IMPACT OF BORON, SULPHUR, AND CYTOKININ IN INDIAN MUSTARD (*Brassica juncea* L.) UNDER SPATIAL DYNAMICS

Thesis Submitted for the Award of the Degree of

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in

AGRONOMY

By

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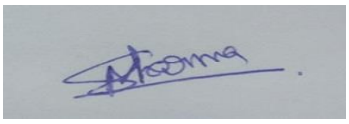
DECLARATION

I, hereby declared that the presented work in the thesis entitled **"IMPACT OF BORON, SULPHUR, AND CYTOKININ IN INDIAN MUSTARD (*Brassica juncea* L.) UNDER SPATIAL DYNAMICS"** in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of ____Dr. Prasann Kumar___, working as _Assistant Professor, in the _Agronomy, School of Agriculture of Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of another investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.



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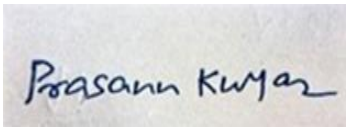
CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled **"IMPACT OF BORON, SULPHUR, AND CYTOKININ IN INDIAN MUSTARD (*Brassica juncea* L.) UNDER SPATIAL DYNAMICS"** submitted in fulfillment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the Agronomy, School of Agriculture , is a research work carried out by Monika Sharma, (12106542) , is bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.



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ABSTRACT

Quality production is the main important factor which plays a significant role in the health of individuals as well as economy of a nation. Among oilseed crops, mustard (*Brassica juncea* L.) is an important crop which ranks third as the most crucial oilseed crop of the world and in India, it is the second most widely grown edible oilseed after groundnut. With the increasing population day by day, the land area is decreasing which is affecting the yield production of the crop. Another cause of mustard's low production is an unbalanced fertilizer application which decreases the micronutrient uptake inside the plant. So, this study is practiced seeing the effect of different agrochemicals on mustard crops under reduced row-to-row spacing to balance or hasten the yield production than its production under recommended spacing. Mustard reacts differently to foliar application of boron, sulphur, and Phytohormones; therefore, it is essential to check the morpho-physiological and biochemical characteristics of the mustard plant to know the effect of these nutrients on growth, development and yield of the mustard crop. The study aimed to assess the effects of boron, sulphur, and cytokinin under the limiting spacing of the mustard crop to hasten the quality yield and production under a limited ground area. The present study, **"Impact of Boron, Sulphur, and Cytokinin in Indian mustard (Brassica juncea L.) under Spatial Dynamics"** was carried out in an open environment. A field experiment was conducted at Lovely Professional University field, Jalandhar, Punjab with one variety of mustard crop- NB-RIMUL-2019 (Nandi Bull). Different levels of boron (0.5-1.5%), sulphur (0.10-0.25%) were applied individually or in combination with plant growth hormone cytokinin (0.003-0.0045%) as a foliar application under split plot design to know their effect on growth, physiology, biochemical and yield attributing characters of the mustard crop. Different morphological, physiological, biochemical, and yield attributing parameters of the mustard crop were studied on evidence-based observations at different intervals of time under this experiment. Application of boron, sulphur, and cytokinin enhances the morpho-physiological, biochemical as well as quality parameters of the mustard crop grown under limiting spacing and shows a positive impact on quality production of the crop.

Keywords: Agrochemicals, Mustard, Phytohormone, Spatial dynamics, Sustainability, Quality yield, Zero hunger

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Place: LPU, Phagwara

Dated: **(Monika Sharma)**

TABLE OF CONTENT

List of Tables

List of Figures

LIST OF ABBREVIATIONS

Symbols used	Abbreviation
;	Semicolon
%	Per cent
:	Colon
^{0}C	Degree Celsius
CAT	Catalase
Chl a	Chlorophyll a
Chl b	Chlorophyll b
cm^{2}	Square Centimeter
cm	Centimetre
CO^{2}	Carbon dioxide
SPD	Split plot Design
Conc.	Concentration
DAS	Days after sowing
DDW	Double distilled water
EC	Electrical Conductivity
EDTA	Ethylenediaminetetraacetic acid
et al.,	Co-worker
FW	Fresh Weight
DW	Dry Weight
g	Gram
$g\ cm^{-3}$	Gram per cubic centimeter

HCl	Hydrochloric Acid
H_2SO_4	Sulphuric Acid
H_2O_2	Hydrogen Peroxide
q ha^{-1}	Quintal per hectare
kg ha^{-1}	Kilogram per hectare
lakh ha	Lakh hectare
lakh tons	Lakh tons
M	Molar
t ha^{-1}	Tonne per hectare
mg	Milligram
mg kg^{-1}	Milligram per kilogram
ml	Milliliter
mM	Millimolar
N	Normal
NaOH	Sodium Hydroxide
Nm	Nano Meter
NO	Nitrous Oxide
POD	Peroxidase
ppm	Parts Per Million
R	Replication
rpm	Rotation Per Minute
RWC	Relative Water Content
PAL	Phenylalanine ammonia lyase
T	Treatment

R	Replication
B	Boron
S	Sulphur
Cyt.	Cytokinin
UV	Ultra Violet
w/v	weight by volume
MSI	Membrane Stability Index
MII	Membrane Injury Index
v/v	volume by volume
OP	Osmotic Potential
PV	Peroxide value
H.I.	Harvest index
E.Y.	Economic yield
B.Y.	Biological yield

CHAPTER 1

INTRODUCTION

Oils are the primary source of energy, which may be obtained through the cultivation of oilseed crops. Mustard is a vital oilseed crop that is cultivated and consumed worldwide. After soybean and oil palm, it ranks third in the most important oilseed crop in the world. The mustard crop belongs to the family Brassicaceae. Brassica genus has six species, namely: *B. carinata, B. juncea, B. oleracea, B. napus, B. nigra,* and *B. rapa*). It ranks second in the most crucial oilseed crop after soybean (FAO, 2010). Brassica compestris is referred to by the names such as sarson, toria, summer turnip rape, polish rape, etc. Oilseed production is in short supply in the state. Toria (*Brassica rapa*), raya (*Brassica juncea*), Gobhi sarson (*B. napus*), taramira (*Erucea sativa*), and African sarson are all varieties of rapeseed mustard (*B. carinata*). Toria and taramira are mostly cross-pollinated, while Raya, Gobhi Carson, and African Sarson are mostly self-pollinated. Toria, Gobhi Carson, and taramira are classified as rapeseed in the trade, whereas raya and African sarson are classified as mustard. 59.7 million tons of Brassica oilseeds are globally produced from 32.5 m ha areas (Kapila et al., 2012). In the world, India ranks 2nd and 3rd for area and production, respectively, with 26.5% and 16.6% of total hectares and production of Rapeseed-Mustard. In India, the oilseed crop and the Rapeseed-Mustard species account for 14.1% and 3% of gross cropped area, respectively.

The mustard crop is grown effectively in areas of modern agronomy. It can be grown under different climatic conditions. Raya sarson can be cultivated in both irrigated and rain-fed environments. Generally, mustard is a Rabi season crop that can be grown in a relaxed environment. Mustard is a C3 plant and performs various pathways to activate carbon assimilation and other secondary metabolites. It requires 15-20◦C temperature for conducting photosynthesis and other processes of plant life. Indian mustard contains a lot of proteins, carbohydrates, vitamins, lipids, and minerals. Carbohydrates contribute about 85% of the total

calorie requirement in the mustard plant, whereas fats and oils only account for about 4-12% (Ullah, 1989).

Mustard crops can be grown and consumed worldwide. In India, it is grown in different states. It may be planted as a catch crop in Punjab, Haryana, and Himachal Pradesh. Mustard is a *Rabi* season crop and can be grown in a temperate environment. Grown in a temperate climate, mustard is a crop of the Rabi season. As a cold-growing crop, it can also be grown in tropical or subtropical climates. Its tolerance for 500–4200 mm of precipitation annually and 5–25°C is enough for its healthy growth and development in rainfed and irrigated environments. Heat stress has hugely damaging effects because of India's tropical climate. Rain is necessary for the sowing of *Brassica* because it is a Rabi crop native to arid and semi-arid areas. When heat stress occurs during the sowing season, fewer seeds germinate, and more seedlings die. Better development requires a pH of 4.3 to 8.3. This pathway is used by mustard, a C3 plant, to assimilate carbon and other metabolites. The edible oil content of mustard seeds ranges from 37 to 49 per cent (Singh *et al.,* 2009). Gobhi sarson is a new emergent oilseed, a long-duration crop that may be grown in Punjab, Haryana, and Himachal Pradesh. The young leaves of the mustard crop are utilized as greens or leafy vegetables, and the oil cakes are fed to cattle.

In India, mustard is the second most widely grown edible oilseed after groundnut, accounting for 23.7 and 26.0 per cent of total oilseed acreage and production, respectively. The chromosome number of Indian mustard is 2n=36. Generally, it is a self-pollinated crop; however, insects and other pollination methods may also occur. Nearly 5-10% of pollination is done by insects or other factors. The origins of the Mustard crop are China and northeast India, from where it goes up to Punjab, Haryana, etc.

The leading cause of mustard's low production is an unbalanced fertilizer application. Mustard is more sensitive to sulphur than other crops. As the sulphur is applied to the mustard crop, the oil content of the mustard seeds also increases, which results in a higher net return (Singh *et al.,* 2015). The sulphur-containing glucosinolates are responsible for the aroma and

pungent flavour. Indian mustard yields rise when sulphur fertilizer is used (Piri *et al.,* 2011). Sulphur considerably impacts the concentration of oil, fatty acids, and glucosinolates in mustard seeds (Falk *et al.,* 2007). Sulphur levels improve sulphur uptake, stover yield, and seed yield (Sharma *et al.,* 2009).

Sulphur

Sulphur (S) is a secondary plant nutrient for crop growth and development. It is essential for protein synthesis and performs the activities of different enzymes. In the mustard plant system, sulphur also plays a vital role in the defence mechanism, protecting the plant from various pathogens and environmental conditions. As we know, sulphur is an essential constituent of seed protein, different amino acids, enzymes, and glucosinolates, so it can benefit the crop in reduced spacing and help balance or hasten the yield compared to recommended spacing. Sulphur helps enhance chlorophyll content and oil synthesis; thus, it significantly improves the quality yield and production of the mustard crop. The deficiency of sulphur in the plants can lead to the yellowing of the leaves or the pale green colour of the mustard plant. It can also show chlorosis symptoms on the shoot tip of the plants. The roots absorb the primary sources of sulphur in the form of sulphate. The plant's shooting portion absorbs sulphur gas, which is then used by various plant sections. It shields the plant from various biotic and abiotic stresses. It is essential to the overall growth of the plant, and its absence can result in stunted plant growth. Different activities are observed in rapeseed mustard when sulphur and boron are applied. B and S foliar application enhances yield production by inducing various physiological and biological processes in the plant. The best ways to determine the activity of B and S in the mustard plant are to measure various photosynthetic activities, chlorophyll content (Rohacek et al., 2008), and leaf area index (Kulig et al., 2014). After nitrogen, phosphorus, and potassium, sulphur is the fourth most important plant nutrient. Plant tissue has a total sulphur content ranging from 0.3 per cent to 7.6 per cent; plants grown in gypsum soils have a concentration of 7.6 per cent. After

potassium, phosphorus, and nitrogen, sulphur is the fourth most important plant nutrient. Most of the sulphate the plant absorbs is reduced and transformed into the amino acids cysteine and methionine, which are necessary to synthesise proteins (Haneklaus et al., 2007). Amino acids, chloroplasts, sulfatides, vitamins, coenzymes, and prosthetic groups (lipoic acid, thiamine, coenzyme A, iron–S clusters, and so forth) are all found in sulphur. Therefore, S is necessary for respiration, photosynthesis, and the development of cell membrane structures in plants (Nakai et al., 2020; Yoshimoto & Saito, 2019). For plants to respond to biotic and abiotic stress, S is necessary. Among the S-containing substances connected to plant stress tolerance are glutathione (GSH), phytochelatin, S-containing proteins, and glucosinolates. These molecules that contain Sulphur can aid plants in overcoming a range of stressors. For instance, glutathione (GSH) is a vital antioxidant. It may impact the relative oxygen species that oxidative stress produces. Crop yield and quality correlate with S (Henriet et al., 2019). Sulphur participates in the plant's overall development, and its deficiency can stunt its growth. The availability of sulphur helps regulate N-use efficiency and promotes photosynthesis, dry matter accumulation, and so on in the plant. Plant growth hormones like GA3, auxin and cytokinin play a vital role in s-assimilation and physiological and biochemical responses of the plant under optimum and constrained climatic conditions (Khan & Khan, 2014).

Boron

Indian mustard (*Brassica juncea*) is very sensitive to boron deficiency. Boron is a metalloid of group III and has the properties of both metals and non-metals (Warington1923). In nature, it has a higher scattering in the lithosphere and hydrosphere despite having a low fixation. In rocks, the B concentration ranges from 5 to 10 mg kg^{-1}; in rivers, 3 to 30μgl^{-1}; and in seas, it is approximately 4.0 mg-1. Among the micronutrients that stimulate different processes in plants, such as the formation of cell walls, membrane stability, and the maintenance of

structural and physiological processes, boron is the main component. It is essential for pollination and photosynthetic translocation in plants. As a micronutrient, boron is needed in trace amounts. One ppm is adequate for plants growing in soil, and two ppm in the soil test may be detrimental. According to Brown P.H. *et al.* (2002), B is one of the nutrients that crops need to grow, develop, yield, and quality at their best. Plants in the crucifer family are susceptible to boron deficiency, and a hollow heart, characterised by blasting and necrosis of the stem centre, is one of the most prevalent symptoms. This is essential to the growth and physiological processes of the mustard plant. Cell wall integrity, cell division, plasma membranes, phenol metabolism, and the requirement for nitrogen fixation and plant reproductive growth are just a few of the processes that make boron significant. One of the essential elements for plants to grow to their full potential is boric acid. It has previously been established how crucial B is for increased plant growth and development. Critical functions of boron include the development of hormones, fruit and seed formation, cell division, sugar transport, and wall growth and strength. The actions of phosphorus, potassium, calcium, nitrogen, and boron in plants are interdependent. The two most essential functions of boron in plants are its structural role in cell wall growth and the stimulation or inhibition of specific metabolic pathways. It is an essential micronutrient that plays a significant role in the growth and development of the plant. It plays a significant role in improving yield and oil content in mustard crops. The exogenous application of boron significantly improves the yield-attributing characteristics of the mustard crop and thus influences production quality (Kumararaja et al., 2015). Boron is a micronutrient with a significant role in forming cell walls, membrane stability, and other plant morpho-physiological functions. Boron has a role

in the translocation of photosynthates and pollination in plants. It also takes part in oil and protein synthesis.

Cytokinin

Phytohormones are essential in improving the yield and oil formation of the mustard plant. Plant growth hormones like auxin, cytokinin, and Gibberellin are considered growth promoters, which have a role in the overall development of the plant. Cytokinin plays a significant role in forming lateral buds and increases the branching to enhance quality production. The foliar application of plant growth hormone cytokinin helps increase photosynthesis efficiency and promotes the plant's yield and production. Plant growth regulators are vital for plant development, increasing yield, and boosting seed quality. Cytokinin has been shown in studies to promote mustard growth, flowering, photosynthesis, nutrient transfer, and yield (Khan *et al*., 2005).

Cytokinin is a phytohormone required for plant growth and development in small amounts at low concentrations. So, favourable conditions can be generated in a specific crop by exogenously injecting growth regulators like Cytokinin in the right concentration at the right time. The study aimed to assess the effects of boron, sulphur, and cytokinin under the limiting spacing of the mustard crop to hasten the quality yield and production under a limited ground area. In this study, modern instruments will be used to carry out the molecular study inside the plant system and determine the chemical components from the extract of the seeds and leaves of Indian mustard. Plant growth hormone significantly influences the growth and quality of a plant's yield. Gibberellins, auxin, and cytokinin are the growth promoters that greatly accelerate the plant's development. Cytokinin is a critical factor in the development of lateral buds and an increase in branching that maximises yield potential. A particular element or nutrient is produced due to growth and development and is necessary for the organism to function normally. Exogenous application of B, S, and Cytokinin can boost photosynthetic capacity and encourage plant growth and yield. When plants are stressed by drought, cytokinins are known to help them hold onto their flowers. It is well known that cytokinins

keep plants from sensing disease, resulting in a higher-quality and more abundant harvest. According to C. Dervinis (2010), plant hormones are naturally occurring organic molecules that aid in the growth and development of plants as well as the reduction of biotic and abiotic stressors. Auxins, cytokinins (CK), gibberellins, abscisic acid, ethylene, brassinosteroids, salicylic acid, jasmonic acid, and strigolactones are examples of plant hormones. Phytohormones are categorised into nine categories (Su et al., 2017). It is only recently that the role of the conventional growth-stimulating hormone cytokinin has been identified. There is more significant activity in natural sources than in artificial ones. Cytokinins (CKs) regulate various aspects of plant growth, development, and physiology, including seed germination, apical dominance, flower and fruit development, leaf senescence, and plant-pathogen interactions. Cytokinins are isoprenoid-substituted adenines. Different plant species, tissues, developmental stages, and environmental factors have very different types and activities of CK molecules.

Importance of spacing

Spacing is the most crucial factor that influences the plant's morpho-physiological development and affects crop growth and yield. The crop sowing under varying spacing and environmental conditions is supposed to affect the plant growth and yield. To obtain a higher yield, spacing plays an important role, and in Punjab, sowing of mustard (different genotypes) with spacing 30 x 10 cm is generally practised. However, as we all know, with the increasing population day by day, it is impossible to increase the production area. We have to take action to increase the production per unit area. As the land area is decreasing daily, we should invent new methods to achieve higher production in a limited land area. So, this study is practised to see the effect of different agrochemicals on mustard crops under reduced row-to-row spacing to balance or hasten the yield production than its production under recommended spacing. Planting patterns significantly impact how effectively incoming solar radiation is intercepted, absorbed, penetrated, and utilised, which increases crop productivity overall. Plant density is another crucial factor that can be adjusted to get the most output per unit of land area. Agroclimatic conditions, growth habits, and variety influence the ideal plant density and

planting geometry. Furthermore, it is a fact that certain varieties do not always display the same phenotypic traits in all environmental circumstances. A better cultivar is a valuable instrument that has influenced production in numerous nations around the globe. Apart from numerous other elements, cultivars with a more significant potential for yield and a broad range of climatic and edaphic adaptation conditions are necessary to raise the yield per unit area, thus increasing overall production (Singh & Kaur, 2011). Numerous new cultivars have been created, and each location-specific assessment of agro-input is required. Plant spacing is the most critical aspect of management.

A common oilseed crop, mustard, exhibits a high demand for fertilisers such as boron and sulphur (Sienkiewicz-Cholewa et al., (2015). This is so because the production of sulphur amino acids and glucosinolates requires both B and S. The best way to supply B to plants is by adding B fertilisers to the soil; however, foliar treatment can be very successful, mainly if insufficiency is discovered during the growing season; and seed and band placement may be detrimental (Varga et al., 2014). Foliar fertilisation is a great way to give plants B when dry soil inhibits root activity. Despite growth trends in sulphur-fertilized soil facilities compared to objects on which sulphur was sprayed, no discernible difference in seed output was found between objects on which sulphur was supplied foliar and sulfur-fertilized soil facilities. It is found that foliar fertilisation and top dressing combined with sulphur feeding result in a higher yield of rape seeds. Since mustard responds differently to foliar applications of S and B, it is imperative to recognise an early cue that indicates the direction of the response. For this, measurements like the chlorophyll index (SPAD) and chlorophyll fluorescence, which characterise how well the photosynthetic machinery works, are helpful (Kulig et al., 2014). Chlorophyll fluorescence measurements are straightforward, transparent, and highly sensitive (Kalaji *et al.,* 2014). Chlorophyll values, crop yield, specific physiological markers, and plant biometric or yield attributes correlate significantly. Therefore, this study was conducted to understand the impact of micronutrients and plant growth hormones on mustard growth and productivity under spatial dynamics.

Research gap identification:

Boron, Sulphur and Cytokinin mediated changes in the morpho-physiological, and yield attributes of mustard.

Hypothesis

- Evaluation of spatial dynamic responses in Indian mustard under the influence of Boron, Sulphur and Cytokinin.

RESEARCH OBJECTIVES

- Evaluation of spatial dynamics and B, S and Cytokinin on growth, yield, and quality of Indian mustard.
- Impact of spatial dynamics and B, S and Cytokinin on physiological responses of Indian mustard.
- Assessment of boron, sulphur, and cytokinin on biochemical behavior of Indian mustard.
- To study the impact of different treatments on the economic feasibility of the Indian mustard.

CHAPTER 2

REVIEW OF LITERATURE

Boron plays an influential role in the translocation of sugar and photosynthates. Sulphur plays a significant role in oilseed crops and performs various tasks inside the plant. Cytokinin is an essential plant growth hormone that plays a significant role in the growth and development of the plant. It shows significant results when applied to the crop plant species as a foliar application. When the combined application of Boron sulphur and Cytokinin is applied on the mustard plant under limiting spacing, an increase in the photosynthetic material will be found, which will be translocated in various parts of the plant to enhance the quality yield and production even under spatial variations.

Sumi et al. (2021) conducted a research trial at the research farm of Rajasthan College of Agriculture, Udaipur. He makes four levels of sulphur, i.e. 0, 20, 40, 60 kg S/ha and four levels of GA3, i.e. 0, 25, 50, and 75 ppm, respectively. His findings show that a significant increase in plant height, dry weight, number of branches plant-1, number of siliqua plant-1, number of seeds siliqua-1, test weight, seed yield, stover yield, and biological yield of mustard was observed with the application of 40kgS/ha and 50ppm GA3 respectively.

Khan & Mobin (2005) experimented and showed that the exogenous application of GA3 increases shoots growth, photosynthesis, and soil nitrogen (N) utilisation in mustard. He reported that mustard has a high sulphur requirement, and the assimilatory pathway is well coordinated with N. In his study, he reported that the application of sulphur, along with GA3, improves photosynthetic production, and there is an increase in the crop's N and S use efficiency. The dose of GA3 he used in his experiment was ten μM. He carried out his research to see the effect of foliar application of GA3 on leaf area, plant dry mass, leaf carbon dioxide exchange rate (CER), plant growth rate (PGR), relative growth rate (RGR), net assimilation rate (NAR) and S-use efficiency (SUE) of mustard treated with 0, 100 or 200 mg S kg−1 soil levels. He shows that the plants receiving 100mgS/kg soil and GA3 show higher

specific leaf area and accumulation of dry mass compared to its control. The application of GA3 also increases the concentration of nitrogen and sulphur in the plant.

Puzynska et al. (2018) conducted a research trial and found that Brassica sp. reacts differently to the exogenous application of boron and sulphur. He stated that the exogenous application of boron and sulphur increases the mustard plant species' quality yield and oil content.

Malhi et al. (2005) conducted a field experiment and stated that applying sulphur @15-30 kg/ha helps control the sulphur deficiency in oilseed crops such as mustard. In his study, he reported that applying sulphur can restore seed yield and tolerate plant deficiency at the flowering stage.

Rohacek et al. (2008) experimented and showed that rapeseed mustard shows significant changes in its activities under the foliar application of sulphur and boron. Exogenous boron and sulphur application implements various physiological and biochemical processes in the mustard plant and enhances yield production. Different morphological and physiological parameters like photosynthetic activity, chlorophyll content, and leaf area index show the effect of boron and sulphur in the mustard plant.

Begum et al. (2012) experimented with the Central Research Station of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. The author tested different doses of sulphur, like 0, 20, 40, 60, and 80 kg/ha, on mustard. The result from the study shows a significant increase in the growth parameters and yield attributing characters of mustard where the application of sulphur was there. All the growth and yield attributing characters show a significant increase where sulphur fertiliser up to 60 kg S/ha is provided to the crop. All the morphological and physiological characteristics like plant height, leaf area, NAR, CGR, and yield parameters like no. of siliquae per plant, seeds per siliqua and seed yield per plant show a great increase when S @60kg/ha is applied to the crop. The highest seed yield was found when S was used @ 60 kg/ha compared to its control.

Masood et al. (2016) reported the effect of Gibberellic acid (GA3) and Sulphur in the stimulation of photosynthesis in the mustard crop. This study shows the involvement of ethylene in S-assimilation in GA3-treated mustard crops. Application of GA3 and S lowers oxidative stress of crop plants and decreases stress ethylene formation to the range suitable for promoting sulphur use efficiency, glutathione production, and photosynthesis. By blocking ethylene production with aminoethoxy vinyl glycine, the role of ethylene in GA-induced S-assimilation and restoration of photosynthetic suppression by Cd was demonstrated (AVG). This study shows that inhibiting ethylene in GA plus S-treated plants under Cd stress suppressed S-assimilation and photosynthetic responses, indicating that ethylene is involved in GA-induced S-assimilation and Cd stress alleviation. The study's findings are critical in understanding the interplay between GA and ethylene and their involvement in plant Cd tolerance.

Sosnowski et al. (2019) experimented to see the effect of auxin and cytokinin (6-Benzyl aminopurine (BAP) on the physiological and morphological characteristics of Medicago x varia sp. This study shows an increase caused an increase in plastid pigment content in alfalfa leaves where plant growth hormone cytokinin was applied as a foliar application.

Sosnowski et al. (2019) reported that an exogenous application of Cytokinin at the vegetative and development stages of the plant shows significant effects on the aboveground parts. A significant increase is found in nitrate reductase activity and plastid pigment content in the shooting part of the plants. He conducted a study in which cytokinin was sprayed as synthetic 6-benzyl amino purine (BAP—concentration of 30 $mgdm^{-3}$).

Su & Howell (1995) reported that the effect of Cytokinin and light shows its practical results in hypocotyl elongation. He shows that light is essential in stimulating hypocotyl development under cytokinin-treated plant species.

Kadam et al. (2018) experimented with extracting zeatin (cytokinin) from Moringa oleifera leaves and showed its effects on the mustard plant. He shows that Zeatin's application increases the Brassica species' shooting part when applied externally on the plant surface.

Aremu et al. (2020) specify the mode of action and biological effects of cytokinin on horticultural crops. He also specifies the role of cytokinin in different phases of plant development.

Chang et al., (2016) reported that the exogenous application of Cytokinin (trans-zeatin riboside) at a different concentration, such as 0, 10, and 100µM delays the wilting of the leaf and senescence under drought stress conditions.

Luo et al. (2017) experimented to show that the foliar application of cytokinin leads to an increase in the biomass yield. The main advantage of this plant growth hormone is to enhance the plant species' growth and productivity by accelerating the transpiration rate and inducing minimum leaching.

Hotumalani et al. (2016) experimented to determine the effect of cytokinin on Brassica species. He used two concentrations of Kinetin, i.e. 2.5 and 5.0µM, and states that this shows effective results compared to control in the mustard plant.

Puzynska et al. (2018) conducted an experiment in which Brassica species react differently to the foliar application of Boron and Sulfur. He reported that the foliar application of Boron and Sulfur significantly increases the yield and oil content of the mustard plant.

Masum et al. (2002). Boron is an essential micronutrient that plays a significant role in the growth and development of the mustard plant. He experimented to determine the response of boron to the yield-attributing characteristics of the mustard plant.

Hossain et al. (2011) reported the effect of boron on the yield and mineral nutrition of the mustard plant. He conducted a field experiment to determine the optimum boron dose to enhance the mustard plant's yield.

Malhi et al. (2005) reported that applying sulphur @15-30 kg/ha effectively prevents the deficiency of sulphur in oilseed crops such as mustard. He also reported that applying Sulphur at bolting can restore the seed yield, and at the flowering stage, it can tolerate the deficiency and damage done to the plant.

Shorrocks (1997) states that **the** concentration of B ranges from 5 to 10 mg kg−1 in rocks, 3 to 30μgl−1 in rivers, and about 4.0 mg−1 in seas.

Rohacek et al. (2008) state that rapeseed mustard shows different activities under the application of Boron and Sulfur. The foliar application of B and S induces different physiological and biological processes in the plant and improves yield production. The measurement of different photosynthetic activity, chlorophyll content, and leaf area index are suitable parameters to determine the activity of B and S in the mustard plant.

Shen et al. (1993). B plays a vital role in nitrogen metabolism as it increases nitrate and nitrate reductase activity levels under limited B conditions.

Akhtar (2019) shows that the application of Cytokinin influences plant growth, development, seed germination, apical dominance, flower and fruit formation, and other plant system development.

Sulphur

Sulfur is an essential component for plant development and growth. The crop requires Sulfur, like phosphorus because it contains several critical components that help crop growth and productivity. It is a necessary component of many crops, mainly oilseed crops. Earlier, this element received little attention because fertilisers supplied to the soil gave adequate S, and there was no need for external treatment. However, due to various circumstances and climate change, a widespread S deficiency has been discovered in soils due to high-analysis low-S fertilisers. The use of high-yielding varieties, intensive agricultural practices, and farmyard manure with lower S returns worldwide can cause a deficiency in the soil. S deficiency can decrease the need for protein and enzyme synthesis, resulting in poor crop growth and quality.

S depletion also affects amino acid constituents, including methionine, cysteine, and cystine. To solve the problem of S deficiency, industrial processes provide various S-containing fertilisers and byproducts. The impact of sulfur on oilseed crop growth and yield-attributing traits is studied and reviewed in this study.

The Biological History of Sulphur

Sulfur is a nutrient that all living species require, including plants. The biological importance of sulfur can be traced back to the origin of life when catalytic reactions on iron sulfide surfaces occurred under anaerobic, hydrothermal circumstances **(Wachtershauser, 2000).** Sulfur occurs primarily in the + 6 valence state in the form of sulfate due to the aerobic environment of modern Earth. Sulfur in various oxidation states can be found in anaerobic or volcanic conditions and within living cells. Plants and microorganisms use assimilative reduction to change the valence of sulfate to -sulfide. The process is assimilative because sulfide is utilised mainly for synthesising cysteine, methionine, and other metabolites. The simplest way to understand this approach is to compare it to the dissimilatory process used by anaerobic bacteria that utilise sulfate as the terminal electron acceptor for respiration. In aerobic organisms, the equivalent function of S is oxygen respiration and CO_2 emission.

The functions of Sulphur

Sulphur is found in a wide variety of biological substances. Sulphur can be present in vitamins like biotin and thiamine, as well as cofactors such as S-adenosyl-L-methionine, coenzyme A, molybdenum cofactor (MoCo), and lipoic acid, and a variety of secondary chemicals. In the form of the tripeptide glutathione and specific proteins such as thioredoxin, glutaredoxin, and protein disulfide isomerase, it also fulfils significant structural, regulatory, and catalytic functions in proteins, as well as functioning as a significant cellular redox buffer. The sulfur moiety in many sulfur-containing compounds is often directly involved in the catalytic or chemical reactiveness of the compound. The formation of covalent disulfide bonds between cysteine residues in proteins is a great example. The creation of disulfide

bonds regulates the activity of several enzymes. Many carbon dioxide fixation enzymes are regulated in this way to ensure that their activity is coordinated with photosynthesis light responses. In this case, the regulating molecule is thioredoxin, which uses electrons from ferrous iron to decrease target enzymes. Sulfur seems to increase and decrease due to the low and high soil moisture content **(Gupta & Germida, 1989; Ghani et al., 1990).**

Mishra (2001) conducted a field experiment to see how the different quantities of sulfur (0, 20, 40, and 60 kg ha-1) influenced mustard seed and stover yields. Compared to the control, applying 40 kg S ha-1 resulted in significantly increased seed and stover yields. Mustard's maximum speed and stover yields were 20.63 q ha-1 and 58.26 q ha-1, respectively, which were 36.17 and 39.08 per cent higher than the control values.

Kumar et al. (2001) conducted a field experiment on sulfur levels during winter. For this study, they used 0, 20, 40, and 60 kg ha-1 of sulfur to determine its impact on the growth and yield of the mustard crop. They found that applying 40 and 60 kg S ha-1 simultaneously generated significantly better mustard seed yields than applying 20 kg ha-1 and the control.

Sharma and Jalali (2001) also found that increasing S levels up to 60 kg ha-1 significantly boosted seed and stover production.

Verma et al. (2002) conducted a field experiment to see how different sulphur application rates (15, 30, and 40 kg ha-1) affected Indian mustard yield. Only up to 30 kg ha-1 of seed and stover yield was significantly improved by sulphur application. Meanwhile, Om Prakash et al. (2002) found that increasing sulphur rates by up to 40 kg ha-1 boosted seed yield.

According to **Singh et al. (2002),** using the appropriate amount of NPK in combination with sulphur @ 30 kg ha-1 can enhance mustard yield by 31.4 per cent over using NPK alone. Compared to the control, **Kumawat and Pathan (2002)** found that sulphur spraying at 75 kg ha-1 considerably enhanced plant height and the number of primary and secondary branches per plant.

In a systematic review, **Singh and Meena (2004)** found that with 60 kg sulphur per ha, siliqua plant-1, length of siliqua, seed per siliqua, and test weight of mustard seeds were at their highest. According to **Singh and Singh (2005),** mustard seed output increased by 35.50 per cent when 60 kg S ha-1 was used as a control.

During the winter season, **Rana et al. (2005)** investigated the effects of phosphorus, sulphur, and boron on Indian mustard growth, yield, nutrient uptake, and economics under rainfed conditions with three levels of sulphur. Sulphur significantly influences growth, seed, and biological yield at 20 and 40 kg S ha-1 compared to the control.

Dongarkar et al. (2005) experimented on the effect of nitrogen and sulphur on mustard growth and yield. He concludes that sulphur application significantly influenced mustard growth and yield and observed that plant height, number of branches, dry matter production, number of siliquae, test weight, and seed weight were all significantly influenced by sulphur application.

Kumar et al. (2006) studied the effect of iron and sulphur levels on yield, oil content, and uptake by Indian mustard (*Brassica et al.*) during the winter season (*Rabi)*. They found that applying 40 kg S ha-1 resulted in the highest seed yield (18.37 g/ha), which was 28.1 per cent higher than control.

Karthikeyan and Shukla (2008) conducted a greenhouse experiment on the effect of a combined application of boron and sulphur on mustard (Brassica juncea) and sunflower absorption and quality characteristics (Helianthus annum L.). They discovered that sulphur application raised the mustard dry matter yield to 36.8 g pot^{-1} (@60 mg kg-1 of S application).

Sources of Sulfur

Various S-containing fertilisers, including mined and raw materials, are available in India. Single super phosphate, ammonium sulphate, ammonium phosphate sulphate, gypsum, and pyrite are all notable sources of S. Gypsum has been described as a cheap and superior source

of S when compared to other S sources in increasing the production of oilseeds grown on neutral to slightly alkaline soils **(Subbaiah & Singh, 1970; Arora et al., 1983; Nad & Goswami, 1983).**

Effect of sulphur on the growth and development of mustard:

Kumar *et al.* (2000) conducted an experiment to determine the effect of sulfur on the morphological parameters of the mustard plant. From his study, he reported that the application of sulfur significantly increases the height of the plant, the number of primary and secondary branches, and the leaf area when compared to its control. The application of S to the oilseed mustard shows an increase in seed yield.

Dongarkar *et al.* (2005) studied different doses of sulfur and nitrogen, which significantly affect the growth and development of the mustard crop. He applied different doses of sulfur, like 0, 20, and 40kg ha-1, and nitrogen is 0, 25, 50, and 75 kg ha^{-1}. This study's results show a significant increase in the morphological parameters like plant height, no. of branches, dry matter production, etc. when the applied dose of nutrients is S @25kg ha^{-1} and N @ 75kg ha^{-1}. According to his study, this dose is more effective than any other.

Singh and Dhiman (2005) show that sulfur application on mustard plants increases leaf area, dry weight $plant^{-1}$, and no. of primary and secondary branches $plant^{-1}$.

Giri *et al.* (2006) reported an effective increase in the morphological and yield characteristics of the mustard crop after sulfur was applied.

Ramesh *et al.* (2006) conducted a field experiment to study four different doses of sulfur: 0, 32.5, 65, and 97.5 kg-1. The study shows an increase in the primary and secondary branches of the mustard crop.

Kumar and Kumar (2008) reported the effect of different doses of sulphur in different development stages of mustard on various developmental characteristics of mustard. They showed a possible earlyness in the time of flower initiation and pod formation stage, whereas

it does not affect the number of days to maturity of the mustard crop. This study shows excellent results when compared to its control.

Rajput *et al.* (2018) conducted a research trial on the effect of different levels of S on the mustard crop. From his study, it can be observed that there is a significant result in all the morphological and yield characteristics, like the number of branches, plant height, etc., in the areas where a slightly higher dose of sulphur is applied to the crop.

Verma *et al*. (2018) conducted a field experiment on the mustard crop. His study found an increase in leaf area index and chlorophyll intensity at the mustard crop's pre-flowering stage, i.e., at 30, 60, and 90 DAS.

Effect of sulphur on yield attributes of mustard:

Kumar *et al.* (2000) conducted a field trial on sulfur application. He used different sulfur levels to determine the effect of sulfur on yield-attributing characters of the mustard crop. The results of his study show that there is a significant increase in the number of seed siliquae-1, 1000 seed weight, and harvesting index of the crop when compared to its control.

Kumar *et al.* (2001) conducted an experiment to determine the effect of sulfur on the yield production of the mustard crop. He used different doses of sulfur to determine its effect on quality yield production and yield parameters. As a result, he shows that the treatments where S is applied greatly enhance yield production and other yielding characteristics like the number of seed siliquae^{-1}, seed weight, and biological yield.

Choudhary *et al.* (2003), in their field study, found that the mustard crop acts differently when sulfur is applied to it as soil or foliar. From their experiment, a significant increase is reported in the seed and stover yield of the crop. Sulfur @60kg ha-1 shows a higher increase in seed, stover, and other yield-attributing characters as compared to its control.

Singh and Mukherjee (2004) used S @45kg ha-1 in their experiment, which proved to be very helpful in enhancing the growth and yield of the mustard crop. From his study, an

efficient increase in the number of siliquae plant-1, seed weight, seed yield, and harvesting index is shown when compared to its control or other treatments.

Singh *et al*. (2012) from his experiment observed that there is a significant increase in the morphological as well as yield parameters of the mustard crop when sulfur is applied to the crop.

Kumar *et al*. (2017) conducted a field trial to determine the effect of sulfur on the morphological characteristics of the mustard crop. He observed the maximum increase in the plant's height, leaf area index, fresh weight, dry weight, and yield-attributing characters.

Dongarkar *et al.* (2005) conducted an experiment to observe the effect of sulfur on mustard yield-attributing characters. He observed a significant increase in yield quality attributes compared to the control.

Giri *et al.* (2005) conducted a research trial and studied the effect of phosphorus and sulfur on the growth and seed yield of mustard crops. The experiment was conducted using an RBD design, with twelve treatments applied. In his study, he shows an increase in the seed yield of the mustard crop when sulfur is applied to the crop.

Harendra *et al.* (2005) applied sulfur @60kg ha^{-1} in their field experiment and observed a significant increase in siliquae $plant^{-1}$, seed $siliquae^{-1,}$ and stover yield of mustard.

Rajiv *et al.* (2005) observed that 30kg S ha^{-1} can enhance the mustard yield under salinity stress conditions. His experiment shows that the application of sulfur can significantly enhance the growth, development, and yield of the mustard crop under salinity stress conditions.

Ramesh *et al.* (2005) reported that the application of sulfur maximises the seed yield and 1000 seed weight by 25% compared to its control. It also increases the stover yield and dry matter content of the mustard crop.

Rana *et al.* (2005) studied the yield characteristics of the mustard plant and found an increase in seed yield, oil content, number of siliquae, etc. when sulfur is applied to the crop.

Yadav *et al.* (2005) observed higher yield production under basal application of elemental sulfur @ 30kg ha^{-1} and 66kg P ha^{-1} compared to the control.

Issa and Sharma (2006) conducted an experiment to check the effect of different levels of sulphur on mustard crops and found that higher levels of sulphur can increase yields.

Mehdi and Singh (2006) reported that S treatment up to 40 kg ha^{-1} effectively increased yield contributing parameters such as 1000-seed weight, seed weight $plant^{-1}$, number of siliquae $plant^{-1}$, siliqua length, and seed and straw yields.

Chaubey *et al.* (2008) observed the highest seed yield of mustard with the application of 100% NPK + 5t FYM + 40 kg S ha^{-1} + Azotobactor.

Jat *et al.* (2008) observed in a study that applying S + ZnSO4 + FeSO4 significantly improved the number of siliquae plant-1, number of seed siliqua-1, test weight, seed yield, and stover yield of mustard.

Thuan *et al.* (2010) IARI, New Delhi, in their study, show that applying sulphur @ 40 kg ha^{-1} produces a 19.3% increase in seed yield compared to the control plot.

Mohiuddin *et al.* (2011), in their experiment, show that the application of sulfur significantly increases the quality yield characteristics of the mustard crop.

Verma *et al.* (2012) observed that in mustard, fertiliser applications of 120 kg N + 45 kg S ha^{-1} resulted in significantly higher plant height, number of primary branches $plant^{-1}$, number of secondary branches $plant^{-1}$, dry matter accumulation $plant^{-1}$, siliquae length, number of siliquae $plant^{-1}$, number of seeds $siliqua^{-1}$, 1000-seed weight, grain yield, stover yield, harvest index, and protein content than other levels of fertiliser application.

Debnath *et al.* (2014) found that the seed yield in elemental S was 14.5 percent greater on average in Kalyani (West Bengal) than in control, and this increased to 30.6 percent with inoculation S oxidisers in mustard.

Singh *et al*. (2017) observed the effect of sulphur levels (0, 20, and 60 kg ha-1) and four levels of boron (0, 1, 2, and 3 kg ha-1) on mustard yield, protein, and oil content. It was found that 60 kg S ha-1 produced the highest seed yield, protein, and oil content of mustard, which was statistically comparable to 40 kg S ha-1 and significantly superior to other levels of sulphur. Mustard seed production was increased by 19.1 per cent above control at 40 kg S ha-1. Stover yield increased significantly, up to 60 kg S ha^{-1}. A 51.1 per cent increase was observed in the above study over control.

Kumar *et al.* (2017) show that mustard is a significant oilseed crop used chiefly in cooking worldwide. His study described the higher seed yield of mustard at 40 kg S ha^{-1}.

Singh et al. (2021) conducted a field experiment on sandy loam soil during winter. He studied the effect of foliar application of sulfur on the yield and quality of the mustard crop. Mustard variety GSC-7 is sown in the spacing 30×10cm in RBD design. His study shows a significant increase in the treatment with higher S application than in the control group. An increase in test weight, protein content, and no. of seeds siliquae $plant^{-1}$ is shown in the experiment.

Wright et al. (2015) conducted a field trial to determine the efficiency of wetttable sulfur, which is applied to the vegetable crop as a foliar application. From his experiment, he observed that foliar application of sulfur reduces the insect population from the crop and acts as an insecticide to increase the crop's yield potential. Foliar application of sulfur reduces the number of nymphs from the foliage of potatoes and increases the efficacy of yield production.

Moradi et al. (2021) show that sulfur is an important nutrient, and it plays an essential role in the quality of oilseed crops. A field experiment was done at different sulfur concentrations (0, 1, 2, 4, and 8mg L^{-1}) on sesame cultivars. Applying 6-8mgL-1 concentration of S proves to be

efficient in increasing seed and oil yield. From the study, maximum seed and oil yield was observed in the treatment @ 8 mg L^{-1} of Sulfur application. There is an increase in the fatty acid content and yield of the crop.

Belikova et al. (2017) conducted a two-year experiment on the effect of foliar application of fertilizers with different forms and concentrations of sulfur on the Malus Domestica. He applied different doses of sulfur along with different mineral elements to determine its effect. The experiment showed a possible increase in the growth, development, and yield of the crop. It also increased the concentration of boron in the leaves and fruits of the plant.

Boron

Boron (B) is essential for plant growth, development, and quality **(Brown et al., 2002)**. It is required to complete the various processes of plant life. Plants require it in relatively small amounts (<10 m mole kg^{-1} dry mass) to maintain average growth and development. Its primary function is cell wall synthesis and structural integration in plants, and it also performs a variety of essential processes in the plant system.

Boron in plants

Boron is an essential element for the growth and development of the plant. The concentration of boron is different for different plants, which carry out different life processes. Based on its requirements for the plants, the plants are divided into three groups. Plants that require boron in the lowest amount are graminaceous plants, and other plants, primarily dicots and monocots, require boron in moderate amounts. The third group of plants, generally latex-forming, requires boron in higher amounts. **Shkolnik (1984)** divided monocots and dicots based on growth and deficiency symptoms of boron.

The exact function of B in the plant is not fully understood **(Raisanen et al., 2007)**, but it does appear to play a critical role in cell wall structure **(Hull, 2002)**. Each crop group also presents a different genetic capability, which can be more or less responsive to fertilisation due to active and passive mechanisms of B absorption (**Leite et al., 2007)**. It is estimated that

the threshold B concentration for early vegetative growth in wheat is around 1 mg B kg-1 dry matter **(Asad et al., 2001)**.

Hossain et al. (2011) experimented to find the perfect rate of B application for enhancing the uptake of nutrients and yield of mustard in Bangladesh. The levels of Boron application were 0, 1, and 2 kg/ha. He used mustard variety BARI Sarisha-8 to check the effect of his treatments in the experiment. From this experiment, he observed that the effect of B plays a significant role in mustard growth and development. Its effect was shown in the yield and uptake of nutrients. He found 1-2kg B ha^{-1} optimum to enhance the quality of the seed and stover yield of the crop. The concentration of boron in grains and stover of the mustard crop shows that the B plays a vital role in protein synthesis.

Boron transport in plants

Various molecular aspects of B uptake by root cells and its allocation in plants have been discovered in recent years, which has aided in understanding the process of B transport and usage in plants. It could be an innovative technique for improving crop plants' nutrient transport properties, allowing them to survive boron stress. Boron is primarily found in soil solutions as soluble boric acid (H3BO3), which plants absorb in this form. Plant roots have long been assumed to passively absorb B from the soil solution by simple diffusion across lipid bilayers **(Brown *et al.*, 2002; Tanaka & Fujiwara, 2007).** Takano et al. (2006) recently discovered a new boric acid channel (NIP5;1) in A. thaliana. NIP5;1, a transporter similar to aquaporin, is found in the plasma membrane of root epidermal, cortical, and endodermal cells and is needed for effective uptake of B. B is transported towards the shoot after being put into the xylem by a process mediated by the transpiration stream **(Shelp et al., 1995)**. Although B mobility differs among species, it can be delivered by phloem to vegetative and reproductive organs. The development of boron-diol complexes with sugar alcohols as transport molecules is required for B transport through the phloem **(Brown & Hu, 1996; Hu et al., 1997)**.

Effect of boron on photosynthesis and related attributes

Even though it has been shown that excess B suppresses photosynthesis, information on the effects of B on the photosynthetic process is still lacking (Han et al., 2008; Guidi et al., 2011; Chen et al., 2012). Under B stress, CO_2 assimilation may be reduced for several reasons, including increased oxidative burden, decreased photosynthetic enzyme activity, and a slowed electron transport rate (Han et al., 2009). However, the mechanisms underlying B stress's photosynthesis change have yet to be discovered. It has been found that in plants exposed to high B, the reduction in the photosynthetic rate was accompanied by an increase in intercellular CO2 concentration, whereas stomatal conductance remains unaffected (Sotiropoulos *et al.*, 2002).

Cytokinin

Cytokinins are known to help retain plant flowers when they are exposed to drought stress. Cytokinins help keep plants from sensesciencing, allowing for less disease and greater harvest and quality. Plant hormones are naturally occurring organic molecules that function in the growth and development of plants and help decrease biotic and abiotic stresses **(Dervinis, 2010).** Phytohormones have been classified into nine categories, which include auxins, cytokinins (CK), gibberellins, abscisic acid, ethylene, brassinosteroids, salicylic acid, jasmonic acid, and strigolactones examples of plant hormones **(Su et al., 2017)**. The function of the traditional growth-stimulating hormone cytokinin has only recently been discovered.

Pavlu et al. (2018) reported that cytokinin is a complex plant hormone involved in a variety of plant growth and development processes and stress responses. We review what we know about its metabolism, transport, and signalling in response to variations in macronutrient (nitrogen, phosphorus, potassium, sulphur) and micronutrient (nitrogen, phosphorus, potassium, sulphur) levels (boron, iron, silicon, selenium).

Sosnowski et al. (2019) reported the effects of synthetic auxin and cytokinin on the growth and physiology of the Medicago x varia T. Martyn. The auxin was sprayed as synthetic

indole-3-butyric acid, while the cytokinin was sprayed as synthetic 6-benzyl amino purine. The study revealed that alfalfa plants' responses to cytokinin and auxin treatment were not homogeneous. Using a mixture, but only during the vegetative stage, appears to be the most beneficial. In addition, cytokinin increased the number of plastid pigments in alfalfa leaves. A combination of auxin and cytokinin, on the other hand, induced the maximum nitrate reductase activity in alfalfa roots and increased the ratio of total chlorophyll to carotenoids.

Hasnain et al. (2018) extracted plant growth hormone zeatin (cytokinin) in Moringa oleifera leaves. An 80 per cent methanolic extract was produced from Moringa oleifera (drumstick plant) leaves (cytokinin) to extract and detect zeatin. Using methyl acetate and water-saturated n-butanol, the methanolic extract was further partitioned into Zeatin (cytokinin) fraction. By measuring the Retardation factor (Rf) value using the Thin-layer chromatography (TLC) technique, the presence of the plant growth hormone zeatin in the extracted sample from Moringa oleifera leaves was discovered and detected. The growth of Brassica nigra (black mustard) was studied using a crude extract of Moringa oleifera leaves.

Aremu et al. (2020) reported that Cytokinins (CKs) are a chemically varied class of plant growth regulators that have a wide range of effects on plant growth and development, which is why they are used in agriculture to improve and control crops. Their complex regulatory e and crosstalk interactions with other phytohormones and signalling networks elicit and control various biological activities from the cellular to the organismal level. Even within the same species, the effects of CKs on fruit set, development, maturation, and ripening are not always general, indicating the extent of still unknown complicated biochemical and genetic systems governing these processes.

Chang et al. (2016) reported that Cytokinin (CK) is an important plant hormone that regulates plant growth and development. Nitrogen (N) is an essential macronutrient for plants and one of the most critical growth-limiting factors. This study aimed to see how CK and N affect the visual turf quality and antioxidant metabolism of drought-stressed creeping

bentgrass (Agrostis stolonifera L.). Under drought stress, exogenous CK increased lawn quality and delayed leaf wilting, especially when N levels were high.

Thu et al. (2017) reported that CKs, like auxin, are plant hormones identified about 60 years ago. The role of CKs in driving cell division and differentiation in the shoot meristem was first discovered. According to subsequent research, other essential processes of plant development that CKs regulate include apical dominance, lateral bud growth, root growth suppression, shoot meristem creation and maintenance, nitrogen (N) signalling, phyllotaxis, leaf expansion, and leaf senescence. More recently, CKs have been shown to have a role in developing plant immunity to biotic and abiotic stressors.

Zahir et al. (2001) conducted a field experiment. He reported that Phytohormones are well-known for regulating plant growth and development, and cytokinins are among the most well-known phytohormone groups. An experiment was undertaken to see how synthetic cytokinin (kinetin) and its physiological precursors affected rice growth and yield. Treatments were administered by immersing the roots of rice seedlings for an hour in cytokinin or its precursors soon before transplantation. In comparison to pure cytokinin, the precursors were more potent. The findings support that an exogenous infusion of cytokinin or its precursors in the root zone could boost the treated plant's growth and yield.

Cytokinin transport in plants

The xylem transports cytokinins from roots to shoots, while the phloem transports them reversely. Transported cytokinins may play a role in root and shoot development coordination, for example, by transmitting information on nutrient availability. Although little is known about cytokinin transporters, many cellular importers and exporters are necessary to facilitate effective mobilisation and tailored translocation of cytokinin transporters.

Hotumalani et al. (2016) show that the current study looked at the effects of oxytocin on plant growth metrics such as per cent seed germination, root length and hypocotyl length in Brassica campestris, a commercially important spice and source of vegetable oil. The function

of oxytocin, a neurotransmitter, in plant growth indices, as well as auxin (IAA) and cytokinin, was investigated in a comparative study (kinetin). The results of this study show that oxytocin increased mustard germination and hypocotyl length while having a minor negative effect on root length. These findings suggest that Oxytocin has a growth-stimulating effect comparable to IAA and that it can be administered in the same way.

Effect of spacing on different oilseed crops

Spacing determines the uniform distribution of plants in the field. It directly affects the interception of solar radiation by plants and indirectly affects WUE. **Borger *et al*. (2010)** reported that the crop canopy can be increased by manipulating the row spacing by reducing the light interception to weeds.

Ali *et al*. (2007) conducted a field experiment on different row spacing (45cm, 60 cm, 75cm) and plant spacings (10,20, 30 cm) in sunflowers. The results revealed that maximum achene yield and oil content were obtained at 60×20 cm spacing. They concluded that spacing also influenced the seed and oil content in sunflowers.

Arif *et al*. (2012) carried out a field experiment on three different plant spacing (5,10, and 15 cm) and three different row spacing (10,20,30 cm) in white mustard. Results revealed that seed yield increased with the increasing number of pods per plant in 10x15cm spacing or 20x15 cm spacing.

Malik *et al*. (2001) carried out a field experiment on different row spacings (30,45 and 60 cm), and the results revealed that maximum yield was obtained in 30cm row spacing.

Mulvaney *et al*. (2019) revealed no spacing and seed rate effect on oil concentration. At the same time, spacing affected the seed and oil yield. The highest yield was obtained in 36cm spacing, followed by 18, 53, and 89 cm. They concluded that row spacing significantly influenced the seed and oil yield of *Brassica carinata.*

Kaur *et al*. (2019) conducted a field experiment during the Rabi season at Ludhiana, Punjab, to study the effect of N and four different spacings on different genotypes of Ethiopian mustard. The results revealed that high seed and stover yield is obtained in 25×15cm spacing with more N and protein content in the seed. Tewari and Singh (2011) observed the effect of three different spacing and S levels on the growth and yield of spring sunflowers. The results revealed that maximum yield, oil content, and oil yield are found in 45×30 cm spacing.

CHAPTER 3

MATERIALS AND METHODS

The project work entitled **"Impact of Boron, Sulphur, and Cytokinin in Indian Mustard (*Brassica juncea* L.) under Spatial Dynamics"** was conducted during the *Rabi* season of the year 2021-2022 and 2022-2023 at Lovely Professional University field, Jalandhar, Punjab. The materials and methods used in this investigation are described below in this chapter. A brief description of all materials and methods, like the location of the experiments, properties of soil, climatic conditions, treatments, and all agronomical operations, are given in this chapter. Statistical analysis by SPSS data collected from the experimental field is described in a tabular and graphical form.

3.1 Description of the experimental site

3.1.1 Location of the Experiments

This experiment was conducted at the agricultural research farm of Lovely Professional University, Phagwara, Punjab, during the Rabi seasons of 2021-22 and 2022-23. The research field is situated at a latitude of 31°22'31.8" North and a longitude of 75°23'03.02" East, at an altitude of 252 meters above sea level.

3.1.2 Cropping history

Maize was grown in the preceding season. Immediately after the maize harvest, the field was properly levelled, and mustard was planted in the same field.

Year	Crop rotation
2019-20	Mustard-chick pea
2020-21	Fallow
2021-22	Mustard
2022-23	Mustard

3.2 Experimental design and Treatments details

3.2.1 Experimental Design:

The experiment was arranged using a Split Plot Design, covering an approximate area of 1200 m². It involved two spacing treatments, ten different treatments, and three replications, resulting in a total of 60 plots. The treatments were randomly assigned to the plots to ensure unbiased results. Each subplot measured 5 m × 3 m, equating to 15 m². Separate irrigation channels, each 1 meter wide, were installed to provide irrigation to each plot individually.

3.2.2 Weather and Climate

The research site experienced varying hot and cold climate conditions, with average temperatures reaching up to approximately 50ºC. Weather parameters, including average minimum and maximum temperatures, rainfall, and evaporation rates, were monitored throughout the season from sowing to harvesting. These data were obtained from a relevant weather website.

Table: 3.2.2 Weather and climate data

Month		Temperature		Relative Humidity (%) (RH)		Cloud (%)		Average Wind Speed (Kmph)		Rainfall (mm)		Rainy Days		Pressure (mb)	
		Max. ºC	Min. ºC												
				1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
1.	November	28	24	26	27	15	12	6.6	6.7	17.4	16.5	2	3	1016.3	1026.4
		Av.=17													
2.	December	23	19	37	36	11	10	7.3	6.9	11	12.2	4	2	1015.6	1015.4
		Av.=13													
3.	January	21	17	53	55	15	13	7.1	7.3	1.7	2.1	5	4	1014.3	1013.5
		Av.=11													
4.	February	28	23	39	42	10	12	7.3	7.2	2.7	2.4	4	4	1013.5	1014.3
		Av.=15													
5.	March	35	29	26	28	13	14	11.1	12	16.9	12.3	8	7	1009.5	1013.4
		Av.=19													
6.	April	38	33	21	24	19	17	11.4	11	8	6	6	5	1006.2	1006.4
		Av.=24													

3.2.3 Properties of soil

Soil properties were determined using various standard procedures, including measuring soil pH, electrical conductivity (EC), and macronutrient content.

