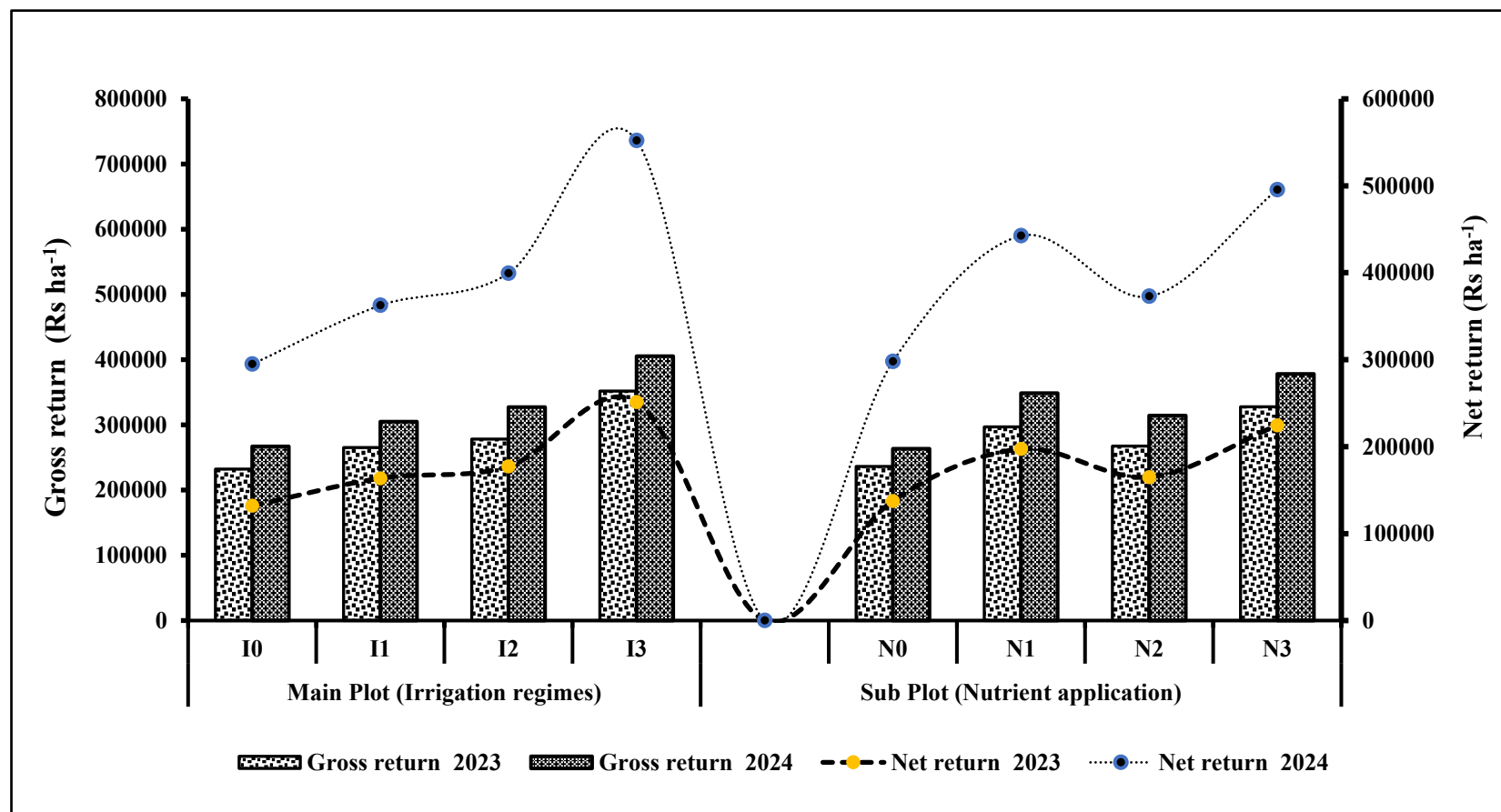


Figure 4.13 Effect of different irrigation regimes and different nutrient management on gross return and net return of Indian mustard of 2022-23 and 2023-24