- **Soil pH:** Soil pH was measured using a pH meter. Soil samples were collected from different field layers and placed into polythene bags. A 12.5 g sample of soil was added to a 150 ml beaker. To this, 50 ml of distilled water was added, and the mixture was stirred for 30 minutes. The pH meter was calibrated with buffer pH solutions. The pH meter rod was then dipped into the soil solution, and the pH readings were recorded (Sawarkar, 2012).
- **Soil EC (Electrical Conductivity):** Soil electrical conductivity was assessed using an EC meter. Ten grams of air-dried soil were placed into a bottle, and 50 ml of distilled water was added. The mixture was shaken on a mechanical shaker for 1 hour to dissolve soluble salts. The EC meter was calibrated with a 0.01 M KCl solution before measuring the sample. The electrode was inserted into the soil solution without disturbing the sample, and the electrical conductivity readings were recorded (Sawarkar, 2012).
- **Nitrogen Content**: The nitrogen content in soil and plant samples was determined through a three-part process: digestion, distillation, and titration.

Digestion: One gram of soil sample was transferred to a digestion tube, and 10 ml of concentrated sulfuric acid and 5 g of catalyst mixture were added. Digestion was initiated at 100ºC, progressing to 360ºC. The process was complete when the solution changed to a light green colour and then became colourless.

Distillation: After cooling, the digested material was subjected to distillation. A hose was placed in 20 ml of 4% boric acid solution, and 40 ml of NaOH was added to the distillation unit. Ammonia gas released during heating was absorbed in the boric acid, changing the solution from pinkish to green. A blank sample was run simultaneously.

Titration: The final step involved titration with 0.02 N sulfuric acid, noting the volume used when the colour changed from greenish to pink (Upadhyay and Sahu, 2012).

- **Available Phosphorus**: Available phosphorus was estimated using Olsen's method. One gram of soil was placed in a 150 ml conical flask, and a pinch of charcoal was added. Next, 0.5 N $NaHCO_3$ was added, and the mixture was shaken for 30 minutes. The content was then filtered through Whatman No. 1

filter paper. Five milliliters of the filtrate were pipetted into a 25 ml volumetric flask. A blank solution was prepared simultaneously. To the flask, 0.5 ml of 5 N H_2SO_4 was added, followed by 4 ml of ascorbic acid, and the volume was adjusted with distilled water. The blue color intensity of the solution was measured using a colorimeter at 760 nm wavelength (Thakur, 2012).

- **Available Potassium:** Exchangeable potassium was determined using a flame photometer. Five grams of soil were placed in a 50 ml conical flask, and 25 ml of ammonium acetate solution was added. The mixture was shaken for 5 minutes and filtered through Whatman No. 1 filter paper. Five millilitres of the filtrate were transferred to a 25 ml volumetric flask. Standard potassium solutions with varying concentrations (0, 2, 4, 6, 8, 10 ppm) were prepared. The solutions were analyzed using a flame photometer, and the readings were recorded (Baghel, 2012).

Table 3.2.3: Soil data

Sr No.	**Particulars**	**Initial reading**	
Chemical Properties			
		1st trial	**2nd trial**
1	pH	6.82	6.7
2	E.C (dS/m)	0.417	0.431
Nutrient Availability			
		1st trial	**2nd trial**
1	Available N	186.6 kg/ha	202.3 kg/ha
2	Available P	11.97 kg/ha	14.01 kg/ha
3	Available K	63.68 kg/ha	67.14 kg/ha

3.3 Experimental Site and details of the experiment

3.3.1 Experimental Site

The field experiment was conducted at the Agronomy Research Farm, Department of Agronomy, Lovely Professional University, during the Rabi seasons of 2021-2022 and 2022-2023. The experimental plots were characterized by fertile soil with uniform

topography and consistent textural composition. They were well-connected to the main irrigation channel, which is linked to a tube well, ensuring prompt and efficient irrigation. Additionally, the plots featured an effective drainage system to remove excess water during the experimental period.

3.3.2 Details of the experiment:

The field experiment was conducted during Rabi 2021-2022 and 2022-2023 by Split Plot Design (SPD) with three replications and nine treatments. Experimental details are given below:

Title: Impact of Boron, Sulphur, and Cytokinin in Indian mustard (*Brassica juncea* L.) under Spatial Dynamics.

Experimental Design: Split Plot design (SPD)

Spacings-2

Replications- 3

Treatment- 20

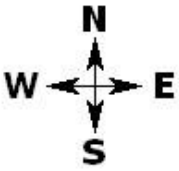
3.3.3: TREATMENT DETAILS

Symbol	Treatment	Symbol	Treatment
T1	**M1S0**	**T11**	**M1S5**
T2	**M2S0**	**T12**	**M2S5**
T3	**M1S1**	**T13**	**M1S6**
T3	**M2S1**	**T14**	**M2S6**
T4	**M1S2**	**T15**	**M1S7**
T6	**M2S2**	**T16**	**M2S7**
T7	**M1S3**	**T17**	**M1S8**
T8	**M2S3**	**T18**	**M2S8**
T9	**M1S4**	**T19**	**M1S9**
T10	**M2S4**	**T20**	**M2S9**

Table 3.3.3: Details of Treatment

A. Main plot	**Spacing**
M1	30*10
M2	20*10
B. Sub plot	
S0	Control
S1	Boron @1%
S2	Sulphur @ 0.15%
S3	BAP @0.003%
S4	Boron @0.5% +Sulphur @0.25%
S5	Boron @ 1.5%+ Sulphur @0.075%
S6	Boron @ 0.5% + BAP (@0.0045%)
S7	Boron @ 1.5%+ BAP (@0.0015%)
S8	Sulphur @ 0.075%+ BAP (@0.0045%)
S9	Sulphur @0.25%+ BAP (@0.0015%)

3.3.4: LAYOUT: -



R1				R2				R3		
M1S0	IRRIGATION CHANNEL	M2S0	PATH	M1S9	IRRIGATION CHANNEL	M2S9	PATH	M1S2	IRRIGATION CHANNEL	M2S2
M1S1		M2S1		M1S8		M2S8		M1S7		M2S7
M1S2		M2S2		M1S7		M2S7		M1S6		M2S6
M1S3		M2S3		M1S6		M2S6		M1S0		M2S0
M1S4		M2S4		M1S5		M2S5		M1S1		M2S1
M1S5		M2S5		M1S4		M2S4		M1S8		M2S8
M1S6		M2S6		M1S3		M2S3		M1S9		M2S9
M1S7		M2S7		M1S2		M2S2		M1S5		M2S5
M1S8		M2S8		M1S1		M2S1		M1S4		M2S4
M1S9		M2S9		M1S0		M2S0		M1S3		M2S3

3.3.5: Details of layout:

Table 3.3.5: Details of layout

S. No	Particulars	Remark
1	Design	Split Plot design
2	No. of treatments	20
3	No. of replications	3
4	No. of spacing	2
5	Total no. of plots	10x6= 60
6	Gross plot size	1200m^2
7	Net plot size	3x5m^2
8	Sowing method	Line sowing
9	Replication border	1.0m
10	Plot border	0.5m
11	Row to row spacing	30cm and 20cm
12	Plant to plant spacing	10cm
13	Variety	NB-RIMUL-2019 (Nandi Bull)
14	Date of sowing (1^{st} trial)	03/11/2021
15	Date of harvest (1^{st} trial)	05/04/2022
16	Date of sowing (2^{nd} trial)	20/11/2022
17	Date of harvest (2^{nd} trial)	24/04/2023

3.3.5: Cultivation Details:

Table-3.3.5: Cultivation Details

S. No	Operations	Date
1	Preparatory tillage	
	[a] Ploughing with tractor-drawn disc plough (1^{st} trial)	30-10-2021

	(2nd trial)	10-10-2022
	[b] Followed by disc harrow and rotavator (1st trial) (2nd trial)	30-10-2021 10-11-2022
	[c] Planking (1st trial) (2nd trial)	01-11-2021 12-11-2022
2	Layout (1st trial) (2nd trial)	01-11-2021 12-11-2022
3	Fertilizer Application (1st trial) (2nd trial)	03-11-2021 20-11-2022
4	Sowing (1st trial) (2nd trial)	03-11-2021 20-11-2022
	Thinning (1st trial) (2nd trial)	20-11-2021 10-12-2022
	1st Top dressing (1st trial) (2nd trial)	26-12-2021 31-12-2022
	2nd Top dressing (1st trial) (2nd trial)	16-01-2022 26-01-2023
5	Intercultural operations (1st trial) (2nd trial)	30-12-2021 13-01-2023
6	Irrigation	
	1. First (1st trial) (2nd trial)	 26-11-2021 13-12-2022
	2. Second (1st trial) (2nd trial)	 12-01-2022 22-01-2023
7	Treatment Spray: (1st trial) • 15DAS	 18-11-2021

	• 45DAS • 75DAS • 105DAS Treatment Spray: (2nd trial) 1. 15DAS 2. 45DAS 3. 75DAS 4. 105DAS	18-12-2021 17-01-2022 16-02-2022 05-12-2022 20-12-2022 04-01-2023 19-01-2023
8	Observation Taken: (1st trial) • 30DAS • 60DAS • 90DAS • 120DAS Observation Taken: (2nd trial) 1. 30DAS 2. 60DAS 3. 90DAS 4. 120DAS	 03-12-2021 02-01-2022 01-02-2022 03-03-2022 20-12-2022 19-01-2023 18-02-2023 19-03-2023
9	Plant protection measures: First (Thiamethoxam) Second (Thiamethoxam)	 28-12-2021 15-01-2022
10	Harvesting (1st trial) (2nd trial)	05-04-2022 24-04-2023

3.5: Cultivation details:

The cultivation practices followed during the experiment are detailed below.

3.5.1: Selection and preparation of field:

A plot with uniform fertility and levelled topography was selected for the experiment. Two deep ploughings and two cross harrowing with tractor-drawn disc harrows prepared the land. After that, it was levelled and divided into plots according to layout and requirements.

3.5.2: Layout preparation:

Prepared the experimental layout according to the requirement and plan by dividing plots uniformly with a disc plough.

3.5.3: Planting material:

The mustard variety "NB-RIMUL-2019 (Nandi Bull)" was selected for this research. It was obtained from an authorized certified seed producer, Good Grow', in Phagwara, Punjab. Healthy and undamaged seeds were used for sowing.

Fig. 3.5.3: Description of the variety

3.5.4: Sowing:

Seeds were sown in lines 3-4 cm deep, with a 30x10 and 20x10 cm planting distance.

3.5.5: Description of variety:

NB-RIMUL-2019 (Nandi Bull): The plants produced were tall, erect, and compact with green foliage. They matured within 120 to 140 days, yielding approximately 6.74 quintals per acre. The seeds were round, bold, and brown and appeared uniform. This variety was resistant to various soil and seed-borne diseases.

3.5.6: Application of fertilizers:

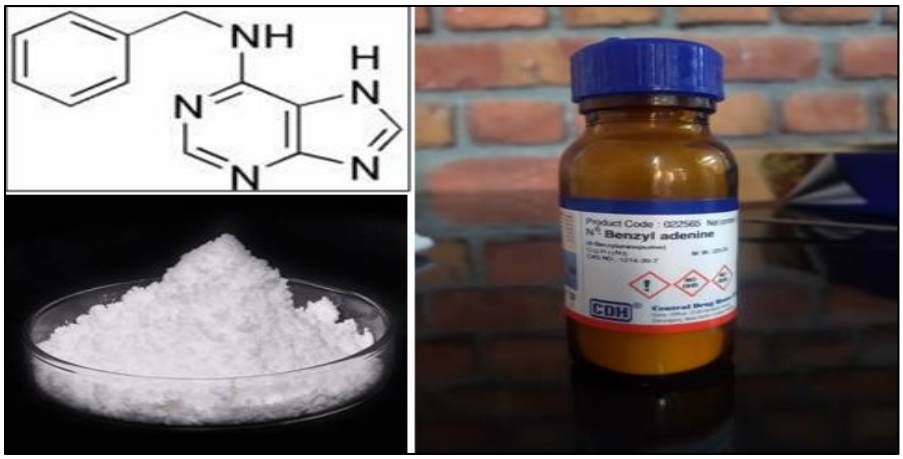
Uniform quantities of nitrogen and phosphorus were applied to the experimental plots, excluding the control plots, using urea (46% N) and Single Superphosphate (16% P_2O_5). Half the nitrogen dose and full phosphorus and potassium doses were applied basally at

the time of seed sowing in the rows. The remaining half of the nitrogen was used in two stages: 25% at 30 days after sowing (DAS) and the remaining 25% following irrigation.

3.5.7: Treatment application:

The treatments were applied as foliar sprays using a knapsack sprayer at 30-day intervals. Applications were made at 15 days after sowing (DAS), 45 DAS, 75 DAS, 105 DAS, and 135 DAS. Observations were recorded at 30 DAS, 60 DAS, 90 DAS, and 120 DAS. At various intervals, sulfur, boron, and cytokinin were applied to the crop canopy as foliar sprays. The specifics of the cytokinin, Boron and sulphur used are detailed below:

Fig. 3.5.7 (a): Treatment Used



Cytokinin (6-benzyl-amino-purine (BAP)

Sulphur

Boron

Fig. 3.5.7 (b): Treatment application

3.6: CULTURAL OPERATIONS AFTER SOWING:

3.6.1: Weeding: Manual weeding was performed two to three times during the crop period. The first weeding was conducted at 30 days after sowing (DAS), followed by a second weeding at 70 DAS. The third weeding, carried out at 120 DAS, was specifically aimed at cleaning the bunds.

Fig. 3.6.1: Showing weeding at different intervals

3.6.2: Irrigation:

The mustard crop requires minimal irrigation. Two irrigations were provided throughout its growth cycle. The first irrigation occurred after the initial weeding, using flood irrigation to water each plot. The second irrigation was applied at the flowering stage to promote effective pod formation.

3.6.3: Plant protection measures:

Meticulous plant protection measures were implemented throughout the crop's growth period. Severe various insect pests and diseases were observed during the season. Careful attention was given to the mustard crop at regular intervals, addressing pest infestations as they occurred in the experimental field.

Fig. 3.6.3: Disease and Insect pest during the experiment

Plant protection measures	Date of spraying
Thiamethoxam	28-12-2021
Thiamethoxam	9-01-2022
Metalaxyl+Mancozeb	31-01-2022

3.6.4: Harvesting:

The crop was harvested 140 days after sowing (DAS), when the pods matured and the foliage became completely dry.

3.6.5: Sampling technique:

Three plants were randomly selected and tagged in each plot for data sampling to facilitate intervallic observations. Treatments were applied at 15, 45, 75, and 105 days after sowing (DAS). Observations were first recorded at 30 DAS, with subsequent observations taken at 30-day intervals, including 60, 90, and 120 DAS. The average values of the recorded data were calculated, and the final observations were statistically analyzed using Statistix software.

Fig: 3.6.5 Experimental field

3.7: OBSERVATIONS RECORDED:

A. Pre-harvest studies:

3.7.1: MORPHOLOGICAL PARAMETERS:

3.7.1.1: Plant emergence percent: The number of plants that emerged was counted at 10 DAS. In each row, emerged plants were recorded for every plot and expressed in percentage. The following formulae calculated the emergence per cent:

$$\text{Percent emergence} = \frac{\text{The total number of seeds emerges}}{\text{Total number of seeds sown}} \times 100$$

3.7.1.2: Plant height (cm): Initially, three plants were randomly selected and tagged in each plot for observation. The main shoot length, measured from the ground level to the growing point tip, was recorded using a meter scale at 30, 60, 90, and 120 days after sowing (DAS) and at harvest time.

The same was repeated for the 2nd trial as well.

Fig. 3.7.1.2: Measuring Plant height

3.7.1.3: Number of leaves per plant: Three plants were randomly selected and tagged in each plot for observation. The number of leaves per plant was counted 30, 60, 90, and 120 days after sowing (DAS) and at harvest time.

3.7.1.4: Stem diameter (cm): The stem girth was recorded from the base of the plant to the tip of the stem of the plant at 30, 60, 90, and 120 DAS intervals by using the instrument Vernier calliper. The mean stem girth of the tagged plant of each replicate was calculated and expressed in the cm.

Fig. 3.7.1.4: Measuring Stem girth

3.7.1.5: Leaf area: The area covered by a leaf from each plant within its canopy was measured in the laboratory using a leaf area meter. Leaves were collected at 30, 60, 90, and 120 days after sowing (DAS) for this calculation.

3.7.1.6: Fresh weight of plant (g): The fresh weight of the plants was recorded by removing three plants from each plot using a sickle at 30, 60, 90, and 120 days after sowing (DAS) and at harvest time. The plants were weighed immediately in grams.

Fig.3.7.1.6: Air drying of plant samples

3.7.1.7: Dry weight of plant (g): The dry weight was measured following the fresh weight determination. The plants were first air-dried in the sun for 3-4 days.

Subsequently, they were placed in a hot air oven at 50°C for 36 hours to remove moisture. The average dry matter accumulation was calculated based on the measurements from three plants at different stages of crop growth. After drying, the plants were weighed, and observations were recorded.

PHYSIOLOGICAL PARAMETERS:

3.7.2.1: Specific Leaf Area: Specific Leaf Area (SLA) measures the leaf area relative to the leaf's dry weight, expressed in cm^2/g. It is calculated using the formula:

$$\boldsymbol{S}LA = \frac{\text{Leaf Area}}{\text{Leaf Weight}}$$

This metric, as proposed by Kvet et al. (1971), provides insight into leaf productivity and is useful for understanding how efficiently plants produce leaf area relative to their biomass.

3.7.2.2: Specific Leaf Weight: It is a measure of leaf weight per unit leaf area. Hence, it is a ratio expressed as g cm^{-2} and the term was suggested by Pearce *et al.* (1968). More SLW/unit leaf area indicates more biomass and a positive relationship with yield can be expected.

$$\text{SLA} = \frac{\text{Leaf Weight}}{\text{Leaf Area}}$$

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

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

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

$$NAR = \frac{(W2 - W1)}{(t2 - t1)} \times \frac{(\log e\ L2 - \log e\ L1)}{(L2 - L1)}$$

Where, $W_1$and W_2 is the dry weight of the whole plant at time t_1 and t_2 respectivelyL_1 and L_2 are leaf weights of leaf area at t_1 and t_2 respectively

NAR is expressed as the grams of dry weight increase per unit dry weight or area per unit time(g $g^{-1}day^{-1}$).

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

$$RGR = \frac{\log e\ W2 - \log e\ W1}{t2 - t1}$$

Where, W1 and W_2 are whole plant dry weight at t_1 and t_2 respectivelyt_1 and t_2 are time interval in days

3.7.2.6: Crop Growth Rate: The method was suggested by Watson (1956). The CGR explains the dry matteraccumulated per unit land area per unit time (g m^{-2} day^{-1}).

$$CGR = \frac{(W2 - W1)}{ (t2 - t1)}$$

Where W2 & W2 are plant dry weight at time T1 & T2.

3.7.3: BIOCHEMICAL PARAMETERS:

3.7.3.1: Chlorophyll content (mg g^{-1} fresh weight)

The chlorophyll content in the leaf of the Mustard crop was estimated using Arnon DI. (1949).

Principle: Chlorophyll was extracted in 80% acetone, and the absorbance was measured at 645nm and 663nm. The amount of chlorophyll was calculated using the absorbance coefficient.

Reagent:

- Acetone (80%, pre-chilled)

Instrument used: Visible Spectrophotometer

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

Chlorophyll 'a' (mg/g Fresh Weight) = 12.7(A663)-2.69(A645) x $\mathbf{\frac{V}{1000xW}}$

Chlorophyll 'b' (mg/g Fresh Weight) = 22.9(A645)-4.68(A663) x $\mathbf{\frac{V}{1000xW}}$

Total chlorophyll (mg/g Fresh Weight) = 20.2(A645) +8.02(A663) x $\mathbf{\frac{V}{1000xW}}$

Where V= Final volume of the extract

W= Fresh weight of the leaves

A= Absorbance at the specific wavelength

The value is expressed as the mg/g fresh weight

Fig. 3.7.3.1: Estimating Chlorophyll content

3.7.3.2: MSI and MII

The MSI was calculated using the formula described by Premchandra et al (1990).

Principle: Membrane damage can be evaluated indirectly by measuring solute leakage (electrolyte leakage) from cells and the MSI. The stimulation effect of stress on Electro Leyte leakage might be attributed to the injury of the plasma membrane.

Reagent:

1. Double Distilled Water

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

Max Electronics, India). The MSI and MII were calculated using the formula described below:

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

$$MSI = 100 - MII$$

Fig. 3.7.3.2: Estimating MSI and MII

3.7.3.3: Total soluble sugar

Principle: The method for estimating total soluble sugar content in plant samples is based on the Anthrone reaction. In this assay, carbohydrates are dehydrated by concentrated sulfuric acid to form furfural. Furfural then reacts with Anthrone to produce a blue-green complex, quantified calorimetrically at 630 nm.

Reagents:

1. **Ethanol (80%)**
2. **Anthrone Reagent:** Dissolve 200 mg of anthrone in 100 ml of ice-cold 95% sulfuric acid. Prepare fresh before use.
3. **Standard Glucose:**

- **Stock Solution:** Dissolve 100 mg of glucose in 100 ml of distilled water.
- **Working Standard:** Dilute 10 ml of the glucose stock solution to 100 ml with distilled water.

Procedure:

1. **Sample Preparation:**
    - Homogenized 100 mg of leaf tissue with 10 ml of 80% ethanol until complete digestion of the tissue was achieved.
    - Centrifuged the homogenate at 5000 rpm for 15 minutes.
    - Transferred the supernatant to a volumetric flask and diluted it to a final volume of 100 ml with distilled water.
2. **Assay:**
    - Transferred 1 ml of the diluted extract to a test tube.
    - Added 6 ml of Anthrone reagent to the test tube.
    - Incubated the mixture in a boiling water bath for 10 minutes.
    - Cooled the test tube under running water.
    - Prepared a blank control in parallel, omitting the leaf sample.
    - After cooling, a blue coloration developed. The intensity of this color was measured at 620 nm using a spectrophotometer.
    - The sugar content in the leaf sample was quantified by referencing a standard curve.

Preparation of the Standard Curve for Estimation of Total Soluble Sugar:

1. A standard glucose solution was prepared by dissolving 100 mg of glucose in 100 ml of distilled water.
2. To create a working standard, the glucose stock solution was diluted by adding 10 ml to 100 ml of distilled water.
3. Sugar solutions of varying concentrations were prepared by transferring 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the working standard into separate test tubes.
4. The final volume of each test tube was adjusted to 3 ml with distilled water, and 6 ml of Anthrone reagent was added.

5. The test tubes were boiled in a water bath as described for the samples.
6. After cooling, the absorbance of each solution was measured at 620 nm using a spectrophotometer.
7. Absorbance values were plotted against the corresponding sugar concentrations to construct the standard curve, which was used to determine the sugar content in the plant samples.

3.7.3.4: Total soluble protein

Bradford's method (1976) was followed.

The assay is based on the principle that the absorbance maximum of an acidic Coomassie Brilliant Blue G-250 solution shifts from 465 nm to 595 nm upon binding to proteins. This shift occurs due to hydrophobic and ionic interactions that stabilize the anionic form of the dye, resulting in a visible colour change from red to blue. The assay is particularly useful because the extinction coefficient of the dye-protein complex remains constant over a 10-fold concentration range, allowing for accurate protein quantification.

Reagents:

1. Sodium Phosphate Buffer (pH 7.4): a. Solution A: Dissolved 13.9 g of sodium dihydrogen phosphate (NaH_2PO_4) in distilled water and adjusted the volume to 1000 ml to prepare a 0.1 M solution. b. Solution B: Dissolved 26.82 g of disodium hydrogen phosphate (Na_2HPO_4) in distilled water and adjusted the volume to 1000 ml to prepare a 0.1 M solution. c. Buffer Preparation: Mixed Solution A and Solution B in a 19:81 ratio and adjusted the final pH to 7.4 using a pH meter.

2. Dye Concentration: a. Dissolved 100 mg of Coomassie Brilliant Blue G-250 in 50 ml of 95% ethanol. b. Added 100 ml of concentrated orthophosphoric acid, then diluted the solution with distilled water to a final volume of 200 ml. c. Stored the dye solution in an amber bottle in the refrigerator; it remained stable for at least six months. d. Before use, diluted the concentrated dye solution with distilled water at a 1:4 ratio. Filtered the solution using Whatman No. 1 paper if any precipitate formed.

Procedure:

1. Transferred 100 mg of plant tissue sample into a mortar.

2. Added 10 ml of cold extraction buffer to the mortar.
3. Placed the mortar in an ice bucket.
4. Ground the sample with a pestle until a fine slurry was obtained.
5. Centrifuged the resulting homogenate at 15,000 rpm for 15 minutes.
6. Collected the supernatant containing the crude protein extract for further analysis.
7. In a test tube, mixed 5 ml of diluted Coomassie Brilliant Blue G-250 dye solution with 0.2 ml of the crude protein extract and 0.8 ml of distilled water.
8. Allowed the mixture to develop colour for at least 5 minutes but not more than 30 minutes.
9. Measured the absorbance of the solution at 595 nm using a spectrophotometer.

Preparation of the Standard Curve for Estimation of Total Soluble Protein:

1. Prepared a series of Bovine Serum Albumin (BSA) solutions with concentrations ranging from 0.1 to 1.0 ml.
2. Added these BSA solutions to separate test tubes.
3. Each BSA solution was mixed with the Coomassie Brilliant Blue G-250 dye solution and followed the same procedure described for the sample analysis.
4. Allowed the mixtures to develop colour for at least 5 minutes but not more than 30 minutes.
5. Measured the absorbance of each solution at 595 nm using a spectrophotometer.
6. Plotted the absorbance values on the y-axis against the BSA concentrations on the x-axis to construct the standard curve.
7. The standard curve was used to quantify the total soluble protein content in the plant samples, expressed as mg/g of sample.

3.7.3.5: Estimation of Total Phenol

Principle:

The total phenol content is determined based on the reaction of phenolic compounds with an oxidizing agent, phosphomolybdate, present in the Folin-Ciocalteu reagent under

alkaline conditions. This reaction forms a blue-coloured complex known as molybdenum blue, which can be quantitatively measured using a spectrophotometer at 650 nm.

Reagents:

1. 80% Ethanol
2. Folin-Ciocalteu Reagent (FCR)
3. 20% Sodium Carbonate (Na_2CO_3)
4. Stock Standard Solution: 100 mg of catechol dissolved in 100 ml of distilled water. A working standard is prepared by diluting the stock solution tenfold.

Procedure:

1. **Sample Preparation:**
   - Crush 500 mg of leaf samples in 3 ml of 80% ethanol.
   - Centrifuge the mixture at 10,000 rpm for 20 minutes.
   - Separate the residue from the supernatant and retain the supernatant.
   - Wash the residue with 2 ml of 80% ethanol and combine this supernatant with the first.
   - Adjust the final volume of the combined supernatants to 5 ml using 80% ethanol.
2. **Assay:**
   - Take 1 ml of the prepared supernatant (extract) and add 1 ml of Folin-Ciocalteu reagent and 2 ml of 20% sodium carbonate.
   - Heat the mixture briefly for 1 minute.
   - Measure the absorbance of the resulting, blue-coloured complex at 650 nm using a spectrophotometer.
   - Calculate the total phenol content by comparing the absorbance with a standard curve and express the result as mg of phenols per gram of fresh weight.

Preparation of the Standard Curve for Estimation of Total Phenol:

(a) Dissolve 100 mg of catechol in 100 ml of distilled water to prepare the stock solution.

(b) Dilute the stock solution 1:10 to prepare the working standard.

(c) Prepare a series of catechol dilutions by taking 0.2, 0.4, 0.6, 0.8, and 1.0-ml aliquots of the working standard into different test tubes, adjusting the final volume to 1 ml with distilled water.

(d) Develop the pink colour following the same procedure used for the sample, then measure the absorbance at 650 nm using a spectrophotometer.

(e) Plot the absorbance values against the corresponding concentrations to create the standard curve, which will be used to estimate the total phenol content in the sample.

Fig. 3.7.3.5: Estimating of Total Phenol

3.7.3.6: Estimation of Total Lipid

The total lipid content in the leaves was determined using the method described by Jayaraman (1981).

Principle: Leaf tissue was extracted using a 3:1 (v/v) ethyl ether and ethanol mixture. The homogenate was then subjected to centrifugation. To facilitate layer separation and prevent emulsification, 0.05 M KCl solution was added. The lipid layer was subsequently dried and weighed, and the total lipid content was calculated.

Reagents: (a) Ethyl Ether: Ethanol (3:1, v/v) (b) 0.05 M KCl solution

Procedure:

1. Homogenized 1.0 g of fresh leaf sample with 10 ml of a 3:1 (v/v) mixture of ethyl ether and ethanol.
2. Centrifuged the homogenate at 2000g for 10 minutes.
3. Transferred the supernatant to a separatory funnel.
4. Added 2 ml of 0.05 M KCl solution to the extract and mixed well.
5. Allowed the mixture to separate into two distinct layers: lipid and water.
6. Carefully decanted both layers separately.
7. Dried the lipid layer.
8. Weighed the dried lipid layer.
9. Calculate the total lipid content based on the weight of the lipid layer.

3.7.3.7: Estimation of Ascorbic Acid

The amount of ascorbic acid was measured using the method described by Mukherjee and Choudhary (1983).

Principle: Ascorbic acid was dehydrogenated by chlorination to form dehydroascorbic acid. This dehydroascorbic acid reacted with 2,4-dinitrophenyl hydrazine to produce osazone. The osazone was then dissolved in sulfuric acid, resulting in an orange-red solution. The absorbance of this solution was measured at 540 nm.

Reagents: (a) 6% TCA (b) 2% Dinitrophenyl hydrazine (c) 10% Thiourea (d) 70% Ethanol (e) 80% Sulfuric Acid (f) **Stock Standard Solution:** Dissolved 100 mg of

ascorbic acid in 100 ml of 4% oxalic acid solution (1 mg/ml). (g) **Working Standard:** Diluted 10 ml of the stock solution to 100 ml with 4% oxalic acid, resulting in a concentration of 100 µg/ml.

Procedure:

1. Extracted 100 mg of leaf sample with 5 ml of 6% TCA.
2. Mixed 3 ml of the enzyme extract with 2 ml of 2% dinitrophenyl hydrazine (in acidic medium).
3. Added 1 drop of 10% thiourea (in 70% ethanol) to the mixture.
4. Boiled the mixture for 15 minutes in a water bath.
5. After cooling to room temperature, added 5 ml of 80% (v/v) sulfuric acid to the mixture while keeping it in an ice bath.
6. Measured the absorbance at 530 nm.
7. Calculated the concentration of ascorbic acid from a standard curve plotted with known concentrations of ascorbic acid.

Preparation of the Standard Curve for Estimation of Ascorbic Acid:

1. Dissolved 100 mg of ascorbic acid in 100 ml of 4% oxalic acid solution (1 mg/ml) to prepare the stock solution.
2. Prepared the working standard by diluting the stock solution 1:10 to achieve a concentration of 100 µg/ml.
3. Transferred 0.2, 0.4, 0.6, 0.8, and 1.0 ml aliquots of the working standard into separate test tubes.
4. Adjusted the volume in each test tube to 1 ml with 4% oxalic acid solution.
5. Measured the absorbance of each solution at 530 nm.

3.7.3.8: Estimation of Total Free Amino Acid

The total free amino acid content in the plant sample was estimated using the method described by Moore and Stein (1948).

Principle: Ninhydrin, a powerful oxidizing agent, reacts with α-amino acids in a pH range of 4 to 8. This reaction leads to decarboxylation and forms a bluish-purple colored compound.

Reagents: (a) 80% Ethanol

(b) 0.2M Citrate buffer, pH 5.0

(c) **Ninhydrin reagent:** Dissolved 0.8 g of stannous chloride in 500 ml of 0.2M citrate buffer, pH 5.0. Added this solution to 20 g of ninhydrin in 500 ml of methyl cellosolve (2-methoxy ethanol).

(d) **Diluent solvent:** Mixed equal volumes of water and n-propanol.

(e) **Stock standard leucine solution:** Dissolved 50 mg of leucine in 50 ml of water.

(f) **Working standard leucine solution:** Diluted 10 ml of the stock leucine solution to 100 ml with water.

Procedure:

1. Homogenized 500 mg of leaf sample in a pestle and mortar with a small quantity of acid-washed sand.
2. Added 5-10 ml of 80% ethanol to the homogenate.
3. Centrifuged the mixture at 5000 rpm.
4. Repeated the extraction twice, combining all the supernatants.
5. Transferred 0.1 ml of the extract into a test tube.
6. Added 1 ml of ninhydrin reagent to the test tube and mixed thoroughly.
7. Made up the final volume to 2 ml with distilled water.

8. Boiled the reaction mixture in a water bath for 20 minutes.
9. Added 5 ml of water and n-propanol while still in the water bath.
10. After 15 minutes of cooling, read the absorbance of the purple color against a blank at 570 nm.
11. Calculated the total free amino acid content from a standard curve prepared using leucine.

3.7.3.9: Estimation of L-Phenylalanine Ammonia Lyase (PAL) Activity

The activity of the L-Phenylalanine Ammonia Lyase (PAL) enzyme was measured using the method described by Subba Rao et al. (1970).

Principle: The enzyme activity is assessed by measuring the appearance of trans-cinnamic acid from phenylalanine, which is quantified spectrophotometrically at 650 nm.

Reagents:

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

Procedure:

1. Ground 3.0 grams of fresh leaf sample in 2.6 ml of sodium borate buffer containing 2-mercaptoethanol (0.8 ml/liter).
2. Centrifuge the mixture at 7000 g for 10 minutes at 2-4°C. Collect the supernatant.

3. Adjust the pH of the supernatant to 5.5 using 1M acetic acid.
4. Incubate 1 ml of 0.05M Tris-HCl buffer, 0.5 ml of 0.01M L-phenylalanine, and 0.4 ml of water at 30°C for 5 minutes.
5. Initiate the reaction by adding 0.1 ml of the enzyme extract and incubate for 60 minutes at 30°C. Prepare a blank sample without phenylalanine.
6. Stop the reaction by adding 0.5 ml of 1N HCl.
7. Extract the mixture with 3.5 ml of ether twice. Remove the ether phase, pool the residue, and dry it under a stream of air.
8. Dissolve the residue in 3 ml of 0.05N NaOH and keep it at room temperature overnight.
9. Centrifuge the mixture at 2000 g for 15 minutes and collect the supernatant.
10. Take 1 ml of the supernatant (extract) and add 1 ml of Folin-Ciocalteu reagent. Heat the mixture for 1 minute and measure the absorbance at 650 nm.
11. Prepare a calibration curve using known dilutions of cinnamic acid and follow the same procedure as for the sample. Express the PAL activity as µmoles of cinnamic acid produced/min/mg protein.

Preparation of the Standard Curve for Estimation of PAL Activity:

1. Dissolve 100 mg of cinnamic acid in 100 ml of borate buffer to prepare the stock solution.
2. Dilute the stock solution 1:10.
3. Transfer 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the working standard solution into different test tubes.
4. Adjust the volume to 1 ml with borate buffer.
5. Measure the absorbance at 650 nm using a spectrophotometer

3.7.3.10: Estimation of Free Proline

The free proline content in the leaves was determined using the method described by Bates et al. (1973).

Principle: Proteins are precipitated as a protein-sulphosalicylic acid complex during tissue extraction with sulphosalicylic acid. The extracted proline reacts with ninhydrin under acidic conditions to form a red color.

Reagents:

- **Acidic Ninhydrin Reagent:** Dissolved 1.25 g of ninhydrin in a mixture of 30 ml of warm glacial acetic acid and 20 ml of 6M phosphoric acid (pH 1.0) with agitation until dissolved. Store at 4°C and use within 24 hours.
- 3% Aqueous Sulphosalicylic Acid
- Glacial Acetic Acid
- Toluene
- Standard Proline Solution

Procedure:

1. Homogenize 100 mg of leaf sample in 10 ml of 3% aqueous sulphosalicylic acid using a mortar and pestle.
2. Centrifuge the homogenate at 6000 rpm for 10 minutes and collect the supernatant.
3. Transfer 2.0 ml of the extract into a test tube.
4. Add 2 ml each of glacial acetic acid and acidic ninhydrin reagent to the test tube.
5. Boil the reaction mixture in a water bath at 100°C for 30 minutes until a brick red color develops.
6. Allow the mixture to cool.
7. Add 5 ml of toluene to the reaction mixture and transfer to a separating funnel.

8. Measure the absorbance of the toluene layer at 520 nm using a spectrophotometer, with toluene as the blank.

Preparation of the Standard Curve for Estimation of Proline:

1. Dissolve 10 mg of proline in 3% aqueous sulphosalicylic acid and dilute to a final volume of 100 ml.
2. Prepare aliquots of 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the standard solution in separate test tubes.
3. Adjust the volume in each test tube to 2 ml by adding 3% aqueous sulphosalicylic acid solution.
4. Develop color in the same manner as for the sample and measure the absorbance using a spectrophotometer.

3.7.3.11: Estimation of Total Flavonol content

Principle: The Aluminium Chloride Colorimetric Method is based on the formation of acid-stable complexes between aluminium chloride and the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavonols and flavonoids.

Reagents:

- Methanol 80%
- Sodium Acetate
- Aluminium Chloride

Procedure:

1. Extract 0.05 g of the plant sample with boiling 80% methanol for 3 hours.
2. Mix 1 ml of the methanol extract with 3 ml of sodium acetate and 1 ml of aluminium chloride solution.
3. Allow the mixture to develop color for 2.5 hours.
4. Record the absorbance at 445 nm.

3.10.12 Total Flavonoid Content

Principle: The Aluminium Chloride Colorimetric Method relies on the formation of acid-stable complexes between aluminium chloride and the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavanols. Aluminium chloride also forms acid-labile complexes with ortho-dihydroxyl groups in the A- or B-ring of flavonoids. Quercetin is used as the standard material to build the calibration curve.

Reagents:

- Quercetin
- Methanol
- Aluminium Chloride
- Potassium Acetate

Procedure:

1. Prepare the stock quercetin solution by dissolving 5.0 mg of quercetin in 1.0 mL of methanol.
2. Prepare standard quercetin solutions by serial dilutions of the stock solution in methanol to obtain concentrations ranging from 5 to 200 μg/mL.
3. Mix 0.6 mL of each diluted quercetin standard solution or extract with 0.6 mL of 2% aluminium chloride solution.
4. Incubate the mixtures at room temperature for 60 minutes.
5. Measure the absorbance of the reaction mixtures at 420 nm using a Varian UV-Vis spectrophotometer (Cary 50 Bio UV-Vis Spectrophotometer).
6. Plot the absorbance values against the concentration of quercetin to create a calibration curve.
7. Calculate the total flavonoid content in the test samples from the calibration plot and express it as mg quercetin equivalent (QE) per gram of dried plant material.
8. Perform all determinations in triplicate.

3.7.3.13: Relative Water Content (%)

Principle: Relative Water Content (RWC) effectively measures plant water status, reflecting the physiological consequences of cellular water deficits. It provides insight into the energy status of water within plants, which is useful for understanding water transport through the soil-plant-atmosphere continuum.

Reagents:

- Distilled Water

Procedure:

Principle: Relative Water Content (RWC) was used to measure plant water status. This measure reflects the physiological consequences of cellular water deficits and provides insight into the energy status of water within plants.

Reagents:

- Distilled Water

Procedure:

1. Fresh leaf samples were collected from each cultivar.
2. 500 mg of the leaf samples were placed into a 500 mL beaker containing 500 mL of distilled water.
3. The samples were fully submerged in the water and left for 30 minutes to achieve full turgidity.
4. The samples were then removed, gently blotted to remove excess water, and weighed to obtain the turgid weight.
5. The samples were dried thoroughly (e.g., using an oven) and the dry weight was measured.
6. The Relative Water Content (RWC) was calculated using the formula:

   RWC (%) = Fresh Weight-Dry weight/Turgid Weight-Dry weight×100

Fig. 3.7.3.13: Estimating RWC

3.7.3.14: Chl. Index (SPAD Unit)

Principle: The SPAD (Soil Plant Analysis Development) meter measures the chlorophyll content in leaves based on the light absorption properties of chlorophyll. The instrument provides a quantitative value, known as the SPAD index, which correlates with the chlorophyll concentration in the leaf tissue.

Equipment and Reagents:

- SPAD meter (e.g., SPAD-502)
- Plant leaves (fresh samples)

Procedure:

1. **Preparation:**
    - Ensure the SPAD meter is calibrated according to the manufacturer's instructions.
    - Select healthy, fully developed leaves from the plant for measurement.
2. **Measurement:**

- Select a leaf from the plant sample to be measured.
- Place the leaf between the measurement heads of the SPAD meter. Ensure the leaf is flat and properly aligned with the measurement area of the meter.
- Close the meter's sensor head to take a measurement. The meter will automatically apply a light source and measure the absorbance of the leaf in the red and near-infrared wavelengths.

3. **Recording:**
    - Wait for the SPAD meter to display the chlorophyll index value. This value, known as the SPAD index, represents the relative chlorophyll content of the leaf.
    - Record the SPAD index value displayed on the meter.
4. **Repetition:**
    - Repeat the measurement on multiple leaves from different plants or areas to obtain an average chlorophyll index for the sample.
    - Ensure consistent measurement conditions (e.g., same time of day, similar leaf age and condition) to reduce variability.
5. **Cleaning and Maintenance:**
    - After measurements, clean the SPAD meter's sensor heads with a soft, dry cloth to remove any leaf residues.
    - Store the SPAD meter in a dry, protective case when not in use.

Data Analysis:

- Calculate the average SPAD index value from the repeated measurements.

- Use the SPAD index values to assess the chlorophyll content in the plant samples and correlate with plant health or nutrient status if applicable.

Notes:

- Ensure the leaf is not damaged or wet, as this can affect the accuracy of the measurement.
- Follow the manufacturer's guidelines for properly calibrating and maintaining the SPAD meter to ensure reliable results.

Fig. 3.7.3.14: Measuring Chl. Index with SPAD Unit

B. Post-harvest studies:

3.7.4: OIL QUALITY PARAMETERS

3.7.4.1: Peroxide value (PV):

The Peroxide values were predicted using the standard of ISO 3960-2007. 5g of the oil sample was dissolved in glacial acetic acid: chloroform (3:2, v/v, 30 ml) mixture, followed by 1ml of saturated potassium iodide solution was added. Further on that the desired amount of distilled water was added then titrated gradually against sodium

thiosulphate solution (0.01ml), where the starch solution (1%) has been used as an indicator (Sharma et al., 2006).

Formula: -

$$\text{POV (meq per1000g)} = \frac{(VS-Vb)\times F\times N\times 1000}{W} = \frac{(VS-Vb)\times F\times 1\times 1000}{W\times 100}$$

$$= \frac{\mathbf{(VS-Vb)\times F\times 10}}{\mathbf{W}}$$

Where, V_s= Titration volume of sample (ml)

V_b = Titration volume of blank (ml)

F= Factor of 0.01N $Na_2S_2O_3$ solution

W= Weight of oil

N= Normality of $Na_2S_2O_3$ solution

3.7.4.2: p-Anisidine value (p-AV)

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

Procedure:

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

Formula: $\frac{\mathbf{10ml\times(1.2\times(AS2-AB2)-(AS1-AB1)}}{\mathbf{W\ sample}}$

3.7.4.3: Totox value (TV)

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

Formula: $\mathbf{T\ v = (2 * P\ v) + p\text{-}Av}$

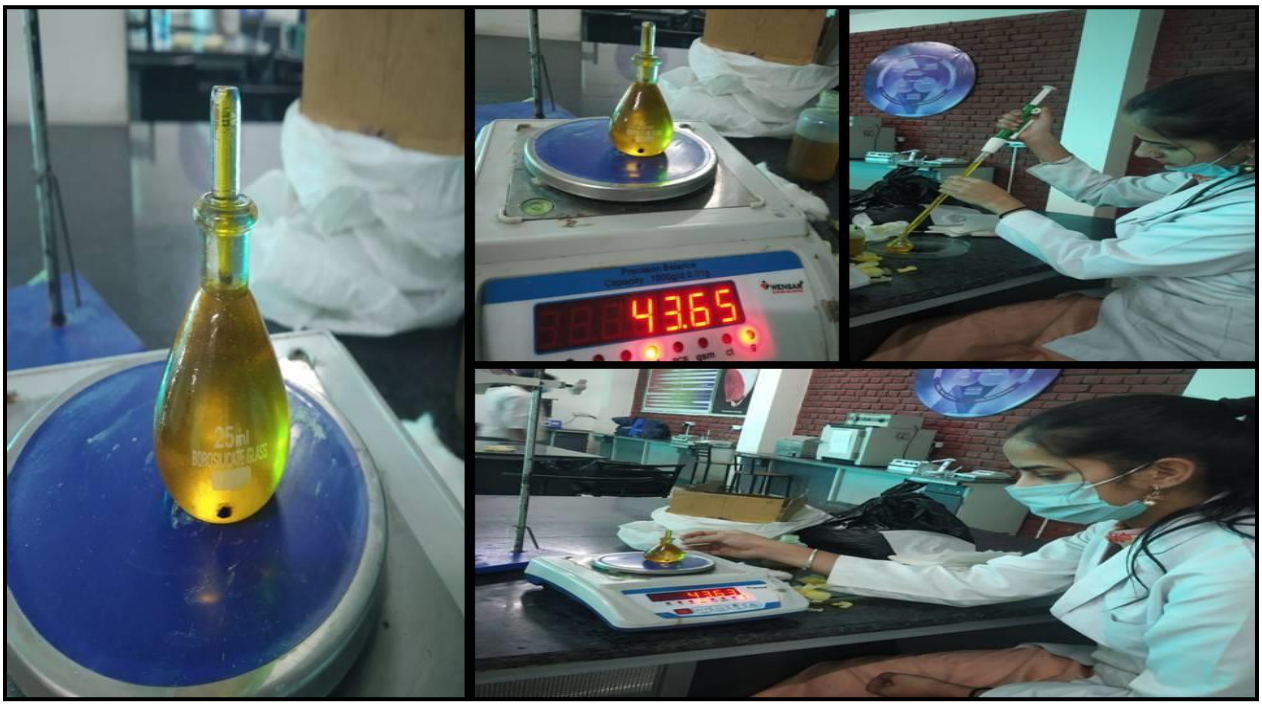
Pv = Peroxide value,

p-Av = para Anisidine value

3.7.4.4: Density

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

Fig. 3.7.4.4: Estimating Density of oil



3.7.4.5: Viscosity

By using Cannon - Fenske (Fisher Scientific, Pitts-burgh, PA) glass capillary kinematic viscometers in a steady- temperature bath. A programmable water bath (Model F25-HE, Julabo USA Inc. Allentown, PA) was utilized to confirm the exact and concurrent results. The trials have been made with ASTM D445 for viscosity determination. The difference in viscosity as a function of temperature was calculated using this formula (Noureddine et al., 1992), $V = c \times t$

Where,

c = Viscometer Constant (mm2/s2)

t = Time

$\mu = v \times p$

Where,

v = Viscosity in mm^2/s^2

p = Density of the oil

3.7.4.6: Saponification value

The known amount of oil sample is mixed with 10 mL of 1 N KOH and 10 mL of de-ionized water. The resultant combination is heated below the reserved condenser for 30–40 min and chilled. It is titrated against 0.5 M of HCl, using an indicator to get the pale pink color. The same conditions were followed for the blank (Firestone, 2007).

Calculation: Saponification Value = $\frac{56.1\ (B-S)N}{W}$

Where, B = Volume in ml of standard hydrochloric acid required for the blank.

S = Volume in ml of standard hydrochloric acid required for the sample

N = Normality of the standard hydrochloric acid and

W = Weight in g of the oil/fat taken for the test.

3.7.4.7: Iodine value

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

Reagents:

1. Iodine monochloride Reagent
2. Potassium Iodide
3. 0.1N Sod. thiosulphate

Procedure: 1. Take 10ml of oil sample.

2. Add 20ml Iodine monochloride reagent in flask and mix it.

3. Incubate for ½ an hr. in dark.

4. Make blank by adding 10ml chloroform to the flask.

5. Add 10ml of potassium iodide solution.

6. Rinse sides of the flask using 10ml distilled water.

7. Titrate against sod. Thiosulphate solution until pale straw colour.

8. Add 1ml starch indicator (purple color observed).

9. Titrate until solution turns colorless.

10. Follow same for blank and observe colour.

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

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

126.9 is the molecular weight of iodine

B = quantity of sodium thiosulphate used for blank,

S = quantity of thiosulphate for sample,

N = normality of thiosulphate solution,

w = weight of the oil sample

3.7.4.8: Acid value

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

Reagents:

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

Procedure:

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

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

3.7.4.9: Refractive index

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

Procedure:

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

Fig. 3.7.4.9: Measuring Refractive index of mustard oil

3.7.4.10: Oil content

Oil content from mustard seeds was extracted using an oil expeller machine. The machine was thoroughly cleaned before use. Mustard seeds were placed in the top bowl of the machine. The oil was extracted automatically through a tube attached to the bottom of the machine. The oil and oilcake were collected separately from different openings: the oil was collected in a beaker and poured into a measuring cylinder to determine the oil content, while the oilcake was collected in a bowl and weighed using a weighing balance to determine its weight.

Fig. 3.7.4.10: Estimating Oil content

3.7.5: Oil cake parameters: -

3.7.5.1: Oil cake wt. /100g seed:

The weight of the oil cake was estimated by measuring the amount obtained from 100 g of mustard seeds during the oil extraction process. The oil cake was weighed using a weighing balance.

3.7.5.2: Glucosinolates

Spectrophotometric estimation was performed using a methanolic extract prepared from the same genotypes. A 0.1 g sample of defatted seed meal was homogenized in a 2 mL vial with 80% methanol. This homogenate was allowed to stand overnight at room temperature and centrifuged at 3000 rpm for 4 minutes. The supernatant was collected and adjusted to a final volume of 2 mL with 80% methanol. A 100 µL aliquot of this extract was used for the estimation. It was mixed with 0.3 mL of double-distilled water and 3 mL of 2 mM sodium tetrachloropalladate solution (prepared by dissolving 58.8 mg sodium tetrachloropalladate in 100 mL double-distilled water with 170 µL concentrated HCl). After incubating the mixture at room temperature for 1 hour, the absorbance was measured at 425 nm using a spectrophotometer. A blank was prepared using the same procedure without the extract.

Total glucosinolates were calculated using the following formula, where the OD at 425 nm (A425) was used:

$$y=1.40+118.86\times A425$$

Post-harvest studies:

3.7.6 Yield Parameters:

3.7.6.1 Number of Primary Branches:

- **Measurement:** The number of primary branches was counted manually.
- **Timing:** This was done at 90 days after sowing (DAS).
- **Recording:** The data was noted in a notebook.

3.7.6.2 Number of Secondary Branches:

- **Measurement:** The number of secondary branches was counted manually.
- **Timing:** This was done at 120 days after sowing (DAS).
- **Recording:** The data was noted in a notebook.

3.7.6.3 Number of Siliquae per Plant:

- **Measurement:** The number of siliquae per plant was counted manually.
- **Timing:** This was done at 120 days after sowing (DAS) and at harvest.
- **Recording:** The data was noted in a notebook.

3.7.6.4 Length of Siliquae:

- **Measurement:** The length of siliquae was measured using a 15 cm ruler.
- **Recording:** The data was recorded in a notebook for further use.

3.7.6.5 Number of Seeds per Siliqua:

- **Measurement:** The number of seeds per siliqua was counted manually by opening each siliqua (pod) into two halves.
- **Recording:** The data was noted in a notebook for further use.

3.7.6.6 Seed Yield per Square Meter:

- **Measurement:** Plants from a 1 m² area were harvested, and grains were separated after threshing.
- **Calculation:** The seed yield per m² area was determined. The yield per plot was converted into quintals per hectare (q/ha).

3.7.6.7 Stover Yield per Square Meter:

- **Measurement:** Plants were weighed after threshing to determine stover yield.
- **Calculation:** The stover yield per m² was calculated from the harvested plants and expressed per plot.

3.7.6.8 Harvest Index (%):

- **Calculation:** The harvest index was calculated as the ratio of economic yield to biological yield using the following formula:

It was given by Fisher in 1962.

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

CHAPTER 4

RESULTS AND DISCUSSION

The present research work entitled **"Impact of Boron, Sulphur, and Cytokinin in Indian mustard (Brassica juncea L.) under Spatial Dynamics"** was conducted during the *Rabi* season of the year 2021-2022 and 2022-2023 at the agricultural research farm of Lovely Professional University field, Jalandhar, Punjab. All results obtained are presented and discussed in this chapter.

The present study was carried out to estimate the Impact of B, S, and BAP on growth and yield attributing characters of mustard crop variety NB-RIMUL-2019 (Nandi Bull) at 30, 60, 90, and 120 DAS under spatial dynamics. Mustard seeds were taken from an authorized certified seed producer, 'Good grow', from Phagwara, Punjab. The plot size selected for the experiment is $5\times3=15m^2$. Sowing of seeds has been done successfully in research fields in two different spacings i.e. 30×10cm and 20×10. According to the plan of work, the experiment was arranged in statistical design SPD, and treatments were applied at 15, 45, 75, and 105 DAS. The source of treatments applied was arranged from the local market in Phagwara. The exogenous application of B, S, and Cyt. was applied by selecting the best concentration used in earlier studies. The concentrations applied were B @1%, S @0.15%, and Cyt.@0.003% as a foliar spray at the interval of fifteen days after sowing. The various observations were taken at four stages such as 30 DAS, 60 DAS, 90 DAS, and 120DAS in all the treatments. The detailed plan of treatments are: T1-M1S0:RDF, T2-M2S0: RDF, T3-M1S1: Boron @1%, T4-M2S1: Boron @1%, T5-M1S2: Sulphur @ 0.15%, T6-M2S2: Sulphur @ 0.15%, T7-M1S3: BAP @0.003%, T8-M2S3: BAP @0.003%, T9-M1S4: Boron @0.5% +Sulphur @0.25%, T10-M2S4: Boron @0.5% +Sulphur @0.25%, T11-M1S5: Boron @ 1.5%+ Sulphur @0.075%, T12-M2S5: Boron @ 1.5%+ Sulphur @0.075%, T13-M1S6: Boron @ 0.5% + BAP (@0.0045%), T14-M2S6: Boron @ 0.5% + BAP (@0.0045%), T15-M1S7: Boron @ 1.5%+ BAP (@0.0015%), T16-M2S7 :Boron @ 1.5%+ BAP

(@0.0015%), T17-M1S8: Sulphur @ 0.075%+ BAP (@0.0045%), T18-M2S8: Sulphur @ 0.075%+ BAP (@0.0045%), T19-M1S9: Sulphur @0.25%+ BAP (@0.0015%), T20-M2S9: Sulphur @0.25%+ BAP (@0.0015%)

Basic nutrients to the crop were applied at the time of sowing and as a top dressing. 2-3 weeding was carried out and 2 irrigations were provided to attain good growth and production. The results obtained after the experiment during the years 2021-22 and 2022-23 are presented in this chapter. This includes all observations recorded on the crop's morphological, biochemical, and yield attributes.

There were two major concerns in this experiment. In the first section, the investigation was developed to determine the morpho-physiological and yield attributes in the mustard crop in main and sub-treatments at 30DAS, 60DAS, 90DAS, and 120DAS. The second part represents the impact of Boron, Sulphur, and Cytokinin on mustard crop and biochemical responses at 30DAS, 60DAS, 90DAS, and 120DAS under all treatments. All experimental details and procedures of all biochemical experiments performed in the experiment are given in Chapter 3. In this chapter, an attempt has been made to illustrate and explain the recorded data. The findings of the two-year research experiments are presented under the following heading.

In our study associated with micronutrients and plant growth hormones, we found that the treatments applied show greater yield and an increase in morphological and biochemical parameters, which were discussed in each section. The effect of applied nutrients and plant growth hormones shows better quality, yield, and productivity production. The treated plots show a higher increase in growth and output than the controlled plot.

We have tested my research on the mustard crop, and different results have been obtained from the effects of sulphur, boron, and cytokinin. We found that mustard crops produce a better production and yield when nutrients were applied to the crop with plant growth hormone. These findings indicate that applying micro and secondary nutrients along with primary nutrients can

give a higher yield and productivity in terms of the mustard crop's quality and quantity. Foliar application of plant growth hormone, i.e. Cytokinin, can increase the quality yield of the mustard when applied in accurate quantity.

4.1 Morphological observations

4.1.1 Plant height (cm):

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in plant height were observed at 30DAS, 60DAS, 90DAS and 120 DAS, shown in Table 4.1, Fig 4.1. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the plant heights in each treatment compared to Control of both the spacings at 30, 60, 90 and 120 DAS. The percentage increase was calculated by comparing all the treatments with control and comparing both the spacings together. Thus, the pattern of percentage increase in the plant height was observed at 30, 60, 90, and 120 DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, main plot M2 shows better plant height as compared to M1 with values of 11.36 cm (M2) and 10.92cm (M1), respectively. A percentage increase of 3.87% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in plant height was found in S7, where Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop as a foliar spray.

Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S7, followed by S6>S8>S5>S2>S1>S9>S3, and the per cent values were 23.47%, 21.54%, 20.20%, 17.97%, 16.99%, 15.87%, 14.84% and 14.06% respectively. At 60DAS, the main plot M2 shows a better plant height than M1, with 96.53cm (M2) and 86.01cm (M1) values, respectively. A percentage increase of 10.89% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S4 with a value of 99.16cm, where Boron @0.5% + sulphur @0.25% was applied to the crop as a foliar application. The per cent increase was found highest in S4, followed by S2> S7> S5> S6> S9> S3, and the per cent values were 15.02%, 14.95%, 10.59%, 7.99%, 7.83% and 6.12%, respectively when it is compared with its control (S0). At 90DAS, main plot M2 shows better plant height than M1 with values 171.39 cm (M2) and 174.76cm (M1), respectively. A percentage increase of 1.92% was found in M2,

where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S6 with a value of 180.56cm where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S6 followed by S8> S2> S9> S7> S3> S4> S5, and the per cent values were 15.75%, 14.80%, 13.97%, 13.66%, 13.26%, 13.21%, 13.13% and 12.37% respectively when it is compared with its control (S0). At 120DAS, main plot M2 shows better plant height than M1 with values 177.19 cm (M2) and 174.28cm (M1), respectively. A percentage increase of 1.64% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S6 with a value of 182.67cm, where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S6, followed by S8> S9> S2> S7> S3> S4> S5, and the per cent values were 15.17%, 14.52%, 13.79%, 13.54%, 13.16%, 12.88%, 12.81% and 11.72% respectively when it is compared with its control (S0).

The study shows a significant increase with 21.54%, 7.99%, 15.75% and 15.17% per cent values at 30DAS, 60DAS, 90DAS, and 120DAS when comparing S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 90DAS and 120DAS, a significant increase in the plant height is found in treatment S6, where the combined application of Boron and Cytokinin is applied to the crop when compared to its control (S0) followed by S8, where sulphur and Cytokinin are used in combination to the crop i.e. (Sulphur @ 0.075%+ BAP (@0.0045%).

The lowest increase was found in treatment S3, i.e. 14.06% at 30DAS, compared to its control (S0), where the application of Cytokinin (Rec. dose @30mg dm-3) is provided alone. At 60DAS, 90DAS, and 120DAS, the lowest increase was found in treatment S1, i.e. 0.39%, 9.02%, and 8.84%, compared to its control (S0), where the single application of boron (Rec. dose @1%) is provided to the crop.

In the year (2022-2023) at 30DAS, main plot M2 shows better plant height as compared to M1 with values of 11.59 cm (M2) and 11.56cm (M1), respectively. A percentage increase of 0.25% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, the significant increase in plant height was found in S8, where Sulphur @ 0.075%+ BAP (@0.0045% was applied to the crop as a foliar spray.

Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S8 followed by S7>S2>S6>S9&S5>S4>S1>S3, and the percent values were 24.44%, 24.03%, 22.48%, 22.16%, 20.53%, 20.53%, 17.29%, 13.11% and 11.90% respectively. At 60DAS, the

main plot M2 shows a better plant height than M1, with values of 99.51cm (M2) and 88.89cm (M1), respectively. A percentage increase of 10.67% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S4 with a value of 101.98cm where Boron @0.5% + sulphur @0.25% was applied to the crop as a foliar application. The per cent increase was found highest in S4, followed by S2> S7> S5> S6> S9> S3, and the per cent values were 13.53%, 12.76%, 9.20%, 7.975, 6.78%, 6.32% and 3.78% respectively when it is compared with its control (S0). At 90DAS, main plot M2 shows better plant height than M1 with values 177.20 cm (M2) and 173.70cm (M1), respectively. A percentage increase of 1.97% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S6 with a value of 182.64cm where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S6 followed by S8> S2> S9> S4> S7> S3> S5, and the per cent values were 15.79%, 14.93%, 14.37%, 13.98%, 13.63%, 13.25%, 13.28% and 12.85% respectively when it is compared with its control (S0). At 120DAS, the main plot M2 shows a better plant height than M1, with 179.54cm (M2) and 176.68cm (M1) values. A percentage increase of 1.59% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S6 with a value of 185.72cm, where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S6 followed by S8> S9> S2&S7> S4> S3> S5, and the per cent values were 15.70%, 14.50%, 13.90%, 13.72%, 13.72%, 13.02%, 12.90% and 12.10% respectively when it is compared with its control (S0).

The study shows a significant increase with 22.16%, 6.78%, 15.79% and 15.70% per cent values at 30DAS, 60DAS, 90DAS, and 120DAS when comparing S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 90DAS and 120DAS, a significant increase in the plant height is found in treatment S6, where the combined application of Boron and Cytokinin is applied to the crop when compared to its control (S0) followed by S8, where sulphur and Cytokinin is used in combination to the crop, i.e. (Sulphur @ 0.075%+ BAP (@0.0045%).

At 30DAS, 60DAS, 90DAS, and 120DAS, the lowest increase was found in treatment S1, i.e. 13.11%, 0.67%, 9.39%, and 9.53%, when compared to its control (S0), where the crop received a single application of boron (Rec. dose @1%). According to the results observed from the study, cytokinin shows better results when applied in combination with boron and sulphur than results obtained from its single application. The highest plant height was recorded with a recommended dose of fertilizers + sulphur and boron (Sharma, S.et al. 2020). Because sulphur increases the

activity of meristematic tissue, increasing plant height and cell elongation. Similarly, boron also helps in cell elongation, photosynthesis, and translocation of photosynthates.

In mustard crops cultivated under restricted spacing, the influence of nutrient application on plant height can be ascribed to the function of vital nutrients in facilitating ideal growth and development. Spacing constraints often hinder the growth capacity of plants by intensifying competition for resources such as light, water, and nutrients. By implementing focused nutrient treatments, plants are more effectively prepared to surmount these constraints and optimize their capacity for growth. The essential nutrients Nitrogen, Phosphorus, and Potassium, together with the secondary nutrients Boron and Sulphur, are vital for enhancing physiological processes, promoting cell elongation, and maintaining overall plant health. Consequently, adequately fed vegetation can attain higher heights even in limited space, enhancing agricultural productivity and yield. This highlights the significance of nutrient management in maximizing plant development and guaranteeing effective use of the available area.

Table 4.1 (a): Effect of spacing and nutrients on Plant height (cm) of mustard crop during *rabi* 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS		120DAS	
Spacing								
M1 (30×10)	10.92	11.56	86.35	88.69	171.39	173.7	174.28	176.68
M2 (20×10)	11.36	11.59	96.33	99.11	174.76	177.2	177.19	179.54
C.D. at p<0.05	NS	NS	NS	NS	NS	NS	NS	NS
SEM±	0.48	0.54	2.96	2.43	1.77	1.78	1.59	1.81
Nutrients foliar application								
S0-Control	9.31	9.47	86	88.18	152.11	153.79	154.94	156.55
S1-Boron @1%	11.06	10.9	84.66	88.78	167.19	169.73	169.98	173.05
S2-Sulphur @ 0.15%	11.21	12.21	99.16	101.08	176.82	179.61	179.23	181.45
S3-BAP @0.003%	10.83	10.75	89.83	91.65	175.28	177.35	177.87	179.74
S4-Boron @0.5% +Sulphur @0.25%	11.05	11.45	99.25	101.98	175.1	178.06	177.71	180
S5-Boron @ 1.5%+ Sulphur @0.075%	11.35	11.91	93.16	95.81	173.6	176.48	175.52	178.11
S6-Boron @ 0.5% + BAP (@0.0045%)	11.86	12.16	91.66	94.6	180.56	182.64	182.67	185.72
S7-Boron @ 1.5%+ BAP (@0.0015%)	12.16	12.46	94.33	97.11	175.36	177.28	178.43	181.46
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	11.66	12.53	83.83	85.7	178.55	180.79	181.27	183.11
S9-Sulphur @0.25%+ BAP (@0.0015%)	10.93	11.91	91.5	94.13	176.19	178.79	179.73	181.95
C.D. at p<0.05	1.15	1.13	9.29	8.18	9.53	9.74	9.53	8.39
SEM±	0.4	0.39	3.22	2.84	3.31	3.83	3.31	2.91
C.D. S×M at p<0.05	NS	NS	NS	NS	NS	NS	NS	NS
SEM±	1.52	0.78	6.45	5.68	6.62	6.76	6.62	5.83
C.D. M×S at p<0.05	NS	NS	NS	NS	NS	NS	NS	NS
SEM±	0.72	1.07	7.42	6.39	6.76	6.89	6.67	6.09

Where, C.D. represents critical difference, SEM± represents standard error of mean.

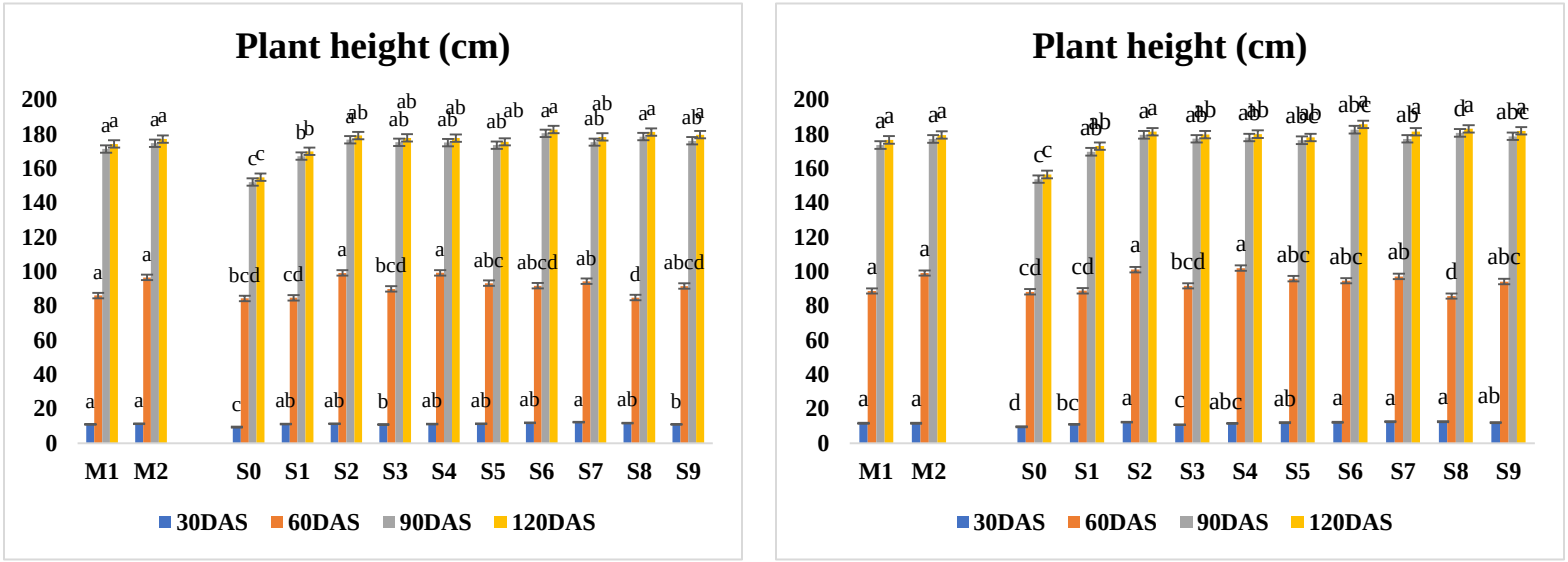


Fig.4.1. (a): Effect of spacing and nutrient on Plant height of mustard crop during *Rabi* season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%).

4.1.2: Leaf number (No. Plant^{-1}):

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in the number of leaves were observed at 30DAS, 60DAS, 90DAS and 120 DAS, as shown in Table 4.2, Fig 4.2. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the number of leaves in each treatment compared to the control of both the spacings at 30, 60, 90 and 120 DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the number of leaves was observed at 30, 60, 90, and 120 DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, the main plot M2 shows the maximum leaf number as compared to M1 with values of 5.96 (M2) and 5.63 (M1), respectively. A percentage increase of 5.53% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in the number of leaves was not observed at 30DAS. Maximum number of leaves was found in S5, S6 and S7, i.e. 6. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S5, S6, and S7 followed by S1, S3, S8, S9>S2&S4 and the per cent values were 11.16%, 11.16%, 11.16%, 8.62%, 8.62%, 8.62%, 8.62%, 5.94%, 5.94% respectively. At 60DAS, main plot M1 shows the maximum leaf number compared to M2 with values 25.7 (M1) and 25.2 (M2), respectively. A percentage increase of 0.77% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase was found in S4 with a value of 32.33 where Boron @0.5% + sulphur @0.25% was applied to the crop as a foliar application. The per cent increase was found highest in S4 followed by S7> S9> S6> S8> S3> S2> S1, and the per cent values were 45.38%, 37.78%, 36.16%, 35.78%, 35.39%, 31.63%, 30.28% and 21.51% respectively when it is compared with its control (S0). At 90DAS, main plot M1 shows the maximum leaf number compared to M2 with values 29.9 (M1) and 29.7 (M2), respectively. A percentage increase of 0.66% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S4 with a value of 36.33 where Boron @0.5% +Sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S4, followed by S7> S6&S9> S3&S8> S2> S5, and the per cent values were 44.04%, 37.76%, 37.12%, 37.12%, 35.11%, 35.11%, 31.47% and 21.80% respectively when it is compared with its control (S0). At 120DAS, no such difference was shown in the values of the main plots. An average value, i.e.

31.8, was found in M1 and M2, respectively. In subplots, significant results were observed in S4 with a value of 36.33, where Boron @0.5% + sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S4 followed by S7&S9> S3> S6&S8> S2> S1, and the per cent values were 36.69%, 33.00%, 33.00%, 32.35%, 31.68%, 31.68%, 28.12% and 21.59% respectively when it is compared with its control (S0).

The study showed a significant increase with 5.94%, 45.38%, 44.04% and 36.69% per cent values at 30DAS, 60DAS, 90DAS, and 120DAS when a comparison was made between S4 and S0 (control). In treatment S4, the foliar application of Boron @0.5% + sulphur @0.25% was applied to the mustard crop. At 90DAS and 120DAS, a significant increase in the plant height is found in treatment S4, where the combined application of Boron and sulphur is applied to the crop when compared to its control (S0) followed by S7, where boron and Cytokinin is used in combination to the crop, i.e. Boron @ 1.5%+ BAP (@0.0015%)

The lowest increase was found in treatment S2, i.e. 5.94% at 30DAS, when compared to its control (S0), where the application of Sulphur at its recommended dose, i.e. @ 0.15%, is provided alone. At 60DAS and 90DAS, the lowest increase was found in the treatment S1, i.e. 21.51% and 21.30%, when compared to its control (S0), where the single application of boron (Rec. dose @1%) is provided to the crop.

In the year (2022-2023) at 30DAS, the main plot M2 shows the maximum number of leaves as compared to M1, with values of 6.3 (M2) and 5.93 (M1), respectively. A percentage increase of 0.25% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in plant height was found in S6, where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray.

Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S6, followed by S9>S8&S2>S4&S5>S7>S1>S3, and the per cent values were 17.5%, 15.38%, 13.15%, 13.15%, 10.81%, 10.81%, 8.33%, 5.71% and 2.94% respectively. At 60DAS, main plot M1 shows the maximum leaf number compared to M2 with values 27.1 (M1) and 26.9 (M2), respectively. A percentage increase of 0.73% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase was found in S4 with value 34 where Boron @0.5% + sulphur @0.25% was applied to the crop as a foliar application. The per cent increase was found highest in S4, followed by S9> S7> S8> S6> S3> S2, and the per cent values were 47.55%, 40.56%, 40.23%, 38.86%, 37.80%, 34.76% and 32.29% respectively when it is compared with its control (S0). At 90DAS, the main plot M1 shows the maximum leaf number

compared to M2, which is 32.53 (M1) and 32.06 (M2), respectively. A percentage increase of 1.44% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S4 with a value of 43.33 where Boron @0.5% +Sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S4 followed by S9> S7> S6> S8> S3> S2> S5, and the per cent values were 47.70%, 37.05%, 34%, 33.35%, 32.02%, 29.91%, 28.44% and 19.07% respectively when it is compared with its control (S0). At 120DAS, main plot M2 shows the maximum leaf number compared to M1 with values 33.93 (M1) and 33.73 (M2), respectively. A percentage increase of 0.58% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S4 with a value of 38.33 where Boron @0.5% +Sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S4, followed by S7&S9> S3> S6> S8> S2> S1, and the per cent values were 33.06%, 29.37%, 29.37%, 28.72%, 28.05%, 26.68, 24.52% and 18.10% respectively when it is compared with its control (S0).

The study showed a significant increase with 10.81%, 47.55%, 47.70% and 33.06% per cent values at 30DAS, 60DAS, 90DAS, and 120DAS when a comparison was made between S4 and S0 (control). In treatment S4, the foliar application of Boron @0.5% + sulphur @0.25% was applied to the mustard crop. At 90DAS and 120DAS, a significant increase in the plant height is found in treatment S6, where the combined application of Boron and Cytokinin is applied to the crop when compared to its control (S0) followed by S8, where sulphur and Cytokinin are used in combination to the crop i.e. (Sulphur @ 0.075%+ BAP (@0.0045%).

At 30DAS, the lowest increase was found in S3, i.e. 2.94%, where application of cytokinin alone @ 0.003% was provided to the crop. At 60DAS, 90DAS and 120DAS, the lowest increase was found in the treatment S1, i.e. 24.66%, 17.09% and 18.10 when it is compared to its control (S0) where the single application of boron (Rec. dose @1%) is provided to the crop. The maximum number of leaves contributes to the maximum photosynthetic ability. The same results were reported by Awal et al. (2020). The influence of nutrient application on mustard crops cultivated in restricted spacing conditions is substantial, especially regarding the leaf yield. Mustard, a crop with high nutritional requirements, necessitates an ideal provision of both macronutrients and micronutrients to attain robust growth and development. Under conditions of restricted spacing, the competition for resources such as light, water, and nutrients intensifies, underscoring the need to ensure that the plants get sufficient nourishment. Effective nutrient management can optimise the physiological parameters contributing to leaf development, essential for photosynthesis and plant vitality. The plant's capacity to generate new leaves is directly affected by nutrient

availability under restricted spacing conditions. Nitrogen is a crucial nutrient that stimulates vegetative growth and the production of leaves. Optimal nitrogen supply under such circumstances can enhance leaf proliferation, thus enhancing the plant's ability to carry out photosynthesis and absorb nutrients. Furthermore, essential nutrients such as phosphorus and potassium are crucial for facilitating energy transfer and regulating water levels, enhancing the plant's general development and capacity to generate a more significant number of leaves, even in limited areas. Implementing foliar application of nutrients can be incredibly efficient in situations with restricted spacing since it enables direct absorption through the leaves, circumventing the competition in the root zone. This approach guarantees every plant obtains the essential nutrients required to sustain leaf development, even with restricted root spread and soil nutrient availability. Phytohormones such as cytokines and nutrients can enhance cellular division and leaf development, increasing leaf density per plant. Effective nutrient management in mustard crops with restricted spacing is vital for optimising leaf count. The negative impacts of competition and limited space can be counteracted by ensuring a sufficient and well-balanced provision of essential nutrients, thus enhancing plant growth and productivity.

Table-4.2 (a): Effect of spacing and nutrient on Leaf number (No. plant^{-1}) of mustard crop during rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS		120DAS	
Spacing								
M1 (30×10)	5.63	5.93	25.73	27.10	29.90	32.53	31.86	33.73
M2 (20×10)	5.96	6.30	25.50	26.93	29.73	32.06	31.80	33.93
C.D. at p<0.05	NS	NS	NS	NS	NS	NS	NS	NS
SEM±	0.04	0.41	0.39	0.47	0.06	0.51	1.12	0.35
Nutrients foliar application								
S0-Control	5.33	5.50	17.66	17.83	20.33	22.66	23.00	25.66
S1-Boron @1%	5.83	5.83	22.50	23.66	25.83	27.33	29.33	31.33
S2-Sulphur @ 0.15%	5.66	6.33	25.33	26.33	29.66	31.66	32.00	34.00
S3-BAP @0.003%	5.83	5.66	25.83	27.33	31.33	32.33	34.00	36.00
S4-Boron @0.5% +Sulphur @0.25%	5.66	6.16	32.33	34.00	36.33	43.33	36.33	38.33
S5-Boron @ 1.5%+ Sulphur @0.075%	6.00	1.16	21.66	23.33	26.00	28.00	27.66	29.66
S6-Boron @ 0.5% + BAP (@0.0045%)	6.00	6.66	27.50	28.66	32.33	34.00	33.66	35.66
S7-Boron @ 1.5%+ BAP (@0.0015%)	6.00	6.00	28.33	29.83	32.66	34.33	34.33	36.33
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	5.83	6.33	27.33	29.16	31.33	33.33	33.66	35.00
S9-Sulphur @0.25%+ BAP (@0.0015%)	5.83	6.50	27.66	30.00	32.33	36.00	34.33	36.33
C.D. at p<0.05	NS	NS	5.62	4.46	5.11	5.84	4.61	4.51
SEM±	0.15	0.33	1.95	1.55	1.77	2.03	1.60	1.56
C.D. S×M at p<0.05	NS	NS	NS	NS	NS	NS	NS	NS
SEM±	0.14	0.19	1.30	1.61	1.24	3.56	1.51	1.12
C.D. M×S at p<0.05	NS	NS	NS	NS	NS	NS	NS	NS
SEM±	0.21	0.45	2.65	2.14	2.41	2.94	2.20	2.13

Where, C.D. represents critical difference, SE (m) represents standard error of mean.

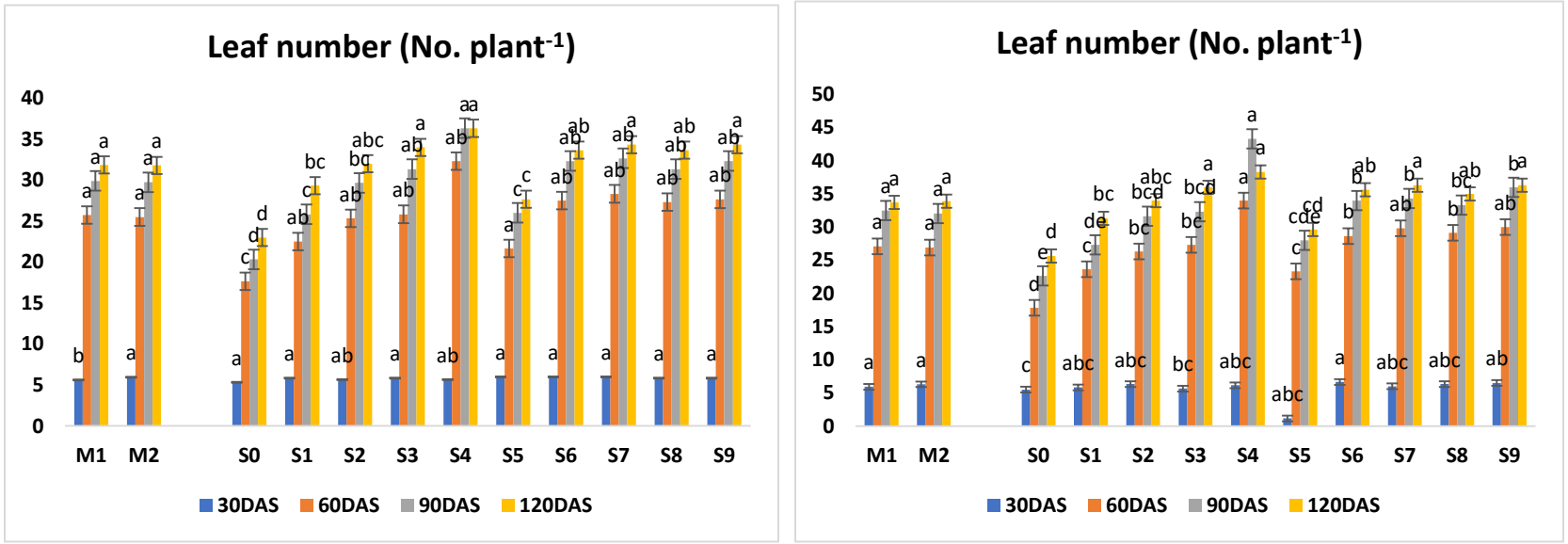


Fig. 4.2 (a): Effect of spacing and nutrient on Leaf no. of mustard crop during rabi season of 2021-22 and 2022-23

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%) 1

4.1.3 Leaf Area (cm^2)

Mustard crop shows different changes when grown under different nutrient levels. In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in leaf area were observed at 30DAS, 60DAS, 90DAS and 120 DAS in Table 4.3, Fig 4.3. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the leaf area in each treatment as compared to control of both the spacings at 30, 60, 90 and 120 DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the number of leaves was observed at 30, 60, 90, and 120 DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, main plot M1 shows maximum leaf area as compared to M2 with values 17.1 (M1) and 16.15 (M2), respectively. A percentage increase of 9.77% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase in leaf area was observed in S6 at 30DAS with a value of 21.95cm2, where Boron @ 0.5% + BAP (@0.0045%) was provided to the crop as a foliar application. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S6, followed by S5, S7, S9, S8, S4>S3> S1, and the per cent values were 52.39%, 48.18%, 46.90%, 46.54%, 44.06%, 36.08%, 30.79% and 26.58% respectively.

At 60DAS, main plot M1 shows maximum leaf area compared to M2 with values 31.45 (M1) and 30.89 (M2), respectively. A percentage increase of 1.78% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase was found in S5 with a value of 36.31cm2 where Boron @ 1.5%+ Sulphur @0.075% was applied to the crop as a foliar application. The per cent increase was found highest in S5 followed by S9> S1> S3> S2> S8>

S6> S7, and the per cent values were 29.37%, 25.25%, 19.76%, 19.38%, 19.17%, 18.22%, 14.87% and 13.14% respectively when it is compared with its control (S0). At 90DAS, the main plot M1 shows maximum leaf area compared to M2, with values of 40.87 (M1) and 40.29 (M2), respectively. A percentage increase of 1.41% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S9 with a value of 41.93 where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent increase was found highest in S9 followed by S6> S5> S1> S8&S3> S2> S7, and the per cent values were 41.9%, 41.5%, 41.2%, 41.26%, 40.73%, 40.73%, 40.66%, 40.13% respectively when it is compared with its control (S0). At 120DAS, the main plot M1 shows maximum leaf area compared to M2, with values of 41.88 (M1) and 41.19 (M2), respectively. A percentage increase of 1.64% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S9 with a value of 42.92 where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent increase was found highest in S9, followed by S1> S5> S6> S3> S8> S2> S7> S4, and the per cent values were 11.58%, 10.09%, 9.90%, 9.17%, 9.10%, 8.94%, 7.79% and 7.03% respectively when it is compared with its control (S0).

The study showed a significant increase with 19.55%, 34.31%, 41.9% and 42.95% per cent values at 30DAS, 60DAS, 90DAS and 120DAS when a comparison was made between S9 and S0 (control). In treatment S9, the foliar application of Sulphur @0.25%+ BAP (@0.0015%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the leaf area is found in treatment S9, where the combined application of sulphur and cytokinin is applied to the crop when compared to its control (S0), followed by S6, where boron and Cytokinin have applied in combination to the crop, i.e. Boron @ 0.5% + BAP (@0.0045%)

The lowest increase was found in treatment S2, i.e. 25.79% at 30DAS when compared to its control (S0), where the application of Sulphur at its rec. dose i.e. @ 0.15%is provided alone. At 60DAS and 90DAS, the lowest increase was found in the treatment S4, i.e. 11.34% and 4.52%

when it is compared to its control (S0) where the application of Boron @0.5% +Sulphur @0.25% is provided to the crop.

In the year (2022-2023) at 30DAS, main plot M1 shows maximum leaf area as compared to M2 with values 19.11 (M1) and 17.49 (M2), respectively. A percentage increase of 8.47% was found in M1, where the crop was grown in spacing (30*10). In subplots, a leaf area was significantly increased in S6 at 30DAS with a value of 23.03cm2, where Boron @ 0.5% + BAP (@0.0045%) was provided to the crop as a foliar application. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S6, followed by S5, S7&S9, S8, S4>S3> S2, and the per cent values were 46.33%, 42.60%, 40.38%, 40.38%, 38.45%, 31.01%, 23.46 and 19.47% respectively.

At 60DAS, the main plot M1 shows the maximum leaf area compared to M2, which is 32.53 (M1) and 32.16 (M2), respectively. A percentage increase of 1.13% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase was found in S5 with a value of 37.21cm2, where Boron @ 1.5%+ Sulphur @0.075% was applied to the crop as a foliar application. The per cent increase was found highest in S5, followed by S9> S3> S1> S8> S2> S6> S7, and the per cent values were 27.45%, 23.80%, 18.95%, 17.72%, 17.43%, 17.38%, 14.28% and 11.37% respectively when it is compared with its control (S0). At 90DAS, main plot M1 shows maximum leaf area compared to M2 with values 42.02 (M1) and 41.83 (M2), respectively. A percentage increase of 0.45% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S9 with a value of 42.90cm2 where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent increase was found highest in S9, followed by S1> S5> S6> S3> S8> S2> S7, and the per cent values were 7.84%, 7.08%, 7.05%, 6.90%, 6.17%, 5.87%, 5.79% and 4.77% respectively when it is compared with its control (S0). At 120DAS, the main plot M1 shows maximum leaf area compared to M2, with values of 43.24(M1) and 43.06 (M2), respectively. A percentage

increase of 0.41% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S9 with a value of 44.02cm2 where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent increase was found highest in S9, followed by S6> S3> S5> S1> S8> S2> S4, and the per cent values were 6.63%, 6.67%, 5.93%, 5.84%, 5.78%, 4.55%, 4.49% and 3.89% respectively when it is compared with its control (S0). The study showed a significant increase with 40.38%, 23.80%, 7.84% and 6.63% per cent values at 30DAS, 60DAS, 90DAS and 120DAS when a comparison was made between S9 and S0 (control). In treatment S9, the foliar application of Sulphur @0.25%+ BAP (@0.0015%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the leaf area is found in treatment S9, where the combined application of sulphur and cytokinin is applied to the crop when compared to its control (S0) followed by S6, where boron and Cytokinin are used in combination to the crop i.e. Boron @ 0.5% + BAP (@0.0045%). The lowest increase was found in the treatment S1, i.e. 18.32% at 30DAS when compared to its control (S0), where the application of boron at its rec. Dose, i.e. @ 1%, is provided alone. The substantial expansion of leaf area seen in mustard crops when subjected to combined nutrient treatments, namely the synergistic impact of Sulphur and cytokines (S9 treatment), can be elucidated by their respective cellular functions in plant physiology. The class of plant hormones known as cytokinins stimulates cell division, particularly in the shoot apical meristem, where active cell proliferation occurs. The transition of cells from the G1 phase to the S phase of the cell cycle is facilitated by cytokinin, which promotes DNA replication and subsequent mitosis. This augmented cellular division generates additional cells within the leaf tissue, enlarging the leaf surface area. At the molecular level, cytokinins control the expression of essential genes related to the advancement of the cell cycle, including cyclins and cyclin-dependent kinases (CDKs). These proteins facilitate the timely and regulated division of cells, contributing to the enlargement of leaves. Furthermore, cytokinins facilitate the process of cell differentiation into different leaf tissues, contributing further to the growth and dilation of leaves. Sulphur is a primary macronutrient necessary for

synthesizing amino acids (such as cysteine and methionine) and proteins, which are crucial for the growth and development of plants. One vital function of Sulphur is in the synthesis of chlorophyll, the pigment that is responsible for the process of photosynthesis. Sulphur availability is crucial for chlorophyll synthesis as it is a constituent of many enzymes which participate in the chlorophyll biosynthesis pathway. Sufficient availability of sulphur guarantees that the plant can generate plentiful chlorophyll, augmenting its photosynthetic capability and resulting in improved energy generation and biomass accumulation. In addition, Sulphur is an essential constituent of glutathione, a tripeptide that safeguards plant cells against oxidative stress by its ability to detoxify reactive oxygen species (ROS). By regulating cellular redox equilibrium, Sulphur enables continuous cellular function, such as photosynthesis and growth, in different environmental circumstances. The concurrent application of Sulphur and Cytokinin has a synergistic impact on the enlargement of leaf area, which can be ascribed to their complementary functions in cellular processes. Cytokinin-induced cell division promotes cell proliferation, while Sulphur guarantees that these cells are metabolically active and capable of synthesizing essential proteins and chlorophyll for optimal biochemical activity. This synergy amplifies leaves' overall growth and expansion, as evidenced by the larger leaf areas observed in the S9 treatment. The rationale for this synergistic interaction can be further supported by the fact that both nutrients enhance the efficiency of photograph synthesis. Cytokinin promotes the proliferation of cells capable of housing chloroplasts, the organoids responsible for photosynthesis, while Sulphur augments chlorophyll concentration within these chloroplasts. The outcome is an increased photosynthetic rate per unit leaf area, contributing to the observed growth and biomass in the mustard crops. The cellular processes outlined above offer a strong rationale for the observed phenomenon of increased leaf area when Sulphur and cytokines are applied together. Enhancements in cell division, chlorophyll synthesis, and photosynthetic efficiency, crucial for optimal leaf development and overall plant growth, are directly responsible for the substantial percentage increase in leaf area at different growth stages under the S9 treatment. The findings

emphasize the need for strategic nutrient management in mustard farming, namely the synchronous application of Sulphur and cytokines to optimize leaf area and crop productivity. The cellular mechanisms also elucidate the reason for the suboptimal effectiveness of treatments involving individual nutrients, such as Sulphur alone in S2 or cytokines alone in S7. These treatments failed to exploit the synergistic effects that promote optimal leaf growth. Hence, the results of this study align with established cellular mechanisms and emphasize the possibility of improving the performance of mustard crops by concentrated nutrient applications.

Table 4.3 (a): Effect of spacing and nutrient on Leaf area (cm^2) of mustard crop during rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS		120DAS	
Spacing								
M1 (30×10)	17.90	19.11	31.45	32.53	40.87	42.02	41.89	43.25
M2 (20×10)	16.15	17.49	30.89	32.16	40.29	41.83	41.19	43.07
C.D. at p<0.05	NS	NS	NS	NS	NS	NS	NS	NS
SEM±	0.86	0.84	1.81	1.73	0.39	0.49	0.29	0.25
Nutrients foliar application								
S0-Control	10.45	12.36	25.65	27.00	37.95	39.58	38.66	41.10
S1-Boron @1%	14.23	15.13	31.96	32.81	41.26	42.60	42.28	43.63
S2-Sulphur @ 0.15%	14.08	15.35	31.73	32.68	40.66	42.01	41.68	43.04
S3-BAP @0.003%	15.10	16.15	31.81	33.31	40.73	42.18	41.79	43.70
S4-Boron @0.5% +Sulphur @0.25%	16.35	17.91	28.93	30.35	39.75	41.23	40.82	42.77
S5-Boron @ 1.5%+ Sulphur @0.075%	20.16	21.53	36.31	37.21	41.20	42.58	42.21	43.65
S6-Boron @ 0.5% + BAP (@0.0045%)	21.95	23.03	30.13	31.50	41.50	42.51	42.12	44.04
S7-Boron @ 1.5%+ BAP (@0.0015%)	19.68	20.73	29.53	30.46	40.13	41.56	41.16	42.59
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	18.68	20.08	31.36	32.70	40.73	42.05	41.75	43.06
S9-Sulphur @0.25%+ BAP (@0.0015%)	19.55	20.73	34.31	35.43	41.90	42.95	42.93	44.02
C.D. at p<0.05	NS	NS	NS	NS	NS	NS	NS	NS
SEM±	1.57	1.57	1.99	2.00	0.93	0.97	0.91	0.91
C.D. S×M at p<0.05	NS	NS	NS	NS	NS	NS	NS	NS
SEM±	2.74	2.67	5.74	5.48	1.24	1.57	1.26	1.58
C.D. M×S at p<0.05	NS	NS	NS	NS	NS	NS	NS	NS
SEM±	2.28	2.27	3.23	3.20	1.31	1.39	1.33	1.40

Where, C.D. represents critical difference, SE (m) represents standard error of mean.

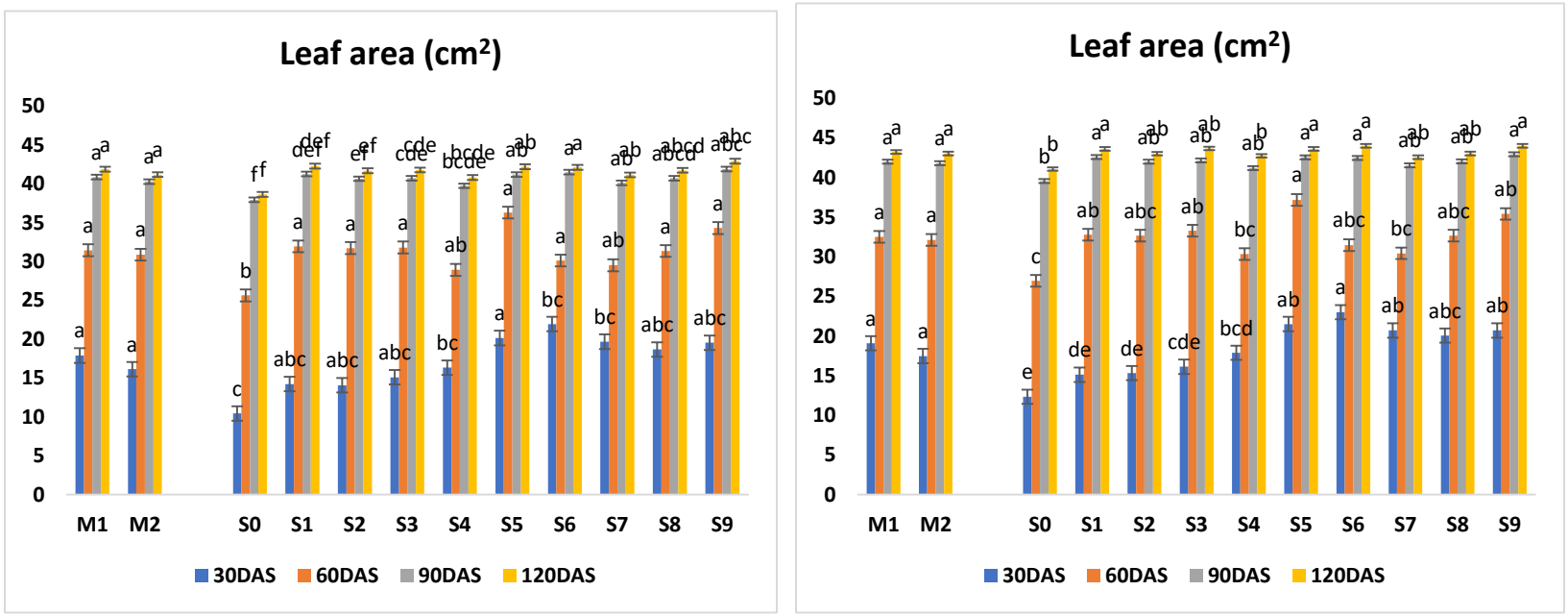


Fig. 4.3 (a): Effect of spacing and nutrient on Leaf area of mustard crop during rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.1.4 Stem diameter (cm)

Mustard crop shows different changes when grown under different nutrient levels. In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in stem diameter were observed at 30DAS, 60DAS, 90DAS and 120 DAS, as shown in Table 4.4, Fig 4.4. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the stem diameter in each treatment as compared to control of both the spacings at 30, 60, 90 and 120 DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the stem diameter was observed at 30, 60, 90, and 120 DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, main plot M1 shows maximum stem diameter as compared to M2 with values 0.46cm (M1) and 0.33cm (M2), respectively. A percentage increase of 28.26% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase in stem diameter was observed in S9, i.e. 0.5cm at 30DAS. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S9, followed by S8, S4, S5&S3> S2> S7> S6&S1, and the per cent values were 48%, 46.20%, 42.22%, 37.6%, 37.6%, 35%, 29.09%, 25.71% and 25.71% respectively. At 60DAS, the main plot M1 shows maximum stem diameter compared to M2, with 1.29cm (M1) and 1.14cm (M2) values, respectively. A percentage increase of 11.62% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase was found in S8 with a value of 1.35cm where Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop as a foliar application. The per cent increase was found highest in S8, followed by S6> S3& S1> S9> S2> S4> S5> S7, and the per cent values were 33.33%, 32.5%, 31.64%, 31.64%, 28.94%, 26.02%, 25%, 23.94% and

20.58% respectively when it is compared with its control (S0). At 90DAS, the main plot M1 shows maximum stem diameter compared to M2, with values of 1.49cm (M1) and 1.44cm (M2), respectively. A percentage increase of 3.35% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S6 with a value of 1.65cm where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S6, followed by S1> S2&S8> S4> S3> S5> S7, and the per cent values were 31.51%, 28.63%, 27.87%, 27.87%, 27.48%, 24.66%, 22.95% and 16.29% respectively when it is compared with its control (S0). At 120DAS, the main plot M1 shows maximum stem diameter compared to M2, with values of 1.54cm (M1) and 1.48cm (M2), respectively. A percentage increase of 3.89% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S6 with a value of 1.7cm where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S6 followed by S1&S8> S2&S4> S3> S5> S7, and the per cent values were 29.41%, 26.53%, 26.53%, 25%, 25%, 21.73%, 20% and 14.28% respectively when it is compared with its control (S0).

The study showed a significant increase with 25.71%, 32.5%, 31.51% and 29.41% per cent values at 30DAS, 60DAS, 90DAS, and 120DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 90DAS and 120DAS, a significant increase in the stem diameter is found in treatment S6, where the combined application of Boron and cytokinin is applied to the crop compared to its control (S0).

The lowest increase was found in treatment S1, i.e. 25.71% at 30DAS, when compared to its control (S0), where the application of boron at its rec. Dose, i.e. @ 1%, is provided alone. At 60DAS and 90DAS, the lowest increase was found in treatment S4, i.e. 25% and 27%, when

compared to its control (S0), where the single application of cytokinin (Rec. dose @0.003%) is provided to the crop.

In the year (2022-23), at 30DAS, main plot M1 shows maximum stem diameter as compared to M2 with values 0.56cm (M1) and 0.42cm (M2), respectively. A percentage increase of 25% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase in stem diameter was observed in S8, i.e. 0.58cm at 30DAS. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S8, followed by S4&S9, S3&S5> S2> S7> S1&S6, and the per cent values were 38.28%, 34.54%, 34.54%, 30.32%, 30.32%, 28%, 22.85%, 20% and 20% respectively. At 60DAS, the main plot M1 shows maximum stem diameter compared to M2, with values of 1.4cm (M1) and 1.24cm (M2), respectively. A percentage increase of 11.42% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase was found in S8 with a value of 1.45cm where Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop as a foliar application. The per cent increase was found highest in S8, followed by S6> S3& S1> S9> S2> S4> S5> S7, and the per cent values were 30.34%, 29.53%, 28.70%, 28.70%, 26.09%, 23.29%, 22.30%, 21.29% and 18.10% respectively when it is compared with its control (S0). At 90DAS, the main plot M1 shows maximum stem diameter compared to M2, with values of 1.59cm (M1) and 1.55cm (M2), respectively. A percentage increase of 2.51% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S6 with a value of 1.75cm where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S6, followed by S1> S2&S8> S4> S3> S5> S7, and the per cent values were 28%, 25.14%, 24.4%, 24.4%, 23.63%, 21.25%, 19.57% and 13.10% respectively when it is compared with its control (S0). At 120DAS, the main plot M1 shows maximum stem diameter compared to M2, with values of 1.61cm (M1) and 1.58cm (M2), respectively. A percentage increase of 1.86% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results

were observed in S6 with a value of 1.8cm where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S6 followed by S1&S8> S4> S3> S5> S2> S7, and the per cent values were 27.77%, 25%, 25%, 22.77%, 20.40%, 18.75%, 15.21% and 13.33% respectively when it is compared with its control (S0).

The study showed a significant increase with 20%, 29.53%, 28% and 27.77% per cent values at 30DAS, 60DAS, 90DAS, and 120DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 90DAS and 120DAS, a significant increase in the plant height is found in treatment S6, where the combined application of Boron and cytokinin is applied to the crop when compared to its control (S0).

The lowest increase was found in treatment S1, i.e. 20% at 30DAS, when compared to its control (S0), where the application of boron at its rec. Dose, i.e. @ 1%, is provided alone. At 60DAS and 90DAS, the lowest increase was found in the treatment S4, i.e. 22.30% and 23.63%, when it is compared to its control (S0) where the single application of cytokinin (Rec. dose @0.003%) is provided to the crop. The stem girth was reduced slightly at the crop's harvest stage due to the plant's drying at harvesting maturity. The accumulation of cytokinin enlarges the cambium cells and transfers the photosynthate from roots to shoot via a transpiration stream through the xylem (Kiba et al. 2011). Gu et al. (2018) suggest the same results in their experiment.

In mustard crops, the stem diameter is crucial to the plant's general health and structural soundness. Several physiological processes dictate it, such as cell division, cell enlargement, lignification, and nutrient transport. The presence and equilibrium of nutrients like Boron, Sulphur, and cytokines directly impact these. Cytokinin is a phytohormone that stimulates embryonic division (cytokinesis) in the cambium, the stratum of actively proliferating cells responsible for stem thickening. The stimulation of cambial cell proliferation by cytokinin results in enhanced synthesis of xylem and phloem tissues, so contributing to an augmentation in stem

diameter. Furthermore, cytokinin stimulates cell proliferation by augmenting the production of cell wall constituents, facilitating the radial enlargement of stem cells. Sulphur plays a vital role in the synthesis of lignin, an intricate organic compound that enhances the structural integrity of cell walls, especially in the xylem. The lignification process is crucial for maintaining the structural integrity of the stem by imparting rigidity and resistance to external stresses. Sulphur is an essential constituent of amino acids such as cysteine and methionine, which serve as precursors in lignin biosynthesis. Sufficient availability of Sulphur guarantees the optimization of lignification conditions, resulting in the development of thicker and more robust stems. Boron is indispensable for preserving the structural integrity of cellular walls and membranes. It promotes cross-linking of pectin molecules in the cell wall, increasing its stiffness and structural integrity. Furthermore, boron is involved in transporting sugars and nutrients via the phloem, a vital component for facilitating the growth and development of the stem. Boron facilitates the overall thickening of the stem through its ability to stabilize cell walls and promote efficient nutrient transport. The combined application of Boron, Sulphur, and Cytokinin synergistically augments cell division, enlargement, lignification, and nutrient transport. The phenomenon of synergy results in a more significant augmentation in stem diameter compared to the individual application of each nutrient. Cytokinin stimulates fast cell division and subsequent growth, while Sulphur and Boron enhance the newly generated cells by strengthening their cell walls and facilitating the transportation of nutrients. Cytokinin-induced stimulation of cambial cell division results in enhanced synthesis of xylem and phloem tissues, directly promoting the thickening of stems. Cytokinin guarantees the sustained activity of the cambium during the entire growth phase, facilitating uninterrupted expansion of the stem. The involvement of sulfur in the lignin biosynthesis provides the stem with improved mechanical strength, enabling it to sustain higher biomass levels without compromising its stability. The lignification process reinforces the stem's ability to endure environmental pressures, such as wind and weight from reproductive structures, increasing its diameter. The participation of boron in the process of pectin cross-linking within

cell walls stabilizes the cells and enhances the transport of nutrients throughout the vascular system. Adequate transportation of nutrients is crucial for maintaining the growth processes that contribute to the thickening of stem fibers. The synergistic use of these nutrients capitalizes on their advantages, leading to a more significant augmentation in stem diameter. Specifically, the structural reinforcement supplied by Sulphur and Boron facilitates the fast growth and elongation induced by Cytokinin, resulting in a strong and thicker stem. The observed growth in stem diameter in the mustard crop can be attributed to cellular processes such as increased cambial activity, lignification, and nutrient transport. The coordinated application of Boron, Sulphur, and Cytokinin influences these processes. By promoting cell division, strengthening cell walls, and enhancing nutrient flow, these nutrients synergistically contribute to developing a thicker and more resilient stem that supports the plant's growth and yield potential. This interpretation of the cellular processes provides a compelling rationale for the enlarged stem diameter and emphasizes the need for well-balanced nutrient management in maximizing crop productivity.

Table 4.4 (a): Effect of spacing and nutrient on Stem diameter (cm) of mustard crop during rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS		120DAS	
Spacing								
M1 (30×10)	0.46	0.56	1.29	1.40	1.49	1.59	1.54	1.61
M2 (20×10)	0.33	0.42	1.14	1.24	1.44	1.55	1.48	1.58
C.D. at p<0.05	0.04	0.05	0.08	0.09	0.03	0.04	0.06	0.07
SEM±	0.01	0.00	0.01	0.01	0.01	0.00	0.01	0.03
Nutrients foliar application								
S0-Control	0.26	0.36	0.90	1.01	1.13	1.26	1.20	1.30
S1-Boron @1%	0.35	0.45	1.31	1.41	1.58	1.68	1.63	1.73
S2-Sulphur @ 0.15%	0.40	0.50	1.21	1.31	1.56	1.66	1.60	1.53
S3-BAP @0.003%	0.41	0.51	1.31	1.41	1.50	1.60	1.53	1.63
S4-Boron @0.5% +Sulphur @0.25%	0.45	0.55	1.20	1.30	1.55	1.65	1.60	1.68
S5-Boron @ 1.5%+ Sulphur @0.075%	0.41	0.51	1.18	1.28	1.46	1.56	1.50	1.60
S6-Boron @ 0.5% + BAP (@0.0045%)	0.35	0.45	1.33	1.43	1.65	1.75	1.70	1.80
S7-Boron @ 1.5%+ BAP (@0.0015%)	0.36	0.46	1.13	1.23	1.35	1.45	1.40	1.50
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	0.48	0.58	1.35	1.45	1.56	1.66	1.63	1.73
S9-Sulphur @0.25%+ BAP (@0.0015%)	0.60	0.55	1.26	1.36	1.31	1.43	1.35	1.45
C.D. at p<0.05	0.12	0.09	0.23	0.23	0.28	0.27	0.26	0.29
SEM±	0.04	0.03	0.08	0.08	0.10	0.09	0.09	0.10
C.D. S×M at p<0.05	NS	NS	NS	NS	NS	NS	NS	NS
SEM±	0.02	0.02	0.03	0.04	0.03	0.02	0.03	0.11
C.D. M×S at p<0.05	NS	NS	NS	NS	NS	NS	NS	NS
SEM±	0.06	0.05	0.01	0.10	0.13	0.13	0.12	0.14

Where, C.D. represents critical difference, SE (m) represents standard error of mean.

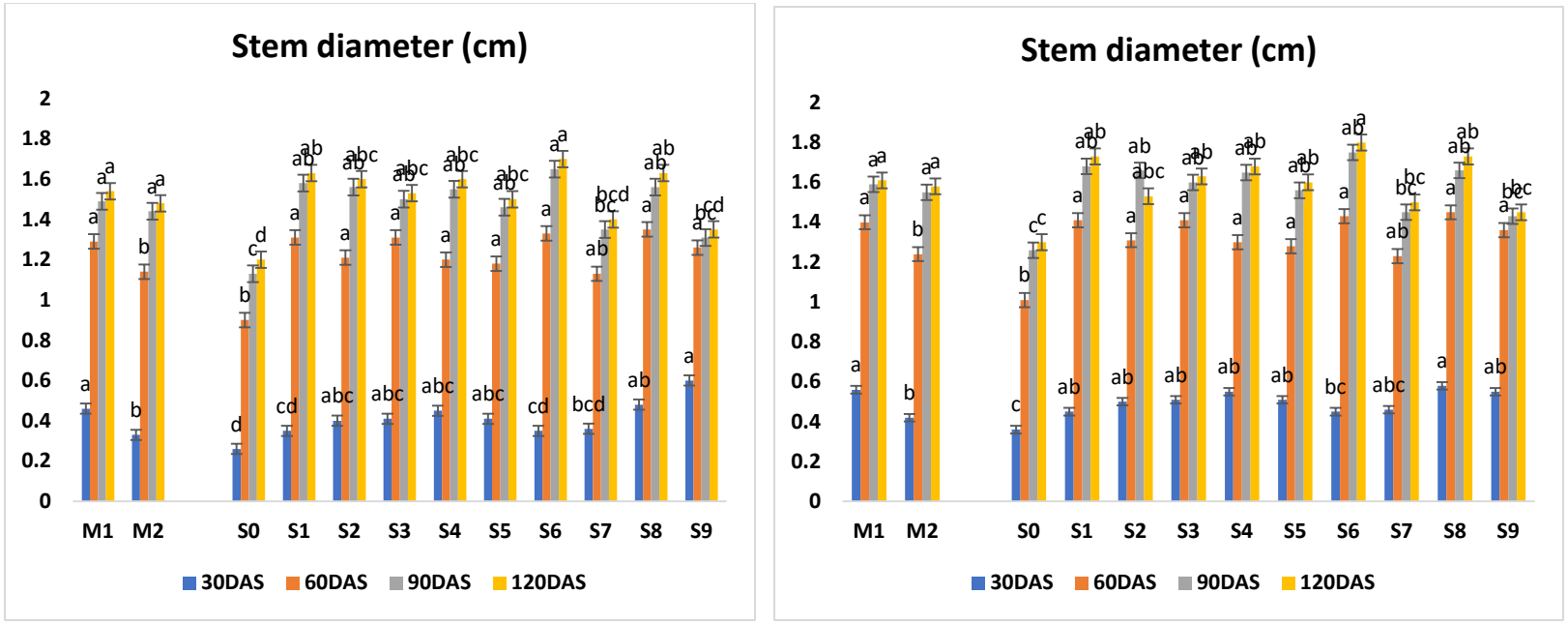


Fig. 4.4 (a): Effect of spacing and nutrient on Stem diameter of mustard crop during rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.2 Physiological observations

4.2.1 Leaf Area Index (LAI):

The effect of micro and secondary nutrients (Boron and Sulphur) and their combination with plant growth hormone (Cytokinin) on leaf area index was studied in the Mustard crop under spatial dynamics with variety NB-RIMUL-2019 (Nandi Bull) during the years 2021-22 and 2022-23.

Changes in leaf area index were observed at 30DAS, 60DAS, 90DAS, and 120 DAS, which are shown in Table 4.5, Fig 4.5. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the leaf area index in each treatment as compared to control of both the spacings at 30, 60, 90 and 120 DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the leaf area index was observed at 30, 60, 90, and 120 DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, the main plot M2 shows the maximum leaf area index as compared to M1 with values of 0.48 (M2) and 0.33 (M1), respectively. A percentage increase of 31.25% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in leaf area index was observed in S6, i.e. 0.54 at 30DAS. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S6, followed by S5, S7, S9> S8> S4> S1> S3> S2, and the per cent values were 57.93%, 53.09%, 52.99%, 51.27%, 49.76%, 40.06%, 35.01%, 34.30% and 29.18% respectively. At 60DAS, main plot M2 shows a maximum leaf area index compared to M1 with values of 3.95 (M2) and 2.69 (M1), respectively. A percentage increase of 31.89% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S9 with a value of 3.89, where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar application. The per cent increase

was found highest in S9, followed by S4> S7> S8> S6> S2> S3> S5> S1, and the per cent values were 51.22%, 51.07%, 47.79%, 47.78%, 44.44%, 43.86%, 42.65%, 41.435 and 35.52% respectively when it is compared with its control (S0). At 90DAS, main plot M2 shows a maximum leaf area index compared to M1 with values of 5.99 (M2) and 4.08 (M1), respectively. A percentage increase of 31.88% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S4 with a value of 5.99 where Boron @0.5% + sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S4, followed by S9> S7> S6> S8> S3> S2> S5, and the per cent values were 46.27%, 42.62%, 41.45%, 41.38%, 39.42%, 39.05%, 35.43% and 28.58% respectively when it is compared with its control (S0). At 120DAS, main plot M2 shows a maximum leaf area index compared to M1 with values of 6.51 (M2) and 4.28 (M1), respectively. A percentage increase of 34.25% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S4 with a value of 6.15 where Boron @0.5% + sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S4, followed by S9> S3> S7> S8> S2> S6> S1, and the per cent values were 40.51%, 39.23%, 38.06%, 37.32%, 37.04%, 32.87%, 29.58% and 28.31% respectively when it is compared with its control (S0).

The study showed a significant increase with 57.93%, 44.44%, 41.38% and 29.58% per cent values at 30DAS, 60DAS, 90DAS, and 120DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 90DAS and 120DAS, a significant increase in the leaf area index is found in treatment S4, where the combined application of Boron and sulphur is applied to the crop when compared to its control (S0).

The lowest increase was found in treatment S2, i.e. 29.18% at 30DAS, when compared to its control (S0), where the application of sulphur at its rec. Dose, i.e. @ 0.15%, is provided alone. At

60DAS, 90DAS and 120DAS, the lowest increase was found in the treatment S1, i.e. 35.52%, 27.60% and 28.31% when it is compared to its control (S0) where the single application of boron (Rec. dose @1%) is provided to the crop.

In the year (2022-23), at 30DAS, the main plot M2 shows the maximum leaf area index as compared to M1 with values of 0.49 (M2) and 0.35 (M1), respectively. A percentage increase of 28.57% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in leaf area index was observed in S6, i.e. 0.56 at 30DAS. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S6, followed by S5&S7, S9> S8> S4> S1> S3> S2 and the per cent values were 55.35%, 50.49%, 50.49%, 48.45%, 46.80%, 37.5%, 32.43%, 31.50% and 26.47% respectively. At 60DAS, main plot M2 shows a maximum leaf area index compared to M1 with values of 3.97 (M2) and 2.72 (M1), respectively. A percentage increase of 31.48% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S9 with a value of 3.92, where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar application. The per cent increase was found highest in S9 followed by S4> S7&S8> S6> S2> S3> S5> S1, and the per cent values were 51.02%, 50.76%, 47.46%, 47.46%, 44.18%, 43.52%, 43.02%, 41.10% and 35.13% respectively when it is compared with its control (S0). At 90DAS, main plot M2 shows a maximum leaf area index compared to M1 with values of 6.00 (M2) and 3.89 (M1), respectively. A percentage increase of 35.16% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S9 with a value of 6.12 where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent increase was found highest in S9, followed by S4> S7> S6> S8> S2> S5> S1, and the per cent values were 47.10%, 46.13%, 41.25%, 41.14%, 39.21%, 35.2%, 28.39% and 27.43% respectively when it is compared with its control (S0). At 120DAS, main plot M2 shows a maximum leaf area index compared to M1 with values of 6.53 (M2) and 4.30 (M1), respectively.