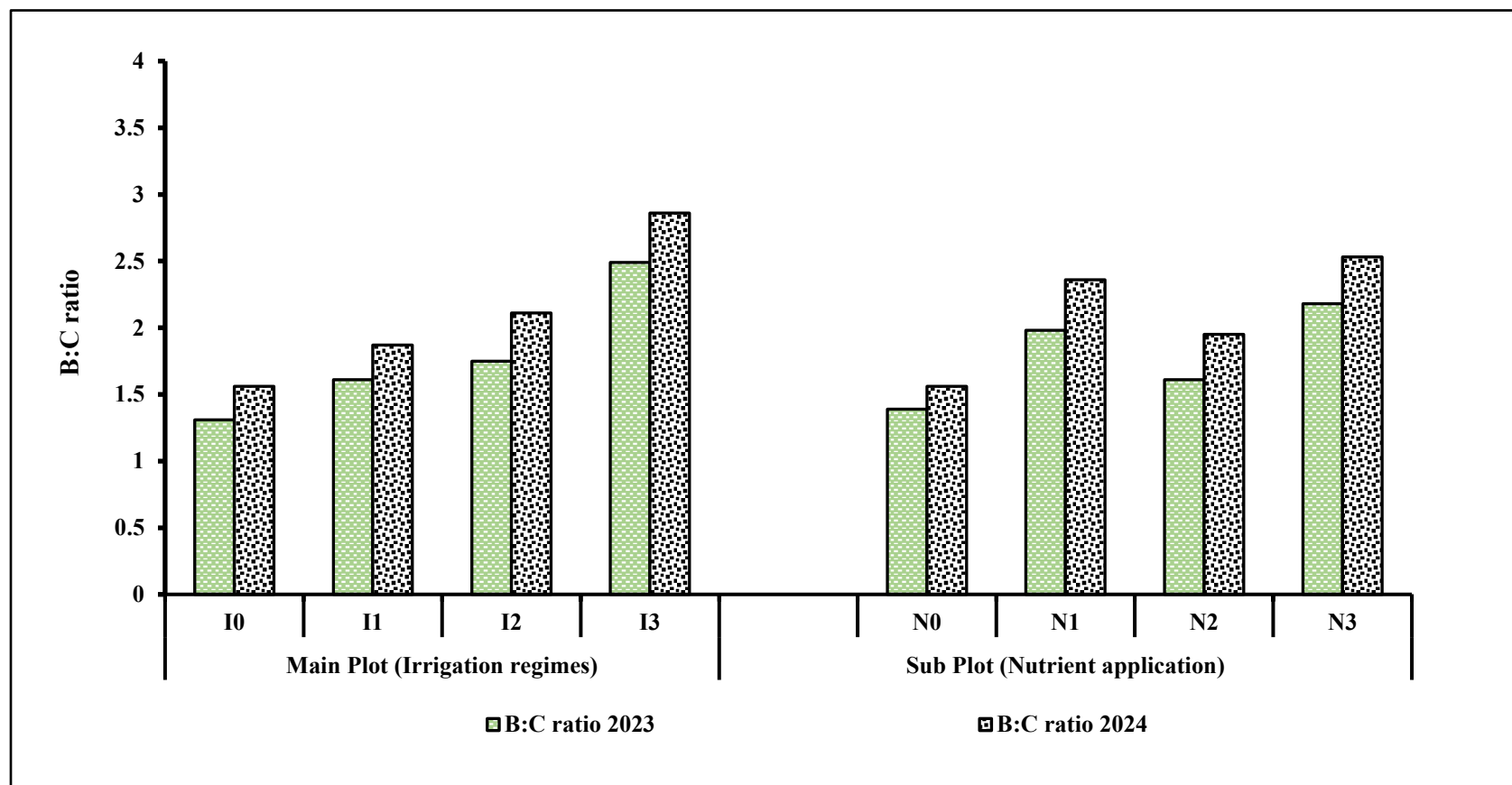


Figure 4.14 Effect of different irrigation regimes and different nutrient management on the benefit-cost ratio in Indian mustard