A percentage increase of 34.15% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S4 with a value of 6.17, where Boron @0.5% + sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S4, followed by S9> S3> S7> S8> S2> S6> S1, and the per cent values were 40.40%, 39.07%, 37.89%, 37.14%, 36.87%, 32.72%, 29.43% and 28.26% respectively when it is compared with its control (S0).

The study showed a significant increase with 37.5%, 50.76%, 46.13% and 40.40% per cent values at 30DAS, 60DAS, 90DAS, and 120DAS when a comparison was made between S4 and S0 (control). In treatment S4, the foliar application of Boron @0.5% + sulphur @0.25% was applied to the mustard crop. At 90DAS and 120DAS, a significant increase in the leaf area index is found in treatment S4, where the combined application of Boron and sulphur is applied to the crop when compared to its control (S0).

The lowest increase was found in treatment S2, i.e. 26.47% at 30DAS when compared to its control (S0), where the application of sulphur at its rec. Dose, i.e. @ 0.15%, is provided alone. At 60DAS, 90DAS and 120DAS, the lowest increase was found in the treatment S1, i.e. 35.13%, 27.43% and 28.26% when it is compared to its control (S0) where the single application of boron (Rec. dose @1%) is provided to the crop. Ravikumar et al. 2021 show significant results on leaf area index by applying micronutrients in sunflower crops. Leaf area index (LAI) in plants, such as mustard crops, is directly affected by various cellular and physiological mechanisms that control leaf development and enlargement. Two fundamental processes at the cellular level are the main drivers of the increase in leaf area: cell division and cell expansion. The cytokinin hormone is essential for stimulating cell division, especially in the meristematic areas of the plant, including the shoot apical meristem and leaf primordia. Cytokinin coordinates the shift from the G1 phase to the S phase in the cell cycle, characterized by DNA replication and subsequent mitosis. An elevated rate of cellular division leads to a greater quantity of cells in the growing

leaf, contributing to an expansion in leaf surface area. Indirectly, sulphur, a vital constituent of amino acids such as cysteine and methionine, promotes cell division by enabling protein synthesis. These essential amino acids are crucial for developing new cells and tissues, guaranteeing the functionality of the newly divided cells and their contribution to leaf growth. The formation and stability of cell walls are crucially dependent on boron. It plays a vital role in cross-linking pectic polysaccharides in the cell wall, ensuring the integrity and flexibility of all cell walls. Sufficient boron levels guarantee that cells can efficiently expand without compromising their structural integrity when they absorb water (turgor pressure). This cellular proliferation is crucial for the overall augmentation of leaf surface area. Moreover, cytokinin modulates the expression of genes related to cell wall loosening, such as expansions, affecting cell growth. Expansions disrupt the hydrogen bonds among cellulose microfibrils, enabling the cell wall to elongate and accommodate the growing volume of the cell. The size and surface area of leaves are crucially dependent on this process. Sulphur is an essential constituent of many coenzymes and proteins that play a crucial role in the photosynthetic process, including ferredoxin and thioredoxin. Enhanced photosynthesis leads to an increased availability of carbohydrates, which function as energy sources and fundamental components for subsequent cell division and expansion. Consequently, this facilitates the growth of higher-sized leaves, contributing to an elevated leaf area index. The augmented metabolic activity facilitated by these nutrients guarantees the fulfilment of the energy requirements of the growing cells, so enabling continuous growth and development of the leaves. The leaf area index is an essential agronomic parameter that indicates the structure of a crop's canopy and its potential productivity. A higher leaf area index (LAI) signifies a larger leaf surface area per unit ground area, greatly improving the photosynthetic capacity of the crop and, as a result, its yield. The concurrent administration of Sulphur and Cytokinin (S9 treatment) showed the most notable enhancement in LAI, especially 60 days after sowing (DAS). The interaction between the function of Sulphur in photosynthesis and the stimulation of cell division and expansion by cytokines leads to the development of larger

and more resilient leaves. By directly increasing the number and size of the leaves, this combination offers a compelling explanation for the observed rise in leaf area index (LAI). The utilization of Boron, especially when combined with Cytokinin, promotes vigorous cell proliferation by enhancing the integrity of the cell wall. This structural improvement enables the sustained development of bigger leaves, contributing to an increased Leaf Area Index (LAI). Treatments containing Boron (e.g., S6) consistently demonstrated substantial growth in leaf area, confirming the significance of Boron in attaining an ideal Leaf Area Index (LAI). The closer planting spacing (30 × 10 cm) led to a greater Leaf Area Index (LAI) because of the heightened competition for light, consequently promoting leaf development. Plants adjust within a more compact canopy by enlarging their leaves to intercept more light, enhancing the Leaf Area Index (LAI). The observed increase in leaf area index (LAI) in these conditions can be attributed to a crucial adaptation mechanism that enhances the photosynthetic efficiency of the canopy. The temporal rise in Leaf Area Index (LAI), especially during the initial growth phases (30 and 60 days after sowing), indicates the crucial periods of leaf area enlargement. During these stages, the cells exhibit their highest level of activity in division and expansion, which is primarily influenced by the nutrient treatments administered. The consistent rise in Leaf Area Index (LAI) during later stages (90 and 120 days after sowing) suggests that the initial nutrient treatments had a long-lasting effect on the plant's growth pathway, underscoring the need for early and suitable nutrient control. The observed increases in the leaf area index of mustard crops can be strongly justified by the biochemical processes of cell division, expansion, and photosynthesis, which are facilitated by specific nutrient applications. The Leaf Area Index (LAI) functions as a dependable measure of the crop's potential productivity by reflecting the efficacy of the nutrient treatments.

Table-4.5 (a): Effect of spacing and nutrient on LAI of mustard crop during rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS		120DAS	
Spacing								
M1 (30×10)	0.34	0.35	2.69	2.72	4.08	3.90	4.28	4.30
M2 (20×10)	0.48	0.49	3.95	3.97	5.99	6.01	6.51	6.53
C.D. at p<0.05	NS	NS	NS	NS	0.72	0.72	0.29	0.29
SEM±	0.02	0.02	0.23	0.23	0.11	0.10	0.04	0.03
Nutrients foliar application								
S0-Control	0.23	0.26	1.90	1.92	3.22	3.25	3.66	3.68
S1-Boron @1%	0.35	0.37	2.94	2.96	4.44	4.47	5.10	5.13
S2-Sulphur @ 0.15%	0.32	0.34	3.38	3.40	4.98	5.00	5.45	5.47
S3-BAP @0.003%	0.35	0.37	3.31	3.37	5.28	3.80	5.90	5.93
S4-Boron @0.5% +Sulphur @0.25%	0.38	0.40	3.88	3.90	5.99	6.02	6.15	6.18
S5-Boron @ 1.5%+ Sulphur @0.075%	0.49	0.51	3.24	3.26	4.50	4.53	4.83	4.86
S6-Boron @ 0.5% + BAP (@0.0045%)	0.54	0.56	3.42	3.44	5.49	5.51	5.19	5.22
S7-Boron @ 1.5%+ BAP (@0.0015%)	0.48	0.51	3.64	3.66	5.50	5.52	5.83	5.86
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	0.45	0.47	3.63	3.66	5.31	5.33	5.81	5.83
S9-Sulphur @0.25%+ BAP (@0.0015%)	0.47	0.49	3.89	3.92	5.61	6.13	6.02	6.04
C.D. at p<0.05	0.11	0.10	0.99	0.99	1.00	1.00	1.09	1.09
SEM±	0.03	0.03	0.34	0.34	0.34	0.34	0.38	0.39
C.D. S×M at p<0.05	0.15	0.16	1.20	1.22	1.22	1.22	1.26	1.24
SEM±	0.08	0.07	0.74	0.74	0.34	0.34	0.14	0.10
C.D. M×S at p<0.05	0.16	0.15	1.24	1.24	1.24	1.24	0.28	1.22
SEM±	0.05	0.05	0.51	0.51	0.47	0.47	0.51	0.52

Where, C.D. represents critical difference, SE (m) represents standard error of mean.

Table 4.5 (b): Interaction effect of spacing and nutrient on LAI of mustard crop during rabi season at 30DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	0.164	0.253	0.281	0.338	0.305	0.459	0.447	0.405	0.349	0.397	0.34
M2	0.312	0.455	0.369	0.362	0.463	0.522	0.647	0.574	0.567	0.547	0.482
Mean B	0.238	0.354	0.325	0.35	0.384	0.49	0.547	0.489	0.458	0.472	
C.D. S×M at p<0.05						0.15					
SEM±						0.08					
C.D. M×S at p<0.05						0.16					
SEM±						0.05					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	0.203	0.259	0.288	0.341	0.316	0.469	0.451	0.41	0.356	0.411	0.35
M2	0.319	0.449	0.377	0.368	0.473	0.526	0.652	0.579	0.578	0.553	0.487
Mean B	0.261	0.354	0.333	0.354	0.395	0.497	0.551	0.494	0.467	0.482	
C.D. S×M at p<0.05						0.16					
SEM±						0.07					
C.D. M×S at p<0.05						0.15					
SEM±						0.05					

Table 4.5 (c): Interaction effect of spacing and nutrient on LAI of mustard crop during rabi season at 60DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.547	2.549	2.608	3.041	3.204	2.551	3.306	2.425	2.652	3.087	2.697
M2	2.266	3.346	4.162	3.585	4.563	3.938	3.534	4.855	4.625	4.705	3.958
Mean B	1.906	2.947	3.385	3.313	3.884	3.245	3.42	3.64	3.639	3.896	
C.D. S×M at p<0.05						1.20					
SEM±						0.74					
C.D. M×S at p<0.05						1.24					
SEM±						0.51					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.555	2.552	2.615	3.048	3.216	2.555	3.311	2.431	2.655	3.093	2.703
M2	2.274	3.35	4.166	3.591	4.571	3.943	3.537	4.859	4.632	4.713	3.964
Mean B	1.914	2.951	3.391	3.32	3.893	3.249	3.424	3.645	3.644	3.903	
C.D. S×M at p<0.05						1.22					
SEM±						0.74					
C.D. M×S at p<0.05						1.24					
SEM±						0.51					

Table 4.5 (d): Interaction effect of spacing and nutrient on LAI of mustard crop during rabi season at 90DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	2.503	3.504	4.178	4.568	4.92	3.324	4.857	4.239	4.185	4.555	4.083
M2	3.955	5.391	5.797	5.999	7.067	5.693	6.129	6.761	6.446	6.669	5.991
Mean B	3.229	4.448	4.988	5.283	5.994	4.509	5.493	5.5	5.316	5.612	
C.D. S×M at p<0.05						1.22					
SEM±						0.34					
C.D. M×S at p<0.05						1.24					
SEM±						0.47					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	2.51	3.51	4.183	4.573	4.927	3.328	4.863	4.245	4.214	4.561	4.091
M2	3.957	5.396	5.804	6.009	7.073	5.698	6.132	6.768	6.451	6.673	5.996
Mean B	3.234	4.453	4.994	5.291	6	4.513	5.497	5.506	5.332	5.617	
C.D. S×M at p<0.05						1.22					
SEM±						0.34					
C.D. M×S at p<0.05						1.24					
SEM±						0.47					

Table 4.5 (e): Interaction effect of spacing and nutrient on LAI of mustard crop during rabi season at 120DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	2.911	4.009	4.504	5.174	4.997	3.619	3.694	4.556	4.483	4.884	4.283
M2	4.418	6.202	6.401	6.644	7.308	6.051	6.702	7.123	7.144	7.162	6.516
Mean B	3.665	5.106	5.452	5.909	6.152	4.835	5.198	5.839	5.814	6.023	
C.D. S×M at p<0.05						1.26					
SEM±						0.14					
C.D. M×S at p<0.05						0.28					
SEM±						0.51					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	2.917	3.681	4.51	5.192	5.004	3.624	3.702	4.561	4.487	4.891	4.257
M2	4.426	6.208	6.396	6.649	7.311	6.058	6.712	7.133	7.15	7.173	6.522
Mean B	3.672	4.945	5.453	5.921	6.158	4.841	5.207	5.847	5.819	6.032	
C.D. S×M at p<0.05						1.24					
SEM±						0.10					
C.D. M×S at p<0.05						1.22					
SEM±						0.52					

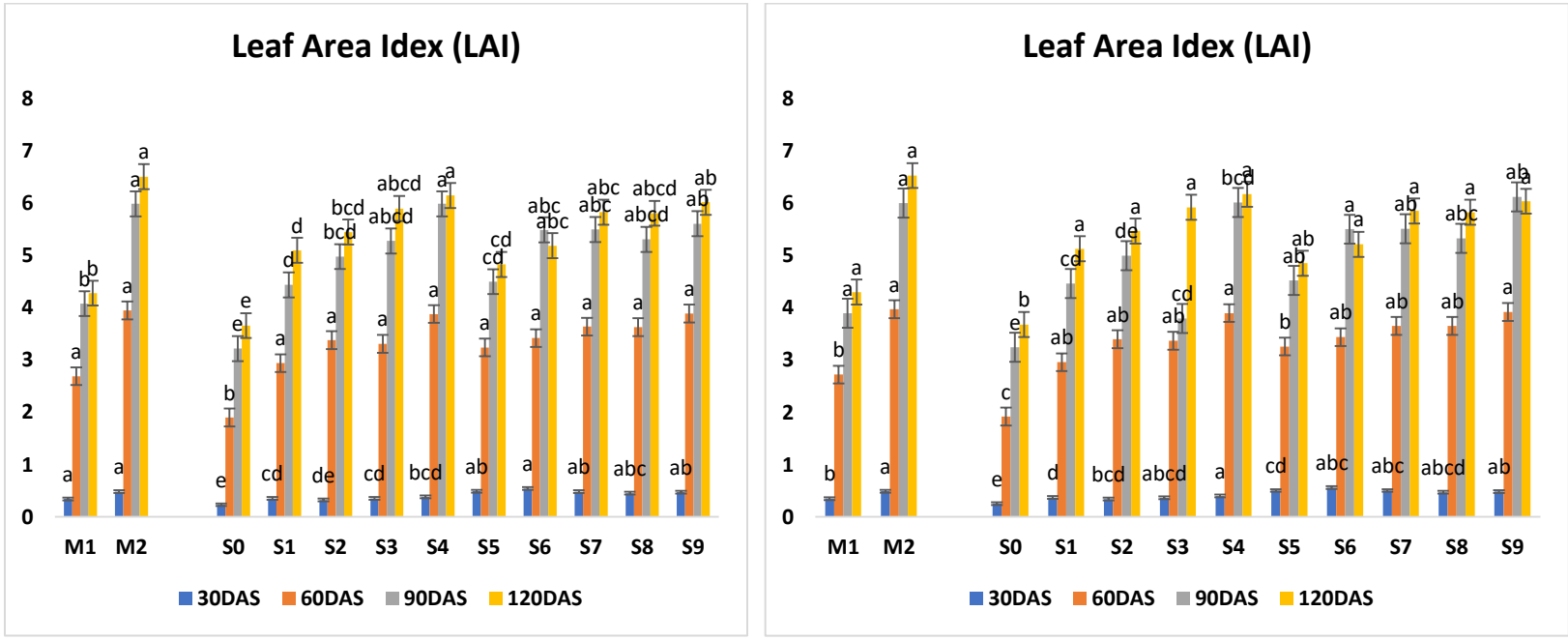


Fig-4.5 (a): Effect of spacing and nutrient on LAI of mustard crop during rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.2.2 Dry matter accumulation (DMA)(g)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in dry matter accumulation were observed at 30DAS, 60DAS and 90DAS, as shown in Table 4.6, Fig 4.6. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the dry matter accumulation in each treatment compared to control of both the spacings at 30, 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the dry matter accumulation was observed at 30, 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, main plot M2 shows maximum dry matter accumulation as compared to M1 with values 8.08 (M2) and 7.20 (M1), respectively. A percentage increase of 10.89% was found in M1, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in dry matter accumulation was observed in S7, i.e. 9.37 at 30DAS, where in S7, Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S7, followed by S3> S6> S4> S2> S8> S1> S5 and the per cent values were 39.11%, 33.60%, 32.17%, 32.03%, 22.85%, 22.82%, 21.53% and 20.71% respectively. At 60DAS, the main plot M2 shows maximum dry matter accumulation compared to M1, with values of 28.87 (M2) and 28.44 (M1), respectively. A percentage increase of 1.48% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S4 with a value of 34.3, where Boron @0.5% + sulphur @0.25% was applied to the crop as a foliar application. The per cent increase was found highest in S4, followed by S8> S3> S2> S5> S6> S9> S7, and the per cent values were 52.71%, 48.54%, 47.91%, 47.195, 46.64%, 46.55%,

45.29% and 44.29% respectively when it is compared with its control (S0). At 90DAS, the main plot M2 shows maximum dry matter accumulation compared to M1, with 152.18 (M2) and 120.18 (M1) values, respectively. A percentage increase of 21.42% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S1 with a value of 168.63, where Boron @1% was applied to the crop as a foliar spray. The per cent increase was found highest in S1, followed by S9> S7> S8> S6> S4> S3> S5, and the per cent values were 39.38%, 37.63%, 33.52%, 30.12%, 27.16%, 20.04%, 19.69% and 14.47% respectively when it is compared with its control (S0).

The study showed a significant increase with 32.17%, 46.55% and 1.44% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant dry matter accumulation was observed in treatment S4, where the combined application of aqueous formulation of Boron @0.5% + sulphur @0.25% was applied to the crop.

In the year (2022-23), at 30DAS, main plot M2 shows maximum dry matter accumulation as compared to M1 with values 9.31 (M2) and 7.91 (M1), respectively. A percentage increase of 15.03% was found in M1, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in dry matter accumulation was observed in S7, i.e. 10.34 at 30DAS, where in S7, Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S7, followed by S3> S4> S6> S2> S8> S1> S5 and the per cent values were 34.04%, 28.02%, 28.76%, 25.19%, 19.17%, 13.94%, 18.51% and 16.43% respectively. At 60DAS, the main plot M2 shows maximum dry matter accumulation compared to M1, with values of 30.05 (M2) and 29.80 (M1), respectively. A percentage increase of 0.83% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S4 with a value of 34.3 where Boron

@0.5% + sulphur @0.25% was applied to the crop as a foliar application. The per cent increase was found highest in S4, followed by S8> S3> S2> S5> S6> S9> S7, and the per cent values were 51.06%, 46.14%, 45.47%, 44.75%, 44.55%, 44.23%, 42.97% and 42.17% respectively when it is compared with its control (S0). At 90DAS, the main plot M2 shows maximum dry matter accumulation compared to M1, with values of 155.41 (M2) and 122.59 (M1), respectively. A percentage increase of 21.11% was found in M2, where the crop was grown in reduced spacing (20*10). Significant results were observed in S1 with a value of 170.72 in subplots where Boron @1% was applied to the crop as a foliar spray. The percent increase was found highest in S1 followed by S9> S7> S8> S6> S4> S3> S5 and the percent values were 39.90%, 37.75%, 33.88%, 30.38%, 27.57%, 20.51%, 20.10% and 15.44% % respectively when it is compared with its control (S0).

The study showed a significant increase with 25.19%, 44.23% and 27.57% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant dry matter accumulation was observed in treatment S4, where the combined application of aqueous formulation of Boron @0.5% + sulphur @0.25% was applied to the crop. Dry matter accumulation in mustard crops is a critical factor determining the overall growth and yield of the plant. This accumulation is primarily driven by the plant's photosynthetic activity, where carbon dioxide is fixed into organic compounds that contribute to the plant's biomass. Various factors, including nutrient availability, hormonal regulation, and environmental conditions influence this process. The primary mechanism driving dry matter accumulation is photosynthesis. In the chloroplasts of mustard leaves, light energy is captured by chlorophyll pigments and used to convert carbon dioxide and water into glucose and oxygen. The glucose produced is used immediately for energy or stored as starch in the plant. Over time, this contributes to the plant's overall biomass and dry matter content. Boron is

essential for cell wall formation and the development of meristematic tissues. It aids in synthesizing structural carbohydrates, contributing to cell wall strength and dry matter content. Boron also plays a role in transporting photosynthates from leaves to other parts of the plant, facilitating the distribution of assimilates and promoting overall growth. Sulphur is a critical component of amino acids, proteins, and enzymes involved in photosynthesis. Adequate sulphur levels enhance chlorophyll synthesis, increasing the plant's photosynthetic efficiency and dry matter accumulation. Sulphur also supports the formation of essential oils and glucosinolates necessary for mustard crop quality. Cytokinin is a plant hormone that promotes cell division and differentiation, particularly in the meristematic regions of the plant. By enhancing cell proliferation, cytokinin contributes to the growth of new tissues, leading to increased dry matter accumulation. It also plays a role in delaying leaf senescence, thereby extending the photosynthetically active period of the plant. The interaction between nutrients and plant hormones like cytokinin is crucial for coordinating growth and dry matter accumulation. Cytokinins influence the distribution of nutrients and assimilate within the plant, directing them towards areas of active growth, such as young leaves and developing seeds. This hormonal regulation ensures that the plant maximises its biomass production. The observed increase in dry matter accumulation under different nutrient treatments can be attributed to the enhanced photosynthetic activity and efficient nutrient utilization facilitated by the combined application of Boron, Sulphur, and Cytokinin. A synergistic effect was observed in treatments where these nutrients were applied in combination, leading to a significant increase in dry matter content compared to control treatments. For example, the combination of Sulphur and cytokines (as observed in treatment S9) enhanced chlorophyll synthesis and photosynthetic efficiency and promoted cell division and the development of new tissues. This resulted in a higher accumulation of biomass, which was reflected in the increased dry matter content. Similarly, the combination of boron and cytokinin (as observed in treatment S6) improved cell wall formation and the transport of photosynthates, further contributing to the accumulation of dry matter. The

increased leaf area observed in these treatments also played a crucial role, as it provided a larger surface area for photosynthesis, leading to greater carbon assimilation and storage. This, in turn, translated into higher dry matter accumulation in the mustard crops, justifying the observed differences across the treatments. The enhanced dry matter accumulation in mustard crops under the influence of Boron, Sulphur, and Cytokinin results from improved photosynthetic activity, nutrient utilization, and hormonal regulation, contributing to increased biomass production and overall plant growth.

Table 4.6 (a): Effect of spacing and nutrient on DMA (g) of the mustard crop during the rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	7.21	7.92	28.45	29.40	120.18	122.60
M2 (20×10)	8.08	9.31	28.88	30.05	152.95	155.41
C.D. at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.40	0.33	0.82	0.77	11.38	10.98
Nutrients foliar application						
S0-Control	5.71	6.82	16.22	17.58	102.22	103.63
S1-Boron @1%	7.28	8.37	23.18	22.59	168.64	170.73
S2-Sulphur @ 0.15%	7.40	8.44	30.72	31.82	115.81	117.88
S3-BAP @0.003%	8.60	9.48	31.14	32.24	127.29	129.72
S4-Boron @0.5% +Sulphur @0.25%	8.40	9.57	34.30	35.92	127.85	130.38
S5-Boron @ 1.5%+ Sulphur @0.075%	7.20	8.16	30.40	31.70	119.53	122.56
S6-Boron @ 0.5% + BAP (@0.0045%)	8.42	9.12	30.35	31.52	140.35	143.08
S7-Boron @ 1.5%+ BAP (@0.0015%)	9.38	10.34	29.12	30.40	153.78	156.74
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	7.40	7.93	31.52	32.64	146.30	148.87
S9-Sulphur @0.25%+ BAP (@0.0015%)	6.67	7.95	29.65	30.82	163.91	166.48
C.D. at p<0.05	NS	NS	5.66	5.51	8.85	8.89
SEM±	0.74	0.73	1.96	1.91	16.93	16.93
C.D. S×M at p<0.05	NS	NS	8.90	8.63	8.87	8.89
SEM±	1.26	1.05	2.59	2.46	13.02	13.13
C.D. M×S at p<0.05	NS	NS	8.85	8.56	8.82	8.74
SEM±	1.08	1.03	2.76	2.78	23.09	23.09

Where, C.D. represents critical difference, SE (m) represents standard error of mean and SE (d) represents the standard error of deviation.

Table 4.6 (b): Interaction effect of spacing and nutrient on DMA of mustard crop during rabi season at 60DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	17.173	13.82	25.527	32.667	37.503	30.57	29.103	28.38	35.5	31.54	28.178
M2	15.94	29.207	35.91	29.617	31.097	30.23	31.593	29.853	27.547	27.763	28.876
Mean B	16.557	21.513	30.718	31.142	34.3	30.4	30.348	29.117	31.523	29.652	
C.D. S×M at p<0.05						8.90					
SEM±						2.59					
C.D. M×S at p<0.05						8.85					
SEM±						2.76					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	18.32	14.957	26.953	33.783	38.97	32.177	30.243	29.643	36.373	32.593	29.401
M2	16.853	30.237	36.687	30.7	32.877	31.237	32.803	31.16	28.917	29.06	30.053
Mean B	17.587	22.597	31.82	32.242	35.923	31.707	31.523	30.402	32.645	30.827	
C.D. S×M at p<0.05						8.63					
SEM±						2.46					
C.D. M×S at p<0.05						8.56					
SEM±						2.78					

Table 4.6 (c): Interaction effect of spacing and nutrient on DMA of mustard crop during rabi season at 90DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	82.543	147.47	109.377	105.62	127.397	106.51	118.86	137.803	124.983	141.263	120.183
M2	121.9	189.8	115.58	148.95	128.3	132.54	161.833	169.753	167.613	186.553	152.282
Mean B	102.222	168.635	112.478	127.285	127.848	119.525	140.347	153.778	146.298	163.908	
C.D. S×M at p<0.05						8.87					
SEM±						13.02					
C.D. M×S at p<0.05						8.82					
SEM±						23.09					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	84.09	148.97	111.403	108.793	129.867	109.223	121.4	141.157	127.717	143.36	122.598
M2	123.173	192.48	117.68	150.637	130.887	135.9	164.753	172.33	170.013	189.6	154.745
Mean B	103.632	170.725	114.542	129.715	130.377	122.562	143.077	156.743	148.865	166.48	
C.D. S×M at p<0.05						8.89					
SEM±						13.13					
C.D. M×S at p<0.05						8.74					
SEM±						23.09					

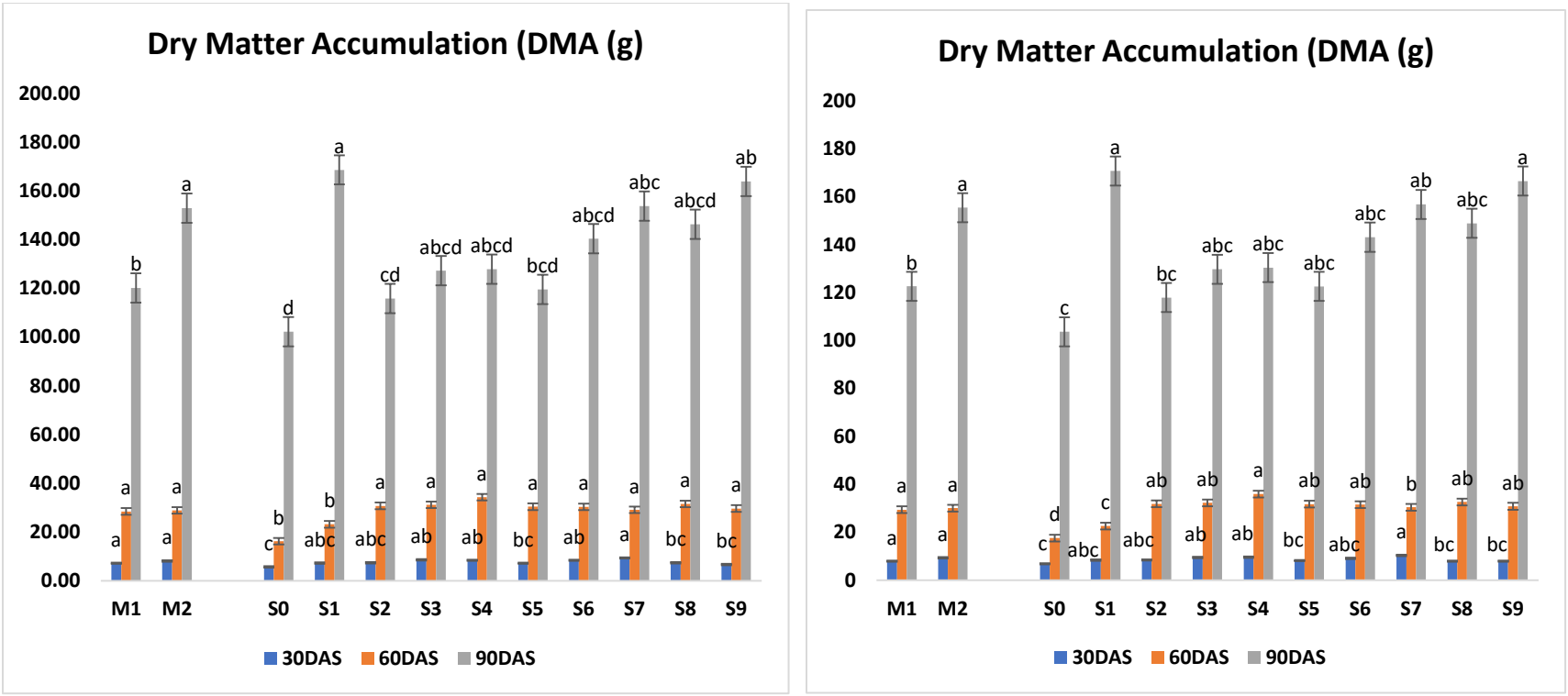


Fig-4.6 (a): Effect of spacing and nutrient on DMA of mustard crop during rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.2.3 Crop Growth Rate (CGR) (g m^{-2} day^{-1}):

The effect of micronutrients and secondary nutrients (Boron and Sulphur) and their combination with plant growth hormone (Cytokinin) on crop growth rate (RGR) was studied in Mustard crop under spatial dynamics with variety NB-RIMUL-2019 (Nandi Bull) during the years 2021-22 and 2022-23. Variations in CGR were observed at 30DAS, 60DAS and 90DAS, as shown in Table 4.7, Fig 4.7. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the CGR in each treatment as compared to control of both the spacings at 30, 60 and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the CGR was observed at 30, 60 and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30-60DAS, main plot M1 shows maximum CGR as compared to M2 with values 0.061 (M1) and 0.060 (M2), respectively. A percentage increase of 1.63% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant rise in CGR was observed in S2, i.e. 0.065 at 30-60DAS. Therefore, at 30-60 DAS, the percentage increase as compared to S0 was found to be highest in S2, followed by S4> S1> S3> S9> S8>S5> S6, and the per cent values were 17.76%, 17.62%, 16.42%, 14.51%, 12.19%, 13.6%, 9.24% and 7.86% respectively. At 60-90DAS, the main plot M2 shows maximum CGR compared to M1, with values of 0.68 (M2) and 0.64 (M1), respectively. A percentage increase of 5.88% was found in M2, where the crop was grown under reduced spacing (20*10). Therefore, at 60-90DAS, a significant increase was found in S1 with a value of 46.27, where Boron @ 1% was applied to the crop as a foliar application. The per cent increase was found highest in S1, followed by S7> S9> S6> S8> S3> S4> S2> S5 and the per cent values were 46.27%, 45.49%, 45.32%, 39.38%, 31.28%, 30.85%, 30.75%, 28.98% and 27.53% respectively when it is compared with its control (S0).

The study shows a significant increase, with 7.86% and 39.38% per cent values at 30-60DAS and 60-90DAS, when comparing S6 and S0 (control). In treatment S6, the mustard crop was treated with a foliar application of Boron @ 0.5% + BAP (@0.0045%).

The lowest increase was found in treatment S2, i.e. 28.98% at 60-90DAS when it is compared to its control (S0), where the application of sulphur at its rec. Dose, i.e. @ 0.15%, is provided alone.

In the year (2022-23), at 30-60DAS, main plot M1 shows maximum CGR as compared to M2 with values 0.060 (M1) and 0.067 (M2), respectively. A percentage increase of 10.04% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant rise in CGR was observed in S2 and S4, i.e. 0.067 at 30-60DAS. Therefore, at 30-60, DAS the percentage increase as compared to S0 was found to be highest in S2&S4 followed by S1> S6> S3> S7&S8> S9> S5 and the per cent values were 17.03%, 17.03%, 15.78%, 14.50%, 13.84%, 13.17%, 13.17%, 11.81% and 9.67% respectively. At 60-90DAS, the main plot M2 shows maximum CGR compared to M1, with values of 0.68 (M2) and 0.64 (M1), respectively. A percentage increase of 5.88% was found in M2, where the crop was grown under reduced spacing (20*10). Therefore, at 60-90DAS, a significant increase was seen in S1 with a value of 47.01, where Boron @ 1% was applied to the crop as a foliar application. The per cent increase was found highest in S1, followed by S7> S9> S6> S8> S3> S4> S2> S5, and the per cent values were 47.01%, 46.28%, 46.11%, 40.23%, 32.28%, 31.90%, 31.74%, 30.02% and 28.63% respectively when it is compared with its control (S0).

The study shows a significant increase, with 14.50% and 40.23% per cent values at 30-60DAS and 60-90DAS when comparing S6 and S0 (control). In treatment S6, the mustard crop was treated with a foliar application of Boron @ 0.5% + BAP (@0.0045%). The lowest increase was found in treatment S2, i.e. 30.02% at 60-90DAS when compared to its control (S0), where the application of sulphur at its rec. Dose, i.e. @ 0.15%, is provided alone. The crop growth rate was more adversely affected by 50% shading at 71–90 DAS, as Shekhawat et al. (2012) reported.

Table 4.7 (a): Effect of spacing and nutrient on CGR (g m^{-2} day^{-1}) of the mustard crop during the rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23
	30-60DAS		60-90DAS	
Spacing				
M1 (30×10)	0.062	0.068	0.645	0.647
M2 (20×10)	0.06	0.061	0.687	0.689
C.D. at p<0.05	NS	NS	0.206	0.004
SEM±	0.004	0.011	0.052	0.001
Nutrients foliar application				
S0-Control	0.054	0.057	0.435	0.437
S1-Boron @1%	0.065	0.067	0.81	0.812
S2-Sulphur @ 0.15%	0.066	0.068	0.613	0.615
S3-BAP @0.003%	0.063	0.065	0.629	0.632
S4-Boron @0.5% +Sulphur @0.25%	0.066	0.068	0.628	0.63
S5-Boron @ 1.5%+ Sulphur @0.075%	0.060	0.062	0.600	0.603
S6-Boron @ 0.5% + BAP (@0.0045%)	0.059	0.066	0.718	0.72
S7-Boron @ 1.5%+ BAP (@0.0015%)	0.055	0.065	0.798	0.801
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	0.063	0.065	0.633	0.635
S9-Sulphur @0.25%+ BAP (@0.0015%)	0.062	0.064	0.796	0.798
C.D. at p<0.05	NS	NS	0.255	0.004
SEM±	0.009	0.008	0.088	0.001
C.D. S×M at p<0.05	NS	0.04	0.443	0.008
SEM±	0.011	0.013	0.153	0.002
C.D. M×S at p<0.05	NS	0.04	0.451	0.008
SEM±	0.013	0.012	0.149	0.002

Where, C.D. represents critical difference, SE (m) represents standard error of mean.

Table 4.7 (b): Interaction effect of spacing and nutrient on CGR of mustard crop during rabi season at 30-60 DAS

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	0.077	0.067	0.08	0.087	0.107	0.087	0.05	0.05	0.093	0.083	0.078
M2	0.08	0.103	0.08	0.07	0.07	0.083	0.1	0.063	0.087	0.073	0.081
Mean B	0.078	0.085	0.08	0.078	0.088	0.085	0.075	0.057	0.09	0.078	
C.D. S×M at p<0.05						0.04					
SEM±						0.013					
C.D. M×S at p<0.05						0.04					
SEM±						0.012					

Table 4.7 (c): Interaction effect of spacing and nutrient on CGR of mustard crop during rabi season at 60-90 DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	0.293	0.737	0.517	0.597	0.777	0.617	0.687	0.813	0.62	0.79	0.645
M2	0.58	0.883	0.707	0.66	0.48	0.587	0.747	0.78	0.647	0.8	0.687
Mean B	0.437	0.81	0.612	0.628	0.628	0.602	0.717	0.797	0.633	0.795	
C.D. S×M at p<0.05						0.443					
SEM±						0.153					
C.D. M×S at p<0.05						0.451					
SEM±						0.149					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	0.313	0.497	0.54	0.62	0.797	0.383	0.707	0.843	0.667	0.817	0.618
M2	0.61	0.937	0.73	0.45	0.527	0.657	0.793	0.857	0.7	0.65	0.691
Mean B	0.462	0.717	0.635	0.535	0.662	0.52	0.75	0.85	0.683	0.733	
C.D. S×M at p<0.05						0.008					
SEM±						0.002					
C.D. M×S at p<0.05						0.008					
SEM±						0.002					

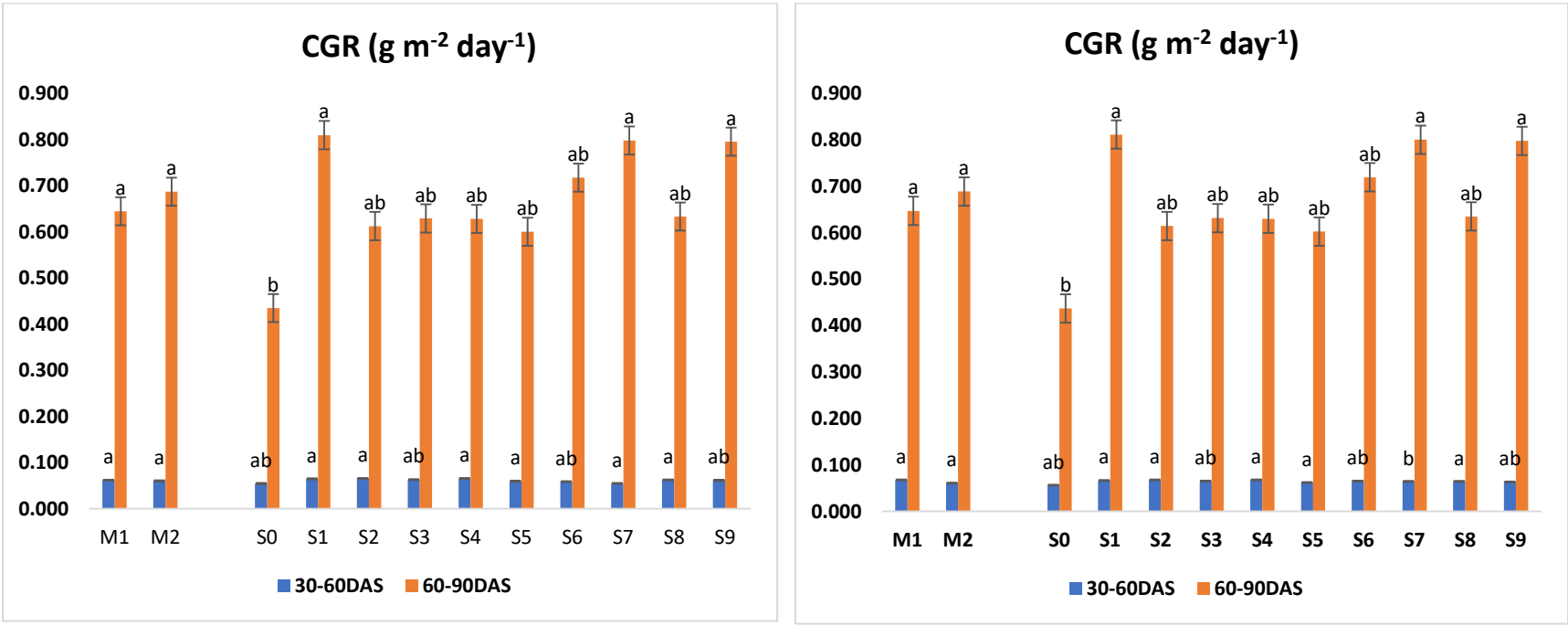


Fig-4.7 (a): Effect of spacing and nutrient on CGR of the mustard crop during rabi season of 2021-22 and 2022-23

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.2.4 Relative Growth Rate (RGR) (g g^{-1} day^{-1}):

The effect of micronutrients and secondary nutrients (Boron and Sulphur) and their combination with plant growth hormone (Cytokinin) on relative growth rate (RGR) was studied in Mustard crop under spatial dynamics with a variety of NB-RIMUL-2019 (Nandi Bull) during the years 2021-22 and 2022-23. Variations in RGR were observed at 30DAS, 60DAS and 90DAS, as shown in Table 4.8 and Fig 4.8. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the RGR in each treatment as compared to control of both the spacings at 30, 60 and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the RGR was observed at 30, 60 and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30-60DAS, main plot M1 shows maximum RGR as compared to M2 with values 0.027 (M1) and 0.025 (M2), respectively. A percentage increase of 7.40% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase in RGR was observed in S2, i.e. 0.028 at 30-60DAS. Therefore, at 30-60 DAS, the percentage increase as compared to S0 was found to be highest in S2 followed by S4, S3, S1> S9> S8>S6> S5, and the per cent values were 18.54%, 18.40%, 17.44%, 17.21%, 15.26%, 14.41%, 11.20 and 10.10% respectively. At 60-90DAS, the main plot M2 shows maximum RGR compared to M1, with values of 0.29 (M2) and 0.27 (M1), respectively. A percentage increase of 6.89% was found in M2, where the crop was grown under reduced spacing (20*10). Therefore, at 60-90DAS, a significant increase was found in S1 with a value of 0.34, where Boron @ 1% was applied to the crop as a foliar application. The per cent increase was found highest in S1, followed by S7> S9> S6> S8> S3> S4> S2> S5, and the per cent values were 48.29%, 47.55%, 47.38%, 41.66%, 33.87%, 33.46%, 33.36%, 31.66% and 30.26% respectively when it is compared with its control (S0).

The study shows a significant increase, with 11.20% and 41.66% per cent values at 30-60DAS and 60-90DAS when comparing S6 and S0 (control). In treatment S6, the mustard crop was treated with a foliar application of Boron @ 0.5% + BAP (@0.0045%).

The lowest increase was found in treatment S2, i.e. 31.66% at 60-90DAS when compared to its control (S0), where the application of sulphur at its rec. Dose, i.e. @ 0.15%, is provided alone.

In the year (2022-23), at 30-60DAS, main plot M1 shows maximum RGR as compared to M2 with values 0.028 (M1) and 0.027 (M2), respectively. A percentage increase of 3.57% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase in RGR was observed in S1, S2, S3 and S4, i.e. 33.33 at 30-60DAS. Therefore, at 30-60 DAS, the percentage increase as compared to S0 was found to be highest in S1, S2, S3 and S4, followed by S9> S5&S6> S7&S8, and the per cent values were 33.33%, 33.33%, 33.33%, 33.33%, 32.20%, 28.57%, 28.57%, 21.56% and 21.56% respectively. At 60-90DAS, the main plot M2 shows maximum RGR compared to M1, with values of 0.29 (M2) and 0.27 (M1), respectively. A percentage increase of 6.89% was found in M2, where the crop was grown under reduced spacing (20*10). Therefore, at 60-90DAS, a significant increase was seen in S1 with a value of 0.35, where Boron @ 1% was applied to the crop as a foliar application. The per cent increase was found highest in S1, followed by S7> S9> S6> S8> S3> S4> S2> S5, and the per cent values were 46%, 45.21%, 45.13%, 39.13%, 31.14%, 30.64%, 30.51%, 28.81% and 26.45% respectively when it is compared with its control (S0). The study showed a significant increase with 28.57% and 39.13% per cent values at 30-60DAS and 60-90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. The lowest increase was found in treatment S2, i.e. 28.81% at 60-90DAS when compared to its control (S0), where the application of sulphur at its rec. Dose, i.e. @ 0.15%, is provided alone. Relative growth rates were more adversely affected by 50% shading at 71–90 DAS, as reported by Shekhawat, K.et al. (2012).

Table-4.8 (a): Effect of spacing and nutrient on RGR (g g^{-1} day^{-1}) of mustard crop during rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23
	30-60DAS		60-90DAS	
Spacing				
M1 (30×10)	0.027	0.029	0.277	0.279
M2 (20×10)	0.026	0.028	0.295	0.298
C.D. at p<0.05	NS	NS	NS	NS
SEM±	0.002	0.015	0.015	0.015
Nutrients foliar application				
S0-Control	0.023	0.026	0.187	0.189
S1-Boron @1%	0.028	0.030	0.348	0.350
S2-Sulphur @ 0.15%	0.028	0.030	0.263	0.266
S3-BAP @0.003%	0.028	0.030	0.271	0.273
S4-Boron @0.5% +Sulphur @0.25%	0.028	0.030	0.270	0.272
S5-Boron @ 1.5%+ Sulphur @0.075%	0.026	0.028	0.258	0.261
S6-Boron @ 0.5% + BAP (@0.0045%)	0.026	0.028	0.309	0.311
S7-Boron @ 1.5%+ BAP (@0.0015%)	0.023	0.026	0.343	0.345
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	0.027	0.026	0.272	0.275
S9-Sulphur @0.25%+ BAP (@0.0015%)	0.027	0.030	0.342	0.345
C.D. at p<0.05	NS	NS	NS	NS
SEM±	0.004	0.049	0.049	0.049
C.D. S×M at p<0.05	NS	NS	NS	NS
SEM±	0.007	0.047	0.047	0.048
C.D. M×S at p<0.05	NS	NS	NS	NS
SEM±	0.006	0.067	0.067	0.067

Where, C.D. represents critical difference, SE (m) represents standard error of mean and SE (d) represents the standard error of deviation.

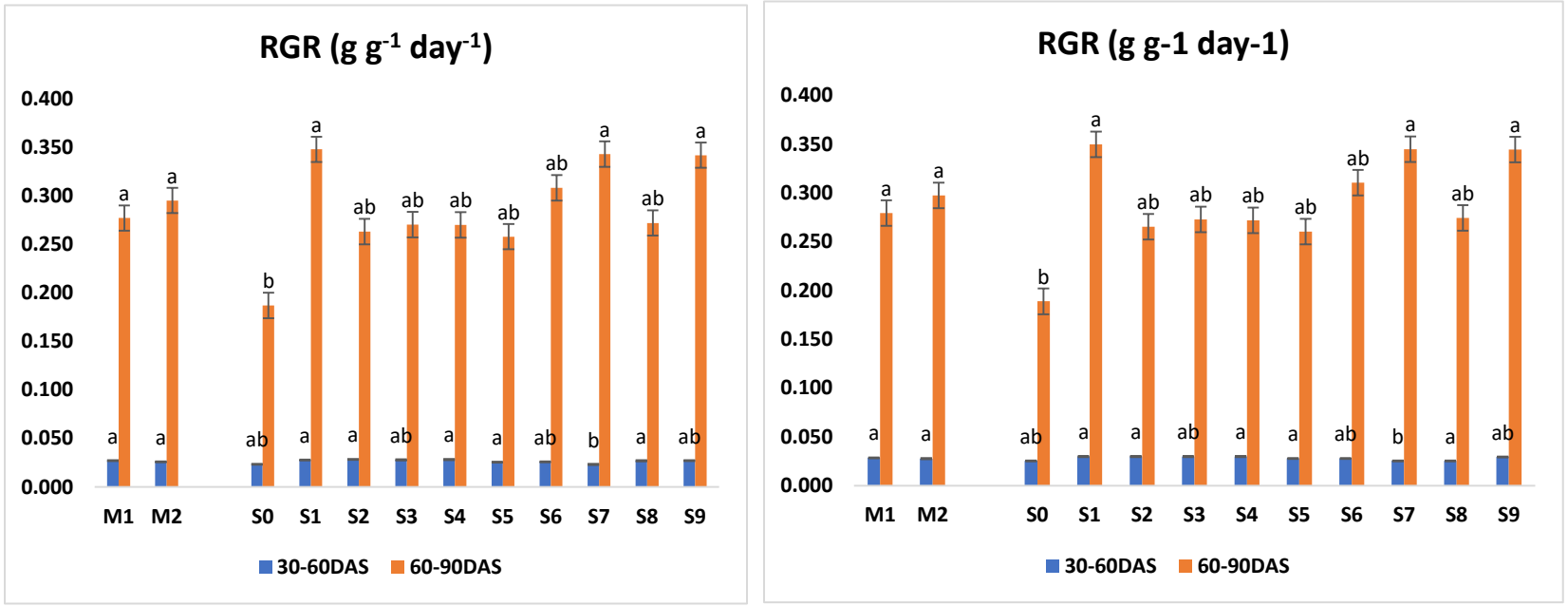


Fig-4.8 (a): Effect of spacing and nutrient on RGR of the mustard crop during rabi season of 2021-22 and 2022-23

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.2.5 Net Assimilation Rate (NAR) (g m^{-2} day^{-1}):

The effect of micronutrients and secondary nutrients (Boron and Sulphur) and their combination with plant growth hormone (Cytokinin) on net assimilation rate was studied in Mustard crop under spatial dynamics with variety NB-RIMUL-2019 (Nandi Bull) during the years 2021-22 and 2022-23. Variations in NAR were observed at 30DAS, 60DAS and 90DAS, as shown in Table 4.9, Fig 4.9. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the NAR in each treatment compared to control of both the spacings at 30, 60 and 90. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the NAR was observed at 30, 60 and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30-60DAS, main plot M1 shows maximum NAR as compared to M2 with values 0.027 (M1) and 0.025 (M2), respectively. A percentage increase of 7.40% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase in NAR was observed in S2, i.e. 0.028 at 30DAS. Therefore, at 30-60 DAS, the percentage increase as compared to S0 was found to be highest in S2, followed by S4, S3, S1> S9> S8>S6> S5, and the per cent values were 18.54%, 18.40%, 17.44%, 17.21%, 15.26%, 14.41%, 11.20 and 10.10% respectively. At 60-90DAS, the main plot M2 shows maximum NAR compared to M1, with values of 0.29 (M2) and 0.27 (M1), respectively. A percentage increase of 6.89% was found in M2, where the crop was grown under reduced spacing (20*10). Therefore, at 60-90DAS, a significant increase was found in S1 with a value of 0.34, where Boron @ 1% was applied to the crop as a foliar application. The per cent increase was found highest in S1, followed by S7> S9> S6> S8> S3> S4> S2> S5, and the per cent values were 48.29%, 47.55%, 47.38%, 41.66%, 33.87%, 33.46%, 33.36%, 31.66% and 30.26% respectively when it is compared with its control (S0).

The study shows a significant increase, with 11.20% and 41.66% per cent values at 30-60DAS and 60-90DAS when comparing S6 and S0 (control). In treatment S6, the mustard crop was treated with a foliar application of Boron @ 0.5% + BAP (@0.0045%). The lowest increase was found in treatment S2, i.e. 31.66% at 60-90DAS when compared to its control (S0), where the application of sulphur at its rec. Dose, i.e. @ 0.15%, is provided alone. Net assimilation rates were more adversely affected by 50% shading at 71–90 DAS, as reported by Shekhawat, K.et al. (2012).

In the year (2022-23), at 30-60DAS, main plot M1 shows maximum NAR as compared to M2 with values 0.029 (M1) and 0.027 (M2), respectively. A percentage increase of 6.89% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase in NAR was observed in S1, S3 and S4, i.e. 33.33 at 30-60DAS. Therefore, at 30-60 DAS, the percentage increase as compared to S0 was found to be highest in S1, S3 and S4, followed by S2&S9> S8> S5&S6, and the per cent values were 33.33%, 33.33%, 33.33%, 32.20%, 32.20%, 31.03%, 28.57%, 28.57% respectively. At 60-90DAS, the main plot M2 shows maximum NAR compared to M1, with values of 0.29 (M2) and 0.27 (M1), respectively. A percentage increase of 6.89% was found in M2, where the crop was grown under reduced spacing (20*10). Therefore, at 60-90DAS, a significant increase was seen in S1 with a value of 0.35, where Boron @ 1% was applied to the crop as a foliar application. The per cent increase was found highest in S1, followed by S7> S9> S6> S8> S3> S4> S2> S5, and the per cent values were 46%, 45.21%, 45.13%, 39.13%, 31.14%, 30.64%, 30.51%, 28.81% and 26.45% respectively when it is compared with its control (S0). The study showed a significant increase with 28.57% and 39.13% per cent values at 30-60DAS and 60-90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop.

The lowest increase was found in treatment S2, i.e. 28.81% at 60-90DAS when compared to its control (S0), where the application of sulphur at its rec. Dose, i.e. @ 0.15%, is provided alone. The Net Assimilation Rate (NAR) is an essential physiological parameter that indicates a plant's effectiveness in transforming absorbed light energy into plant biomass. It is directly affected by cellular physiological processes, including photosynthesis, nutrient absorption, and the equilibrium between carbon sequestration and respiratory excretion. A comprehensive understanding of the cellular processes involved in NAR offers valuable insights into the impact of nutrient treatments on the growth and productivity of mustard crops. Photosynthesis is the primary cellular physiological process that propels NAR. This process entails converting light energy into chemical energy, namely ATP and NADPH, which are subsequently utilized to fix carbon dioxide (CO_2) into organic compounds in the Calvin cycle. Compounds such as Sulphur, Boron, and Cytokinin augment the efficiency of photosynthesis. Sulphur is indispensable for producing chlorophyll and forming crucial amino acids and enzymes that enable electron transport and ATP synthesis. The rate of photosynthesis is directly influenced by chlorophyll, the principal pigment responsible for light absorption. Cytokinin, a phytohormone, stimulates cellular division and enlargement, inducing an increase in chloroplasts per cell and augmenting the total photosynthesis capacity. Nutrient assimilation is a crucial determinant of NAR. Sulphur is essential for synthesizing amino acids such as cysteine and methionine, which are fundamental

components for proteins and enzymes in carbon metabolism. Boron is vital for cell wall development, maintaining membrane integrity, and facilitating the transportation of sugars and other assimilates from the source (leaves) to the sink (growing tissues). This nutrient promotes the effective movement of photosynthates, ensuring that a more significant amount of biomass is directed towards biological growth rather than being wasted through respiration. The equilibrium between photosynthetic carbon gain and respiratory carbon loss determines the nutritional availability ratio (NAR). By enhancing cellular integrity and function, the foliar application of nutrients such as Boron and Sulphur can decrease respiratory losses. Sulphur plays a crucial role in the synthesis of glutathione. This vital antioxidant helps to alleviate cell oxidative stress and diminish the requirement for respiratory energy expenditure to restore cellular damage. This increases net carbon gain, raising the net annual rate (NAR). Cytokinin stimulates cellular division and prolongs leaf senescence by preserving chlorophyll levels and upregulating photosynthetic productivity. This hormone regulates the expression of genes and enzymes associated with photosynthesis, so maintaining a high level of photosynthetic efficiency throughout plant maturation. Through the postponement of senescence, Cytokinin guarantees an extended duration of active photosynthesis, contributing to a consistent net aquaporin (NAR) throughout the growing season. The increased cellular activity can explain the substantial rise in nitrogen attachment ratio (NAR) in the mustard crop when subjected to various nutrient treatments. The concurrent use of Sulphur and cytokines (as seen in treatment S9) optimizes the chlorophyll concentration and photosynthetic efficiency, resulting in increased carbon fixation rates. This augmentation in photosynthesis directly leads to an elevated net acidification rate (NAR). The function of boron in preserving the integrity of the cell wall and promoting the transportation of sugar results in the efficient movement of photosynthates from leaves to developing tissues, thus reducing losses and promoting growth. Adequate transportation and assimilating use contribute to a greater net acidification rate (NAR).

The mitigation of oxidative stress by Sulphur and the improvement of cellular integrity by Boron result in the minimization of respiratory losses, hence promoting a more significant net gain of assimilates. An essential determinant in the rise of NAR is the decrease in respiration compared to photosynthesis. The capacity of cytokinin to postpone leaf senescence guarantees the prolonged activity of the photosynthetic apparatus, so net ammonia requirement (NAR) is maintained throughout the crop's growth cycle. The extended duration of photosynthesis enables ongoing accumulation of biomass, so contributing to an increased net adsorption rate (NAR). The increased nitrogen accumulation rate (NAR) in mustard crops treated with Sulphur, Boron, and Cytokinin is due to their enhanced photosynthetic efficiency, nutrient assimilation, decreased

respiratory losses, and extended photosynthetic activity. These biological processes provide a rationale for the observed enhancements in NAR, resulting in improved growth performance and increased yields in the treated crops.

Table 4.9 (a): Effect of spacing and nutrient on Net Assimilation Rate (g $m^{-2}day^{-1}$) of the mustard crop during the rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23
	30-60DAS		60-90DAS	
Spacing				
M1 (30×10)	0.027	0.029	0.277	0.279
M2 (20×10)	0.026	0.028	0.295	0.298
C.D. at p<0.05	NS	NS	NS	NS
SEM±	0.00	0.00	0.02	0.04
Nutrients foliar application				
S0-Control	0.023	0.026	0.187	0.189
S1-Boron @1%	0.028	0.030	0.348	0.350
S2-Sulphur @ 0.15%	0.028	0.030	0.263	0.266
S3-BAP @0.003%	0.028	0.030	0.271	0.273
S4-Boron @0.5% +Sulphur @0.25%	0.028	0.030	0.270	0.272
S5-Boron @ 1.5%+ Sulphur @0.075%	0.026	0.028	0.258	0.257
S6-Boron @ 0.5% + BAP (@0.0045%)	0.026	0.028	0.309	0.311
S7-Boron @ 1.5%+ BAP (@0.0015%)	0.023	0.026	0.343	0.345
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	0.027	0.029	0.272	0.275
S9-Sulphur @0.25%+ BAP (@0.0015%)	0.027	0.030	0.342	0.345
C.D. at p<0.05	NS	NS	NS	NS
SEM±	0.004	0.005	0.04	0.04
C.D. S×M at p<0.05	NS	NS	NS	NS
SEM±	0.005	0.001	0.046	0.049
C.D. M×S at p<0.05	NS	NS	NS	NS
SEM±	0.006	0.007	0.068	0.068

Where, C.D. represents critical difference, SE (m) represents standard error of mean

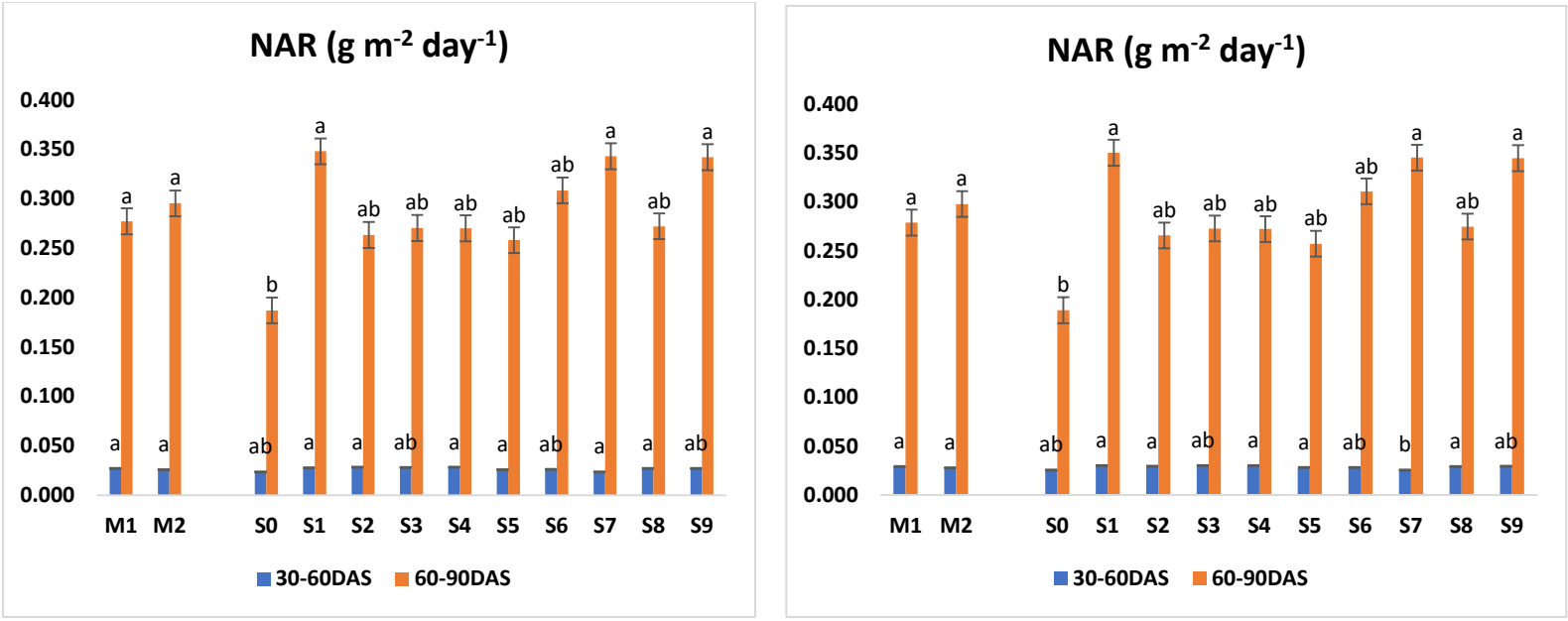


Fig-4.9 (a): Effect of spacing and nutrient on Net Assimilation Rate of mustard crop during rabi season of 2021-22 and 2022-23

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%) .

4.3 Biochemical observations

4.3.1 Chlorophyll 'a' Content (mg g^{-1} Fresh Weight)

Mustard crop shows different variations when grown under different nutrient levels. In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in chlorophyll 'a' were observed at 30DAS, 60DAS and 90DAS, as shown in Table 4.9, Fig 4.9. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the chlorophyll 'a' in each treatment compared to control of both the spacings at 30, 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the chlorophyll 'a' was observed at 30, 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, main plot M1 shows maximum chlorophyll 'a' as compared to M2 with values 1.13 (M1) and 1.08 (M2), respectively. A percentage increase of 4.42% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase in chlorophyll 'a' was observed in S8, i.e. 1.24 at 30DAS. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S8, followed by S2> S7> S9> S1> S6> S4> S3, and the per cent values were 35.82%, 34.06%, 33.74%, 32.10%, 31.69%, 30.00%, 27.36% and 23.86% respectively. At 60DAS, the main plot M2 shows maximum chlorophyll 'a' compared to M1, with values 1.45 (M2) and 1.44 (M1), respectively. A percentage increase of 0.68% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S6 with a value of 1.47, where Boron @ 0.5% + BAP (@0.0045% was applied to the crop as a foliar application. The per cent increase was found highest in S6 followed by S4> S1> S5> S3> S2> S9> S7&S8, and the

per cent values were 3.40%, 3.29%, 2.51%, 2.29%, 1.95%, 1.63%, 1.50%, 1.38% and 1.38% respectively when it is compared with its control (S0). At 90DAS, the main plot M1 shows maximum chlorophyll ‘a’ compared to M2, with values of 2.39 (M1) and 2.12 (M2), respectively. A percentage increase of 11.29% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S8 with a value of 2.49, where Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S8, followed by S3> S5> S1> S4> S2> S6> S7, and the per cent values were 17.77%, 15.73%, 11.63%, 8.61%, 8.53%, 8.49%, 7.34% and 6.81% respectively when it is compared with its control (S0).

The study showed a significant increase with 35.82%, 1.38% and 17.77% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S8 and S0 (control). In treatment S8, the foliar application of Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the chlorophyll ‘a’ content was found in treatment S8, where the combined application of sulphur and cytokinin is applied to the crop compared to its control (S0).

In the year (2022-23), at 30DAS, main plot M1 shows maximum chlorophyll ‘a’ as compared to M2 with values 1.15 (M1) and 1.11 (M2), respectively. A percentage increase of 3.47% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase in chlorophyll ‘a’ was observed in S8, i.e. 1.26 at 30DAS. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S8, followed by S2> S7> S9> S1> S6> S4> S3 and the per cent values were 30.61%, 28.84%, 28.45%, 26.66%, 26.15%, 24.78%, 21.66% and 17.62% respectively. At 60DAS, the main plot M2 shows maximum chlorophyll ‘a’ compared to M1, with values 1.46 (M2) and 1.45 (M1), respectively. A percentage increase of 0.68% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S6 with a value of 1.48, where Boron @ 0.5% + BAP

(@0.0045% was applied to the crop as a foliar application. The per cent increase was found highest in S6, S4 followed by S1> S5> S3> S2> S9> S7&S8 and the per cent values were 3.37%, 3.37%, 2.5%, 2.61%, 2.27%, 1.60%, 1.49%, 1.37, 1.37% respectively when it is compared with its control (S0). At 90DAS, the main plot M1 shows maximum chlorophyll ‘a’ compared to M2, with values of 2.23 (M1) and 2.04 (M2), respectively. A percentage increase of 12.44% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S5 with a value of 2.33, where Boron @ 1.5%+ Sulphur @0.075% was applied to the crop as a foliar spray. The per cent increase was found highest in S5, followed by S1> S2> S6> S7> S9> S8> S4 and the per cent values were 11.65%, 8.71%, 8.44%, 7.23%, 6.85%, 4.40%, 4.33% AND 2.83% respectively when it is compared with its control (S0).

The study showed a significant increase with values of 30.61%, 1.37%, and 4.33% per cent at 30DAS, 60DAS, and 90DAS when a comparison was made between S8 and S0 (control). In treatment S8, the foliar application of Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the chlorophyll ‘a’ content was found in treatment S8, where the combined application of sulphur and cytokinin is applied to the crop compared to its control (S0). Chlorophyll 'a' is a crucial pigment in the photosynthetic machinery of plants, responsible for the absorption of light and the subsequent conversion of light energy into chemical energy. It plays a central role in the light-dependent reactions of photosynthesis, primarily occurring in the thylakoid membranes of chloroplasts. Chlorophyll 'a' mainly absorbs light in the electromagnetic spectrum's blue-violet (around 430 nm) and red (around 662 nm) regions. This absorption is crucial for capturing solar energy, which drives the photosynthetic process. The chlorophyll 'a structure includes a porphyrin ring with a central magnesium ion responsible for its light-absorbing properties. Once chlorophyll 'a' absorbs light energy, the energy is transferred to the reaction centre of photosystems I and II. In Photosystem II, chlorophyll 'a' (P680) absorbs light and becomes excited, leading to the oxidation of water

molecules and the release of oxygen. In Photosystem I, chlorophyll 'a' (P700) also absorbs light, reducing NADP+ to NADPH. The excited electrons from chlorophyll 'a' are transferred through a series of electron carriers in the thylakoid membrane, collectively known as the electron transport chain (ETC). This transfer of electrons is coupled with the pumping of protons across the thylakoid membrane, creating a proton gradient that drives ATP synthesis via ATP synthase. The energy captured by chlorophyll 'a' and converted through the electron transport chain is ultimately used to produce ATP and NADPH, the energy-rich molecules required for the Calvin cycle (light-independent reactions), where carbon dioxide is fixed into organic molecules. In Photosystem II, chlorophyll 'a' is integral in splitting water molecules (photolysis), releasing oxygen and providing electrons for the ETC. In Photosystem I, chlorophyll 'a' reduces NADP+ to NADPH, crucial for carbon assimilation in the Calvin cycle. Chlorophyll 'a' is the primary pigment involved in the light-dependent reactions of photosynthesis, making it indispensable for producing ATP and NADPH, which are necessary for carbon fixation in the Calvin cycle. In mustard crops, as in all photosynthetic organisms, the efficiency of photosynthesis directly influences growth, biomass accumulation, and yield. The concentration of chlorophyll 'a' in mustard leaves directly affects the plant's ability to capture light energy. Higher chlorophyll 'a' content leads to more efficient light absorption, enhancing the plant's photosynthetic capacity. This increases energy availability for growth processes, contributing to larger leaf areas, increased biomass, and potentially higher seed yields. Chlorophyll 'a' levels are often used as an indicator of the nutritional status of plants, particularly nitrogen availability, as nitrogen is a critical component of the chlorophyll molecule. In mustard crops, adequate chlorophyll 'a' levels suggest sufficient nitrogen supply, essential for optimal photosynthetic activity and overall plant health. Environmental factors, including light intensity, temperature, and nutrient availability, can influence chlorophyll 'a' content. Maintaining optimal chlorophyll 'a' levels in mustard crops ensures that the plants can adapt to varying environmental conditions, maintaining robust growth and productivity. Monitoring chlorophyll 'a' levels can provide valuable insights for agronomic

management, such as optimizing fertilization strategies to ensure sufficient nitrogen availability. This is particularly important in mustard crops, where maximizing chlorophyll 'a' content can improve photosynthetic efficiency and, consequently, higher yields. Chlorophyll 'a' is vital for mustard crops' photosynthetic efficiency. Its role in light absorption, energy transfer, and electron transport underscores its importance in the plant's overall energy economy. Adequate chlorophyll 'a' content is a crucial determinant of mustard crop performance, influencing growth, development, and yield outcomes. Ensuring optimal conditions for chlorophyll 'a' synthesis through proper nutrient management and environmental control is essential for maximizing the productivity of mustard crops.

Table-4.9 (a): Effect of spacing and nutrient on Chl. 'a' (mg g^{-1} Fresh Weight) of mustard crop during rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	1.13	1.15	1.44	1.451	2.39	2.33
M2 (20×10)	1.08	1.11	1.46	1.469	2.13	2.04
C.D. at p<0.05	NS	NS	NS	NS	0.26	0.03
SEM±	0.08	0.08	0.005	0.004	0.04	0.006
Nutrients foliar application						
S0-Control	0.84	0.88	1.42	1.44	2.06	2.06
S1-Boron @1%	1.17	1.19	1.46	1.47	2.24	2.25
S2-Sulphur @ 0.15%	1.21	1.23	1.44	1.45	2.24	2.25
S3-BAP @0.003%	1.05	1.06	1.45	1.46	2.43	2.10
S4-Boron @0.5% +Sulphur @0.25%	1.10	1.12	1.47	1.48	2.24	2.12
S5-Boron @ 1.5%+ Sulphur @0.075%	0.94	0.95	1.45	1.47	2.32	2.33
S6-Boron @ 0.5% + BAP (@0.0045%)	1.14	1.17	1.47	1.48	2.21	2.22
S7-Boron @ 1.5%+ BAP (@0.0015%)	1.20	1.23	1.44	1.45	2.20	2.21
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	1.24	1.26	1.44	1.45	2.49	2.00
S9-Sulphur @0.25%+ BAP (@0.0015%)	1.17	1.20	1.44	1.45	2.14	2.15
C.D. at p<0.05	NS	NS	0.02	0.02	0.04	0.03
SEM±	0.11	0.11	0.01	0.01	0.08	0.10
C.D. S×M at p<0.05	NS	NS	0.04	0.04	0.40	0.38
SEM±	0.27	0.27	0.02	0.01	0.13	0.01
C.D. M×S at p<0.05	NS	NS	0.04	0.04	0.40	0.38
SEM±	0.17	0.17	0.01	0.01	0.12	0.14

Where, C.D. represents critical difference, SE (m) represents standard error of mean.

Table 4.9 (b): Interaction effect of spacing and nutrient on Chl. 'a' (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 60DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.427	1.467	1.327	1.423	1.44	1.427	1.47	1.423	1.447	1.45	1.43
M2	1.42	1.447	1.46	1.373	1.497	1.48	1.47	1.457	1.433	1.433	1.447
Mean B	1.423	1.457	1.393	1.398	1.468	1.453	1.47	1.44	1.44	1.442	
C.D. S×M at p<0.05						0.04					
SEM±						0.02					
C.D. M×S at p<0.05						0.04					
SEM±						0.01					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.447	1.477	1.337	1.433	1.45	1.437	1.48	1.433	1.457	1.46	1.441
M2	1.43	1.457	1.47	1.393	1.51	1.5	1.48	1.467	1.443	1.443	1.459
Mean B	1.438	1.467	1.403	1.413	1.48	1.468	1.48	1.45	1.45	1.452	
C.D. S×M at p<0.05						0.04					
SEM±						0.01					
C.D. M×S at p<0.05						0.04					
SEM±						0.01					

Table 4.9 (c): Interaction effect of spacing and nutrient on Chl. 'a' (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 90DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	2.3	2.32	2.34	2.33	2.367	2.41	2.28	2.357	1.9	2.31	2.291
M2	1.813	2.17	2.14	1.533	2.117	2.233	2.143	2.043	2.087	1.977	2.026
Mean B	2.057	2.245	2.24	1.932	2.242	2.322	2.212	2.2	1.993	2.143	
C.D. S×M at p<0.05						0.40					
SEM±						0.13					
C.D. M×S at p<0.05						0.40					
SEM±						0.12					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	2.313	2.333	2.35	2.337	2.377	2.42	2.29	2.367	1.91	2.32	2.302
M2	1.823	2.18	2.15	1.877	1.863	2.243	2.15	2.057	2.097	1.99	2.043
Mean B	2.068	2.257	2.25	2.107	2.12	2.332	2.22	2.212	2.003	2.155	
C.D. S×M at p<0.05						0.38					
SEM±						0.01					
C.D. M×S at p<0.05						0.38					
SEM±						0.14					

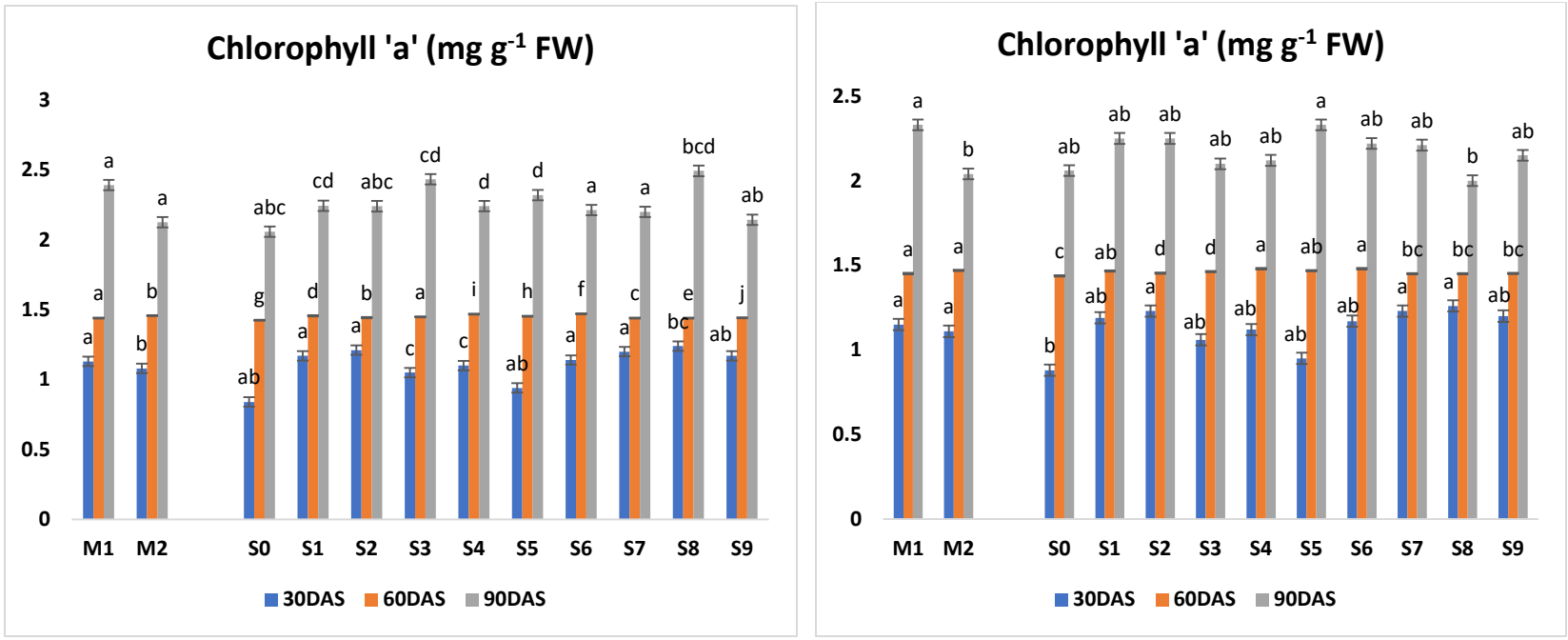


Fig-4.9 (a): Effect of spacing and nutrient on Chl. 'a' of mustard crop during rabi season of 2021-22 and 2022-23

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.3.2 Chlorophyll ‘b’ Content (mg g^{-1} Fresh Weight)

Mustard crop shows different variations when grown under different nutrient levels. In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Chlorophyll ‘b’ changes were observed at 30DAS, 60DAS and 90DAS, shown in Table 4.10, Fig 4.10. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the chlorophyll ‘b’ in each treatment as compared to control of both the spacings at 30, 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the chlorophyll ‘b’ was observed at 30, 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, main plot M1 shows maximum chlorophyll ‘b’ as compared to M2 with values 1.70 (M1) and 1.52 (M2), respectively. A percentage increase of 10.58% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant chlorophyll ‘b’ increase was observed in S4, i.e. 1.70 at 30DAS. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S4, followed by S3> S1> S9> S7> S2> S8> S6 and the per cent values were 13.40%, 13.34%, 12.62%, 11.03%, 10.78%, 10.39%, 8.57% and 0.91% respectively. At 60DAS, the main plot M1 shows maximum chlorophyll ‘b’ compared to M2, with values of 0.59 (M1) and 0.57 (M2), respectively. A percentage increase of 3.38% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase was

found in S2 with a value of 0.48, where Boron @ 1% was applied to the crop as a foliar application. The per cent increase was found highest in S2, followed by S3> S7> S6> S5> S8> S1> S9> S4, and the per cent values were 48.15%, 38.68%, 34.90%, 23.49%, 17.38%, 23.14%, 12.44%, 11.31% and 9.10% respectively when it is compared with its control (S0). At 90DAS, the main plot M1 shows maximum chlorophyll 'b' compared to M2, with values of 0.48 (M1) and 0.36 (M2), respectively. A percentage increase of 16.27% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S2 with a value of 0.57, where boron @1% was applied to the crop as a foliar spray. The per cent increase was found highest in S2, followed by S9> S7> S8> S3> S6> S5> S4, and the per cent values were 40.69%, 18.76%, 17.05%, 16.56%, 123.26%, 9.47%, 8.42% and 7.62% respectively when it is compared with its control (S0).

The study showed a significant increase with 8.57%, 23.14% and 16.56% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S8 and S0 (control). In treatment S8, the foliar application of Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the chlorophyll 'b' content was found in treatment S8, where the combined application of sulphur and cytokinin is applied to the crop compared to its control (S0).

In the year (2022-23), at 30DAS, main plot M1 shows maximum chlorophyll 'b' as compared to M2 with values 1.76 (M1) and 1.45 (M2), respectively. A percentage increase of 17.61% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant chlorophyll 'b' increase was observed in S3, i.e. 1.71 at 30DAS. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest

in S3, followed by S1> S7> S2> S8> S6> S4> S9, and the per cent values were 13.20%, 12.52%, 11.83%, 10.33%, 8.21%, 7.16%, 5.69% and 1.97% respectively. At 60DAS, main plot M2 shows maximum chlorophyll 'b' compared to M1 with values of 0.67 (M2) and 0.65 (M1), respectively. A percentage increase of 2.98% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S2 with a value of 0.86, where Boron @ 1% was applied to the crop as a foliar application. The per cent increase was found highest in S2, followed by S3> S4> S7> S5> S1> S6> S8, and the per cent values were 36.17%, 30.96%, 20.67%, 19.90%, 14.28%, 10.08%, 6.25% and 5.71% respectively when it is compared with its control (S0). At 90DAS, the main plot M1 shows maximum chlorophyll 'b' compared to M2, with values of 0.47 (M1) and 0.41 (M2), respectively. A percentage increase of 12.76% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S2 with a value of 0.58, where sulphur @0.15% was applied to the crop as a foliar spray. The per cent increase was found highest in S2, followed by S3> S5> S1> S4> S6> S9> S7, and the per cent values were 31.42%, 10.78%, 10.44%, 9.77%, 8.74%, 8.39%, 6.61% and 4.76% respectively when it is compared with its control (S0).

The study showed a significant increase with 13.20%, 30.96% and 10.78% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S3 and S0 (control). In treatment S3, the foliar application of Sulphur @ 0.15% was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the chlorophyll 'b' content was found in treatment S3, where sulphur is applied to the crop when compared to its control (S0). Chlorophyll 'b' stands out as a critical pigment in the photosynthetic

apparatus of plants, particularly in the light-harvesting complexes (LHCs) of photosystems I and II. Its unique role significantly expands the range of light wavelengths that plants can absorb, thereby playing a vital role in photosynthesis. Chlorophyll ‘b’ primarily absorbs light in the blue (around 450 nm) and red (around 640 nm) regions of the electromagnetic spectrum, complementing the absorption spectrum of Chlorophyll ‘a,’ which absorbs light more efficiently in the blue-violet and red areas. At the cellular level, Chlorophyll ‘b’ is associated with the protein complexes embedded in the thylakoid membranes of chloroplasts. Its primary function is to capture light energy and transfer it to Chlorophyll ‘a,’ which then uses this energy to drive the photochemical reactions of photosynthesis. The energy transfer from Chlorophyll ‘b’ to Chlorophyll ‘a’ occurs through resonance energy transfer, where the absorbed energy is passed from one chlorophyll molecule to another until it reaches the reaction centre of the photosystem.

Chlorophyll ‘b’ also plays a crucial role in the structural organization of the LHCs. Its role is crucial and pivotal, as it helps stabilize the LHC proteins and maintains the proper orientation of Chlorophyll ‘a’ molecules within the complex. This optimization ensures energy transfer efficiency, allowing plants to effectively capture and utilize light under varying light conditions, such as low-light environments or under a canopy where light intensity and quality are reduced. Chlorophyll ‘b’ broadens the spectrum of light that the photosynthetic machinery can absorb. By absorbing light in wavelengths that Chlorophyll ‘a’ does not efficiently capture, Chlorophyll ‘b’ allows plants to utilize a greater portion of the available light energy. This is particularly important in environments where light intensity is low or quality is altered, such as in shaded conditions. Chlorophyll ‘b’s ability to transfer energy to Chlorophyll ‘a ‘enhances the overall efficiency of the photosynthetic

process. This energy transfer ensures that the light energy captured by Chlorophyll ‘b’ is effectively used in the photochemical reactions, leading to a more efficient conversion of light energy into chemical energy stored in the form of ATP and NADPH. This, in turn, supports higher carbon fixation rates during the Calvin cycle, contributing to improved plant growth and productivity. Chlorophyll ‘b’ gives plants an adaptive advantage in fluctuating light environments. Plants that can efficiently capture and utilize light under various conditions are better equipped to survive and thrive in diverse habitats. Chlorophyll ‘b’ allows plants to adapt to changes in light quality and intensity, making it a key factor in the success of plants in both natural and agricultural settings. The role of Chlorophyll ‘b’ in stabilizing the LHCs and ensuring the proper orientation of Chlorophyll ‘a’ molecules is crucial for the optimal function of the photosystems. This structural support is essential for maintaining the integrity and efficiency of the photosynthetic apparatus, particularly under stress conditions such as high light intensity, drought, or nutrient deficiency. Chlorophyll ‘b’ plays an indispensable role in the photosynthetic machinery of plants. Its ability to enhance light absorption, increase photosynthetic efficiency, provide adaptive advantages, and optimize the structure of the photosystems justifies its critical function in supporting plant growth and productivity. Understanding the cellular mechanisms and the significance of Chlorophyll ‘b’ can inform strategies to improve crop performance, particularly in challenging environmental conditions.

Table 4.10 (a): Effect of spacing and nutrient on Chl. 'b' (mg g^{-1} Fresh Weight) of the mustard crop during the rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	1.71	1.77	0.59	0.65	0.44	0.48
M2 (20×10)	1.52	1.45	0.58	0.67	0.36	0.42
C.D. at p<0.05	0.07	0.07	0.01	0.01	0.01	0.01
SEM±	0.01	0.01	0	0	0	0
Nutrients foliar application						
S0-Control	1.48	1.49	0.45	0.56	0.34	0.40
S1-Boron @1%	1.69	1.70	0.50	0.61	0.35	0.44
S2-Sulphur @ 0.15%	1.65	1.66	0.85	0.86	0.57	0.58
S3-BAP @0.003%	1.71	1.72	0.72	0.80	0.39	0.45
S4-Boron @0.5% +Sulphur @0.25%	1.71	1.58	0.38	0.69	0.37	0.44
S5-Boron @ 1.5%+ Sulphur @0.075%	1.49	1.50	0.53	0.64	0.37	0.45
S6-Boron @ 0.5% + BAP (@0.0045%)	1.49	1.61	0.58	0.59	0.38	0.44
S7-Boron @ 1.5%+ BAP (@0.0015%)	1.66	1.69	0.68	0.69	0.41	0.42
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	1.62	1.62	0.57	0.58	0.41	0.42
S9-Sulphur @0.25%+ BAP (@0.0015%)	1.66	1.52	0.50	0.61	0.42	0.43
C.D. at p<0.05	0.09	0.07	0.02	0.06	0.03	0.03
SEM±	0.03	0.03	0.01	0.02	0.01	0.01
C.D. S×M at p<0.05	0.14	0.14	0.04	0.11	0.05	0.05
SEM±	0.04	0.04	0.01	0.03	0.01	0.01
C.D. M×S at p<0.05	0.14	0.14	0.04	0.10	0.05	0.05
SEM±	0.05	0.05	0.01	0.03	0.02	0.02

Where, C.D. represents critical difference, SE (m) represents standard error of mean.

Table 4.10 (b): Interaction effect of spacing and nutrient on Chl. 'b' (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 30DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.68	1.837	1.943	2.227	1.553	1.6	1.03	1.183	1.22	0.797	1.507
M2	1.283	1.55	1.36	1.187	1.187	1.373	1.69	1.677	1.617	1.223	1.415
Mean B	1.482	1.693	1.652	1.707	1.37	1.487	1.36	1.43	1.418	1.01	
C.D. S×M at p<0.05						0.14					
SEM±						0.04					
C.D. M×S at p<0.05						0.14					
SEM±						0.05					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.69	1.847	1.953	2.237	1.563	1.61	1.04	1.193	1.23	0.807	1.517
M2	1.293	1.56	1.37	1.197	1.197	1.383	1.7	1.687	1.627	1.233	1.425
Mean B	1.492	1.703	1.662	1.717	1.38	1.497	1.37	1.44	1.428	1.02	
C.D. S×M at p<0.05						0.14					
SEM±						0.04					
C.D. M×S at p<0.05						0.14					
SEM±						0.05					

Table 4.10 (c): Interaction effect of spacing and nutrient on Chl. 'b' (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 60DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	0.723	0.59	0.69	0.857	0.36	0.603	0.533	0.64	0.45	0.463	0.591
M2	0.373	0.413	1.007	0.58	0.407	0.46	0.617	0.713	0.697	0.53	0.58
Mean B	0.548	0.502	0.848	0.718	0.383	0.532	0.575	0.677	0.573	0.497	
C.D. S×M at p<0.05						0.04					
SEM±						0.01					
C.D. M×S at p<0.05						0.04					
SEM±						0.01					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	0.733	0.6	0.7	0.867	0.37	0.613	0.543	0.65	0.46	0.473	0.601
M2	0.383	0.423	1.023	0.727	0.417	0.47	0.63	0.723	0.707	0.54	0.604
Mean B	0.558	0.512	0.862	0.797	0.393	0.542	0.587	0.687	0.583	0.507	
C.D. S×M at p<0.05						0.11					
SEM±						0.03					
C.D. M×S at p<0.05						0.10					
SEM±						0.03					

Table 4.10 (d): Interaction effect of spacing and nutrient on Chl. 'b' (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 90DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	0.453	0.333	0.743	0.433	0.247	0.223	0.373	0.41	0.49	0.47	0.418
M2	0.33	0.333	0.403	0.243	0.31	0.35	0.38	0.41	0.323	0.367	0.345
Mean B	0.392	0.333	0.573	0.338	0.278	0.287	0.377	0.41	0.407	0.418	
C.D. S×M at p<0.05						0.05					
SEM±						0.01					
C.D. M×S at p<0.05						0.05					
SEM±						0.02					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	0.463	0.343	0.753	0.443	0.257	0.233	0.383	0.42	0.5	0.48	0.428
M2	0.34	0.343	0.413	0.253	0.32	0.36	0.39	0.42	0.333	0.377	0.355
Mean B	0.402	0.343	0.583	0.348	0.288	0.297	0.387	0.42	0.417	0.428	
C.D. S×M at p<0.05						0.05					
SEM±						0.01					
C.D. M×S at p<0.05						0.05					
SEM±						0.02					

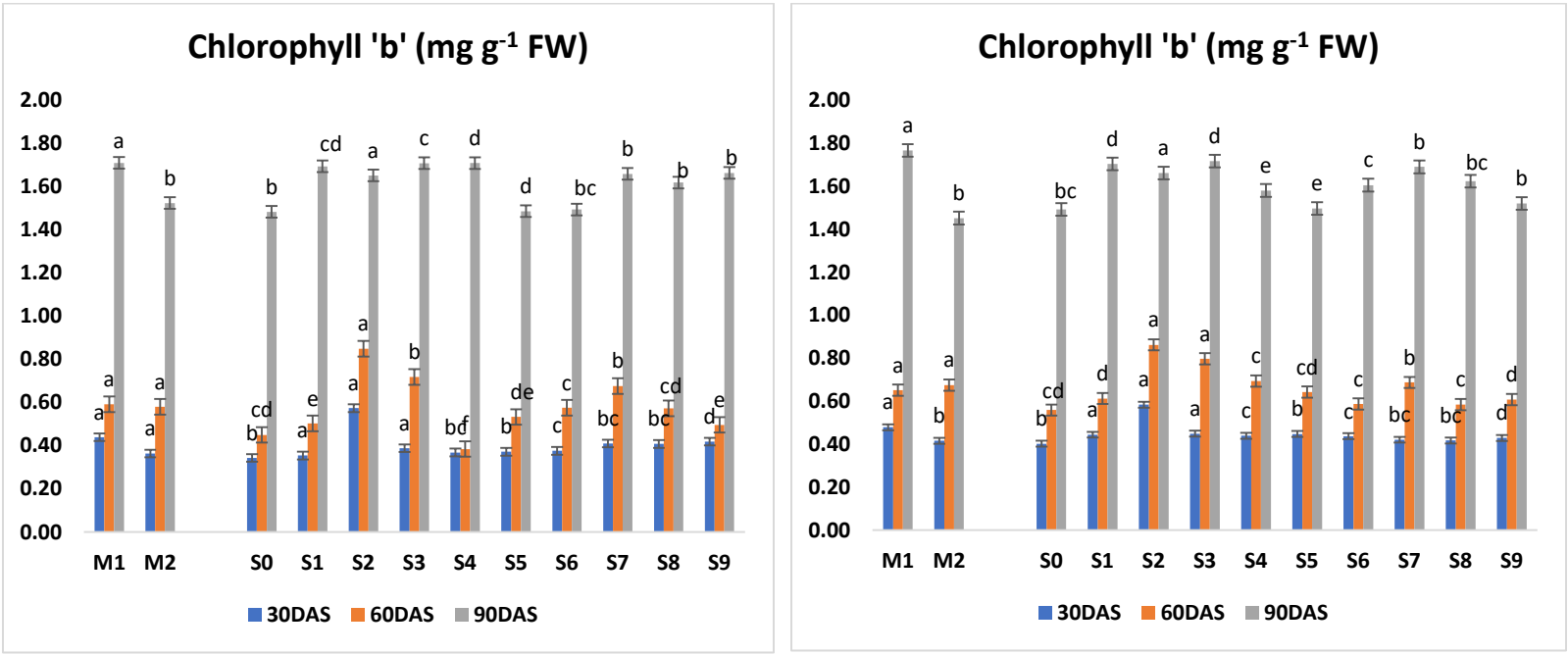


Fig-4.10 (a): Effect of spacing and nutrient on Chl. 'b' of the mustard crop during the rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.3.3 Chlorophyll ‘a:b’ Content

Mustard crops undergo various changes when grown under different nutrient levels. In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Chlorophyll ‘a:b’ changes were observed at 30DAS, 60DAS and 90DAS in Table 4.11, Fig 4.11. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the chlorophyll ‘a:b’ in each treatment as compared to control of both the spacings at 30, 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the chlorophyll ‘a:b’ was observed at 30, 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, the main plot M2 shows maximum chlorophyll ‘a:b’ as compared to M1 with values 0.72 (M2) and 0.67 (M1), respectively. A percentage increase of 6.94% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in chlorophyll ‘a:b’ was observed in S6, i.e. 0.78 at 30DAS, whereas in S6, Boron @ 0.5% + BAP (@0.0045% was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S6, followed by S8> S2> S7> S9> S1> S3> S4> S5, and the per cent values were 27.38%, 25.97%, 21.67%, 19.61%, 18.14%, 14.95%, 13.16% and 12.13% respectively. At 60DAS, the main plot M2 shows maximum chlorophyll ‘a:b’ compared to M1, with values of 2.71 (M2) and 2.50 (M1), respectively. A percentage increase of

7.74% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S1 with a value of 3.98, where Boron @ 1% was applied to the crop as a foliar application. The per cent increase was found highest in S1, followed by S9> S5> S2> S8> S7> S4> S3, and the per cent values were 18.54%, 17.10%, 14.15%, 11.84%, 10.77%, 2.57%, 2.22% and 2.08% respectively when it is compared with its control (S0). At 90DAS, the main plot M2 shows maximum chlorophyll 'a:b' compared to M1, with values of 5.89 (M2) and 5.68 (M1), respectively. A percentage increase of 3.56% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S6 with a value of 6.89, where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S6, followed by S3> S7> S1> S5> S2> S8> S4, and the per cent values were 40.69%, 18.76%, 17.05%, 16.56%, 123.26%, 9.47%, 8.42% and 7.62% respectively when it is compared with its control (S0).

The study showed a significant increase with 27.38%, 8.93% and 13.37% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S8, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the chlorophyll 'a:b' content was found in treatment S6, where the combined application of boron and cytokinin is applied to the crop compared to its control (S0).

In the year (2022-23), at 30DAS, the main plot M2 shows maximum chlorophyll 'a:b' as compared to M1 with values 0.77 (M2) and 0.65 (M1), respectively. A percentage increase of 15.58% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, the significant increase in chlorophyll 'a:b' was observed in S9, i.e.

0.82 at 30DAS, where in S9, Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S9, followed by S8> S2> S6> S7> S4> S1> S3> S5, and the per cent values were 27.16%, 23.22%, 21.38%, 17.62%, 17.56%, 15.71%, 14.83%, 11.47, 7.85% respectively. At 60DAS, the main plot M2 shows maximum chlorophyll 'a:b' compared to M1, with values of 2.299 (M2) and 2.291 (M1), respectively. A percentage increase of 0.34% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S1 with a value of 2.99, where Boron @ 1% was applied to the crop as a foliar application. The per cent increase was found highest in S1, followed by S6> S4> S9> S7> S5> S8> S2, and the per cent values were 4.68%, 2.94%, 2.91%, 2.41%, 2.27%, 1.92%, 1.87% and 0.85% respectively when it is compared with its control (S0). At 90DAS, the main plot M1 shows maximum chlorophyll 'a:b' compared to M2, with values of 5.05 (M2) and 5.00 (M1), respectively. A percentage increase of 0.99% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S3 with a value of 5.70, where cytokinin @ 0.003%) was applied to the crop as a foliar spray. The per cent increase was found highest in S3, followed by S2> S9> S1> S8> S6> S7> S5, and the per cent values were 9.38%, 7.76%, 7.39%, 4.33%, 3.45%, 1.89%, 1.82% and 1.16% respectively when it is compared with its control (S0).

The study shows a significant increase, with 17.62% and 2.94% cent values at 30DAS and 60DAS when comparing S6 and S0 (control). In treatment S6, the mustard crop was treated with a foliar application of Boron @ 0.5% + BAP (@0.0045%). At 120DAS, a significant increase in the chlorophyll 'a:b' content was found in treatment S3, where

cytokinin was applied to the crop compared to its control (S0). Chlorophyll a and chlorophyll b are the two primary pigments involved in photosynthesis. They play distinct but complementary roles in light absorption and energy conversion. The chlorophyll a/b ratio is a critical indicator of the plant's photosynthetic efficiency and adaptation to environmental conditions. Chlorophyll primarily absorbs light in the blue-violet and red regions of the spectrum (wavelengths around 430 nm and 662 nm, respectively). It is the main pigment involved in converting light energy into chemical energy during the light-dependent reactions of photosynthesis**.** Chlorophyll b absorbs light more effectively in the blue and red-orange regions (around 453 nm and 642 nm). It acts as an accessory pigment, expanding the range of light wavelengths the plant can utilize for photosynthesis. Chlorophyll b transfers the absorbed light energy to chlorophyll a, used in the photosynthetic reaction centres. In the chloroplast thylakoid membranes, chlorophyll a is predominantly found in the reaction centres of photosystems I and II, where it plays a direct role in the photochemical reactions leading to ATP and NADPH generation. Chlorophyll b is more abundant in the light-harvesting complexes (LHC) associated with the photosystems. It captures and punches light energy to the reaction centres, optimizing the photosynthetic process. The chlorophyll a/b ratio is dynamically regulated in response to environmental conditions such as light intensity and quality. Under high light conditions, plants increase the chlorophyll a/b ratio, corresponding to a higher proportion of chlorophyll a in the photosynthetic apparatus. This adjustment allows the plant to maximize energy conversion efficiency under intense light. In low light conditions, the ratio typically decreases, meaning that the plant increases the relative amount of chlorophyll b. This adaptation enhances the plant's

ability to capture light in suboptimal conditions, as chlorophyll b broadens the light absorption spectrum, improving the overall light-harvesting capacity. The chlorophyll a/b ratio is crucial for understanding the plant's physiological state and its capacity to adapt to varying environmental conditions. A higher chlorophyll a/b ratio indicates that the plant optimizes its photosynthetic machinery for efficient energy conversion, particularly under high light intensity. Chlorophyll's role in the reaction centres is critical for capturing and converting light into chemical energy. Hence, an increased ratio suggests that the plant is enhancing its ability to perform photosynthesis effectively in bright light conditions. A lower chlorophyll a/b ratio indicates the plant’s adaptation to low-light environments. Increasing the relative amount of chlorophyll b allows the plant to capture a broader range of light wavelengths, which is particularly beneficial when light is a limiting factor. Despite reduced light availability, this adaptation allows the plant to maintain adequate photosynthetic activity. Variations in the chlorophyll a/b ratio can also serve as an indicator of plant stress. Environmental stresses, such as nutrient deficiency, drought, or high salinity, can disrupt the synthesis and balance of chlorophyll pigments. A significant deviation from the typical chlorophyll a/b ratio might suggest that the plant is experiencing stress, which could impair its photosynthetic capacity and overall health. Understanding and manipulating the chlorophyll a/b ratio can be valuable in agricultural practices. For example, selecting crop varieties with an optimal chlorophyll a/b ratio for specific light environments can enhance crop yield and efficiency. Additionally, monitoring changes in this ratio could be an early diagnostic tool for detecting stress conditions, allowing for timely interventions to mitigate potential yield losses. The chlorophyll a/b ratio is not only a reflection of the plant's photosynthetic efficiency but

also a key indicator of its ability to adapt to changing environmental conditions. Its dynamic regulation under different light intensities and stress conditions underscores its importance in maintaining plant health and productivity.

Table-4.11 (a): Effect of spacing and nutrient on Chl. a:b of mustard crop during rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	0.68	0.66	2.51	2.29	5.69	5.06
M2 (20×10)	0.72	0.77	2.72	2.30	5.90	5.00
C.D. at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.01	0.01	0.03	0.03	0.07	0.07
Nutrients foliar application						
S0-Control	0.58	0.6	3.25	2.85	5.98	5.18
S1-Boron @1%	0.70	0.70	3.99	2.99	6.35	5.40
S2-Sulphur @ 0.15%	0.75	0.76	3.69	2.87	6.23	5.61
S3-BAP @0.003%	0.67	0.68	3.32	2.85	6.39	5.71
S4-Boron @0.5% +Sulphur @0.25%	0.66	0.71	3.32	2.94	6.13	5.20
S5-Boron @ 1.5%+ Sulphur @0.075%	0.65	0.65	3.79	2.91	6.26	5.23
S6-Boron @ 0.5% + BAP (@0.0045%)	0.78	0.73	3.57	2.94	6.89	5.27
S7-Boron @ 1.5%+ BAP (@0.0015%)	0.73	0.73	3.34	2.92	6.37	5.27
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	0.77	0.78	3.64	2.9	6.17	5.36
S9-Sulphur @0.25%+ BAP (@0.0015%)	0.71	0.82	3.92	2.92	6.15	5.58
C.D. at p<0.05	0.27	0.27	0.14	0.14	0.34	0.36
SEM±	0.09	0.09	0.05	0.05	0.12	0.12
C.D. S×M at p<0.05	0.39	0.39	0.25	0.25	0.59	0.62
SEM±	0.03	0.03	0.09	0.09	0.22	0.24
C.D. M×S at p<0.05	0.38	0.38	0.26	0.26	0.61	0.65
SEM±	0.13	0.13	0.07	0.07	0.17	0.18

Where, C.D. represents critical difference, SE (m) represents standard error of mean.

Table 4.11 (b): Interaction effect of spacing and nutrient on Chl. 'a:b' (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 30DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.377	1.267	1.203	1.057	1.547	1.52	2.217	2.027	1.37	3.11	1.669
M2	1.42	1.403	1.547	1.037	0.807	1.353	1.28	1.197	1.29	1.53	1.286
Mean B	1.398	1.335	1.375	1.047	1.177	1.437	1.748	1.612	1.33	2.32	
C.D. S×M at p<0.05						0.39					
SEM±						0.03					
C.D. M×S at p<0.05						0.38					
SEM±						0.13					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.387	1.277	1.213	1.067	1.56	1.53	2.227	2.037	1.38	3.12	1.68
M2	1.43	1.413	1.557	1.047	0.82	1.363	1.293	1.21	1.303	1.54	1.298
Mean B	1.408	1.345	1.385	1.057	1.19	1.447	1.76	1.623	1.342	2.33	
C.D. S×M at p<0.05						0.39					
SEM±						0.03					
C.D. M×S at p<0.05						0.38					
SEM±						0.13					

Table 4.11 (c): Interaction effect of spacing and nutrient on Chl. 'a:b' (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 60DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.73	2.643	1.96	2.013	2.617	1.863	2.39	1.767	2.597	1.97	2.155
M2	1.917	2.467	1.663	2.273	2.273	2.04	1.617	2.32	2.017	1.65	2.024
Mean B	1.823	2.555	1.812	2.143	2.445	1.952	2.003	2.043	2.307	1.81	
C.D. S×M at p<0.05						0.25					
SEM±						0.09					
C.D. M×S at p<0.05						0.26					
SEM±						0.07					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.74	2.653	1.97	2.047	2.627	1.88	2.407	1.777	2.607	1.993	2.17
M2	1.933	2.477	1.683	2.29	2.287	2.063	1.633	2.333	2.033	1.66	2.039
Mean B	1.837	2.565	1.827	2.168	2.457	1.972	2.02	2.055	2.32	1.827	
C.D. S×M at p<0.05						0.25					
SEM±						0.09					
C.D. M×S at p<0.05						0.26					
SEM±						0.07					

Table 4.11 (d): Interaction effect of spacing and nutrient on Chl. 'a:b' (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 90DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	2.33	3.163	1.637	2.52	3.49	2.96	3.227	2.56	1.76	2.207	2.585
M2	2.683	2.623	2.117	3.113	2.817	2.907	2.66	2.983	3.16	2.937	2.8
Mean B	2.507	2.893	1.877	2.817	3.153	2.933	2.943	2.772	2.46	2.572	
C.D. S×M at p<0.05						0.59					
SEM±						0.22					
C.D. M×S at p<0.05						0.61					
SEM±						0.17					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	2.34	3.313	1.647	2.53	3.5	2.97	3.237	2.57	1.773	2.22	2.61
M2	2.697	2.637	2.133	3.127	2.83	2.917	2.67	3.003	3.173	2.95	2.814
Mean B	2.518	2.975	1.89	2.828	3.165	2.943	2.953	2.787	2.473	2.585	
C.D. S×M at p<0.05						0.62					
SEM±						0.24					
C.D. M×S at p<0.05						0.65					
SEM±						0.18					

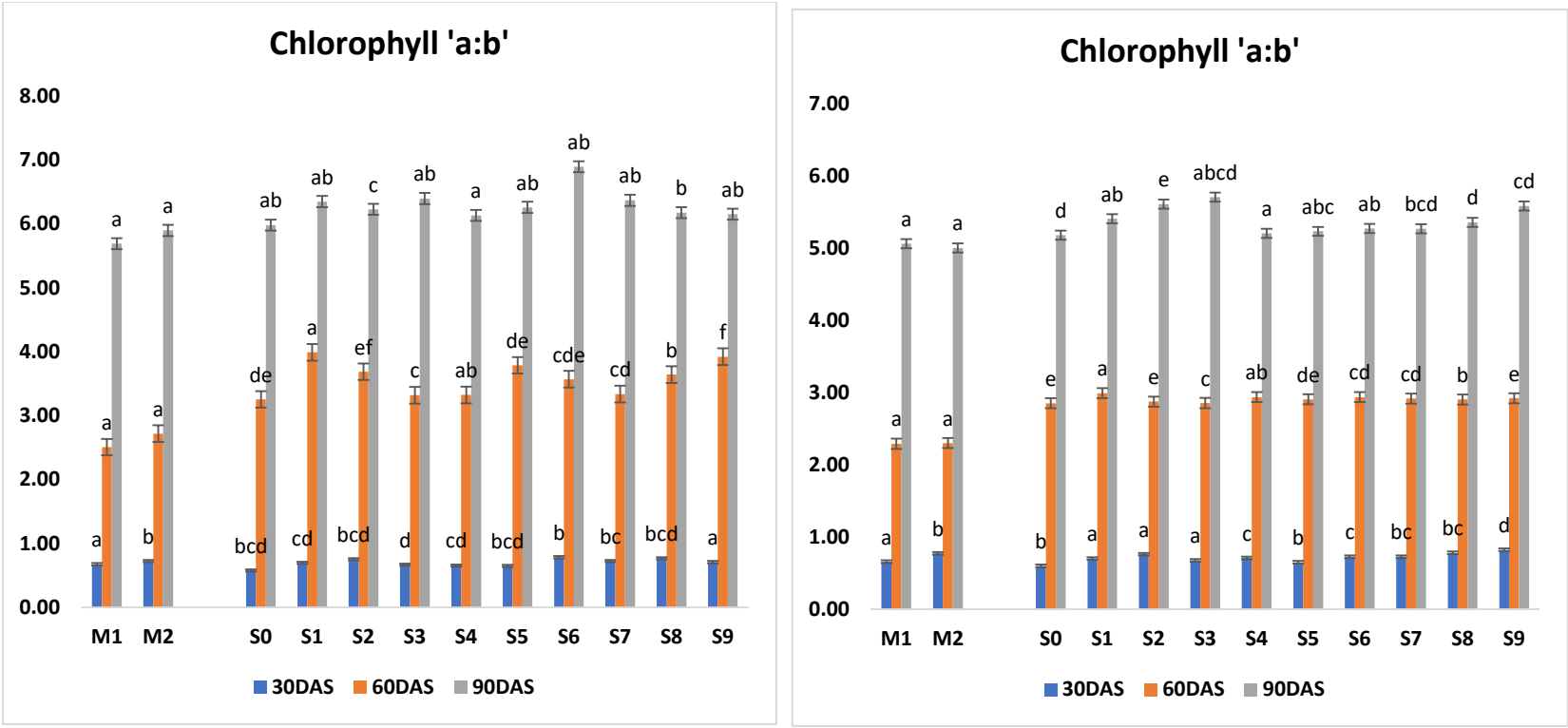


Fig-4.11 (a): Effect of spacing and nutrient on Chl. a:b of mustard crop during rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.3.4 Chlorophyll 'a+b' Content (mg g^{-1} FW)

Mustard crops undergo various changes when grown under different nutrient levels. In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Chlorophyll 'a+b' changes were observed at 30DAS, 60DAS, and 90DAS, as shown in Table 4.12, Fig 4.12. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the chlorophyll 'a+b' in each treatment as compared to control of both the spacings at 30, 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the chlorophyll 'a+b' was observed at 30, 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, main plot M1 shows maximum chlorophyll 'a+b' as compared to M2 with values 2.84 (M1) and 2.61 (M2), respectively. A percentage increase of 8.09% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase in chlorophyll 'a+b' was observed in S7, i.e. 2.86 at 30DAS, whereas in S7, Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S7, followed by S8> S1> S2> S9> S4> S3> S6> S5, and the per cent values were 18.71%, 18.68%, 18.67%, 18.67%, 18.01%, 17.09%, 15.53%, 11.62% and 4.16% respectively. At 60DAS, the main plot M2 shows maximum chlorophyll 'a+b' compared to M1, with values 2.03 (M2) and 2.02 (M1), respectively. A

percentage increase of 0.49 % was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S1 with a value of 3.98, where Boron @ 1% was applied to the crop as a foliar application. The per cent increase was found highest in S1, followed by S9> S5> S2> S8> S7> S4> S3, and the per cent values were 18.54%, 17.10%, 14.15%, 11.84%, 10.77%, 2.57%, 2.22% and 2.08% respectively when it is compared with its control (S0). At 90DAS, the main plot M2 shows maximum chlorophyll 'a+b' compared to M1, with values of 5.89 (M2) and 5.68 (M1), respectively. A percentage increase of 3.56% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S6 with a value of 6.89 where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S6, followed by S3> S7> S1> S5> S2> S8> S4, and the per cent values were 40.69%, 18.76%, 17.05%, 16.56%, 123.26%, 9.47%, 8.42% and 7.62% respectively when it is compared with its control (S0).

The study showed a significant increase with 27.38%, 8.93% and 13.37% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S8, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the chlorophyll 'a+b' content was found in treatment S6, where the combined application of boron and cytokinin is applied to the crop when compared to its control (S0).

In the year (2022-23), at 30DAS, main plot M1 shows maximum chlorophyll 'a+b' as compared to M1 with values 2.91 (M1) and 2.56 (M2), respectively. A percentage increase of 12.02% was found in M1, where the crop was grown in spacing (30*10). In

subplots, a significant increase in chlorophyll ‘a+b’ was observed in S7, i.e. 2.92 at 30DAS, whereas in S7, Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S7, followed by S2> S1> S8> S3> S6> S9> S4> S5, and the per cent values were 18.83%, 18.22%, 18.13%, 18.04%, 14.90%, 14.59%, 12.33% and 3.33% respectively. At 60DAS, the main plot M2 shows maximum chlorophyll ‘a+b’ compared to M1, with values of 2.14 (M2) and 2.10 (M1), respectively. A percentage increase of 1.86 % was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S2 with a value of 2.31, where sulphur @ 0.15% was applied to the crop as a foliar application. The per cent increase was found highest in S2, followed by S3> S4> S7> S5> S1> S6> S9, and the per cent values were 14.03%, 11.94%, 8.43%, 6.86%, 5.68%, 4.25%, 3.70% and 3.31% respectively when it is compared with its control (S0). At 90DAS, the main plot M1 shows maximum chlorophyll ‘a+b’ compared to M2, with values of 2.80 (M1) and 2.45 (M2), respectively. A percentage increase of 12.5% was found in M2, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S2 with a value of 2.83, where Sulphur @ 0.15% was applied to the crop as a foliar spray. The per cent increase was found highest in S2, followed by S5> S1> S6> S7> S9> S8> S4, and the per cent values were 12.82%, 11.09%, 8.51%, 7.04%, 6.14%, 4.38%, 3.89% and 3.45% respectively when it is compared with its control (S0).

The study showed a significant increase with 18.22%, 14.03% and 12.82% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S2 and S0 (control). In treatment S2, the foliar application of Sulphur @ 0.15% was applied to the

mustard crop. At 60DAS and 90DAS, a significant increase in the chlorophyll ‘a+b’ content was found in treatment S2, where the combined application of sulphur is applied to the crop compared to its control (S0). Chlorophylls, specifically chlorophyll a and b, are essential pigments in plants' photosynthetic apparatus. They play a critical role in capturing and converting light energy into chemical energy. The balance and concentration of chlorophyll a and b in the chloroplasts are crucial for optimizing photosynthetic efficiency, particularly under varying environmental conditions and nutrient availability. Chlorophyll a is the primary pigment involved in the light reactions of photosynthesis. It directly converts light into chemical energy by transferring excited electrons to the primary electron acceptor in the photosystem reaction centers (PSI and PSII). Chlorophyll b serves as an accessory pigment, broadening the range of light wavelengths absorbed by the plant. It absorbs light primarily in the blue and red-orange wavelengths, transferring this energy to chlorophyll for the photochemical reactions. Chlorophyll a and b biosynthesis is a tightly regulated process involving multiple enzymatic steps, primarily occurring within the chloroplast. The precursor, 5-aminolevulinic acid (ALA), is synthesized in the plastids and serves as the starting material for the tetrapyrrole pathway, forming protochlorophyllide. Protochlorophyllide is then converted to chlorophyllide by the enzyme protochlorophyllide reductase, a light-dependent reaction. The subsequent addition of a phytyl chain forms chlorophyll a. For chlorophyll b, the enzyme chlorophyllide an oxygenase (CAO) catalyses the conversion of chlorophyll a to chlorophyll b**.** Photosystem I (PSI) and Photosystem II (PSII) contain chlorophyll a and b, with chlorophyll a being the dominant pigment in the reaction centres. In contrast, chlorophyll b is more abundant in the light-harvesting complexes

(LHCs). Chlorophyll's role in the LHCs is to extend the light absorption range, particularly under low-light conditions. This ensures that more light energy is captured and transferred to the reaction centres, where chlorophyll initiates the photochemical reactions. The ratio of chlorophyll a to chlorophyll b is critical for optimizing photosynthetic efficiency. Plants adjust this ratio in response to environmental cues like light intensity and quality. Under high-light conditions, the proportion of chlorophyll increases, enhancing the capacity for direct photochemical conversion. In contrast, the relative increase in chlorophyll b under low light improves light-harvesting efficiency by capturing additional wavelengths. The total content of chlorophyll a + b serves as an indicator of the photosynthetic capacity of the plant. Higher levels of these pigments generally correlate with increased photosynthetic activity and, consequently, more excellent biomass production. Measuring chlorophyll a + b provides insights into the plant's nutrient status, particularly nitrogen availability, as nitrogen is a critical component of chlorophyll molecules. Nitrogen deficiency often leads to a reduction in chlorophyll content, manifesting as chlorosis (yellowing of leaves) and reduced photosynthetic efficiency. The chlorophyll a + b content is sensitive to environmental stresses like drought, salinity, and temperature extremes. These stresses can disrupt chlorophyll synthesis or accelerate chlorophyll degradation, leading to reduced chlorophyll levels and compromised photosynthesis. Chlorophyll a + b measurements at different growth stages (e.g., 30 DAS, 60 DAS, etc.) can provide valuable information on the dynamic changes in the photosynthetic apparatus as the plant develops. This data can help optimize nutrient management practices and maintain the plant's photosynthetic capacity throughout its growth cycle. The combined measurement of chlorophyll a + b

provides a comprehensive understanding of the photosynthetic efficiency and health of the plant. It serves as a critical parameter for assessing the physiological status of the crop under different environmental and nutrient conditions, ultimately guiding agronomic practices to enhance productivity.