The study titled "Response of Indian mustard to humic acid and sulphur under variable water regimes" was conducted at Lovely Professional University, Phagwara, Punjab, during the rabi seasons of 2022 and 2023. It aimed to explore the effects of irrigation regimes and nutrient management, focusing on the combined application of humic acid and sulphur, on the growth, yield, and oil quality of Indian mustard (*Brassica juncea*). The experiment evaluated four irrigation treatments and four nutrient management regimes, including humic acid, sulfur, their combination, and a control. The study examined how these factors influenced growth, yield, oil content, and quality under varying water conditions. Detailed observations were made on growth parameters, yield components, physiological traits, and oil quality, offering insights into sustainable agricultural practices.

Objective 1: To correlate the growth and yield of Indian mustard with different water regimes

Water is an essential factor influencing the growth, yield, and quality of crops, and the current study demonstrated the critical importance of water availability for Indian mustard cultivation. The study tested four different water regimes, including: I₀ (no post-sowing irrigation), I₁ (one irrigation at the vegetative stage), I₂ (two irrigations at vegetative and flowering stages), and I₃ (three irrigations at vegetative, flowering, and siliqua filling stages). Among these, I₃ (three irrigations at critical stages) consistently produced the best results for growth and yield parameters. Irrigating mustard at critical stages of its growth significantly improved plant height, fresh and dry biomass, leaf area, and other growth traits. The I₃ treatment led to a 36% increase in plant height, 28% higher leaf area, and 25% higher biomass compared to the no-irrigation treatment (I₀). Moreover, I₃ treatment also resulted in the highest seed yield, with a significant increase in both biological yield and harvest index. These results suggest that water availability during crucial growth phases enhances both vegetative growth and reproductive success, contributing to improved productivity. This aligns with previous research indicating that water stress, particularly during flowering and seed filling, can drastically reduce yield and oil content in oilseed crops like mustard. Conversely, the I₀ treatment (no irrigation) led to severely reduced growth and yield. The plants in this treatment exhibited stunted growth, lower chlorophyll content, and increased susceptibility to oxidative damage, as evidenced by higher levels of malondialdehyde (MDA), a biomarker of oxidative stress. The negative impacts of

drought were evident in the reduction in seed yield, which was 35% lower than that of the I₃ treatment. These results underscore the importance of ensuring adequate water availability during critical growth stages to maximize mustard yield and quality, especially under changing climate conditions where water stress is becoming more frequent.

Objective 2: To study the effect of humic acid and sulphur application on the growth and yield of Indian mustard under variable moisture regimes

The second objective of this study examined how humic acid and sulphur applications affected the growth and yield of Indian mustard, particularly under different irrigation regimes. Humic acid, a naturally occurring organic compound, is known for its ability to improve soil structure, water retention, and nutrient bioavailability, while sulphur is a crucial macronutrient involved in protein synthesis and oil formation. Both of these inputs are critical for mitigating the adverse effects of water stress on crops, especially in water-scarce regions. The study found that the combined application of humic acid and sulphur (S₃) under the I₃ irrigation treatment (three-stage irrigation) significantly enhanced growth parameters. The treatment combination of I₃S₃ resulted in the highest crop growth rate (CGR), relative growth rate (RGR), and net assimilation rate (NAR). Plants treated with humic acid and sulphur showed improved root architecture, enhanced nutrient uptake, and better tolerance to oxidative stress, which in turn supported better growth and development even under water deficit conditions. This outcome was consistent across both experimental seasons (2022-23 and 2023-24), indicating the robustness of these findings. Humic acid improved nutrient uptake by increasing soil cation exchange capacity (CEC) and solubilizing nutrients, while sulphur directly contributed to the synthesis of essential amino acids, proteins, and fatty acids. The synergistic effect of humic acid and sulphur led to an improvement in chlorophyll content and photosynthetic efficiency. Moreover, the I₃S₃ treatment showed reduced membrane injury (lower membrane injury index, MII) and improved membrane stability index (MSI), indicating that the plants were better equipped to cope with stress and dehydration. Overall, the combination of irrigation and nutrient management (humic acid and sulphur) led to improved growth, increased biomass production, and higher seed yield compared to the control and other treatments.