Table-4.12 (a): Effect of spacing and nutrient on Chl. a+b (mg g^{-1} Fresh Weight) of mustard crop during rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	2.02	2.10	2.83	2.81	2.84	2.92
M2 (20×10)	2.03	2.14	2.49	2.46	2.61	2.56
C.D. at p<0.05	0.02	0.01	0.06	0.02	0.10	0.09
SEM±	0	0	0.01	0	0.01	0.01
Nutrients foliar application						
S0-Control	1.87	2.00	2.40	2.47	2.33	2.38
S1-Boron @1%	1.96	2.08	2.60	2.70	2.87	2.90
S2-Sulphur @ 0.15%	2.24	2.32	2.81	2.83	2.86	2.90
S3-BAP @0.003%	2.12	2.26	2.82	2.56	2.76	2.79
S4-Boron @0.5% +Sulphur @0.25%	1.95	2.17	2.61	2.56	2.81	2.70
S5-Boron @ 1.5%+ Sulphur @0.075%	1.99	2.11	2.69	2.78	2.43	2.45
S6-Boron @ 0.5% + BAP (@0.0045%)	2.05	2.07	2.59	2.66	2.64	2.78
S7-Boron @ 1.5%+ BAP (@0.0015%)	2.12	2.14	2.61	2.63	2.87	2.92
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	2.01	2.03	2.90	2.57	2.87	2.89
S9-Sulphur @0.25%+ BAP (@0.0015%)	1.94	2.06	2.56	2.58	2.84	2.72
C.D. at p<0.05	0.02	0.02	0.09	0.10	0.11	0.11
SEM±	0	0	0.03	0.03	0.04	0.04
C.D. S×M at p<0.05	0.18	0.18	0.04	0.04	0.13	0.15
SEM±	0.05	0.05	0.01	0.01	0.03	0.01
C.D. M×S at p<0.05	0.18	0.18	0.04	0.04	0.14	0.14
SEM±	0.06	0.06	0.01	0.01	0.04	0.05

Where, C.D. represents critical difference, SE (m) represents standard error of mean

Table 4.12 (b): Interaction effect of spacing and nutrient on Chl. 'a+b' (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 30DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	3.997	4.143	4.28	4.563	3.94	4.023	3.307	3.573	2.783	3.213	3.782
M2	3.1	3.72	3.45	2.413	2.137	3.227	3.83	3.663	3.683	3.077	3.23
Mean B	3.548	3.932	3.865	3.488	3.038	3.625	3.568	3.618	3.233	3.145	
C.D. S×M at p<0.05						0.18					
SEM±						0.05					
C.D. M×S at p<0.05						0.18					
SEM±						0.06					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	4.01	4.157	4.29	4.573	3.957	4.06	3.333	3.593	2.803	3.237	3.801
M2	3.12	3.74	3.46	2.427	2.153	3.24	3.847	3.68	3.7	3.097	3.246
Mean B	3.565	3.948	3.875	3.5	3.055	3.65	3.59	3.637	3.252	3.167	
C.D. S×M at p<0.05						0.18					
SEM±						0.05					
C.D. M×S at p<0.05						0.18					
SEM±						0.06					

Table 4.12 (c): Interaction effect of spacing and nutrient on Chl. 'a+b' (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 60DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	2.14	2.15	2.007	2.577	1.303	1.72	1.807	1.75	1.607	1.36	1.842
M2	1.093	1.42	2.67	1.877	1.317	1.4	1.6	2.36	2.087	1.4	1.722
Mean B	1.617	1.785	2.338	2.227	1.31	1.56	1.703	2.055	1.847	1.38	
C.D. S×M at p<0.05						0.04					
SEM±						0.01					
C.D. M×S at p<0.05						0.04					
SEM±						0.01					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	2.15	2.16	2.017	2.587	1.313	1.73	1.817	1.763	1.627	1.373	1.854
M2	1.11	1.43	2.68	1.887	1.34	1.417	1.613	2.37	2.103	1.417	1.737
Mean B	1.63	1.795	2.348	2.237	1.327	1.573	1.715	2.067	1.865	1.395	
C.D. S×M at p<0.05						0.04					
SEM±						0.01					
C.D. M×S at p<0.05						0.04					
SEM±						0.01					

Table 4.12 (d): Interaction effect of spacing and nutrient on Chl. 'a+b' (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 90DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.513	1.35	1.953	1.507	1.057	0.877	1.57	1.46	1.35	1.503	1.414
M2	1.223	1.183	1.25	0.993	1.173	1.35	1.37	1.61	1.34	1.437	1.293
Mean B	1.368	1.267	1.602	1.25	1.115	1.113	1.47	1.535	1.345	1.47	
C.D. S×M at p<0.05						0.13					
SEM±						0.03					
C.D. M×S at p<0.05						0.14					
SEM±						0.04					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.523	1.363	1.967	1.52	1.087	0.893	1.587	1.48	1.367	1.52	1.431
M2	1.24	1.203	1.27	1.057	1.193	1.367	1.383	1.527	1.35	1.453	1.304
Mean B	1.382	1.283	1.618	1.288	1.14	1.13	1.485	1.503	1.358	1.487	
C.D. S×M at p<0.05						0.15					
SEM±						0.01					
C.D. M×S at p<0.05						0.14					
SEM±						0.05					

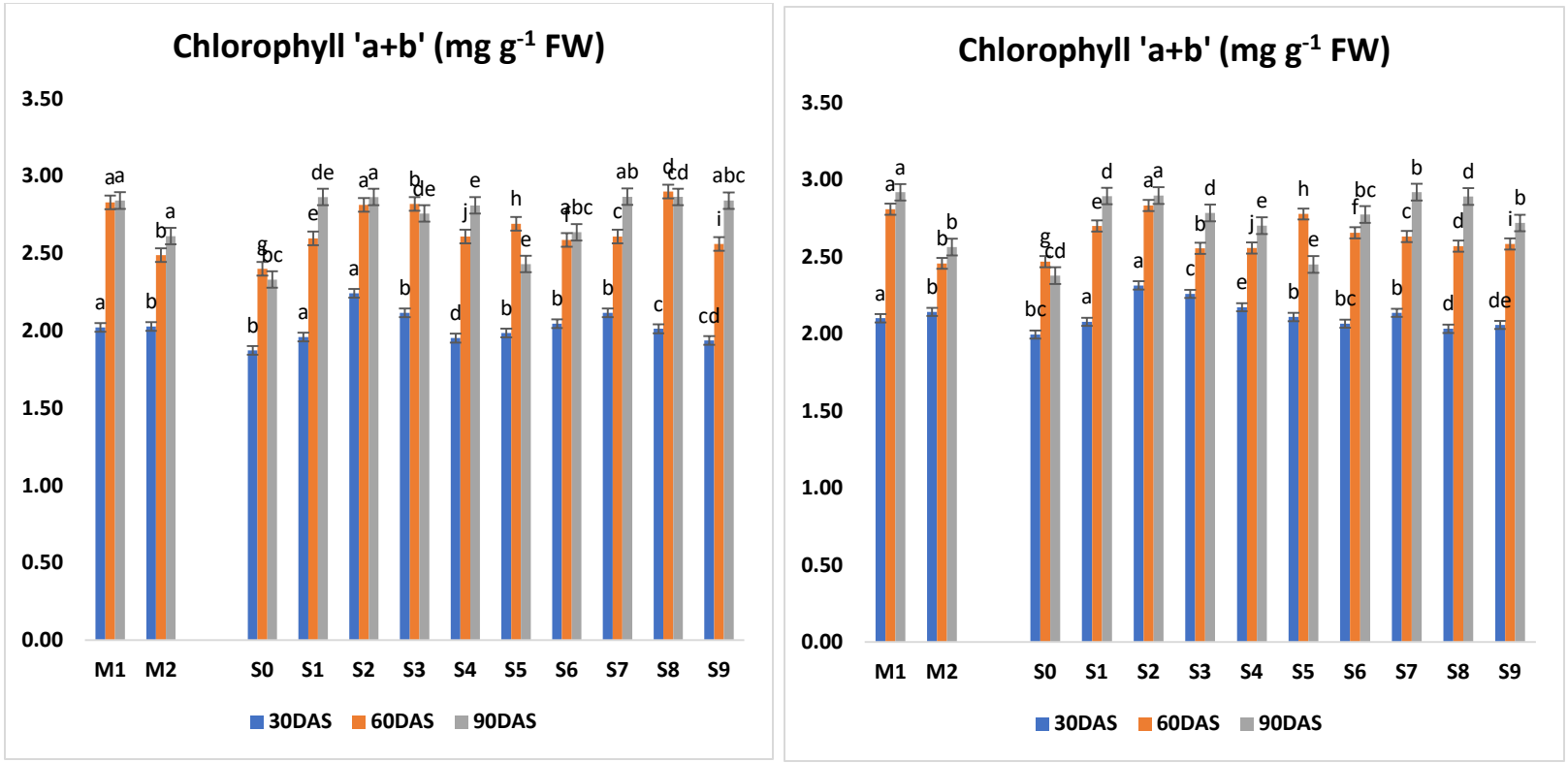


Fig-4.12 (a): Effect of spacing and nutrient on Chl. a+b of mustard crop during rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.3.5 Carotenoids (mg g^{-1} Fresh Weight)

Mustard crops undergo various changes when grown under different nutrient levels. In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in carotenoids were observed at 30DAS, 60DAS, and 90DAS, as shown in Table 4.13, Fig 4.13. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the carotenoids in each treatment compared to control of both the spacings at 30, 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the carotenoids was observed at 30, 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, main plot M1 shows maximum carotenoids as compared to M2 with values 2.80 (M1) and 2.41 (M2), respectively. A percentage increase of 13.92% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase in carotenoids was observed in S3, i.e. 3.73 at 30DAS, whereas in S3, cytokinin (@0.0030%) was applied to the crop. Therefore, at 30 DAS the percentage increase as compared to S0 was found to be highest in S3, followed by S2> S7> S6> S8> S5> S9> S4> S1 and the per cent values were 74.27%, 73.91%, 71.91%, 66.27%, 63.38%, 61.62%, 56.34%, 55.32% and 51.08% respectively. At 60DAS, the main plot M1 shows maximum carotenoids compared to M2, with values of 3.03 (M1) and 2.57 (M2), respectively. A percentage increase of 15.18 % was found in M1, where

the crop was grown in spacing (30*10). In subplots, a significant increase was found in S2 with a value of 3.84, where sulphur @ 0.15% was applied to the crop as a foliar application. The per cent increase was found highest in S2, followed by S7> S1> S6> S3> S8> S4> S5, and the per cent values were 53.67%, 47.70%, 43.97%, 38.18%, 36.38%, 35.21%, 35.18% and 29.37% respectively when it is compared with its control (S0). At 90DAS, the main plot M1 shows maximum carotenoids compared to M2, with values of 2.35 (M1) and 1.94 (M2), respectively. A percentage increase of 17.44% was found in M2, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S1 with a value of 2.72, where Boron @ 1% was applied to the crop as a foliar spray. The per cent increase was found highest in S1, followed by S7> S6> S9> S8> S2> S3> S5, and the per cent values were 48.68%, 44.81%, 44.40%, 42.56%, 40.35%, 35.67%, 32.09% and 22.83% respectively when it is compared with its control (S0). The study showed a significant increase with 66.27%, 38.18% and 44.40% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S8, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the carotenoid content was found in treatment S6, where the combined application of boron and cytokinin is applied to the crop when compared to its control (S0).

In the year (2022-23), at 30DAS, main plot M1 shows maximum carotenoids as compared to M2 with values 2.81 (M1) and 2.43 (M2), respectively. A percentage increase of 13.52% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase in carotenoids was observed in S3, i.e. 3.69 at 30DAS,

whereas in S3, cytokinin (@0.0030%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S3, followed by S2> S7> S6> S8> S5> S9> S4> S1 and the per cent values were 74.12%, 73.74%, 71.74%, 66.10%, 63.35%, 61.50%, 56.40%, 55.19% and 50.96% respectively. At 60DAS, the main plot M1 shows maximum carotenoids compared to M2, with values of 3.06 (M1) and 2.62 (M2), respectively. A percentage increase of 14.37 % was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase was found in S2 with a value of 3.87, where sulphur @ 0.15% was applied to the crop as a foliar application. The per cent increase was found highest in S2, followed by S7> S1> S6> S3> S8> S4> S5, and the per cent values were 53.54%, 47.59%, 43.63%, 38.03%, 36.50%, 35.17%, 357.17% and 29.36% respectively when it is compared with its control (S0). At 90DAS, the main plot M1 shows maximum carotenoids compared to M2, with values of 2.42 (M1) and 1.97 (M2), respectively. A percentage increase of 18.59% was found in M2, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S1 with a value of 2.75, where Boron @ 1% was applied to the crop as a foliar spray. The per cent increase was found highest in S1, followed by S7> S6> S9> S8> S2> S3> S5, and the per cent values were 48.45%, 46.24%, 44.24%, 42.51%, 41.96%, 35.64%, 32.11% and 22.61% respectively when it is compared with its control (S0). The study showed a significant increase with 66.10%, 38.03% and 44.24% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant

increase in the carotenoid content was found in treatment S6, where the combined application of boron and cytokinin is applied to the crop compared to its control (S0).

Carotenoids are a class of pigments found in plants, algae, and photosynthetic bacteria that play a crucial role in various cellular processes. In plants, carotenoids are synthesized in plastids through the isoprenoid pathway, where they are involved in light absorption and protection against photooxidative damage. Carotenoids are integral components of the photosynthetic apparatus, particularly in chloroplasts' light-harvesting complexes (LHC). They absorb light in the blue and green regions of the spectrum (400-500 nm) and transfer the captured energy to chlorophyll molecules, thereby extending the range of light wavelengths that can be used for photosynthesis. Carotenoids play a critical role in photoprotection by quenching excess energy and preventing the formation of reactive oxygen species (ROS). When chlorophyll molecules in the photosystems absorb more light energy than they can process, this excess energy is transferred to carotenoids, dissipating it as heat through non-photochemical quenching (NPQ). This mechanism protects the photosynthetic machinery from oxidative damage caused by high light intensity. Carotenoids are potent antioxidants that scavenge and neutralize ROS, such as singlet oxygen and free radicals, which are generated as byproducts of photosynthesis and other metabolic processes. By preventing oxidative stress, carotenoids protect cellular components, including lipids, proteins, and nucleic acids, from damage. This antioxidant function is essential for maintaining cellular homeostasis and ensuring the longevity and health of plant tissues. Carotenoids are also involved in the regulation of gene expression related to stress responses and development. Their degradation products, such as apocarotenoids, can act as signalling molecules that modulate the expression of

genes involved in stress tolerance, growth, and development. This signalling function allows plants to adapt to changing environmental conditions, such as fluctuations in light intensity, temperature, and nutrient availability. Beyond their photosynthesis and stress protection functions, carotenoids are precursors to essential plant hormones like abscisic acid (ABA) and strigolactones. ABA is crucial for regulating plant responses to drought and other abiotic stresses, while strigolactones play a role in root development, shoot branching, and symbiotic interactions with mycorrhizal fungi. These hormones are derived from the oxidative cleavage of carotenoids, highlighting their importance in plant development and adaptation. Carotenoids are indispensable for maximizing photosynthetic efficiency by extending the light absorption range and protecting the photosystems from light-induced damage. Without carotenoids, plants would be less efficient at capturing light energy and more susceptible to photoinhibition and oxidative stress, leading to reduced growth and productivity. The antioxidant properties of carotenoids are vital for protecting plants from the damaging effects of environmental stresses, such as high light intensity, drought, and temperature extremes. By scavenging ROS, carotenoids help maintain cellular integrity and prevent premature senescence, supporting the plant's overall health and resilience. The role of carotenoids in hormone biosynthesis underscores their importance in regulating key developmental processes and stress responses. The production of ABA and strigolactones from carotenoids links their presence to essential physiological functions, such as water use efficiency, root architecture, and plant-microbe interactions. This connection highlights the multifaceted roles of carotenoids in ensuring optimal plant growth and adaptation. Understanding the cellular mechanisms of carotenoids provides valuable insights for crop improvement

strategies. Enhancing carotenoid content in crops through genetic or agronomic approaches can improve photosynthetic efficiency, stress tolerance, and overall yield. This is particularly important in climate change, where plants face increasing environmental challenges. Carotenoids are central to the efficient functioning of the photosynthetic apparatus, protection against oxidative stress, and regulating plant growth and development. Their diverse roles make them essential for plant survival and productivity, justifying their critical importance in plant biology.

Table-4.13 (a): Effect of spacing and nutrients on Carotenoids (mg g^{-1} Fresh Weight) of the mustard crop during the rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	2.35	2.42	2.80	2.81	3.03	3.06
M2 (20×10)	1.94	1.97	2.41	2.43	2.57	2.62
C.D. at p<0.05	0.03	0.23	0.29	0.28	0.007	0.10
SEM±	0.00	0.03	0.04	0.04	0.001	0.01
Nutrients foliar application						
S0-Control	1.40	1.42	0.97	0.97	1.78	1.80
S1-Boron @1%	2.72	2.75	1.96	1.97	3.17	3.19
S2-Sulphur @ 0.15%	2.17	2.20	3.68	3.69	3.84	3.87
S3-BAP @0.003%	2.06	2.09	3.73	3.74	2.79	2.83
S4-Boron @0.5% +Sulphur @0.25%	1.50	1.60	2.15	2.16	2.74	2.86
S5-Boron @ 1.5%+ Sulphur @0.075%	1.81	1.83	2.50	2.52	2.52	2.54
S6-Boron @ 0.5% + BAP (@0.0045%)	2.51	2.54	2.85	2.86	2.87	2.90
S7-Boron @ 1.5%+ BAP (@0.0015%)	2.53	2.64	3.42	3.43	3.40	3.43
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	2.34	2.44	2.62	2.64	2.74	2.77
S9-Sulphur @0.25%+ BAP (@0.0015%)	2.43	2.47	2.20	2.22	2.14	2.17
C.D. at p<0.05	0.03	0.1	0.31	0.31	0.023	0.07
SEM±	0.01	0.03	0.10	0.10	0.00	0.02
C.D. S×M at p<0.05	0.49	0.49	0.03	0.12	0.05	0.22
SEM±	0.14	0.13	0.00	0.05	0.02	0.12
C.D. M×S at p<0.05	0.49	0.48	0.03	0.13	0.05	0.26
SEM±	0.15	0.15	0.01	0.04	0.02	0.06

Where, C.D. represents critical difference, SE (m) represents standard error of mean and SE (d) represents the standard error of deviation.

Table 4.12 (b): Interaction effect of spacing and nutrient on Carotenoids (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 30DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	0.817	2.277	3.433	4.92	2.18	3.153	3.257	2.96	2.617	2.403	2.802
M2	1.12	1.65	3.923	2.543	2.117	1.85	2.433	3.877	2.627	1.993	2.413
Mean B	0.968	1.963	3.678	3.732	2.148	2.502	2.845	3.418	2.622	2.198	
C.D. S×M at p<0.05						0.49					
SEM±						0.14					
C.D. M×S at p<0.05						0.49					
SEM±						0.15					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	0.827	2.287	3.447	4.93	2.197	3.17	3.27	2.97	2.637	2.42	2.815
M2	1.13	1.67	3.943	2.567	2.133	1.87	2.453	3.897	2.657	2.03	2.435
Mean B	0.978	1.978	3.695	3.748	2.165	2.52	2.862	3.433	2.647	2.225	
C.D. S×M at p<0.05						0.49					
SEM±						0.13					
C.D. M×S at p<0.05						0.48					
SEM±						0.15					

Table 4.12 (c): Interaction effect of spacing and nutrient on Carotenoids (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 60DAS

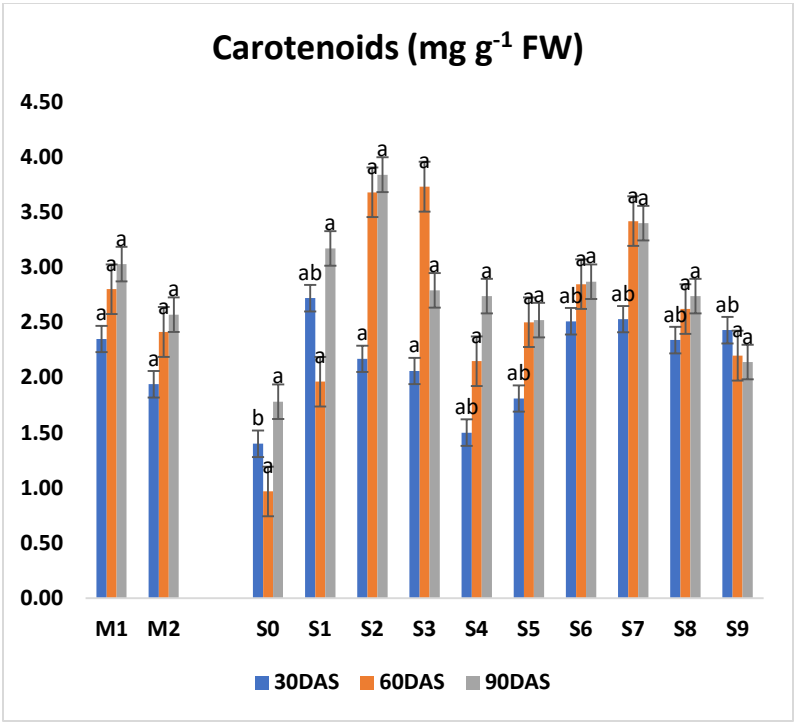
2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	2.143	3.417	3.84	2.977	3.34	3.137	3.32	2.957	2.85	2.35	3.033
M2	1.42	2.937	3.847	2.62	2.153	1.903	2.437	3.85	2.643	1.94	2.575
Mean B	1.782	3.177	3.843	2.798	2.747	2.52	2.878	3.403	2.747	2.145	
C.D. S×M at p<0.05						0.03					
SEM±						0.00					
C.D. M×S at p<0.05						0.03					
SEM±						0.01					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	2.167	3.44	3.867	3.013	3.367	3.163	3.353	2.99	2.88	2.37	3.061
M2	1.437	2.947	3.883	2.657	2.363	1.933	2.457	3.88	2.673	1.98	2.621
Mean B	1.802	3.193	3.875	2.835	2.865	2.548	2.905	3.435	2.777	2.175	
C.D. S×M at p<0.05						0.12					
SEM±						0.05					
C.D. M×S at p<0.05						0.13					
SEM±						0.04					

Table 4.12 (d): Interaction effect of spacing and nutrient on Carotenoids (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 90DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.483	2.733	2.93	2.237	1.547	1.52	3.097	2.54	2.557	2.94	2.358
M2	1.333	2.723	1.423	1.887	1.47	2.11	1.94	2.533	2.137	1.937	1.949
Mean B	1.408	2.728	2.177	2.062	1.508	1.815	2.518	2.537	2.347	2.438	
C.D. S×M at p<0.05						0.05					
SEM±						0.02					
C.D. M×S at p<0.05						0.05					
SEM±						0.02					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.497	2.757	2.953	2.263	1.71	1.54	3.13	2.713	2.737	2.987	2.429
M2	1.357	2.753	1.46	1.92	1.49	2.13	1.963	2.57	2.157	1.953	1.975
Mean B	1.427	2.755	2.207	2.092	1.6	1.835	2.547	2.642	2.447	2.47	
C.D. S×M at p<0.05						0.22					
SEM±						0.12					
C.D. M×S at p<0.05						0.26					
SEM±						0.06					



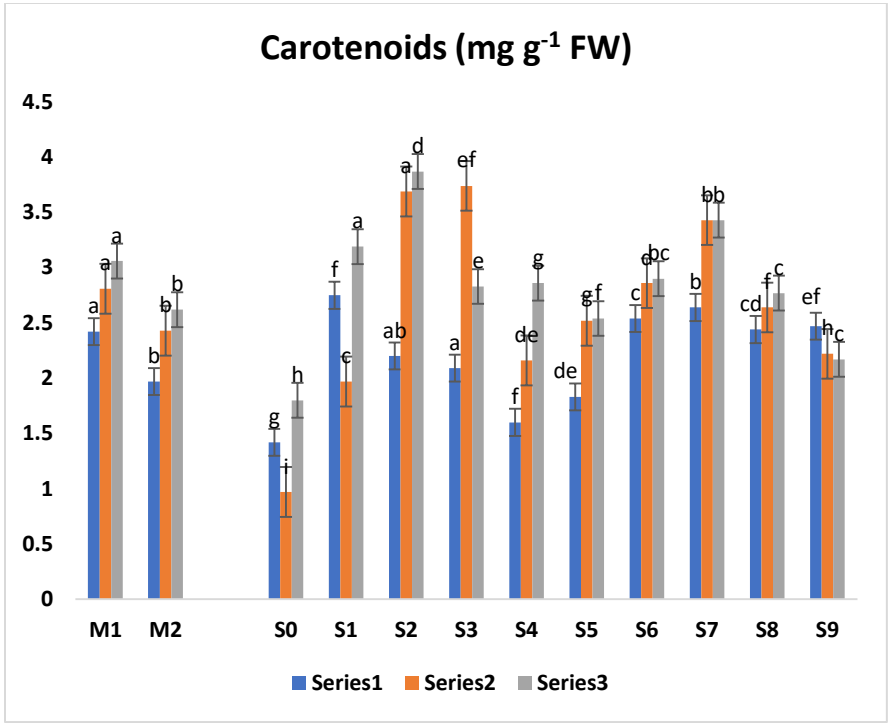


Fig-4.13 (a): Effect of spacing and nutrient on Carotenoids of mustard crop during rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.3.6 Chlorophyll index (SPAD Unit)

Mustard crops undergo various changes when grown under different nutrient levels. In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in chlorophyll index were observed at 30DAS, 60DAS and 90DAS, as shown in Table 4.14, Fig 4.14. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the chlorophyll index in each treatment compared to control of both the spacings at 30, 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the chlorophyll index was observed at 30, 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, main plot M2 shows maximum chlorophyll index as compared to M1 with values 41.33 (M2) and 37.11 (M1), respectively. A percentage increase of 10.21% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in chlorophyll index was observed in S9, i.e. 44.81 at 30DAS, where in S9, Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S9, followed by S8> S2> S1> S4> S3> S6> S5, and the per cent values were 26.90%, 24.66%, 23.39%, 18.54%, 17.44%, 15.42%, 37.8% and 9.04% respectively. At 60DAS, main plot M1 shows a maximum chlorophyll index compared to M2 with values of 36.75 (M1) and 35.72 (M2), respectively. A percentage increase of 2.80% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase was found in S8 with a value of 40.2, where Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop as a foliar application. The per cent increase was found highest in S8, followed by S4> S7> S9> S6> S1> S2> S3 and the per cent values were 19.90%,

16.18%, 14.28%, 11.90%, 11.57%, 9.29%, 8.789% and 8.69% respectively when it is compared with its control (S0). At 90DAS, main plot M1 shows a maximum chlorophyll index compared to M2 with values of 43.7 (M1) and 43.1 (M2), respectively. A percentage increase of 1.37% was found in M2, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S6 with a value of 45.48 where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S6, followed by S2> S7> S1> S3> S8> S5> S9> S4, and the per cent values were 15.66%, 14.88%, 14.75%, 14.37%, 12.42%, 11.51%, 10.89%, 10.65% and 9.02% respectively when it is compared with its control (S0).

The study showed a significant increase with 13.33%, 11.57% and 15.66% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S8, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the carotenoid content was found in treatment S6, where the combined application of boron and cytokinin is applied to the crop when compared to its control (S0).

In the year (2022-23), at 30DAS, main plot M2 shows maximum chlorophyll index as compared to M1 with values 41.79 (M2) and 37.57 (M1), respectively. A percentage increase of 10.09% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in chlorophyll index was observed in S9, i.e. 45.54 at 30DAS, where in S9, Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S9, followed by S8> S2> S1> S4> S3> S6> S5, and the per cent values were 27.23%, 23.19%, 18.45%, 17.43%, 15.43%, 13.95% and 8.71% respectively. At 60DAS, main plot M1 shows a maximum chlorophyll index compared to M2 with values of 37.40 (M1) and 36.21 (M2), respectively. A percentage increase of 3.18% was found in M1, where the crop was grown in spacing (30*10). In subplots, a

significant increase was found in S8 with a value of 40.7, where Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop as a foliar application. The per cent increase was found highest in S8, followed by S4> S7> S9> S6> S1> S3> S2, and the per cent values were 19.60%, 15.72%, 14.15%, 12.44%, 11.18%, 9.41%, 8.74% and 8.47% respectively when it is compared with its control (S0). At 90DAS, main plot M1 shows a maximum chlorophyll index compared to M2 with values of 44.22 (M1) and 43.51 (M2), respectively. A percentage increase of 1.60% was found in M2, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S6 with a value of 45.87 where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S6, followed by S7> S1> S2> S3> S8> S5> S9> S4, and the per cent values were 15.36%, 14.67%, 14.63%, 14.25%, 12.68%, 11.13%, 10.78%, 10.22% and 9.19% respectively when it is compared with its control (S0).

The study showed a significant increase with 13.95%, 11.18% and 15.36% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the carotenoid content was found in treatment S6, where the combined application of boron and cytokinin is applied to the crop compared to its control (S0).

The chlorophyll index is a quantitative assessment of the chlorophyll concentration in plant leaves, which indicates the plant's ability to carry out photosynthesis and its general state of health. The green pigment chlorophyll in chloroplasts is vital in absorbing light energy and its subsequent conversion into chemical energy during photosynthesis. The following is an in-depth examination of the cellular processes implicated: Chlorophyll molecules predominantly absorb light in the blue (430-450 nm) and red (640-680 nm) electromagnetic spectrum wavelengths. The process of photosynthetic formation is initiated by the excitation of electrons in the chlorophyll

molecule through absorption. Excited electrons originating from chlorophyll translocate along the electron transport chain, generating ATP and NADPH. These molecules are crucial in synthesizing carbohydrates from carbon dioxide and water. The process of chlorophyll biosynthesis encompasses several enzymatic stages within the chloroplast. The process initiates with converting glutamate into porphyrins and advances through chlorophyll a and b synthesis. Necessary enzymes included in this group are 5-aminolevulinic acid dehydratase, porphobilinogen deaminase, and chlorophyll synthase. Light conditions, nutrient availability, and plant hormones regulate chlorophyll synthesis. For example, nitrogen and magnesium are indispensable for the synthesis of chlorophyll. As the leaves mature, chlorophyll undergoes degradation, a component of leaf senescence. This degradation leads to the yellowing of leaves as carotenoids accumulate more prominently. Several enzymes, such as chlorophyllase and pheophytinase, regulate this mechanism. Colourimetric assays such as the Arnon method are commonly used to quantify chlorophyll content. This method entails extracting chlorophyll with acetone and the subsequent absorbance measurement at particular wavelengths (645 nm and 663 nm). Spectrum photometric techniques determine the chlorophyll index by quantifying chlorophyll concentrations based on absorbance characteristics. There is a direct correlation between the chlorophyll index and the plant's photosynthetic capacity. A greater chlorophyll concentration signifies a greater capacity to absorb light and, as a result, increased photosynthetic activity. Typically, a higher chlorophyll index indicates superior plant health and growth. Photosynthesis efficiency measures a plant's capacity to carry out this essential process for maximum growth and productivity. Essential nitrogen, magnesium, and iron are indispensable for chlorophyll synthesis. Inadequate nutrient supply can decrease the amount of chlorophyll and hinder the process of photosynthesis, inhibiting plant growth. Hydrological stress, extreme temperatures, and light intensity can impact chlorophyll concentrations. Analysing the chlorophyll index enables the evaluation of the plant's reaction to environmental stress and the subsequent adaptation of management strategies. The chlorophyll index imparts valuable

information regarding the various phases of plant development. An elevated chlorophyll index during the early stages of the growing season is linked to vigorous growth and muscular development. Variations in chlorophyll index during plant maturation can indicate growth phase transitions. A robust correlation exists between the concentration of chlorophyll and the productivity of crops. Monitoring the chlorophyll index can assist in forecasting yield potential and directing management strategy to maximize productivity. Precision agriculture utilizing remote sensing technologies is increasingly incorporating the chlorophyll index. Remote sensing and uncrewed aerial vehicles can quantify chlorophyll concentrations across extensive regions, enabling prompt interventions and effective resource allocation. The chlorophyll index is essential for evaluating plant health, photosynthetic efficiency, and growth potential. Understanding and monitoring chlorophyll content enables farmers and researchers to make well-informed decisions to optimize crop management and improve agricultural productivity.

Table-4.14 (a): Effect of spacing and nutrient on Chl. Index (SPAD unit) of mustard crop during rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	36.75	37.4	37.11	37.57	43.7	44.22
M2 (20×10)	35.72	36.21	41.33	41.79	43.1	43.51
C.D. at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.45	0.49	0.98	1.01	0.59	0.57
Nutrients foliar application						
S0-Control	32.2	32.75	32.76	33.14	38.36	38.83
S1-Boron @1%	35.5	36.15	40.21	40.63	44.8	45.48
S2-Sulphur @ 0.15%	35.3	35.78	42.76	43.15	45.06	45.28
S3-BAP @0.003%	35.26	35.88	38.73	39.19	43.8	44.47
S4-Boron @0.5% +Sulphur @0.25%	38.41	38.86	39.68	40.13	42.16	42.76
S5-Boron @ 1.5%+ Sulphur @0.075%	34.98	35.48	36.01	36.3	43.05	43.52
S6-Boron @ 0.5% + BAP (@0.0045%)	36.41	36.87	37.8	38.51	45.48	45.87
S7-Boron @ 1.5%+ BAP (@0.0015%)	37.56	38.15	35.95	36.32	45	45.5
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	40.2	40.73	43.48	43.92	43.35	43.69
S9-Sulphur @0.25%+ BAP (@0.0015%)	36.55	37.4	44.81	45.54	42.93	43.25
C.D. at p<0.05	NS	NS	4.81	4.74	4.82	4.78
SEM±	1.72	1.69	1.67	1.64	1.5	1.51
C.D. S×M at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	3.11	3.20	1.42	1.57	1.87	1.82
C.D. M×S at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	2.44	2.43	2.35	2.32	2.10	2.10

Where, C.D. represents critical difference, SE (m) represents standard error of mean

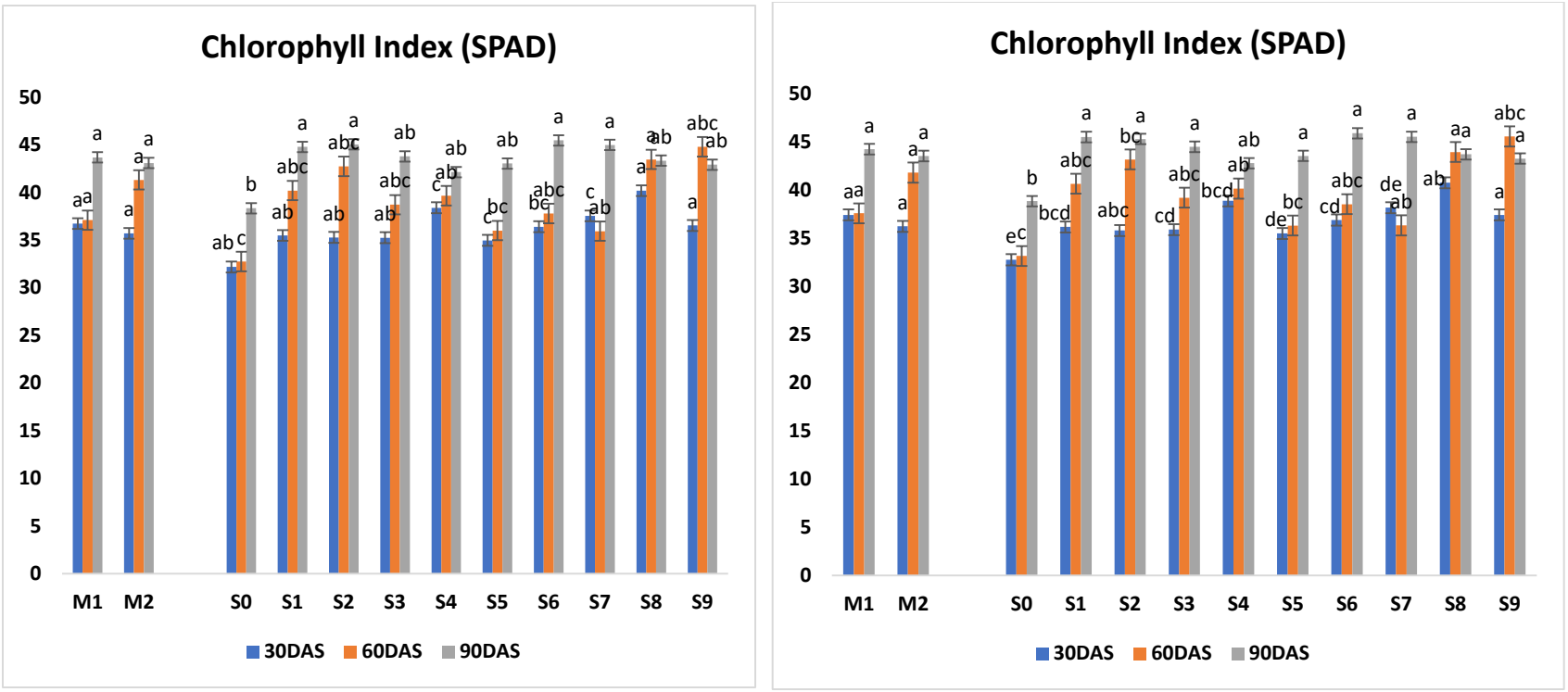


Fig-4.14 (a): Effect of spacing and nutrient on Chl. Index of mustard crop during rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.3.7 Total Soluble Sugars (TSS) (mg/g Fresh Weight)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in total soluble sugars (TSS) were observed at 30DAS, 60DAS and 90DAS, shown in Table 4.15, Fig 4.15. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the total soluble sugars (TSS) in each treatment compared to control of both the spacings at 30, 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the total soluble sugars (TSS) was observed at 30, 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, the main plot M2 shows maximum total soluble sugars (TSS) as compared to M1 with values of 1.43 (M2) and 1.15 (M1), respectively. A percentage increase of 19.58% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total soluble sugars (TSS) was observed in S9, i.e. 1.63 at 30DAS, where in S9, Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S9, followed by S8> S3> S2> S7> S4> S1> S6, and the per cent values were 52.20%, 51.69%, 48.54%, 47.54%, 46.70%, 41.94%, 29.82% and 28.00% respectively. At 60DAS, no such difference was shown in the main plots. The values of TSS obtained in the main plots were 2.39 and 2.39 in M1 (30*10) and M2 (20*10) respectively. In subplots, a significant increase was found in S5 with a value of 2.80, where Boron @ 1.5%+ Sulphur @0.075% was applied to the crop as a foliar application. The per cent increase was found highest in S5, followed by S3> S7> S4> S2> S1> S8> S9, and the per cent values were 36.95%, 36.60%, 31.73%, 26.49%, 26.29%, 23.92%, 23.27% and 22.74% respectively when it is compared with its

control (S0). At 90DAS, no such difference was shown in the main plots. The values of TSS obtained in the main plots were 5.83 and 5.83 in M1 (30*10) and M2 (20*10), respectively. In subplots, significant results were observed in S2 with a value of 6.20, where sulphur @ 0.15% was applied to the crop as a foliar spray. The per cent increase was found highest in S2, followed by S3> S1> S4> S8> S5> S7> S9> S6, and the per cent values were 40.18%, 39.65%, 39.36%, 39.06%, 38.84%, 38.44%, 38.43%, 38.40% and 37.62% respectively when it is compared with its control (S0).

The study showed a significant increase with 28.00%, 20.70% and 37.62% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the TSS content was found in treatment S3, where the application of cytokinin @ 0.0030% is applied to the crop when compared to its control (S0).

In the year (2022-23), at 30DAS, the main plot M2 shows maximum total soluble sugars (TSS) as compared to M1 with values 1.47 (M2) and 1.18 (M1), respectively. A percentage increase of 19.72% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total soluble sugars (TSS) was observed in S9, i.e. 1.64 at 30DAS, whereas in S9, Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S9, followed by S8> S3> S2> S7> S4> S1> S6, and the per cent values were 50.15%, 50%, 46.98%, 45.81%, 45.21%, 40.21%, 28.27% and 26.34% respectively. At 60DAS, the main plot M2 shows the maximum total soluble sugars (TSS) compared to M1, with values of 2.42 (M2) and 2.25 (M1) respectively. A percentage increase of 7.02% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S5 with a value of 2.81 where Boron @ 1.5%+ Sulphur @0.075% was applied to the crop as a foliar application. The per cent increase

was found highest in S5, followed by S3> S7> S4> S2> S1> S8> S9, and the per cent values were 41.38%, 38.35%, 34.95%, 32.28%, 32.51%, 28.20%, 28.77% and 28.31% respectively when it is compared with its control (S0). At 90DAS, the main plot M1 shows the maximum total soluble sugars (TSS) compared to M2, with values of 6.003 (M1) and 5.882 (M2), respectively. A percentage increase of 2.01% was found in M1, where the crop was grown at a reduced (30*10).

In subplots, significant results were observed in S2 with a value of 6.34, where sulphur @ 0.15% was applied to the crop as a foliar spray. The per cent increase was found highest in S2, followed by S1> S3> S4> S8> S5> S9> S7> S6, and the per cent values were 31.30%, 29.18%, 29.08%, 29.04%, 28.60%, 27.77%, 28.38%, 27.77 and 27.29% respectively when it is compared with its control (S0).

The study showed a significant increase with 26.34%, 13.76% and 27.29% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the TSS content was found in treatment S5, where the application of Boron @ 1.5%+ Sulphur @0.075% is applied to the crop when compared to its control (S0). Increased crop growth and development rates and more significant biomass accumulation significantly increase the total sugar content in the mustard crop. Banerjee, A., Datta, J. K., & Mondal, N. K. (2012) show the application of fertilizer along with chemical fertilizer significantly increased the level of total soluble sugar in leaves of crop plants in comparison to the plots without the application of any form of fertilizer. Application of plant growth hormone cytokinin improves the translocation of photosynthates through the phloem to different parts of the plant.

Total soluble sugars (TSS) are critical components of plant metabolism, functioning as essential energy sources and signalling molecules. Many physiological and biochemical processes influence the accumulation and distribution of these substances within plant cells, emphasizing

their importance in the complex network of plant life. Among mustard crops, like other plants, sugars are predominantly synthesized by photosynthesis in the chloroplasts of leaf cells. Within this mechanism, light energy is converted into chemical energy, resulting in the synthesis of glucose and other sugars from carbon dioxide and water. The Calvin cycle is a sequence of enzyme-catalyzed processes in the chloroplast stroma that convert carbon dioxide into a 3-carbon sugar, subsequently transforming into glucose. These glucose molecules can synthesize additional soluble sugars such as sucrose and fructans. Phloem is the conduit through which soluble sugars produced in the leaves are conveyed to other plant tissues. This process entails the utilization of active transport mechanisms, such as sucrose transporters, to transfer sugars from source tissues (e.g., leaves) to sink tissues (e.g., roots and seeds). Once absorbed by sink tissues, sugars are promptly utilized for energy or stored as complex carbohydrates. Endocrine signals and metabolic requirements control the distribution of sugars to various plant tissues. Boron is vital for sugar transport, an essential process in carbohydrate metabolism, by stabilizing cell wall components and improving membrane permeability, thus directing efficient sugar transport. Sulphur is a necessary constituent of amino acids and proteins involved in photosynthesis and carbohydrate metabolism. Sufficient availability of sulphur can improve the efficiency of photosynthesis, hence leading to an increase in sugar productivity. This hormone exerts physiological effects on cell division and expansion, indirectly modulating sugar synthesis and buildup. In addition, cytokinin can influence sugar transport and metabolism by regulating enzyme activities implicated in these processes. Quantification of total soluble sugars provides a direct measure of photosynthetic efficiency and the general health of plants. Higher Total Soluble Solids (TSS) levels often indicate enhanced photosynthetic efficiency, which may be attributed to proper nutrient availability and efficient photosynthesis. Researchers can evaluate the efficiency of light energy conversion into usable carbohydrates in plants through the measurement of Total Secondary Solids (TSS). The measurement of Total Dissolved Solids (TSS) offers valuable information on the impact of various nutrient treatments, such as Boron, Sulphur, and Cytokinin, on the

production and accumulation of carbohydrates. If a specific treatment substantially increases Total Soluble Solids (TSS), the nutrient may improve photosynthesis or sugar transport, which is essential for crop productivity. Total soluble solids (TSS) levels are frequently associated with plant growth parameters and crop productivity. Elevated sugar levels can suggest superior energy availability for growth and development, resulting in augmented biomass and potentially enhanced yields. By monitoring Total Soluble Solids (TSS), scientists can forecast the influence of nutrient management on the whole cultivation performance. Total soluble solids (TSS) levels can also indicate the plant's reaction to stressful circumstances. Altered total soluble solids (TSS) levels may result from the disruption of sugar metabolism caused by nutrient deficiencies or other stress factors. Continuous monitoring of these changes facilitates comprehension of how nutrient treatments alleviate the impact of stress and enhance the adaptation of plants. In crops, especially those cultivated for their seeds or fruits, Total Suspended Solids (TSS) plays a vital role in assessing the quality of the produce. The correlation between higher sugar content and improved taste and quality of the final product underscores the significance of TSS measurement in evaluating yield and quality. The quantification of total soluble sugars in mustard crops yields significant insights into the plant's photosynthetic efficiency, nutrient reactivity, and general well-being. Understanding the cellular processes involved in sugar production and accumulation is crucial for justifying the significance of nutrient management techniques in maximizing crop growth and productivity.

Table 4.15 (a): Effect of spacing and nutrient on TSS (mg g^{-1} Fresh Weight) of mustard crop during rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	1.15	1.18	2.29	2.25	5.83	6
M2 (20×10)	1.44	1.47	2.29	2.32	5.83	5.88
C.D. at p<0.05	NS	NS	0.05	0.06	0.08	0.10
SEM±	0.11	0.11	0.01	0.01	0.13	0.13
Nutrients foliar application						
S0-Control	0.78	0.82	1.77	1.65	3.71	4.36
S1-Boron @1%	1.11	1.14	2.32	2.29	6.11	6.15
S2-Sulphur @ 0.15%	1.48	1.51	2.4	2.44	6.2	6.34
S3-BAP @0.003%	1.51	1.54	2.79	2.67	6.14	6.14
S4-Boron @0.5% +Sulphur @0.25%	1.34	1.37	2.4	2.43	6.08	6.14
S5-Boron @ 1.5%+ Sulphur @0.075%	0.94	0.97	2.8	2.81	6.02	6.03
S6-Boron @ 0.5% + BAP (@0.0045%)	1.08	1.13	1.23	1.41	5.94	5.99
S7-Boron @ 1.5%+ BAP (@0.0015%)	1.46	1.49	2.59	2.53	6.02	6.03
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	1.61	1.64	2.3	2.31	6.06	6.1
S9-Sulphur @0.25%+ BAP (@0.0015%)	1.63	1.64	2.29	2.3	6.02	6.08
C.D. at p<0.05	0.37	0.37	0.16	0.15	0.53	0.54
SEM±	0.13	0.13	0.05	0.05	0.18	0.18
C.D. S×M at p<0.05	0.72	0.74	0.23	0.22	0.92	0.93
SEM±	0.34	0.34	0.02	0.02	0.32	0.32
C.D. M×S at p<0.05	0.81	0.83	0.22	0.21	0.99	0.99
SEM±	0.20	0.20	0.07	0.07	0.32	0.34

Where, C.D. represents critical difference, SE (m) represents standard error of mean and SE (d) represents the standard error of deviation.

Table 4.14 (a): Interaction effect of spacing and nutrient on TSS (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 30DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	0.627	1.123	1.587	1.143	1.313	0.897	1.077	1.42	1.167	1.2	1.155
M2	0.933	1.1	1.387	1.887	1.377	0.987	1.09	1.51	2.06	2.063	1.439
Mean B	0.78	1.112	1.487	1.515	1.345	0.942	1.083	1.465	1.613	1.632	
C.D. S×M at p<0.05						0.72					
SEM±						0.34					
C.D. M×S at p<0.05						0.81					
SEM±						0.20					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	0.667	1.157	1.61	1.173	1.34	0.933	1.11	1.45	1.197	1.21	1.185
M2	0.99	1.13	1.417	1.92	1.403	1.023	1.117	1.543	2.083	2.08	1.471
Mean B	0.828	1.143	1.513	1.547	1.372	0.978	1.113	1.497	1.64	1.645	
C.D. S×M at p<0.05						0.74					
SEM±						0.34					
C.D. M×S at p<0.05						0.83					
SEM±						0.20					

Table 4.14 (a): Interaction effect of spacing and nutrient on TSS (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 60DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.49	2.22	2.43	2.493	2.4	2.777	1.533	2.423	2.27	2.25	2.229
M2	1.78	2.33	2.403	2.793	2.407	2.81	1.233	2.593	2.307	2.29	2.295
Mean B	1.635	2.275	2.417	2.643	2.403	2.793	1.383	2.508	2.288	2.27	
C.D. S×M at p<0.05						0.23					
SEM±						0.02					
C.D. M×S at p<0.05						0.22					
SEM±						0.07					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.513	2.24	2.46	2.527	2.437	2.797	1.553	2.45	2.3	2.28	2.256
M2	1.803	2.357	2.43	2.827	2.437	2.833	1.273	2.623	2.333	2.323	2.324
Mean B	1.658	2.298	2.445	2.677	2.437	2.815	1.413	2.537	2.317	2.302	
C.D. S×M at p<0.05						0.22					
SEM±						0.02					
C.D. M×S at p<0.05						0.21					
SEM±						0.07					

Table 4.14 (a): Interaction effect of spacing and nutrient on TSS (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 90DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	4.96	6.1	6.397	6.073	6.07	5.96	5.953	5.987	6.087	5.98	5.957
M2	3.71	6.117	6.203	6.147	6.09	6.027	5.947	6.027	6.067	6.023	5.836
Mean B	4.335	6.108	6.3	6.11	6.08	5.993	5.95	6.007	6.077	6.002	
C.D. S×M at p<0.05						0.92					
SEM±						0.32					
C.D. M×S at p<0.05						0.99					
SEM±						0.32					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	4.987	6.153	6.46	6.117	6.123	6.01	6.007	6.013	6.117	6.043	6.003
M2	3.74	6.16	6.233	6.18	6.167	6.063	5.987	6.06	6.097	6.133	5.882
Mean B	4.363	6.157	6.347	6.148	6.145	6.037	5.997	6.037	6.107	6.088	
C.D. S×M at p<0.05						0.93					
SEM±						0.32					
C.D. M×S at p<0.05						0.99					
SEM±						0.34					

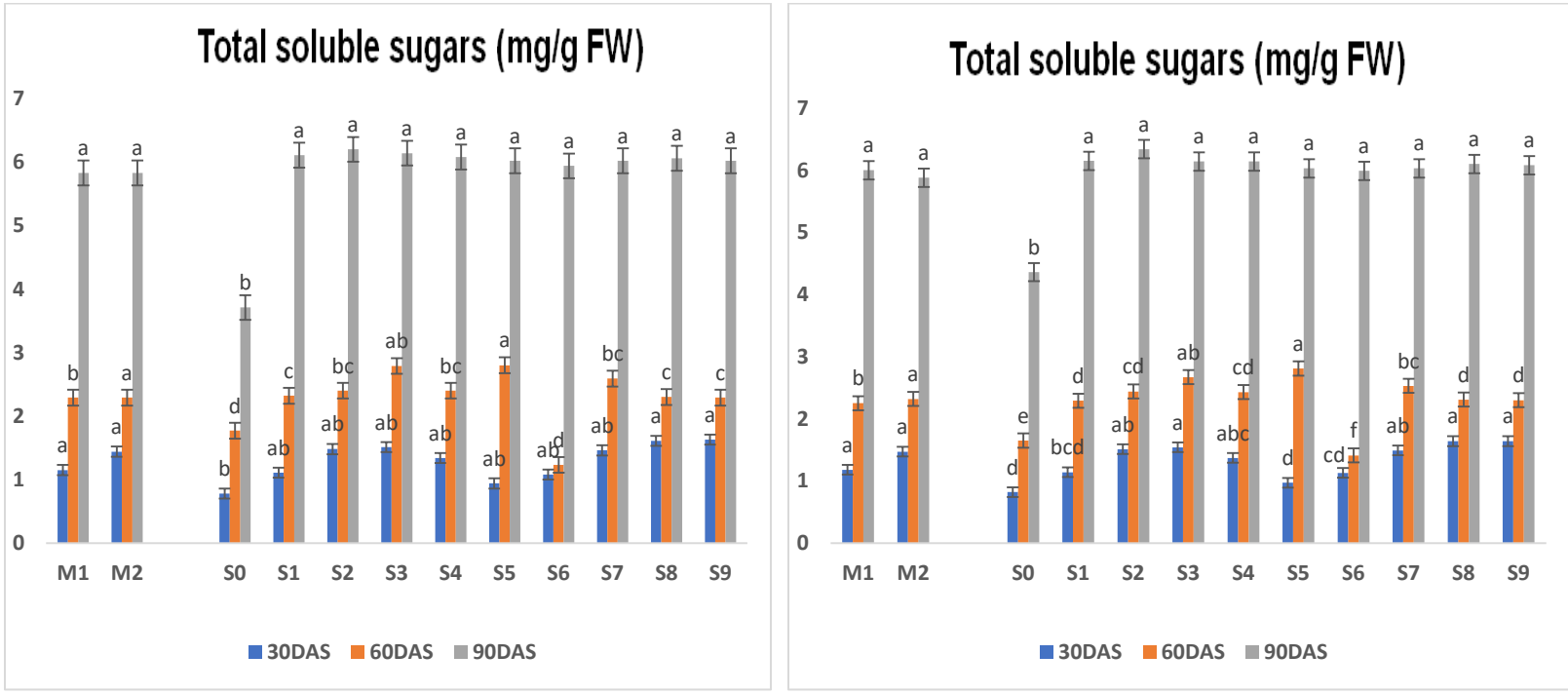


Fig-4.15 (a): Effect of spacing and nutrient on TSS of mustard crop during rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.3.8 Total starch (mg g^{-1} Fresh Weight)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in total starch were observed at 30DAS, 60DAS and 90DAS, shown in Table 4.16, Fig 4.16. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the total starch in each treatment compared to control of both the spacings at 30, 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the total starch was observed at 30, 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, main plot M2 shows maximum total starch as compared to M1 with values 1.44 (M2) and 1.37 (M1), respectively. A percentage increase of 4.86% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total starch was observed in S9, i.e. 1.77 at 30DAS, where in S9, Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S9, followed by S8> S3> S2> S7> S4> S1> S6, and the per cent values were 52.64%, 52.14%, 49.02%, 48.03%, 47.19%, 42.48%, 48.03% and 28.66 respectively. At 60DAS, the main plot M2 shows maximum total starch compared to M1, with values of 2.59 (M2) and 2.52 (M1), respectively. A percentage increase of 2.70% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S5 with a value of 3.03, where Boron @ 1.5%+ Sulphur @0.075% was applied to the crop as a foliar application. The per cent increase was found highest in S5, followed by S3> S7> S4> S2> S1> S8> S9, and the per cent values were 41.67%, 38.37%, 35.04%, 32.26%, 32.59%, 28.37%, 28.88% and 28.27% respectively when it is compared with its control (S0). At 90DAS, the main

plot M1 shows maximum total starch compared to M2, with values of 6.47 (M1) and 6.34 (M2), respectively. A percentage increase of 2.0% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S2 with a value of 6.84, where sulphur @ 0.15% was applied to the crop as a foliar spray. The per cent increase was found highest in S2 followed by S3> S1> S4> S5> S8> S7> S9> S6, and the per cent values were31.21%, 29.09%, 29.08%, 28.72%, 27.70%, 28.68%, 27.84%, 27.81% and 27.18% respectively when it is compared with its control (S0).

The study showed a significant increase with 28.66%, 29.32% and 27.18% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, the total starch content was significantly increased in treatment S5, where boron and sulphur were combined into the crop. The aqueous application of Boron @ 1.5%+ Sulphur @0.075% is applied to the crop when compared to its control (S0).

In the year (2022-23), at 30DAS, main plot M2 shows maximum total starch as compared to M1 with values 1.46 (M2) and 1.38 (M1), respectively. A percentage increase of 5.47% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total starch was observed in S9, i.e. 1.78 at 30DAS, where in S9, Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S9, followed by S8> S3> S2> S7> S4> S1> S6, and the per cent values were 51.82%, 51.41%, 48.19%, 47.23%, 46.58%, 41.69%, 29.79% and 27.42% respectively. At 60DAS, the main plot M2 shows maximum total starch compared to M1, with values of 2.61 (M2) and 2.54 (M1), respectively. A percentage increase of 2.68% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S5 with a value of 3.05, where Boron @ 1.5%+ Sulphur @0.075% was applied to the crop as a foliar application. The per cent increase was found highest in S5, followed by S3> S7>

S2> S4> S8> S1> S9, and the per cent values were 41.40%, 38.06%, 34.79%, 32.32%, 32.06%, 28.68% and 27.96% respectively when it is compared with its control (S0). At 90DAS, the main plot M1 shows maximum total starch compared to M2, with values of 6.49 (M1) and 6.36 (M2), respectively. A percentage increase of 2.0% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S2 with a value of 6.86, where sulphur @ 0.15% was applied to the crop as a foliar spray. The per cent increase was found highest in S2, followed by S3&S1> S4> S5> S8> S7> S9> S6, and the per cent values were 31.04%, 28.97%, 28.97%, 28.65%, 27.62%, 28.65%, 27.78%, 27.73% and 27.11% respectively when it is compared with its control (S0).

The study showed a significant increase with 27.42%, 29.10% and 27.11% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the total starch content was found in treatment S5, where the combined application of boron and sulphur was applied to the crop. The aqueous application of Boron @ 1.5%+ Sulphur @0.075% is applied to the crop compared to its control (S0).

Starch is a crucial carbohydrate reserve in plants, synthesized and stored primarily in plastids, such as chloroplasts in green tissues and amyloplasts in non-green tissues. The synthesis of starch involves a series of biochemical and cellular processes. The primary carbon source for starch synthesis is glucose, produced during photosynthesis. In the chloroplasts, light energy is converted into chemical energy, resulting in the fixation of carbon dioxide into glucose through the Calvin cycle. The glucose produced in the chloroplast is converted into ADP-glucose, the immediate precursor for starch synthesis. This activation involves the enzyme adenosine diphosphate glucose pyrophosphorylase (ADP-glucose pyrophosphorylase), which catalyses the reaction between glucose-1-phosphate and ATP to form ADP-glucose and pyrophosphate. The

enzyme starch synthase then uses the ADP-glucose to extend the glucan chains. Starch synthase catalyses the addition of glucose units from ADP-glucose to the growing starch chain. Starch synthase is responsible for the linear amylose component of starch. The enzyme branching enzyme (also known as branching enzyme or 4:6-α-dextrinotransferase) introduces α-1,6-glycosidic branches into the linear chains, forming amylopectin, the branched component of starch. This branching enhances the solubility and digestibility of starch. The synthesized amylose and amylopectin molecules aggregate to form insoluble starch granules within the plastids. These granules serve as a storage form of glucose, which can be mobilized during periods of energy demand. When the plant requires energy or carbon for growth, the stored starch is broken down into glucose units through the action of enzymes such as amylase and debranching enzymes. The glucose is then transported to various plant parts for metabolism and growth. Total starch content is crucial in assessing plant health, productivity, and nutritional quality. Here are several reasons why measuring total starch is important: Starch serves as the primary carbohydrate reserve in plants. Measuring total starch provides insight into the plant's ability to store energy and overall carbohydrate economy. Higher starch content generally indicates a greater reserve of energy that can be mobilized during periods of stress or growth. Starch accumulation is closely related to plant growth and yield. Plants with higher starch content often show better growth and higher yields, with more energy reserves available for growth and reproduction. Starch content is essential in determining the nutritional quality of plant-based foods. In crops like potatoes and cereals, starch content affects the food's texture, taste, and digestibility. Accurate measurement of total starch helps evaluate and improve the quality of these crops. Total starch content can be influenced by nutrient availability and application. By studying changes in starch content in response to different nutrient treatments, researchers can optimize fertilization practices to enhance crop performance and nutritional value. Plants under abiotic stress (e.g., drought, nutrient deficiency) often alter their starch storage and mobilization patterns. Measuring total starch helps understand how plants adapt to stress and can inform

strategies for improving crop stress resilience. In plant breeding and genetic research, total starch content is a key trait of interest. Breeding programs aimed at strengthening starch content can lead to the development of crops with enhanced yield and nutritional characteristics. Total starch measurement provides valuable information about a plant's carbohydrate reserves, growth potential, nutritional quality, and response to environmental and management conditions. It is a critical parameter for optimizing crop production, improving nutritional quality, and advancing plant research.

Table 4.16 (a): Effect of spacing and nutrients on Total Starch (mg g^{-1} Fresh Weight) of mustard crop during the rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	1.37	1.38	2.42	2.542	6.47	6.495
M2 (20×10)	1.44	1.46	2.49	2.613	6.34	6.363
C.D. at p<0.05	NS	NS	0.05	0.05	0.08	0.10
SEM±	0.04	0.04	0.01	0.01	0.1	0.1
Nutrients foliar application						
S0-Control	0.84	0.86	1.77	1.79	4.71	4.73
S1-Boron @1%	1.2	1.22	2.47	2.49	6.64	6.66
S2-Sulphur @ 0.15%	1.61	1.63	2.62	2.64	6.84	6.86
S3-BAP @0.003%	1.64	1.66	2.87	2.89	6.64	6.66
S4-Boron @0.5% +Sulphur @0.25%	1.45	1.47	2.61	2.63	6.60	6.63
S5-Boron @ 1.5%+ Sulphur @0.075%	1.02	1.04	3.03	3.05	6.51	6.53
S6-Boron @ 0.5% + BAP (@0.0045%)	1.17	1.18	1.5	2.52	6.46	6.49
S7-Boron @ 1.5%+ BAP (@0.0015%)	1.59	1.61	2.72	2.74	6.52	6.55
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	1.75	1.77	2.48	2.51	6.60	6.63
S9-Sulphur @0.25%+ BAP (@0.0015%)	1.77	1.78	2.46	2.48	6.52	6.54
C.D. at p<0.05	0.49	0.49	0.17	0.24	0.68	0.68
SEM±	0.17	0.17	0.06	0.08	0.23	0.23
C.D. S×M at p<0.05	NS	NS	0.24	0.24	0.26	0.28
SEM±	0.13	0.13	0.02	0.02	0.32	0.32
C.D. M×S at p<0.05	NS	NS	0.23	0.23	0.24	0.26
SEM±	0.23	0.23	0.08	0.08	0.33	0.33

Where, C.D. represents critical difference, SE (m) represents standard error of mean and SE (d) represents the standard error of deviation.

Table 4.15 (b): Interaction effect of spacing and nutrient on Total starch (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 60DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.62	2.413	2.643	2.71	2.61	3.017	1.667	2.633	2.47	2.443	2.423
M2	1.93	2.53	2.61	3.033	2.62	3.053	1.34	2.817	2.507	2.49	2.493
Mean B	1.775	2.472	2.627	2.872	2.615	3.035	1.503	2.725	2.488	2.467	
C.D. S×M at p<0.05						0.24					
SEM±						0.02					
C.D. M×S at p<0.05						0.23					
SEM±						0.08					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	1.62	2.413	2.643	2.71	2.61	3.017	1.667	2.633	2.47	2.443	2.423
M2	1.93	2.53	2.61	3.033	2.62	3.053	1.34	2.817	2.507	2.49	2.493
Mean B	1.775	2.472	2.627	2.872	2.615	3.035	1.503	2.725	2.488	2.467	
C.D. S×M at p<0.05						0.24					
SEM±						0.02					
C.D. M×S at p<0.05						0.23					
SEM±						0.08					

Table 4.15 (c): Interaction effect of spacing and nutrient on Total starch (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 90DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	5.393	6.633	6.95	6.603	6.597	6.477	6.47	6.503	6.613	6.503	6.474
M2	4.037	6.65	6.74	6.683	6.617	6.55	6.463	6.547	6.59	6.547	6.342
Mean B	4.715	6.642	6.845	6.643	6.607	6.513	6.467	6.525	6.602	6.525	
C.D. S×M at p<0.05						0.26					
SEM±						0.32					
C.D. M×S at p<0.05						0.24					
SEM±						0.33					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	5.393	6.633	6.95	6.603	6.597	6.477	6.47	6.503	6.613	6.503	6.474
M2	4.037	6.65	6.74	6.683	6.617	6.55	6.463	6.547	6.59	6.547	6.342
Mean B	4.715	6.642	6.845	6.643	6.607	6.513	6.467	6.525	6.602	6.525	
C.D. S×M at p<0.05						0.28					
SEM±						0.32					
C.D. M×S at p<0.05						0.26					
SEM±						0.33					

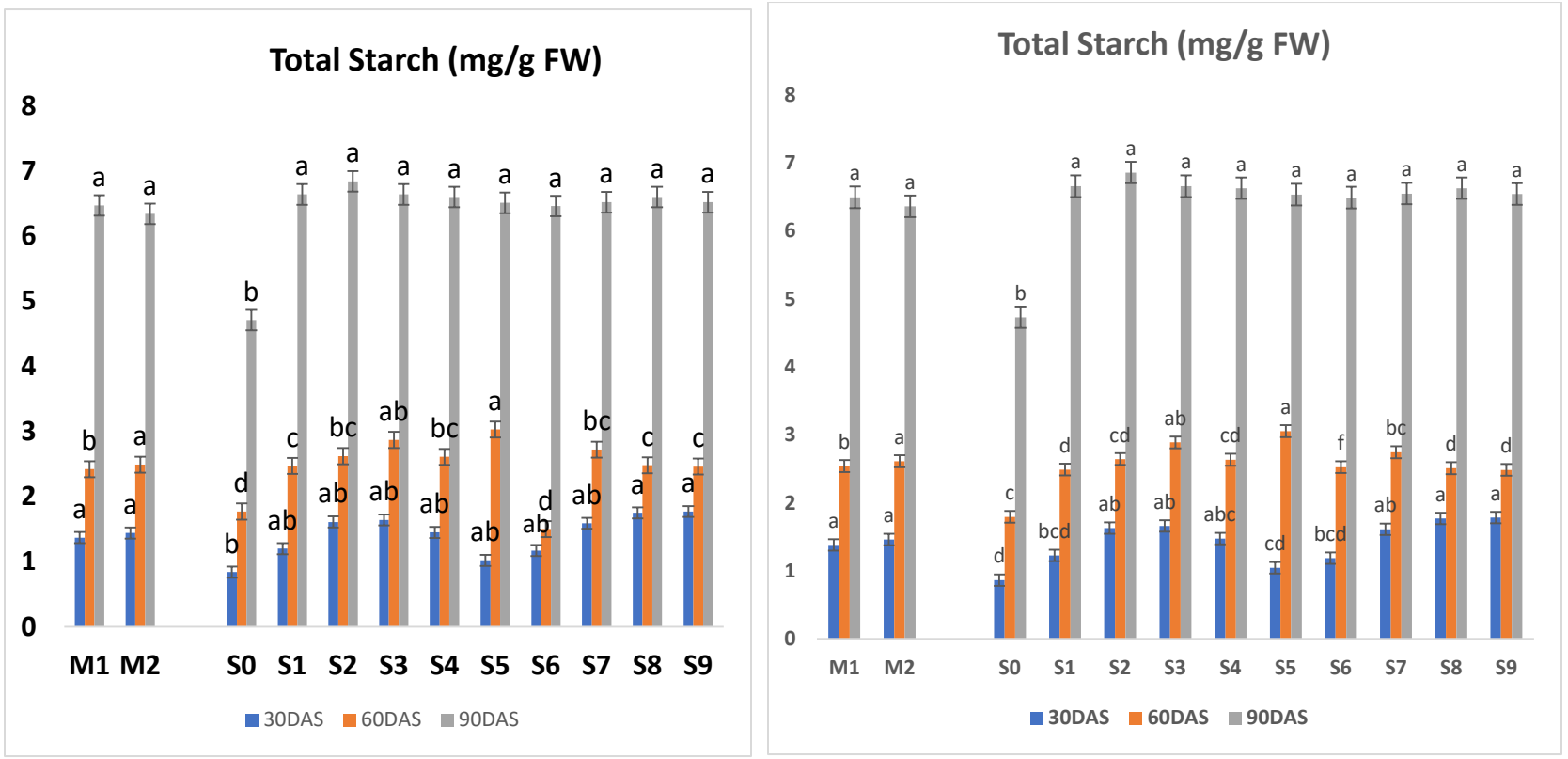


Fig-4.16 (a): Effect of spacing and nutrient on Total Starch of mustard crop during rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.3.9 Total soluble protein (TSP) (mg g^{-1} Fresh Weight)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Total soluble protein (TSP) changes were observed at 30DAS, 60DAS and 90DAS, as shown in Table 4.17, Fig 4.17. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the total soluble protein (TSP) in each treatment compared to control of both the spacings at 30, 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the total soluble protein (TSP) was observed at 30, 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, the main plot M2 shows maximum total soluble protein (TSP) as compared to M1 with values of 52.92 (M2) and 52.13 (M1), respectively. A percentage increase of 1.49% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total soluble protein (TSP) was observed in S8, i.e. 53.87 at 30DAS, whereas in S8, Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S8, followed by S2> S1> S7> S9> S5> S3> S6, and the per cent values were 5.46%, 5.07%, 4.03%, 3.70%, 3.27%, 2.65%, 2.37% and 1.90% respectively. At 60DAS, the main plot M2 shows the maximum total soluble proteins (TSP) compared to M1, with values of 115.49 (M2) and 110.47 (M1), respectively. A percentage increase of 4.28% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S8 with a value of 120.41, where Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop as a foliar application. The per cent increase was found highest in S8, followed by S2> S6> S3> S4> S9> S7> S5, and the per cent values

were 20.41%, 19.77%, 19.48%, 19.30%, 16.87%, 15.95%, 15.84% and 13.96% respectively when it is compared with its control (S0). At 90DAS, the main plot M2 shows the maximum total soluble protein (TSP) compared to M1, with values of 129.48 (M2) and 127.34 (M1), respectively. A percentage increase of 1.65% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S2 with a value of 133.80, where sulphur @ 0.15% was applied to the crop as a foliar spray. The per cent increase was found highest in S2, followed by S1> S3> S4> S8> S9> S5> S6, and the per cent values were 9.05%, 8.96%, 5.82%, 5.53%, 5.30%, 4.92%, 4.20% and 4.14% respectively when it is compared with its control (S0). The study showed a significant increase with 1.90%, 19.48% and 4.14% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the total soluble protein (TSP) was found in treatment S8, where the combined application of sulphur and BAP was applied to the crop. The aqueous application of Sulphur @ 0.075%+ BAP (@0.0045%) is applied to the crop compared to its control (S0).

In the year (2022-23), at 30DAS, the main plot M2 shows maximum total soluble protein (TSP) as compared to M1 with values of 52.95 (M2) and 52.17 (M1), respectively. A percentage increase of 1.47% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total soluble protein (TSP) was observed in S8, i.e. 53.91 at 30DAS, whereas in S8, Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S8, followed by S2> S1> S7> S9> S5> S3> S6, and the per cent values were 5.48%, 5.09%, 3.97%, 3.70%, 3.25%, 2.65%, 2.37% and 1.90% respectively. At 60DAS, the main plot M2 shows the maximum total soluble proteins (TSP) compared to M1, with values of 116.20 (M2) and 110.95 (M1), respectively. A percentage increase of 4.51% was found in M2, where the crop was grown

in reduced spacing (20*10). In subplots, a significant increase was found in S8 with a value of 120.86, where Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop as a foliar application. The per cent increase was found highest in S8, followed by S2> S6> S3> S4> S9> S7> S5, and the per cent values were 42.74%, 42.13%, 42.24%, 42.11%, 40.21%, 40.09%, 39.64% and 38.22% respectively when it is compared with its control (S0). At 90DAS, the main plot M2 shows the maximum total soluble protein (TSP) compared to M1, with values of 130.36 (M2) and 128.23 (M1), respectively. A percentage increase of 1.63% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S2 with a value of 134.72, where sulphur @ 0.15% was applied to the crop as a foliar spray. The per cent increase was found highest in S2, followed by S1> S3> S4> S8> S9> S6> S5, and the per cent values were 8.97%, 8.53%, 5.68%, 5.37%, 5.08%, 4.89%, 4.51% and 4.16% respectively when it is compared with its control (S0). The study showed a significant increase with 1.90%, 42.24% and 4.54% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the total soluble protein (TSP) was found in treatment S8, where the combined application of sulphur and BAP was applied to the crop. The aqueous application of Sulphur @ 0.075%+ BAP (@0.0045%) is applied to the crop compared to its control (S0). Banerjee (2012) reported that the total protein content in leaves varied significantly among the different studied varieties. Applying fertilizer and cycocel significantly promoted considerable variation among the other treatments. The level of total protein content increases when the level of translocated sugars increases in the plant. The application of compost significantly promoted the level of total protein content in leaves compared to control. Plant growth hormone cytokinin also plays a vital role in translocating sugar and carbohydrates to the various parts of the plant through phloem. Total soluble proteins in plants are crucial for multiple physiological processes, including growth, metabolism, and stress response. The synthesis and accumulation of soluble proteins are tightly

regulated at the cellular level and involve several fundamental mechanisms: The synthesis of soluble proteins begins with transcribing specific genes into messenger RNA (mRNA) in the nucleus. This mRNA then migrates to the cytoplasm, serving as a template for protein synthesis on ribosomes. Ribosomes translate the mRNA code into polypeptide chains, which are then folded into functional proteins. The availability of amino acids and other cofactors can influence translation efficiency. Newly synthesized proteins undergo folding and post-translational modifications, such as phosphorylation, glycosylation, and cleavage, which are essential for their functionality and stability. Misfolded or damaged proteins are targeted for degradation by the ubiquitin-proteasome system or the autophagy pathway, ensuring that only functional proteins accumulate in the cell. The availability of amino acids, derived from nutrient uptake and protein degradation, influences protein synthesis. An adequate supply of essential amino acids is necessary for optimal protein production. Plant cells use nutrient signalling pathways to regulate protein synthesis. For example, signalling pathways related to nitrogen and sulfur availability can affect the expression of genes involved in protein synthesis. Under abiotic or biotic stress conditions, plants often increase the synthesis of stress-responsive proteins, such as heat shock proteins (HSPs) and pathogenesis-related proteins. These proteins help mitigate damage and support cellular recovery. Soluble proteins can be stored in vacuoles or other cellular compartments. This storage is essential for maintaining protein reserves that can be mobilized during periods of high demand, such as during seed germination. The total soluble protein content is a crucial indicator of the plant's growth potential and metabolic activity. Higher levels of soluble proteins often correlate with enhanced growth and development, as these proteins play essential roles in cellular processes. The total soluble protein content reflects the plant's nutrient status, particularly nitrogen and sulfur. Adequate nutrient supply promotes optimal protein synthesis, while deficiencies can lead to reduced protein content and impaired growth. The measurement of total soluble proteins can provide insights into the plant's response to stress. An increase in specific stress-related proteins can indicate that the plant is activating defence

mechanisms to cope with adverse conditions. In crops, soluble protein content is directly related to the nutritional quality of the harvest. For example, higher protein content in seeds can enhance their value for human and animal consumption. Soluble protein levels can proxy for overall crop health and yield potential. Crops with higher protein content are often more productive and resilient. Measuring total soluble proteins allows for evaluating different nutrient treatments and their effects on plant physiology. This can guide the optimization of fertilization strategies and improve crop management practices. Total soluble protein measurement is valuable for assessing plant health, nutrient status, stress responses, and overall growth potential. Understanding the cellular mechanisms involved in protein synthesis and accumulation helps interpret the significance of protein levels in plant performance and productivity.

Table 4.17 (a): Effect of spacing and nutrient on TSP (mg g^{-1} Fresh Weight) of the mustard crop during the rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	52.14	52.17	110.47	110.95	127.35	128.23
M2 (20×10)	52.93	52.95	115.41	116.2	129.49	130.36
C.D. at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.1	0.32	1.95	1.89	1.15	1.2
Nutrients foliar application						
S0-Control	50.93	50.96	95.83	96.2	121.71	122.64
S1-Boron @1%	53.07	53.07	101.38	101.81	133.66	134.08
S2-Sulphur @ 0.15%	53.65	53.69	119.44	119.58	133.80	134.72
S3-BAP @0.003%	52.17	52.19	118.74	119.55	129.21	130.03
S4-Boron @0.5% +Sulphur @0.25%	51.85	51.88	115.27	115.75	128.81	129.6
S5-Boron @ 1.5%+ Sulphur @0.075%	52.32	52.35	111.38	112.01	127.03	127.96
S6-Boron @ 0.5% + BAP (@0.0045%)	51.92	51.95	119.02	119.8	126.96	128.44
S7-Boron @ 1.5%+ BAP (@0.0015%)	52.89	52.91	113.88	114.64	126.46	127.35
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	53.88	53.91	120.41	120.86	128.53	129.21
S9-Sulphur @0.25%+ BAP (@0.0015%)	52.65	52.67	114.02	115.52	128.00	128.95
C.D. at p<0.05	0.68	1.31	12.30	11.85	12.32	12.34
SEM±	0.23	0.45	4.27	4.11	3.61	3.42
C.D. S×M at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.32	1.02	6.19	5.99	3.65	3.80
C.D. M×S at p<0.05	NS	2.54	NS	NS	NS	NS
SEM±	0.33	0.69	6.05	5.83	4.98	4.81

Where, C.D. represents critical difference, SE (m) represents standard error of mean and SE (d) represents the standard error of deviation.

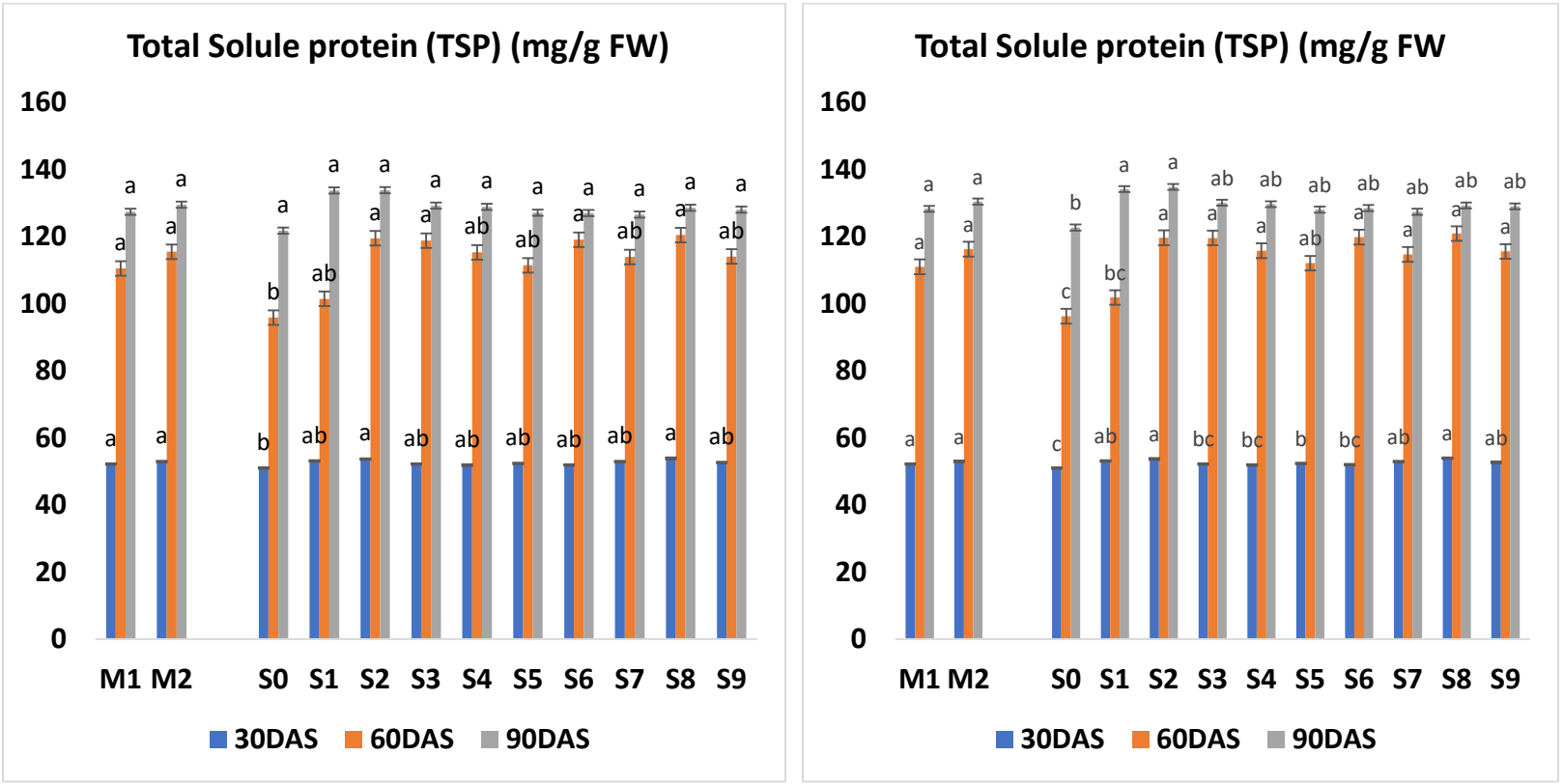


Fig-4.17 (a): Effect of spacing and nutrient on TSP of the mustard crop during rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.3.10 Total phenols (mg/g Fresh Weight)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in total phenols were observed at 30DAS, 60DAS and 90DAS, shown in Table 4.18, Fig 4.18. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the total phenols in each treatment compared to control of both the spacings at 30, 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the total phenols was observed at 30, 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, the main plot M2 shows maximum total phenols as compared to M1 with values of 24.76 (M2) and 24.53 (M1), respectively. A percentage increase of 0.92% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total phenols was observed in S9, i.e. 52.91 at 30DAS, where in S9, Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S9, followed by S8> S6> S4> S2> S3> S1> S5, and the per cent values were 75.69%, 54.89%, 47.63%, 45.47%, 45.46%, 40.51%, 35.88% and 35.02% respectively. At 60DAS, the main plot M2 shows the maximum total phenols compared to M1, with values of 44.84 (M2) and 44.73 (M1), respectively. A percentage increase of 0.24% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S6 with a value of 61.29, where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar application. The per cent increase was found highest in S6, followed by S2> S7> S5> S4> S8> S3> S9, and the per cent values were 51.77%, 48.83%, 38.395, 36.82%, 36.63%, 34.99%, 29.02% and 17.78% respectively when

it is compared with its control (S0). At 90DAS, the main plot M2 shows the maximum total phenols compared to M1, with values of 40.15 (M2) and 39.65 (M1), respectively. A percentage increase of 1.24% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S5 with a value of 54.66, where Boron @ 1.5%+ Sulphur @0.075% was applied to the crop as a foliar spray. The per cent increase was found highest in S5, followed by S1> S7> S3> S6> S9> S4> S2> S8, and the per cent values were 50.17%, 43.46%, 36.18%, 29.925, 28.85%, 28.76%, 27.28%, 26.46% and 25.05% respectively when it is compared with its control (S0). The study showed a significant increase with 47.63%, 51.77% and 28.85% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the total phenols was found in treatment S5, where the combined application of boron and sulphur was applied to the crop. The aqueous application of Boron @ 1.5%+ Sulphur @0.075% is applied to the crop compared to its control (S0). In the year (2022-23), at 30DAS, the main plot M2 shows maximum total phenols as compared to M1 with values of 25.65 (M2) and 25.20 (M1), respectively. A percentage increase of 1.75% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total phenols was observed in S9, i.e. 52.91 at 30DAS, where in S9, Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S9, followed by S8> S6> S4> S2> S3> S1> S5, and the per cent values were 74.86%, 53.54%, 46.20%, 44.78%, 44.38%, 40.64%, 35.48% and 34.06% respectively. At 60DAS, the main plot M2 shows the maximum total phenols compared to M1, with values of 45.40 (M2) and 45.25 (M1), respectively. A percentage increase of 0.33% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S6 with a value of 61.29, where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar application. The per cent increase was found highest in S6, followed by S2> S7> S5> S4> S8> S3> S9, and

the per cent values were 51.00%, 48.01%, 37.50%, 35.94%, 36.12%, 34.50%, 28.61% and 17.33% respectively when it is compared with its control (S0). At 90DAS, the main plot M2 shows the maximum total phenols compared to M1, with values of 41.10 (M2) and 40.62 (M1), respectively. A percentage increase of 1.16% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S5 with a value of 55.74, where Boron @ 1.5%+ Sulphur @0.075% was applied to the crop as a foliar spray. The per cent increase was found highest in S5, followed by S1> S7> S3> S6> S9> S4> S2> S8, and the per cent values were 49.69%, 43.01%, 35.90%, 29.64%, 39.42%, 28.57%, 26.34%, 25.92% and 25.14% respectively when it is compared with its control (S0). The study showed a significant increase with 46.20%, 51.00% and 28.87% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the total phenols was found in treatment S5, where the combined application of boron and sulphur was applied to the crop. The aqueous application of Boron @ 1.5%+ Sulphur @0.075% is applied to the crop compared to its control (S0). The maximum accumulation of phenol in leaves was observed in treatment where nutrients were applied. Enhanced phenol content levels in biofertilizer leaves and cystocele-treated plots were recorded concerning control. The application of compost was found to be stimulatory for phenol accumulation in the leaves of the mustard plant. A higher level of phenolic compounds has frequently been reported in organically grown crops than in conventionally grown crops. (Carbonaro et al., 2002; Lombardi Boccia et al., 2004; Sousa et al., 2005). Total phenols are a heterogeneous family of chemicals containing hydroxyl groups connected to aromatic rings, which have essential functions in plant protection, growth, and development. Investigating the biosynthesis, function, and interaction with other cellular components is necessary to comprehend the cellular mechanisms of these entities. The phenylpropanoid pathway is the main route of synthesis for phenolic compounds. The conversion of the amino acid phenylalanine into cinnamic

acid is initiated by the enzyme phenylalanine ammonia-lyase (PAL). Next, cinnamic acid undergoes additional modifications by enzymatic processes to produce phenolic compounds such as flavonoids, lignins, and tannins. The pivotal enzymes in this pathway are PAL, cinnamate-4-hydroxylase (C4H), 4-coumarate-CoA ligase (4CL), and several others that facilitate the synthesis of particular phenolic compounds. Every individual enzyme facilitates a unique stage in the biosynthesis process, leading to a diverse array of phenolic structures. The antioxidant properties of phenolic compounds are attributed to their ability to neutralize reactive oxygen species (ROS) and free radicals. Consequently, this mechanism safeguards cellular constituents, including lipids, proteins, and DNA, against oxidative harm. The phenolic compounds known as lignins play a crucial role in the composition of cell walls by imparting mechanical strength and rigidity. This phenomenon enhances plant defence by reducing the vulnerability of tissues to penetration by pathogens. Phenomenal compounds contribute to plant defence by repelling herbivores and suppressing the growth of pathogens. In addition, they can augment systemic acquired resistance (SAR) by transmitting signals and synchronizing defence reactions across the entire plant. The accumulation of phenolic compounds often increases in response to environmental stresses such as drought, salinity, and pathogen attacks. The process of accumulation described here is a defensive mechanism that strengthens the plant's ability to withstand unfavourable circumstances. The availability of vital nutrients can influence the synthesis and accumulation of phenolic compounds. The total phenol content is a reliable indicator of the plant's defence capacity. Elevated concentrations of phenolic bioactive compounds frequently correspond to improved defence mechanisms, suggesting that the plant is actively reacting to stress or pathogen aggression. Researchers can evaluate the plant's ability to withstand environmental pressures by quantifying the total phenol content. Increased phenol concentrations in stressed plants can indicate the plant's capacity to handle and adjust to unfavourable circumstances. Quantifying the total phenol content in crops, particularly those intended for human consumption or as medicinal plants, can indicate their quality. Phenolic

compounds are linked to various health advantages, such as their antioxidant and anti-inflammatory characteristics. The determination of total phenol content can also offer valuable information on possible yield results. Plants exhibiting well-balanced phenolic profiles tend to possess greater resilience and can significantly enhance agricultural productivity. The quantification of overall phenol concentration can assist in assessing the influence of nutrient management techniques on the well-being of plants. For instance, the application of specific nutrients could either augment or impede the synthesis of phenolic compounds, impacting the plant's overall performance. The analysis of total phenol content can serve as a criterion for selecting cultivars that exhibit increased resistance to stress and better nutritional characteristics in plant breeding initiatives. Quantifying the total phenol content of a plant yields essential insights into its biochemical condition, reactions to stress, and general wellness. An in-depth knowledge of the cellular processes involved in synthesizing phenolic compounds and their rationale in plant biology is crucial for optimizing crop management techniques, enhancing plant resilience, and increasing agricultural productivity.

Table 4.18 (a): Effect of spacing and nutrients on Total Phenols (mg g^{-1} Fresh Weight) of the mustard crop during the rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	24.53	25.20	44.73	44.25	39.65	40.62
M2 (20×10)	24.76	25.65	44.84	45.40	40.15	41.10
C.D. at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.21	0.22	0.19	6.72	0.1	0.11
Nutrients foliar application						
S0-Control	12.86	13.50	29.56	30.28	27.25	28.04
S1-Boron @1%	20.05	20.92	34.78	29.55	48.18	49.20
S2-Sulphur @ 0.15%	23.58	24.27	57.77	58.24	37.04	37.85
S3-BAP @0.003%	21.62	22.74	41.64	42.42	38.87	39.85
S4-Boron @0.5% +Sulphur @0.25%	23.58	24.45	46.65	47.40	37.46	38.06
S5-Boron @ 1.5%+ Sulphur @0.075%	19.79	20.47	46.79	47.26	54.66	55.74
S6-Boron @ 0.5% + BAP (@0.0045%)	24.55	25.09	61.29	61.80	38.28	39.42
S7-Boron @ 1.5%+ BAP (@0.0015%)	19.04	20.01	47.98	48.44	42.68	43.74
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	28.51	29.06	45.47	46.23	36.34	37.46
S9-Sulphur @0.25%+ BAP (@0.0015%)	52.91	53.70	35.95	36.63	38.24	39.25
C.D. at p<0.05	3.98	4.05	12.19	13.7	13.35	13.31
SEM±	1.38	1.40	4.23	4.75	4.63	4.62
C.D. S×M at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.68	0.71	0.61	2.37	0.34	0.36
C.D. M×S at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	1.87	1.90	5.68	6.42	6.22	6.20

Where, C.D. represents critical difference, SE (m) represents standard error of mean

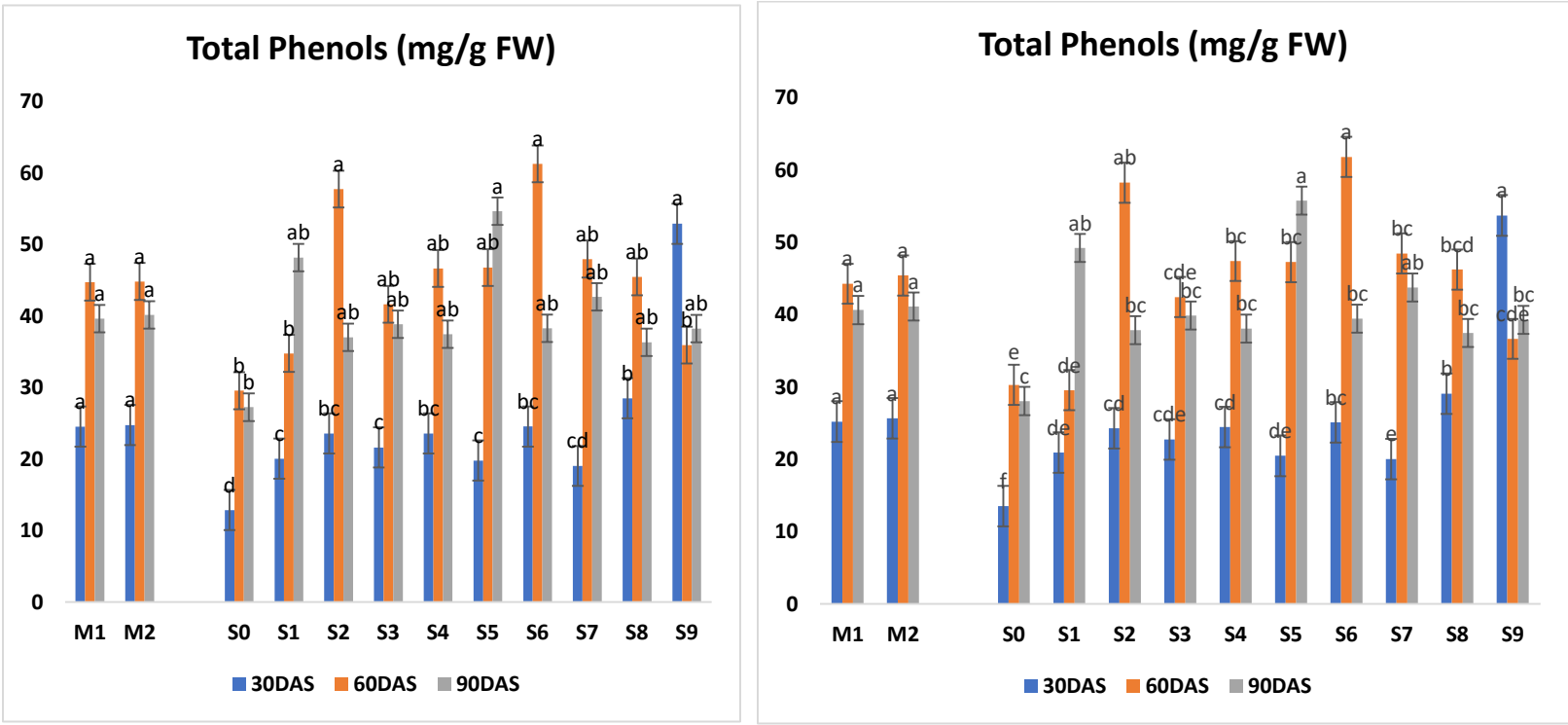


Fig-4.18 (a): Effect of spacing and nutrient on Total Phenols of the mustard crop during rabi season of 2021-22.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.3.11 Total Flavanols (mg/g Fresh Weight)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the years 2021-22 and 2022-23. Flavonols were observed at 30DAS, 60DAS and 90DAS, as shown in Table 4.19, Fig 4.19. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the total Flavonols in each treatment compared to the control of both the spacings at 30, 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the total Flavonols was observed at 30, 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments.

In the year (2021-22), at 30DAS, main plot M2 shows maximum total Flavonols as compared to M1 with values 6.60 (M2) and 6.18 (M1), respectively. A percentage increase of 6.36% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total Flavonols was observed in S6, i.e. 7.20 at 30DAS, where in S6, Boron @ 0.5% + BAP (@0.0045%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S6, followed by S7> S1> S2> S3> S4> S9> S8&S5, and the per cent values were 39.51%, 36.81%, 36.13%, 34.44%, 34.34%, 32.34%, 31.02%, 30.68, 30.68% respectively. At 60DAS, main plot M2 shows the maximum total Flavonols compared to M1 with values of 10.14 (M2) and 9.25 (M1), respectively. A percentage increase of 8.77% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S4 with a value of 13.39, where Boron @0.5% + sulphur @0.25% was applied to the crop as a foliar application. The per cent increase was found highest in S4, followed by S1> S9> S5> S2> S8> S6> S7, and the per cent values were 48.76%, 43.20%, 40.54%, 34.70%, 32.10%, 30.72%, 15.64% and 5.64% respectively when it is compared with its

control (S0). At 90DAS, main plot M2 shows the maximum total Flavonols compared to M1 with values of 30.99 (M2) and 27.26 (M1), respectively. A percentage increase of 12.03% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S4 with a value of 34.25 where Boron @0.5% +Sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S4, followed by S9> S2> S6> S8> S7> S5> S3> S1, and the per cent values were 34.92%, 33.055, 30.09%, 24.71%, 23.49%, 23.28%, 21.61, 20.49% and 11.95% respectively when it is compared with its control (S0).

The study showed a significant increase with 39.51%, 15.64% and 24.71% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the total Flavonols was found in treatment S4, where the combined application of boron and sulphur was applied to the crop. The aqueous application of Boron @0.5% + sulphur @0.25% is applied to the crop compared to its control (S0).

In the year (2022-23), at 30DAS, main plot M2 shows maximum total Flavonols as compared to M1 with values 7.04 (M2) and 6.58 (M1), respectively. A percentage increase of 6.53% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total Flavonols was observed in S6, i.e. 7.60 at 30DAS, where in S6, Boron @ 0.5% + BAP (@0.0045%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S6, followed by S7> S1> S2> S3> S4> S9> S8> S5, and the per cent values were 37.94%, 35.63%, 35.16%, 34.36%, 32.55%, 31.26%, 29.51%, 30.50% and 29.495% respectively. At 60DAS, main plot M2 shows the maximum total Flavonols compared to M1 with values of 10.60 (M2) and 9.63 (M1), respectively. A percentage increase of 9.15% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a

significant increase was found in S4 with a value of 13.65 where Boron @0.5% + sulphur @0.25% was applied to the crop as a foliar application. The per cent increase was found highest in S4, followed by S1> S9> S5> S2> S8> S6> S7 and the per cent values were 47.39%, 42.77%, 40.10%, 33.77%, 31.74%, 30.61%, 14.57% and 7.11% respectively when it is compared with its control (S0). At 90DAS, main plot M2 shows the maximum total Flavonols compared to M1 with values 31.25 (M2) and 27.56 (M1), respectively. A percentage increase of 11.8% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S4 with a value of 34.44 where Boron @0.5% +Sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S4, followed by S9> S2> S6> S8> S7> S5> S3> S1, and the per cent values were 34.75%, 33.17%, 30.33%, 24.83%, 23.52%, 23.40%, 21.78%, 20.67% and 12.24% respectively when it is compared with its control (S0).

The study showed a significant increase with 37.94%, 14.57% and 24.83% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the total Flavonols was found in treatment S4, where the combined application of boron and sulphur was applied to the crop. The aqueous application of Boron @0.5% + sulphur @0.25% is applied to the crop compared to its control (S0). The amount of Flavonols gradually increases with an increase in the phenolic compounds in the plant. Applying micronutrients along with plant growth hormone significantly increases the flavonol content in the mustard crop. Martinovic et al. (2020) reported that flavonol content is an antioxidant that substantially protects the plant from various biotic and abiotic stresses. Applying cytokinin helps protect the plant from different biotic and abiotic stresses and shows a significant increase in the growth and development of the plant.

Flavanols, a group of flavonoids, are bioactive substances well acknowledged for their antioxidant characteristics and involvement in plant immunological processes. In mustard crops

and other plants, the accumulation of flavonols is affected by various environmental factors and the availability of nutrients. A comprehensive knowledge of the cellular processes in producing flavanols and the effects of different nutrient treatments allows for optimizing flavanol levels to improve plant health and nutritional value. The synthesis of flavanols occurs via the phenylpropanoid pathway, which is the primary metabolic route in plants by which different phenolic compounds are produced. The first step involves the conversion of the amino acid phenylalanine to cinnamic acid by the enzyme phenylalanine ammonia-lyase (PAL). The following processes include a sequence of enzymatic reactions facilitated by chalcone synthase (CHS), chalcone isomerase (CHI), and flavanone 3-hydroxylase (F3H), resulting in the synthesis of dihydroflavonols. Proteins such as flavanone 4-reductase (FNR) and anthocyanidin synthase (ANS) catalyse the further conversion of these intermediates into flavanols. Fluorescence, temperature, and nutrient availability are key environmental factors that tightly govern the regulation of flavanol biosynthesis. Significant roles are played by nutrients such as Boron, Sulphur, and Cytokinin in modulating the activity of enzymes in the flavanol biosynthesis pathway. Specifically, Sulphur is necessary for producing glutathione, a crucial molecule in the cellular redox system that affects the function of enzymes involved in synthesizing flavonoids. The plant hormone cytokinin is recognized for controlling gene expression associated with synthesizing secondary metabolites, such as flavonoids. Boron plays a vital role in maintaining the structural integrity of cell walls and membranes. Furthermore, it significantly affects the function of PAL, the enzyme responsible for initiating the initial stage of the phenylpropanoid pathway. Heightened PAL activity in the presence of Boron can result in augmented biosynthesis of flavonoids, including flavonols. Sulphur plays a crucial role in synthesizing glutathione, essential for maintaining cellular metabolic balance. Consequently, this modulates the function of enzymes such as F3H and ANS, which play a role in the later phases of flavanol biosynthesis. Elevated glutathione levels activated by sulphur may improve the stability and activity of these enzymes, resulting in increased flavanol content. Cytokinin modulates the expression of genes

that encode enzymes involved in the biosynthesis of flavonoids. By upregulating the expression of CHS and other essential enzymes, its application can enhance flavanol synthesis. Furthermore, Cytokinin can potentially augment the transportation of flavanols within the plant, assuring their preferential accumulation in particular tissues such as leaves and seeds. The concurrent use of Boron, Sulphur, and Cytokinin may synergistically augment the production of flavanols. Specifically, boron can stabilize cellular membranes, ensuring the optimal operation of enzymes that rely on sulfur. Furthermore, Cytokinin can enhance this effect by increasing the required gene expression, resulting in a more effective transformation of dihydroflavonols into flavanols. This synergy is evident in the observed elevations in flavanol concentration in treatments when these nutrients are administered in combination, as opposed to when they are administered separately. The synergistic functions of these nutrients in simultaneously controlling enzymes and regulating gene expression provide a rationale for their combined application in maximizing the accumulation of flavonols. Flavanols are components of the plant's phytochemical defence system against environmental stressors such as UV radiation and pathogenic assaults. A preparatory mechanism that strengthens the plant's antioxidant capacity and resilience to stress can be observed in the increased synthesis of flavanols in response to nutrient treatments. The use of Sulphur, specifically, can result in enhanced production of flavanols as a component of a more comprehensive stress reduction mechanism. Activating flavonol biosynthesis in abundant nutrients primes the plant to withstand oxidative stress, promoting enhanced growth and productivity. The cellular processes responsible for the accumulation of flavonols in mustard crops are precisely connected to the presence and equilibrium of vital nutrients such as Boron, Sulphur, and cytokines. These nutrients affect the biosynthesis pathway of flavonols by regulating the function of particular enzymes and the expression of associated genes. The observed elevations in overall flavanol concentration following combined nutrient treatments can be ascribed to the synergistic impacts of these nutrients on both enzymatic activity and cellular redox equilibrium. This knowledge offers a compelling rationale for deliberately managing these

nutrients in agricultural methods to improve mustard crops' nutritional quality and stress resistance.

Table-4.19 (a): Effect of spacing and nutrients on Total Flavonols (mg g^{-1} Fresh Weight) of the mustard crop during the rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	6.18	6.58	9.25	9.63	27.26	27.56
M2 (20×10)	6.60	7.04	10.14	10.6	30.99	32.25
C.D. at p<0.05	0.36	0.27	0.81	0.73	3.64	3.84
SEM±	0.05	0.04	0.28	0.11	0.55	0.58
Nutrients foliar application						
S0-Control	4.36	4.72	6.86	7.18	22.29	22.47
S1-Boron @1%	6.82	7.28	12.08	12.54	25.31	25.6
S2-Sulphur @ 0.15%	6.65	7.19	10.1	10.52	31.88	32.25
S3-BAP @0.003%	6.64	6.99	7.21	7.98	28.03	28.32
S4-Boron @0.5% +Sulphur @0.25%	6.44	6.86	13.39	13.65	34.25	34.44
S5-Boron @ 1.5%+ Sulphur @0.075%	6.28	6.69	10.5	10.84	28.43	28.72
S6-Boron @ 0.5% + BAP (@0.0045%)	7.20	7.60	8.13	8.40	29.6	29.89
S7-Boron @ 1.5%+ BAP (@0.0015%)	6.89	7.33	7.27	7.73	29.05	29.33
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	6.92	6.79	9.90	10.34	29.13	29.38
S9-Sulphur @0.25%+ BAP (@0.0015%)	6.32	6.69	11.54	11.98	33.29	33.62
C.D. at p<0.05	0.71	0.78	1.81	1.90	4.98	4.96
SEM±	0.24	0.27	0.63	0.66	1.73	1.72
C.D. S×M at p<0.05	1.04	1.06	1.81	2.74	6.90	6.92
SEM±	0.17	0.13	0.63	0.35	1.76	1.85
C.D. M×S at p<0.05	1.00	1.01	2.56	2.62	7.00	7.01
SEM±	0.33	0.36	0.89	0.89	2.38	2.38

Where, C.D. represents critical difference, SE (m) represents standard error of mean and SE (d) represents the standard error of deviation.

Table 4.19 (b): Interaction effect of spacing and nutrient on Total Flavonols (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 60DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	4.15	6.613	6.44	6.427	6.233	6.077	6.997	6.687	6.08	6.11	6.181
M2	4.573	7.04	6.863	6.853	6.657	6.5	7.42	7.11	6.503	6.53	6.605
Mean B	4.362	6.827	6.652	6.64	6.445	6.288	7.208	6.898	6.292	6.32	
C.D. S×M at p<0.05						1.04					
SEM±						0.17					
C.D. M×S at p<0.05						1.00					
SEM±						0.33					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	4.57	7.017	7.043	6.76	6.607	6.423	7.443	7.1	6.517	6.393	6.587
M2	4.87	7.543	7.34	7.237	7.127	6.967	7.77	7.567	7.067	7.00	7.049
Mean B	4.72	7.28	7.192	6.998	6.867	6.695	7.607	7.333	6.792	6.697	
C.D. S×M at p<0.05						1.06					
SEM±						0.13					
C.D. M×S at p<0.05						1.01					
SEM±						0.36					

Table 4.19 (c): Interaction effect of spacing and nutrient on Total Flavonols (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 60DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	6.933	9.03	9.907	7.163	12.9	10.29	8.05	7.207	9.74	11.363	9.258
M2	6.787	15.13	10.3	7.273	13.88	10.723	8.213	7.337	10.063	11.717	10.142
Mean B	6.86	12.08	10.103	7.218	13.39	10.507	8.132	7.272	9.902	11.54	
C.D. S×M at p<0.05						1.81					
SEM±						0.63					
C.D. M×S at p<0.05						2.56					
SEM±						0.89					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	7.14	9.33	10.333	7.753	13.067	10.583	8.373	7.817	10.11	11.863	9.637
M2	7.237	15.763	10.707	8.217	14.233	11.1	8.437	7.643	10.587	12.11	10.603
Mean B	7.188	12.547	10.52	7.985	13.65	10.842	8.405	7.73	10.348	11.987	
C.D. S×M at p<0.05						2.74					
SEM±						0.35					
C.D. M×S at p<0.05						2.62					
SEM±						0.89					

Table 4.19 (d): Interaction effect of spacing and nutrient on Total Flavonols (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 60DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	19.887	21.22	30.857	26.187	29.687	27.587	28.563	29.393	25.853	33.437	27.267
M2	24.707	29.407	32.913	29.887	38.813	29.29	30.65	28.72	32.417	33.15	30.995
Mean B	22.297	25.313	31.885	28.037	34.25	28.438	29.607	29.057	29.135	33.293	
C.D. S×M at p<0.05						6.90					
SEM±						1.76					
C.D. M×S at p<0.05						7.00					
SEM±						2.38					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	20.1	21.43	31.32	26.52	29.933	27.957	28.817	29.74	26.13	33.683	27.563
M2	24.847	29.78	33.19	30.13	38.95	29.5	30.97	28.93	32.637	33.567	31.25
Mean B	22.473	25.605	32.255	28.325	34.442	28.728	29.893	29.335	29.383	33.625	
C.D. S×M at p<0.05						6.92					
SEM±						1.85					
C.D. M×S at p<0.05						7.01					
SEM±						2.38					

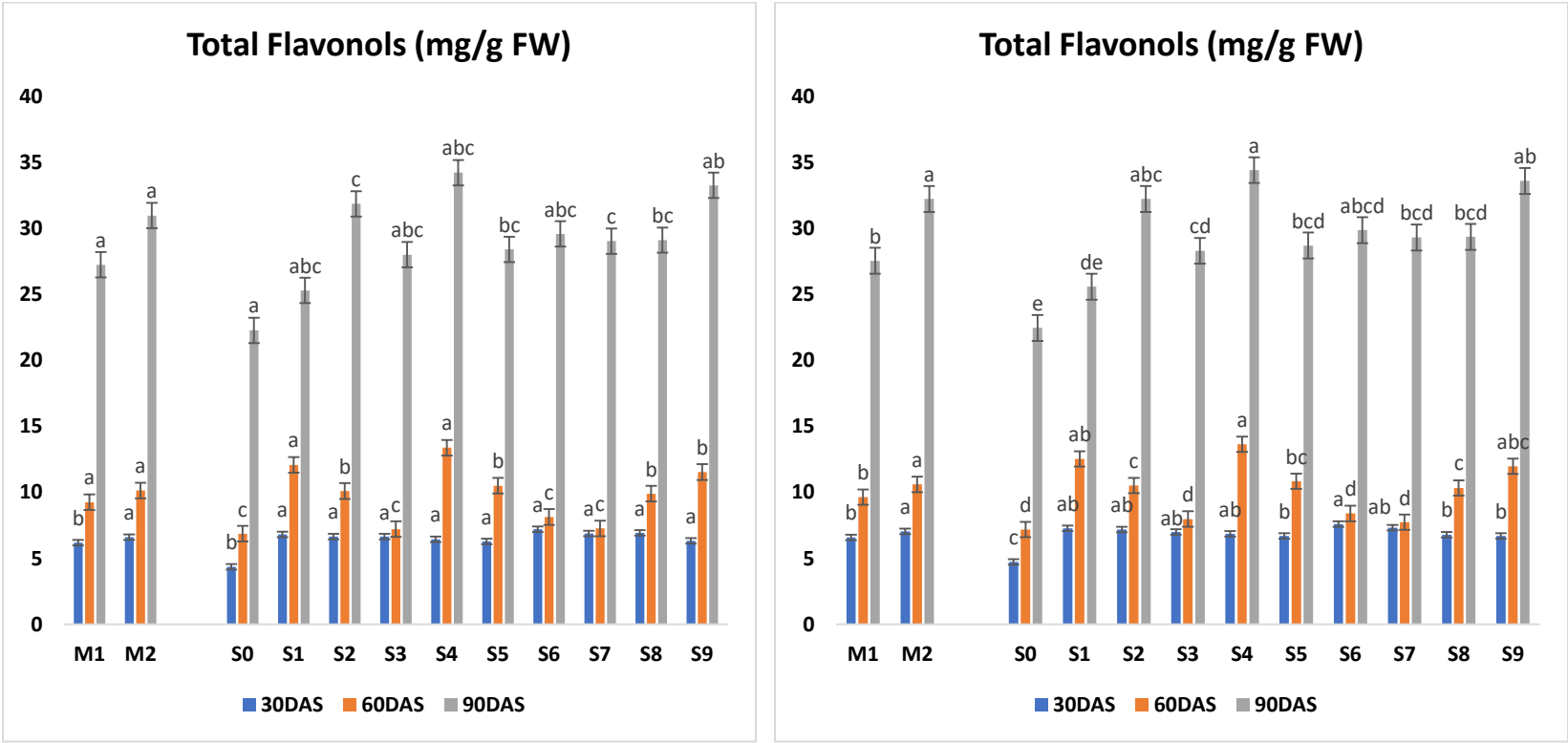


Fig-4.19 (a): Effect of spacing and nutrient on Total Flavonols of mustard crop during rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.3.12 Total Flavonoids (mg/g Fresh Weight)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in total Flavonoids were observed at 30DAS, 60DAS and 90DAS, shown in Table 4.19, Fig 4.19. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the total Flavonoids in each treatment compared to the control of both the spacings at 30, 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the total Flavonoids was observed at 30, 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments.

In the year (2021-22), at 30DAS, main plot M2 shows maximum total Flavonoids as compared to M1 with values of 14.10 (M2) and 13.08 (M1), respectively. A percentage increase of 7.23% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total Flavonoids was observed in S7, i.e. 15.06 at 30DAS, where in S7, Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S7, followed by S9> S6> S5&S8> S4> S2> S3, and the per cent values were 37.34%, 36.91%, 36.69%, 34.66%, 34.66%, 34.19%, 29.36% and 24.71% respectively. At 60DAS, main plot M1 shows the maximum total Flavonoids compared to M2 with values of 38.80 (M1) and 35.59 (M2), respectively. A percentage increase of 8.27% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase was found in S9 with a value of 39.57, where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar application. The per cent increase was found highest in S9, followed by S7> S8> S2> S5> S6> S3> S4, and the per cent values were 16.02%, 15.44%,

14.72%, 14.24%, 11.93%, 11.85%8.98% and 6.61% respectively when it is compared with its control (S0). At 90DAS, the main plot M2 shows the maximum total Flavonoids compared to M1, with values of 74.53 (M2) and 74.33 (M1), respectively. A percentage increase of 0.26% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S2 with a value of 87.31, where Sulphur @ 0.15% was applied to the crop as a foliar spray. The per cent increase was found highest in S2 followed by S7> S6> S3> S5> S1> S8> S9> S4, and the per cent values were 49.41%, 48.36%, 47.645, 47.62%, 42.23%, 41.73%, 36.59%, 36.57% and 34.13% respectively when it is compared with its control (S0).

The study showed a significant increase with 36.69%, 11.85% and 46.64% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the total Flavonoids was found in treatment S7, where the combined application of boron and sulphur was applied to the crop. The aqueous application of Sulphur @ 0.075%+ BAP (@0.0045%) is applied to the crop when compared to its control (S0).