Objective 3: To study the effect of moisture deficit on oil content and quality

Moisture deficit during critical growth phases significantly impacted the oil content and quality of mustard seeds. The study observed that water stress, particularly during flowering and seed filling, led to a reduction in oil content and altered the fatty acid profile of mustard oil. Under water deficit conditions, the oil content decreased by 22% compared to plants that received adequate irrigation. Furthermore, water stress resulted in a higher proportion of saturated fatty acids such as palmitic acid, which decreased the nutritional value and quality of the oil. These findings suggest that optimal water management is critical not only for improving yield but also for enhancing the nutritional quality of mustard oil. Water stress also caused a decrease in seed size and weight, which directly affected the oil yield. In the I_0 treatment (no irrigation), seed yield and oil yield were both significantly lower compared to the I_3 treatment. Additionally, the oil quality parameters, such as iodine value and saponification value, were adversely affected by water stress, indicating that water availability plays a critical role in determining the quality of mustard oil. The I_0 treatment had the lowest iodine and saponification values, suggesting that moisture stress negatively impacts the quality of mustard oil, reducing its shelf life and commercial value.

Objective 4: To study the effect of humic acid and sulphur on oil content and quality

Humic acid and sulphur application had a significant positive effect on oil content and oil quality, especially under moisture deficit conditions. The I_3S_3 treatment (three-stage irrigation with humic acid and sulphur) resulted in higher oil content and improved oil quality compared to the control and other treatments. Humic acid enhanced nutrient availability, improved soil water retention, and mitigated oxidative stress, leading to better seed development and higher oil accumulation. Sulphur played a crucial role in fatty acid synthesis, which improved the oil's fatty acid composition, increasing the proportion of unsaturated fatty acids like oleic acid and linoleic acid, which are beneficial for heart health. Moreover, the combination of humic acid and sulphur improved antioxidant activity and total phenolic content in the oil, enhancing its oxidative stability and shelf life. The I_3S_3 treatment produced oil with the highest total phenolic content (TPC) and antioxidant activity (AA), which are indicators of high-quality mustard oil. In contrast, the I_0 treatment (no irrigation) produced oil with lower antioxidant activity and higher peroxide values, suggesting that water stress

exacerbated the degradation of oil quality. These results underscore the importance of integrating humic acid and sulphur into mustard cultivation to not only increase oil yield but also enhance the oil's nutritional and commercial value.

This research significantly contributes to several United Nations Sustainable Development Goals (SDGs). It supports SDG 2 (Zero Hunger) by enhancing crop productivity and improving the nutritional value of mustard oil, ensuring a stable food supply. It also aligns with SDG 6 (Clean Water and Sanitation) by promoting efficient water use in agriculture, particularly in water-scarce regions, reducing waste, and encouraging sustainable practices. The study further contributes to SDG 12 (Responsible Consumption and Production) by demonstrating how integrated nutrient management, such as using humic acid and sulphur, can optimize production while maintaining environmental sustainability, reducing reliance on chemical fertilizers, and improving soil health. Finally, it addresses SDG 13 (Climate Action) by focusing on improving crop resilience to water stress, helping farmers adapt to the impacts of climate change and extreme weather conditions.

This study highlights the potential of humic acid and sulphur in optimizing Indian mustard cultivation under varying water regimes. Future research should focus on the long-term impact of these practices across different climates and soils, along with an economic analysis to assess their cost-effectiveness, especially in smallholder farming. Integrating these nutrients into water-efficient practices offers a promising solution for boosting mustard production, improving oil quality, and promoting sustainability in oilseed crops, contributing to climate-resilient agricultural systems and food security.

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