In the year (2022-23), at 30DAS, main plot M2 shows maximum total Flavonoids as compared to M1 with values of 14.38 (M2) and 13.39 (M1), respectively. A percentage increase of 6.88% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total Flavonoids was observed in S7, i.e. 15.42 at 30DAS, where in S7, Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S7, followed by S6> S9> S5> S8> S4> S2> S1 and the per cent values were 37.43%, 36.59%, 36.28%, 34.45%, 34.39%, 34.10%, 30.22% and 24.51% respectively. At 60DAS, main plot M1 shows the maximum total Flavonoids compared to M2 with values of 39.04 (M1) and 35.83 (M2), respectively. A percentage increase of 8.22% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant

increase was found in S9 with a value of 39.75, where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar application. The per cent increase was found highest in S9, followed by S7> S8> S2> S6> S5> S3> S4, and the per cent values were 15.10%, 14.60%, 13.74%, 13.50%, 11.39%, 11.16%, 8.07% and 5.47% respectively when it is compared with its control (S0). At 90DAS, the main plot M2 shows the maximum total Flavonoids compared to M1, with 74.91 (M2) and 74.61 (M1) values, respectively. A percentage increase of 0.40% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S2 with a value of 87.31, where Sulphur @ 0.15% was applied to the crop as a foliar spray. The per cent increase was found highest in S2, followed by S7> S6> S3> S5> S1> S8> S9> S4, and the per cent values were 49.17%, 48.14%, 47.50%, 47.41%, 42.07%, 41.52%, 36.39%, 36.27% and 33.90% respectively when it is compared with its control (S0).

The study showed a significant increase with 36.59%, 11.39% and 47.50% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the total Flavonoids was found in treatment S9, where the combined application of sulphur and BAP was applied to the crop. The aqueous application of Sulphur @0.25%+ BAP (@0.0015%) is applied to the crop compared to its control (S0). Flavonoid contents of mustard seed have not been significantly studied previously (Adejumo et al., 2016). Applying micronutrients along with plant growth hormone substantially increases the flavonoid content in the mustard crop. Martinovic et al. (2020) reported that flavonoid content is an antioxidant that protects the plant from various biotic and abiotic stresses. The application of cytokinin helps protect the plant from different biotic and abiotic stresses and shows a significant increase in the growth and development of the plant.

Flavonoids are diverse phytonutrients (plant chemicals) in almost all fruits and vegetables. They play a significant role in plant physiology and offer numerous benefits to human health. Various

environmental and physiological factors influence flavonoid accumulation in plant tissues, including nutrient availability, plant growth regulators, and environmental stresses. Flavonoids are synthesized through the phenylpropanoid pathway, which begins with the amino acid phenylalanine. This enzyme catalyses the deamination of phenylalanine to trans-cinnamic acid, which is the first step in the biosynthesis of flavonoids. Chalcone Synthase (CHS): CHS is a crucial enzyme that catalyses the condensation of p-coumaroyl-CoA with three molecules of malonyl-CoA to form naringenin chalcone, the precursor to various flavonoids. Chalcone Isomerase (CHI): This enzyme converts naringenin chalcone into naringenin, a flavanone that serves as a central intermediate in the biosynthesis of multiple classes of flavonoids, including flavones, flavonols, and anthocyanins. Flavanone 3-Hydroxylase (F3H), Flavonol Synthase (FLS), and Dihydroflavonol 4-Reductase (DFR): These enzymes catalyse subsequent steps that lead to the formation of different flavonoid subgroups. Flavonoids are then stored in plant cell vacuoles, serving several functions, including protection against UV radiation, oxidative stress, and pathogen attack. Additionally, flavonoids regulate auxin transport, contributing to plant growth and development. The availability of nutrients tightly regulates the synthesis and accumulation of flavonoids in plants, particularly those involved in the phenylpropanoid pathway. In the context of the mustard crop study, the application of Boron, Sulphur, and Cytokinin can be justified based on their roles in flavonoid biosynthesis: Boron is essential for the structural integrity of cell walls and membranes and plays a role in the regulation of phenolic metabolism, including the synthesis of flavonoids. Boron deficiency has been shown to reduce phenylalanine ammonia-lyase activity, thereby decreasing flavonoid production. Conversely, adequate boron supply can enhance flavonoid biosynthesis by promoting the activity of key enzymes in the pathway. Sulphur is a critical component of specific amino acids (e.g., cysteine, methionine) and coenzymes involved in the phenylpropanoid pathway. Sulphur-containing compounds like glutathione also protect plant cells from oxidative stress, which can produce flavonoids as part of the plant's defence mechanism. The application of sulphur can thus enhance flavonoid synthesis

by ensuring a sufficient supply of these essential components and modulating the plant's oxidative stress response. Cytokinins are plant hormones that regulate cell division and differentiation. They also modulate secondary metabolite production, including flavonoids. Cytokinins can influence flavonoid biosynthesis by upregulating the expression of critical enzymes like CHS and PAL, thereby increasing the overall flavonoid content in plant tissues. The synergistic effect of cytokinin with boron and sulphur in the mustard crop likely enhanced flavonoid accumulation by stimulating both primary and secondary metabolic processes. The combined application of Boron, Sulphur, and Cytokinin can significantly improve the total flavonoid content in mustard crops by modulating the phenylpropanoid pathway at various levels. Boron supports cell wall integrity and phenolic metabolism, sulphur provides essential building blocks and modulates stress responses, and cytokinin enhances the activity of critical biosynthetic enzymes. This multifaceted approach improves flavonoid biosynthesis and strengthens the plant's defence mechanisms, contributing to better growth and yield. Flavonoids are complex phytochemicals found in nearly all fruits and vegetables. They perform a crucial function in plant physiology and provide many advantages to human health. Several environmental and physiological factors, such as nutrient availability, plant growth regulators, and environmental stresses, influence flavonoid accumulation in plant tissues. The synthesis of flavonoids occurs via the phenylpropanoid pathway, initiated by the amino acid phenylalanine. The deamination of phenylalanine to trans-cinnamic acid is catalysed by this enzyme, marking the initial stage in the biosynthesis of flavonoids. Chalcone Synthase (CHS) is an essential enzyme responsible for catalyzing the condensation reaction between p-coumaroyl-CoA and three molecules of malonyl-CoA. This reaction results in naringenin chalcone, the precursor for several flavonoids. Chalcone Isomerase (CHI) is an enzyme that transforms naringenin chalcone into naringenin, a flavanone that plays a crucial role as an intermediate in the production of several types of flavonoids, such as flavones, flavonols, and anthocyanins compounds. The enzymes Flavanone 3-Hydroxylase (F3H), Flavonol Synthase (FLS), and Dihydroflavonol 4-Reductase (DFR) facilitate the progressive processes involved in the synthesis

of various subgroups of flavonoids. Flavonoids are subsequently stored within the vacuoles of plant cells, thus fulfilling multiple roles, such as safeguarding against UV radiation, oxidative stress, and pathogen invasion. Furthermore, flavonoids control the transportation of auxin, promoting plant growth and development. In plants, the synthesis and accumulation of flavonoids, especially those involved in the phenylpropanoid pathway, are tightly controlled by the availability of nutrients. Justification for using Boron, Sulphur, and Cytokinin in the mustard crop research can be attributed to their respective functions in flavonoid biosynthesis. Boron is indispensable for maintaining the structural integrity of cell walls and membranes and contributing to the control of phenolic metabolism, including flavonoid production. Scientific evidence has demonstrated that a lack of boron leads to a decrease in the activity of phenylalanine ammonia-lyase, hence reducing the production of flavonoids. On the other hand, sufficient boron availability can optimize the production of flavonoids by stimulating the function of crucial enzymes in the process. Sulfur is an essential constituent of specific amino acids (such as cysteine and methionine) and coenzymes in the phenylpropanoid pathway. Compounds containing sulfur, such as glutathione, also protect plant cells against oxidative stress, facilitating the production of flavonoids as a component of the plant's defence mechanism. Thus, applying sulphur can enhance flavonoid synthesis by ensuring an adequate supply of these vital components and regulating the plant's response to oxidative stress. Cytokinins are phytohormones that control the processes of cell division and differentiation. Furthermore, they regulate the synthesis of secondary metabolites, such as flavonoids. By upregulating the expression of crucial enzymes such as CHS and PAL, cytokinins can enhance flavonoid biosynthesis and raise the total flavonoid content in plant tissues. The combined action of cytokinin with boron and sulphur in the mustard crop is expected to increase significantly the accumulation of flavonoids by activating both primary and secondary metabolic pathways. By modulating the phenylpropanoid pathway at different levels, the combined application of Boron, Sulphur, and Cytokinin can significantly enhance the total flavonoid content in mustard crops. Boron promotes the structural integrity of cell walls and

facilitates phenolic metabolism; sulphur provides necessary building blocks and regulates cell stress responses. Cytokinin boosts the activity of critical biosynthetic enzymes. This comprehensive strategy enhances the production of flavonoids and reinforces the plant's defence mechanisms, promoting improved growth and yield.

Table-4.20 (a): Effect of spacing and nutrient on Total Flavonoids (mg/g Fresh Weight) of mustard crop during rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	13.08	13.39	38.8	39.04	74.34	74.61
M2 (20×10)	14.1	14.38	35.59	35.83	74.53	74.91
C.D. at p<0.05	NS	NS	0.48	0.67	0.68	0.69
SEM±	0.17	0.22	0.07	0.10	0.37	0.39
Nutrients foliar application						
S0-Control	9.44	9.65	33.23	33.37	44.17	44.52
S1-Boron @1%	12.43	12.78	34.63	35.09	75.81	76.12
S2-Sulphur @ 0.15%	13.36	13.83	38.75	39.02	87.31	87.59
S3-BAP @0.003%	12.53	12.75	36.51	36.71	84.33	84.66
S4-Boron @0.5% +Sulphur @0.25%	14.34	14.64	35.58	35.7	67.06	67.35
S5-Boron @ 1.5%+ Sulphur @0.075%	14.44	14.72	37.73	37.99	76.46	76.86
S6-Boron @ 0.5% + BAP (@0.0045%)	14.91	15.22	37.7	38.09	84.36	84.81
S7-Boron @ 1.5%+ BAP (@0.0015%)	15.06	15.42	39.29	39.52	85.54	85.86
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	14.44	14.71	38.96	39.13	69.66	69.99
S9-Sulphur @0.25%+ BAP (@0.0015%)	14.96	15.14	39.57	39.75	69.64	69.86
C.D. at p<0.05	1.93	1.89	2.37	2.39	8.83	8.88
SEM±	0.67	0.65	0.82	0.83	3.06	3.08
C.D. S×M at p<0.05	NS	NS	3.37	3.42	9.17	9.25
SEM±	0.56	0.72	0.23	0.32	3.17	3.20
C.D. M×S at p<0.05	NS	NS	3.21	3.26	12.28	12.38
SEM±	0.91	0.91	1.10	1.12	3.72	3.75

Where, C.D. represents critical difference, SE (m) represents standard error of mean and SE (d) represents the standard error of deviation.

Table 4.20 (b): Interaction effect of spacing and nutrient on Total Flavanoids (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 60DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	33.85	34.263	36.533	37.77	35.79	40.247	40.053	43.24	42.517	43.753	38.802
M2	32.61	35.007	40.967	35.253	35.377	35.223	35.347	35.357	35.417	35.387	35.594
Mean B	33.23	34.635	38.75	36.512	35.583	37.735	37.7	39.298	38.967	39.57	
C.D. S×M at p<0.05						3.37					
SEM±						0.23					
C.D. M×S at p<0.05						3.21					
SEM±						1.10					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	34.017	34.883	36.76	38.01	35.877	40.4	40.37	43.447	42.69	43.977	39.043
M2	32.733	35.313	41.28	35.42	35.533	35.587	35.813	35.6	35.57	35.537	35.839
Mean B	33.375	35.098	39.02	36.715	35.705	37.993	38.092	39.523	39.13	39.757	
C.D. S×M at p<0.05						3.42					
SEM±						0.32					
C.D. M×S at p<0.05						3.26					
SEM±						1.12					

Table 4.20 (c): Interaction effect of spacing and nutrient on Total Flavanoids (mg g^{-1} Fresh Weight) of mustard crop during rabi season at 60DAS

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	42.9	79.877	87.97	83.377	66.14	75.717	84.107	85.317	69.02	68.977	74.34
M2	45.447	71.743	86.657	85.283	67.987	77.213	84.613	85.77	70.31	70.307	74.533
Mean B	44.173	75.81	87.313	84.33	67.063	76.465	84.36	85.543	69.665	69.642	
C.D. S×M at p<0.05						9.17					
SEM±						3.17					
C.D. M×S at p<0.05						12.28					
SEM±						3.72					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	43.173	80.157	88.2	83.593	66.443	76.063	84.413	85.72	69.33	69.1	74.619
M2	45.867	72.113	86.983	85.73	68.273	77.663	85.21	86.003	70.653	70.633	74.913
Mean B	44.52	76.135	87.592	84.662	67.358	76.863	84.812	85.862	69.992	69.867	
C.D. S×M at p<0.05						9.25					
SEM±						3.20					
C.D. M×S at p<0.05						12.38					
SEM±						3.75					

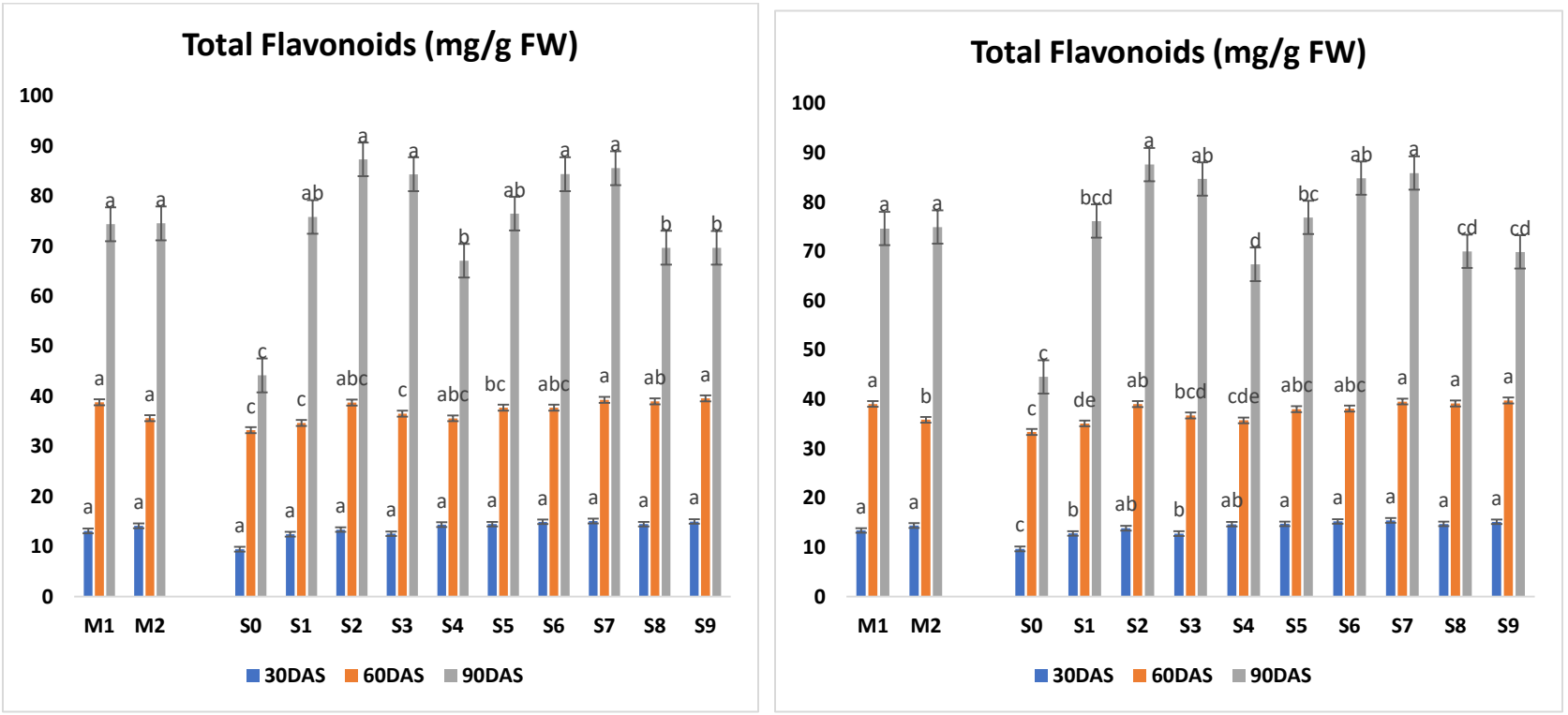


Fig-4.20 (a): Effect of spacing and nutrient on Total Flavonoids of mustard crop during rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.3.13 L-phenyl alanine (PAL) (mg/g Fresh Weight)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in PAL enzyme were observed at 30DAS, 60DAS and 90DAS, shown in Table 4.21, Fig 4.21. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the PAL enzyme in each treatment compared to control of both the spacings at 30, 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the PAL enzyme was observed at 30, 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, no such difference was found in the main plots. The average values obtained in the main plots were 0.17 and 0.17 in M1 (30*10) and M2 (20*10), respectively. In subplots, a significant increase in PAL enzyme was observed in S9, i.e. 0.18 at 30DAS, where in S9, Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S9, followed by S7> S3> S6> S5> S8> S1> S2, and the per cent values were 22.79%, 20.60%, 13.84%, 11.29%, 9.28%, 8.09%, 4.54% and 4.43% respectively. At 60DAS, the main plot M2 shows maximum PAL enzymes compared to M1, with values 0.26 (M2) and 0.24 (M1), respectively. A percentage increase of 7.69% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, the significant increase was found in S7&S8 with a value

of 0.28 where Boron @ 1.5%+ BAP (@0.0015%) and Sulphur @ 0.075%+ BAP (@0.0045%), respectively was applied to the crop as a foliar application. The per cent increase was found highest in S7&S8 followed by S9> S3> S1> S5> S2> S6> S4, and the per cent values were 23.25%, 23.25%, 20.43%, 19.61%, 11.23%, 9.09%, 9.71%, 8.65% and 5.98% respectively when it is compared with its control (S0). At 90DAS, the main plot M2 shows maximum PAL enzyme activity compared to M1, with values of 0.60 (M2) and 0.58 (M1), respectively. A percentage increase of 3.33% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S9 with a value of 0.74, where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent increase was found highest in S9, followed by S8> S6> S7> S1> S4> S2> S5, and the per cent values were 29.19%, 28.66%, 13.77%, 9.73%, 4.64%, 3.13%, 2.33% and 2.24% respectively when it is compared with its control (S0).

The study showed a significant increase with 11.29%, 8.65% and 13.77% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the PAL enzyme activity was found in treatment S9, where the combined application of sulphur and cytokinin was applied to the crop. The aqueous application of Sulphur @0.25%+ BAP (@0.0015%) is applied to the crop compared to its control (S0).

In the year (2022-23), at 30DAS, main plot M1 shows the maximum PAL enzyme as compared to M2 with values 0.26 (M1) and 0.25 (M2), respectively. A percentage increase of 3.84% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant rise in PAL enzyme was observed in S4, i.e. 0.29 at 30DAS, whereas in S4, Boron @0.5% + sulphur @0.25% was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S4, followed by S1&S7> S9> S3> S6> S8> S5> S2, and the per cent values were 22.60%, 16.36%, 16.36%, 14.81%, 13.75%, 12.10%, 9.80%, 9.21% and 2.81% respectively. At 60DAS, the main plot M2 shows maximum PAL enzymes compared to M1, with values 0.317 (M2) and 0.311 (M1), respectively. A percentage increase of 1.89% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S9 with a value of 0.35 where Sulphur @0.25%+ BAP (@0.0015%) respectively was applied to the crop as a foliar application. The per cent increase was found highest in S9 followed by S8> S3> S7> S2> S5> S1> S6> S4, and the per cent values were 28.57%, 27.18%, 25.74%, 25%, 19.78%, 18.03%, 17.58%, 16.66% and 16.20% respectively when it is compared with its control (S0). At 90DAS, the main plot M2 shows maximum PAL enzyme activity compared to M1, with values of 0.63 (M2) and 0.59 (M1), respectively. A percentage increase of 3.33% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S9 with a value of 0.81, where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent increase was found highest in S9 followed by S8> S6> S1> S5> S7&S2> S4, and the per cent values were 27.63%, 26.82%, 6.25%, 5.44%, 4.34% AND 3.50% respectively when it is compared with its control (S0).

The study showed a significant increase with 12.10%, 16.66% and 12.46% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the PAL enzyme activity was found in treatment S4, where the combined application of sulphur and boron was applied to the crop. The aqueous application of Boron @0.5% + sulphur @0.25% is applied to the crop compared to its control (S0). PAL activity was first measured in protein extracts from *Sorghum bicolour* by Koukol and Conn in 1961. It was later detected in many other plant species, and even some fungi and the enzyme protein were purified to homogeneity from various sources. PAL is one of the most intensively studied enzymes of plant secondary metabolism. The formation of phenylpropanoid phytoalexins after fungal infection involves a rapid induction of PAL. PAL is inhibited by its product, trans-cinnamic acid (Zhang & Liu 2015). Applying plant growth hormones and micronutrients helps increase the level of secondary metabolites and protects the plant from various biotic and abiotic stresses.

L-phenylalanine (PAL) is a key amino acid in plant metabolism, crucial for various physiological processes and secondary metabolite biosynthesis. Through its involvement in the phenylpropanoid pathway, it plays a significant role in plant growth, development, and stress responses. Below is a detailed exploration of the cellular mechanisms and justifications for L-phenylalanine's role in plants. The enzyme phenylalanine ammonia-lyase (PAL) catalyses the deamination of L-phenylalanine to cinnamic acid. This reaction is a critical step in the phenylpropanoid pathway, essential for synthesizing various secondary metabolites. Cinnamic acid, produced from L-phenylalanine by PAL, undergoes further enzymatic transformations in the phenylpropanoid pathway. This pathway produces vital compounds such as flavonoids, lignins, coumarins, and tannins. Flavonoids are involved in UV protection, pigmentation, and defence against pathogens. They contribute to plant colouration and can act as antioxidants. Lignins are

structural polymers that provide rigidity and resistance to pathogens. They are essential for cell wall formation and plant mechanical support. The expression of the PAL gene is tightly regulated at the transcriptional level by various factors, including developmental cues, environmental stresses, and signalling molecules. Post-translational modifications, such as phosphorylation or proteolysis, can also modulate PAL activity, which affects enzyme stability and activity. PAL activity is often upregulated in response to biotic and abiotic stresses, such as pathogen attacks, wounding, and environmental stressors. The increased production of phenylpropanoid compounds helps reinforce cell walls, produce antimicrobial agents, and scavenge reactive oxygen species. Besides the phenylpropanoid pathway, L-phenylalanine is a precursor for other metabolic pathways, including synthesizing alkaloids and aromatic amino acids involved in plant defence and signalling. L-phenylalanine is the fundamental building block for synthesizing a wide range of secondary metabolites crucial for plant adaptation, survival, and reproduction. The phenylpropanoid pathway derived from L-phenylalanine is central to producing compounds that contribute to plant defence, stress tolerance, and structural integrity. Plants can enhance their resilience to various stresses by modulating the PAL pathway. Increased PAL activity accumulates protective compounds, which can mitigate damage from environmental stressors and pathogens. Secondary metabolites synthesized from L-phenylalanine, such as flavonoids and lignins, play crucial roles in plant defence by acting as physical barriers, antimicrobial agents, and signalling molecules. Understanding the role of L-phenylalanine and its metabolic products can develop crops with enhanced resistance to pests and diseases, improved stress tolerance, and better nutritional profiles. The phenylpropanoid pathway has biotechnology applications for producing valuable compounds like pharmaceuticals, natural dyes, and flavouring agents. Beyond its role in secondary metabolism, L-phenylalanine is also involved in fundamental physiological processes such as cell division and growth. The synthesis of critical structural and functional compounds contributes to overall plant health and productivity. L-phenylalanine's involvement in the phenylpropanoid pathway underscores its crucial role in plant metabolism. By serving as a

precursor for a range of secondary metabolites, L-phenylalanine contributes to plant growth, stress responses, and adaptation, making it a key component in plant physiological and biochemical processes.

Table 4.21 (a): Effect of spacing and nutrient on PAL (mg/g Fresh Weight) of the mustard crop during rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	0.173	0.26	0.24	0.31	0.59	0.58
M2 (20×10)	0.17	0.25	0.26	0.31	0.6	0.61
C.D. at p<0.05	NS	NS	NS	NS	0.01	0.02
SEM±	0.01	0.01	0.01	0.01	0.01	0.01
Nutrients foliar application						
S0-Control	0.14	0.23	0.22	0.25	0.54	0.55
S1-Boron @1%	0.14	0.27	0.24	0.3	0.56	0.58
S2-Sulphur @ 0.15%	0.14	0.23	0.24	0.31	0.54	0.57
S3-BAP @0.003%	0.16	0.26	0.27	0.33	0.54	0.55
S4-Boron @0.5% +Sulphur @0.25%	0.29	0.29	0.23	0.29	0.55	0.57
S5-Boron @ 1.5%+ Sulphur @0.075%	0.15	0.25	0.24	0.3	0.54	0.53
S6-Boron @ 0.5% + BAP (@0.0045%)	0.15	0.26	0.24	0.3	0.61	0.62
S7-Boron @ 1.5%+ BAP (@0.0015%)	0.17	0.27	0.28	0.33	0.59	0.47
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	0.15	0.25	0.28	0.34	0.74	0.75
S9-Sulphur @0.25%+ BAP (@0.0015%)	0.18	0.27	0.27	0.35	0.75	0.76
C.D. at p<0.05	0.03	0.03	0.04	0.05	0.04	0.04
SEM±	0.01	0.01	0.02	0.01	0.01	0.01
C.D. S×M at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.03	0.02	0.02	0.01	0.00	0.01
C.D. M×S at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.01	0.01	0.03	0.02	0.02	0.02

Where, C.D. represents critical difference, SE (m) represents standard error of mean and SE (d) represents the standard error of deviation.

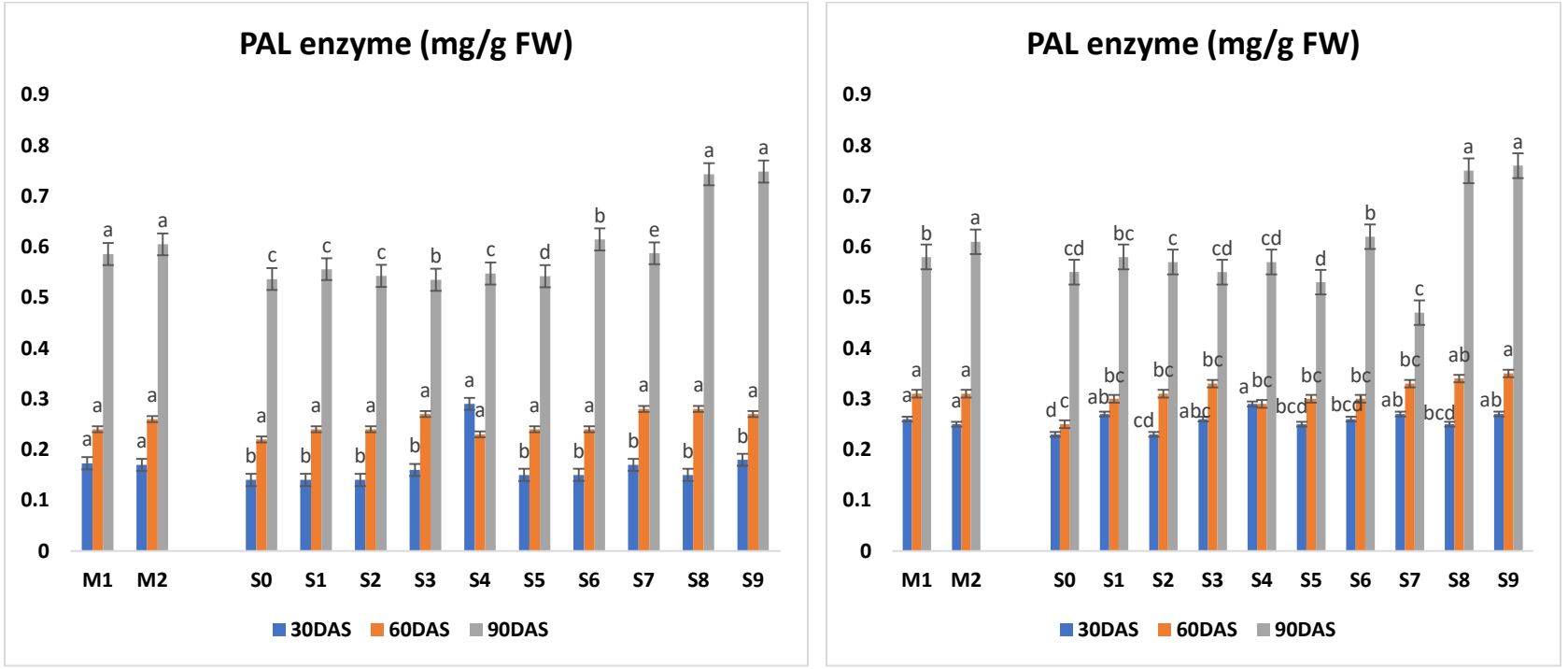


Fig-4.21 (a): Effect of spacing and nutrient on PAL of the mustard crop during rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.3.14 Total Free Amino acids (mg/g Fresh Weight)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in amino acids were observed at 30DAS, 60DAS and 90DAS, shown in Table 4.22, Fig 4.22. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the amino acids in each treatment compared to control of both the spacings at 30, 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the amino acids was observed at 30, 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments.

In the year (2021-22), at 30DAS, the main plot M2 shows maximum amino acid content as compared to M1 with values 0.13 (M2) and 0.12 (M1), respectively. A percentage increase of 7.69% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in amino acid content was observed in S2, i.e. 0.21 at 30DAS, whereas in S2, Sulphur @0.15% was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S2, followed by S6> S1> S5> S7> S9> S3> S4, and the per cent values were 76.97%, 69.81%, 66.17%, 62.02%, 59.83%, 55.02%, 54.68% and 54.47% respectively. At 60DAS, the main plot M2 shows maximum amino acid content compared to M1, with values of 0.13 (M2) and 0.12 (M1), respectively. A percentage increase of 7.69% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S6 with a value of 0.15, where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar application. The per cent increase was found highest in S6, followed by S8> S2> S9> S5> S7> S3> S1, and the per cent values were 33.62%, 31.50%, 29.24%, 26.82%, 25%, 23.66%, 16.20% and 14.77% respectively when it is compared

with its control (S0). At 90DAS, the main plot M2 shows maximum amino acid content compared to M1, with values of 0.50 (M2) and 0.40 (M1), respectively. A percentage increase of 20% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S9 with a value of 0.62, where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent increase was found highest in S9 followed by S8> S5> S1> S4> S7> S6> S2> S3, and the per cent values were 67.88%, 67.58%, 66.31%, 57.90%, 53.65%, 52.24%, 50.67%, 49.32% and 48.47% respectively when it is compared with its control (S0).

The study showed a significant increase with 69.81%, 33.62%, and 50.67% per cent values at 30DAS, 60DAS, and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the mustard crop was treated with a foliar application of Boron @ 0.5% + BAP (@0.0045%). At 60DAS and 90DAS, the amino acid content was significantly increased in treatment S6, where the combined application of aqueous sulphur and boron was applied to the crop.

In the year (2022-23), at 30DAS, the main plot M2 shows maximum amino acid content as compared to M1 with values 0.15 (M2) and 0.13 (M1), respectively. A percentage increase of 13.33% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in amino acid content was observed in S2, i.e. 0.23 at 30DAS, whereas in S2, Sulphur @0.15% was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S2, followed by S6> S1> S5> S7> S9> S3> S4, and the per cent values were 68.30%, 59.09%, 54.08%, 49.43%, 47.67%, 43.75%, 42.30% and 44.44% respectively. At 60DAS, the main plot M2 shows maximum amino acid content compared to M1, with values 0.15 (M2) and 0.14 (M1), respectively. A percentage increase of 6.66% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S6 with a value of 0.17, where Boron @ 0.5% + BAP

(@0.0045%) was applied to the crop as a foliar application. The per cent increase was found highest in S6, followed by S8> S2> S9> S5> S7> S3> S1, and the per cent values were 31.42%, 26.53%, 26.53%, 24.21%, 21.73%, 23.40%, 14.28% and 18.18% respectively when it is compared with its control (S0). At 90DAS, the main plot M2 shows maximum amino acid content compared to M1, with values of 0.51 (M2) and 0.41 (M1), respectively. A percentage increase of 19.60% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S9 with a value of 0.63, where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent increase was found highest in S9 followed by S8> S5> S1> S4> S7> S6> S2> S3, and the per cent values were 65.17%, 65.00%, 63.53%, 55.10%, 50.74%, 49.42%, 48.03%, 45.90% and 45.67% respectively when it is compared with its control (S0).

The study showed a significant increase with 59.09%, 31.42% and 48.03% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the amino acid content was found in treatment S6, where the combined application of aqueous sulphur and boron was applied to the crop. Banerjee et al., (2012) reported that the different studied varieties showed differential responses towards the accumulation of amino acid content in leaves. Maximum accumulation was observed in the case of variety. The impact of applying fertilizer, plant growth hormone, and chemical fertilizer was found to be inhibitive in terms of total free amino acid content in leaves compared to the control. Applying cycocel and compost significantly increased the total free amino acid level in leaves compared to the control. Amino acids are fundamental building blocks of proteins and are synthesized via various metabolic pathways. In plants, amino acids can be synthesized from precursors such as carbohydrates, fatty acids, and organic acids. Enzymatic processes involving key enzymes like glutamine synthetase and aspartate aminotransferase play

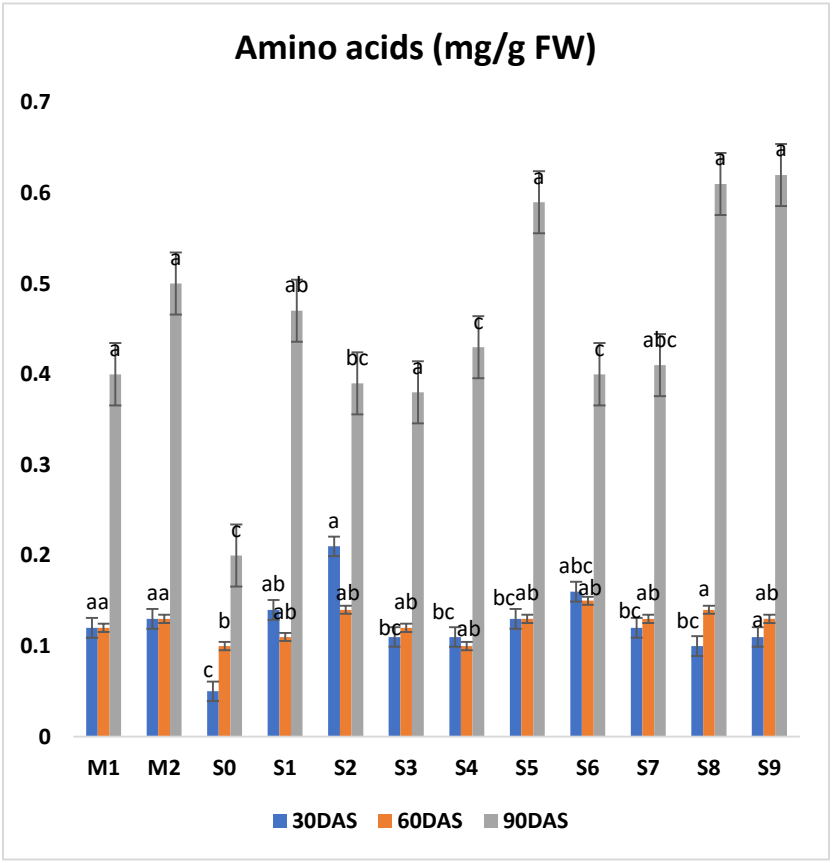
critical roles in the synthesis of amino acids. Essential amino acids are synthesized de novo within the plant cells. For instance, the shikimic acid pathway is crucial for synthesizing aromatic amino acids like phenylalanine, tyrosine, and tryptophan. Plants also mobilize amino acids from protein reserves within vacuoles and plastids to support growth, especially during stress or increased demand for protein synthesis. The availability of nitrogen, sulfur, and other nutrients influences amino acid synthesis. For instance, sulfur is crucial for synthesizing cysteine and methionine, while nitrogen is a critical component of amino acids like glutamine and asparagine. Plant hormones, such as Cytokinin and Auxin, regulate amino acid metabolism by modulating enzyme activities involved in amino acid synthesis and degradation. Cytokinin, for example, enhances amino acid content by promoting cell division and protein synthesis. Under abiotic stress conditions (e.g., drought, salinity), plants accumulate certain free amino acids as part of their stress response mechanisms. These amino acids, such as proline, function as osmoprotectants and stabilize cellular structures. Amino acids are transported into and out of cells through specific transport proteins located in the plasma membrane and tonoplast. These transport systems ensure the proper distribution of amino acids within plant tissues. Amino acids are distributed to various cellular compartments, such as the cytosol, vacuoles, and chloroplasts, where they are involved in protein synthesis, metabolism, and signalling pathways. Amino acids can also be released through the degradation of proteins. Proteolytic enzymes break down proteins into their constituent amino acids, which can then be used for new protein synthesis or other metabolic processes. Excess amino acids are catabolized to yield energy and produce metabolic intermediates. The catabolic pathways include transamination and deamination processes. Free amino acids are essential for protein synthesis and cell growth and development. Adequate levels of free amino acids support the synthesis of structural and functional proteins necessary for plant development. Amino acids contribute to cell division and expansion by providing the precursors needed for protein and nucleic acid synthesis. High free amino acid content supports vigorous growth and development of plant tissues. Amino acids like proline act

as osmoprotectants that help plants manage osmotic stress during adverse conditions such as drought or high salinity. The accumulation of such amino acids enhances stress tolerance and survival. Amino acids also play protective roles by stabilizing cellular structures and scavenging reactive oxygen species (ROS) generated during stress conditions. This helps maintain cellular integrity and function. Adequate free amino acid levels indicate effective utilization of nutrients. For instance, sufficient nitrogen and sulfur availability leads to higher amino acid content, which reflects efficient nutrient uptake and assimilation. A balanced profile of free amino acids ensures that plants access all essential building blocks for growth, improving overall plant health and productivity. High levels of free amino acids are associated with enhanced plant growth and yield. Amino acids improve photosynthetic efficiency and biomass accumulation, leading to higher crop yields. In some crops, the content of free amino acids can influence the nutritional quality of the produce. For example, increased amino acid content can enhance the nutritional value of seeds and fruits. The total free amino acid content is a critical indicator of plant health, growth, and stress tolerance. The synthesis, regulation, and utilization of amino acids are fundamental to various physiological processes, and their levels reflect the plant's overall nutrient status and environmental adaptability.

Table 4.22 (a): Effect of spacing and nutrients on Amino acids (mg/g Fresh Weight) of the mustard crop during the rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	0.12	0.13	0.12	0.14	0.4	0.41
M2 (20×10)	0.13	0.15	0.13	0.15	0.5	0.52
C.D. at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0	0	0	0	0.01	0.01
Nutrients foliar application						
S0-Control	0.05	0.08	0.10	0.12	0.20	0.21
S1-Boron @1%	0.14	0.16	0.11	0.14	0.47	0.49
S2-Sulphur @ 0.15%	0.21	0.23	0.14	0.16	0.39	0.40
S3-BAP @0.003%	0.11	0.13	0.12	0.14	0.38	0.40
S4-Boron @0.5% +Sulphur @0.25%	0.11	0.13	0.10	0.14	0.43	0.44
S5-Boron @ 1.5%+ Sulphur @0.075%	0.13	0.14	0.13	0.15	0.59	0.60
S6-Boron @ 0.5% + BAP (@0.0045%)	0.16	0.18	0.15	0.17	0.40	0.42
S7-Boron @ 1.5%+ BAP (@0.0015%)	0.12	0.14	0.13	0.15	0.41	0.43
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	0.10	0.12	0.14	0.16	0.61	0.62
S9-Sulphur @0.25%+ BAP (@0.0015%)	0.11	0.13	0.13	0.15	0.62	0.63
C.D. at p<0.05	0.04	0.04	0.04	0.04	0.12	0.12
SEM±	0.01	0.01	0.01	0.01	0.04	0.04
C.D. S×M at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.00	0.00	0.01	0.01	0.05	0.05
C.D. M×S at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.02	0.02	0.02	0.01	0.06	0.06

Where, C.D. represents critical difference, SE (m) represents standard error of mean and SE (d) represents the standard error of deviation.



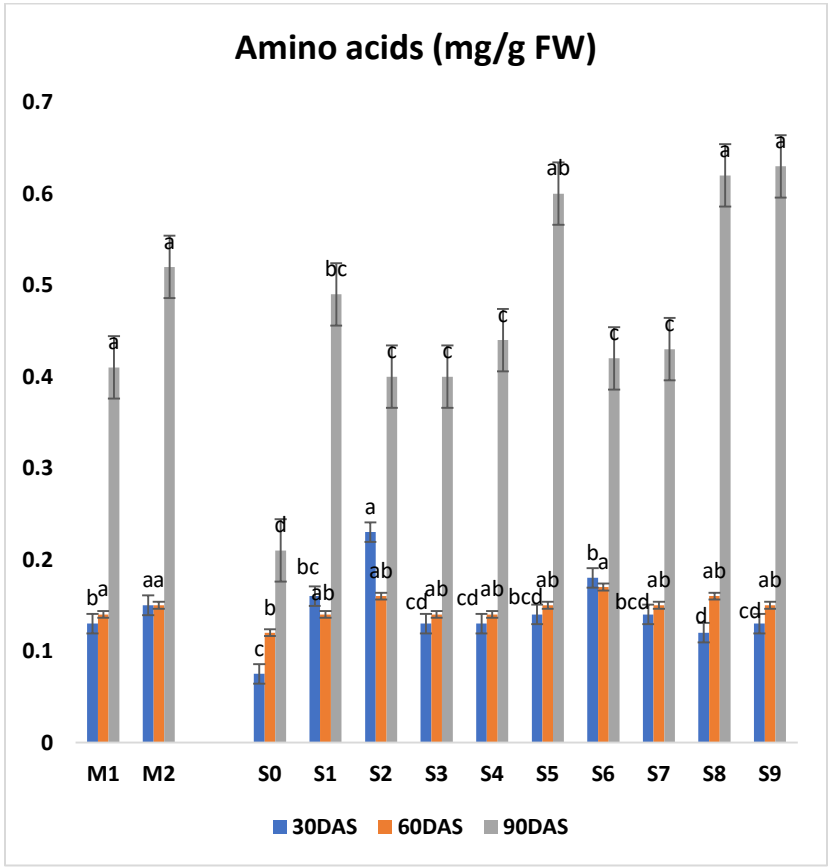


Fig-4.22 (a): Effect of spacing and nutrients on Amino acids of the mustard crop during the rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.3.15 Ascorbic acids (mg/g Fresh Weight)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in ascorbic acid were observed at 30DAS, 60DAS and 90DAS, shown in Table 4.23, Fig 4.23. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the ascorbic acids in each treatment as compared to the control of both the spacings at 30, 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the power and the spacings. Thus, the pattern of percentage increase in the ascorbic acids was observed at 30, 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S4 followed by S6> S9> S2&S7> S5> S8> S3 and the per cent values were 19.71%, 18.55%, 17.68%, 17.36%, 17.36%, 16.50%, 16.38% and 14.86% respectively. At 60DAS, the main plot M2 shows maximum ascorbic acid content compared to M1, with values of 0.25 (M2) and 0.23 (M1), respectively. A percentage increase of 8% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S3 with a value of 0.25, where BAP @0.003% was applied to the crop as a foliar application. The per cent increase was found highest in S3, followed by S1> S4> S7> S9> S8> S5> S6, and the per cent values were 14.00%, 13.89%, 13.72%, 12.69%, 12.06%, 9.59%, 8.27% and 8.07% respectively when it is compared with its control (S0). At 90DAS, the main plot M2 shows maximum ascorbic acid content compared to M1, with values of 0.45 (M2) and 0.44 (M1), respectively. A percentage increase of 2.22% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S7 with a value of 0.55, where Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop as a foliar spray.

The per cent increase was found highest in S7, followed by S9> S8> S4> S6> S3> S1> S5> S2, and the per cent values were 44.14%, 42.46%, 42.05%, 34.50%, 31.36%, 28.04%, 24.36%, 23.42% and 14.36% respectively when it is compared with its control (S0).

The study showed a significant increase with 18.55%, 8.07% and 31.36% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, ascorbic acid content was significantly increased in treatment S7, where the combined application of aqueous formulation of Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop.

In the year (2022-23), at 30DAS, main plot M1 shows maximum ascorbic acid content as compared to M2 with values 0.24 (M1) and 0.23 (M2), respectively. A percentage increase of 4.16% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase in ascorbic acid content was observed in S4, i.e. 0.23 at 30DAS, where in S4, Boron @0.5% + sulphur @0.25% was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S4 followed by S6> S9> S2> S7> S5> S8&S3, and the per cent values were 24.05%, 18.36%, 17.80%, 19.46%, 18.36%, 17.80%, 16.66% and 16.33% respectively. At 60DAS, the main plot M2 shows maximum ascorbic acid content compared to M1, with values of 0.26 (M2) and 0.25 (M1), respectively. A percentage increase of 3.84% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S4 with a value of 0.27 where Boron @0.5% + sulphur @0.25% was applied to the crop as a foliar application. The per cent increase was found highest in S4 followed by S1> S7> S9> S6&S8> S5> S2, and the per cent values were 12.72%, 11.11%, 10%, 9.43%, 7.09%, 7.09%, 6.49% and 3.35% respectively when it is compared with its control (S0). At 90DAS, the main plot M2 shows maximum ascorbic acid content compared to M1, with values of 0.46 (M2) and 0.45 (M1), respectively. A percentage increase of 2.17% was

found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S7 with a value of 0.56, where Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent increase was found highest in S7, followed by S9> S8> S4> S6> S3> S1> S5> S2, and the per cent values were 43.69%, 42.68%, 41.46%, 34.47%, 31.18%, 27.54%, 24.40%, 24.70% and 15.04% respectively when it is compared with its control (S0).

The study showed a significant increase with 18.36%, 7.09% and 31.18% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the ascorbic acid content was found in treatment S7 where the combined application of aqueous formulation of Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop. Similar results were shown by Banerjee et al., (2012) that the Level of ascorbic acid in leaves showed a significant level of variation among the different mustard varieties. The application of micronutrients significantly affected the ascorbic acid content level in crop plants' leaves. There was a steady decrease in the level of ascorbic acid in leaves where Chemical fertilizers were applied alone and then increased significantly in treatment where biofertilizers were applied to the crop. The level of ascorbic acid content in leaves increased in all the fertilizer-treated plots compared to the control. Applying micronutrients and plant growth hormones significantly increased up to 300 ppm and reduced substantially at higher concentrations.

Vitamin C, also known as ascorbic acid, is an essential micronutrient that plays many cellular functions, especially in plants. Its roles in cellular processes are necessary for plant health and productivity, impacting many activities ranging from growth to stress response. A comprehensive examination of its cellular processes and the rationale for its application in plant growth and development is presented here. The antioxidant ascorbic acid is highly effective in safeguarding

cells against oxidative stress by scavenging reactive oxygen species (ROS). In plants, oxidative stress originates from environmental conditions such as drought, intense light, and pathogen invasions. To safeguard cellular components such as lipids, proteins, and nucleic acids from oxidative damage, ascorbic acid counteracts reactive oxygen species (ROS) such as hydrogen peroxide (H_3O_3), hydroxyl radicals (•OH), and singlet oxygen (^$1O_3$). Ascorbic acid is involved in the ascorbate-glutathione (AsA-GSH) cycle, a natural redox system found in plants. During this process, ascorbic acid undergoes oxidation to form dehydroascorbate (DHA), which is subsequently reduced back to ascorbic acid by glutathione. This cell cycle serves to preserve the redox equilibrium within the cell. It facilitates the regeneration of other antioxidants, such as vitamin E, providing additional protection to the plant against oxidative stress. Ascorbic acid functions as a co-factor for many crucial enzymes involved in the biosynthesis of cell walls and the production of secondary metabolites. One essential function of prolyl hydroxylase is to facilitate collagen biosynthesis and stabilize plant cell walls. Moreover, ascorbic acid produces flavonoids and other phenolic compounds that contribute to plant defence mechanisms and pigmentation. Multiple signal transduction pathways in plants are influenced by ascorbic acid. This phenomenon influences the expression of genes that respond to stress and regulates the reactions of plants to environmental stimuli. Ascorbic acid can modulate the function of mitogen-activated protein (MAP) kinases and other signalling molecules integral to stress responses and growth regulation. Ascorbic acid plays a role in the photosynthetic process by safeguarding chloroplasts against radical damage and preserving the structural integrity of the thylakoid membranes. Furthermore, it controls crucial photosynthetic enzymes, impacting the overall efficiency of photosynthesis and the growth of plants. The capacity of ascorbic acid to counteract reactive oxygen species (ROS) and preserve redox equilibrium renders it essential for augmenting plant resilience to diverse environmental hazards, including drought, salinity, and intense light exposure. This application can enhance plant resilience, improving growth and yield in unfavourable conditions. Ascorbic acid enhances plant development and growth through its role

as an enzymatic co-factor and its influence on cell wall biosynthesis. It can stimulate the development of roots and shoots, enhance nutrient absorption, and boost plant biomass accumulation. The function of ascorbic acid in plant defence mechanisms is to produce secondary metabolites and enhance the structural integrity of cell walls. Applying this agent can improve the resistance of plants against pathogen infections and mitigate the consequences of diseases. Ascorbic acid safeguards chloroplasts and is essential for optimizing photosynthetic enzymes. By enhancing photosynthetic efficiency and improving overall plant health, this protection leads to increased crop yields. Ascorbic acid can augment plants' absorption and application of vital nutrients, so it optimizes their nutritional condition. This can be especially advantageous in soils with limited nutrient availability. In plants, ascorbic acid plays a crucial role in several cellular processes, such as antioxidant protection, redox status regulation, enzymatic activity, and signal transduction. The justification for its use in agriculture lies in its capacity to augment stress tolerance, enhance growth and development, amplify disease resistance, and optimize photosynthetic efficiency.

Table 4.23 (a): Effect of spacing and nutrients on Ascorbic acids (mg/g Fresh Weight) of the mustard crop during the rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	0.22	0.25	0.23	0.25	0.44	0.46
M2 (20×10)	0.21	0.24	0.25	0.26	0.45	0.47
C.D. at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.00	0.00	0.00	0.00	0.01	0.01
Nutrients foliar application						
S0-Control	0.18	0.20	0.22	0.24	0.31	0.32
S1-Boron @1%	0.20	0.24	0.25	0.27	0.40	0.42
S2-Sulphur @ 0.15%	0.22	0.25	0.23	0.25	0.36	0.38
S3-BAP @0.003%	0.22	0.24	0.25	0.25	0.43	0.44
S4-Boron @0.5% +Sulphur @0.25%	0.23	0.26	0.25	0.28	0.47	0.49
S5-Boron @ 1.5%+ Sulphur @0.075%	0.25	0.24	0.24	0.26	0.40	0.43
S6-Boron @ 0.5% + BAP (@0.0045%)	0.23	0.25	0.23	0.26	0.45	0.47
S7-Boron @ 1.5%+ BAP (@0.0015%)	0.28	0.25	0.25	0.27	0.55	0.57
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	0.22	0.24	0.24	0.26	0.53	0.55
S9-Sulphur @0.25%+ BAP (@0.0015%)	0.22	0.24	0.25	0.27	0.54	0.56
C.D. at p<0.05	NS	NS	0.02	0.02	0.04	0.04
SEM±	0.01	0.00	0.01	0.00	0.01	0.01
C.D. S×M at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.00	0.00	0.02	0.01	0.03	0.03
C.D. M×S at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.01	0.01	0.01	0.01	0.02	0.02

Where, C.D. represents critical difference, SE (m) represents standard error of mean and SE (d) represents the standard error of deviation.

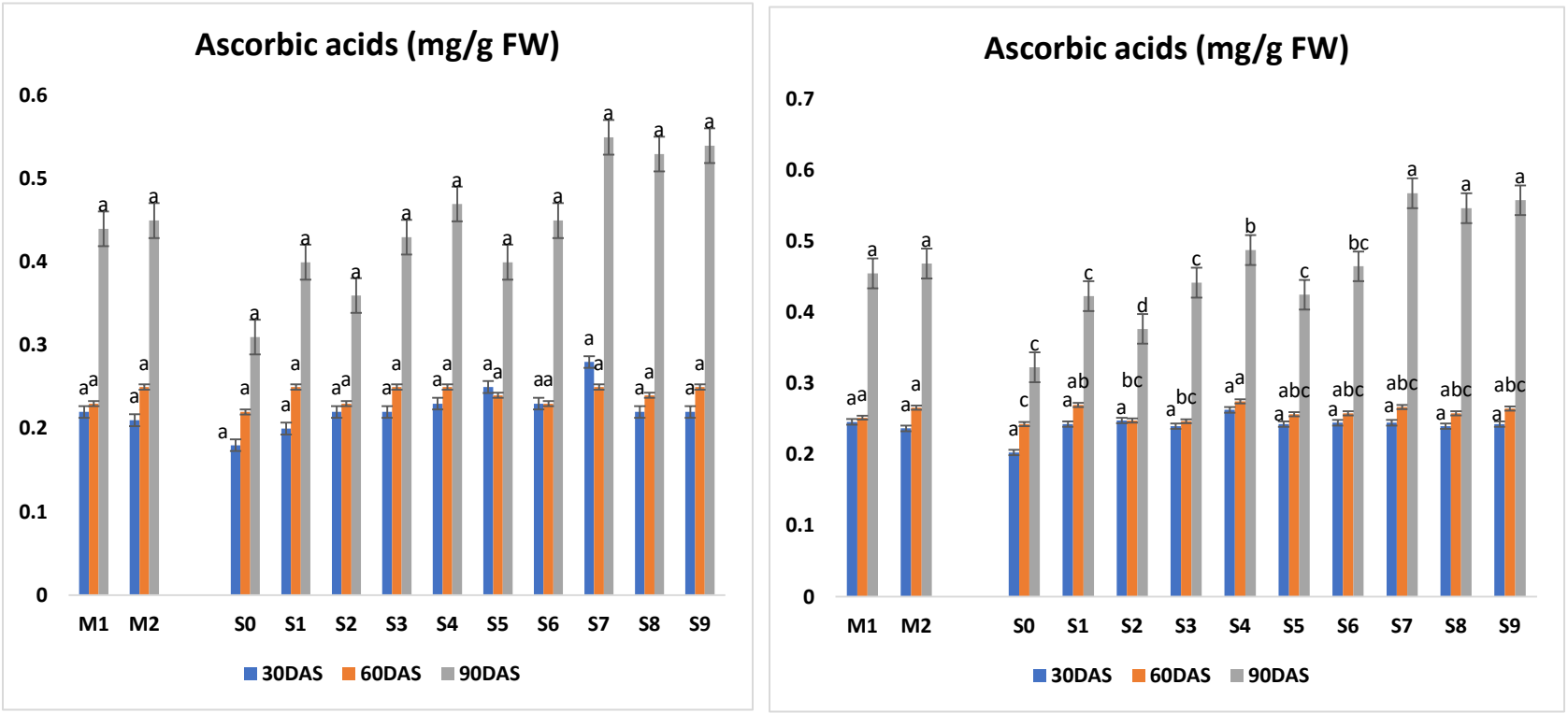


Fig-4.23 (a): Effect of spacing and nutrients on Ascorbic acids of the mustard crop during the rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.3.16 Relative Water Content (RWC) (%)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in relative water content were observed at 30DAS, 60DAS and 90DAS, as shown in Table 4.24, Fig 4.24. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the RWC in each treatment as compared to control of both the spacings at 30, 60DAS and 90DAS. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, the main plot M2 shows maximum RWC as compared to M1 with values 0.46 (M2) and 0.44 (M1), respectively. A percentage increase of 2.34% was found in M1, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in RWC was observed in S7, i.e. 65.17 at 30DAS, whereas in S7, Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S7, followed by S8> S9> S6> S3> S4> S2, and the per cent values were 64.17%, 60.12%, 55.64%, 52.39%, 51.83%, 51.52% and 41.43% respectively. At 60DAS, the main plot M2 shows maximum RWC compared to M1, with 42.43 (M2) and 41.17 (M1), respectively. A percentage increase of 2.96% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S9 with a value of 51.94, where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar application. The per cent increase was found highest in S9, followed by S8> S3> S1> S2> S7> S6> S5, and the per cent values were 36.00%, 35.36%, 22.19%, 21.39%, 16.64%, 16.29%, 14.90% and 14.68% respectively when it is compared with its control (S0). At 90DAS, the main plot M2 shows maximum RWC compared to M1, with values of 60.08 (M2) and 58.69 (M1), respectively. A percentage increase of 2.31% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant

results were observed in S2 with a value of 65.27, where Boron @ 1.5%) was applied to the crop as a foliar spray. The per cent increase was found highest in S2, followed by S6> S7> S3> S1> S4> S9> S8> S5, and the per cent values were 16.06%, 13.82%, 9.65%, 8.32%, 8.19%, 7.27%, 5.11%, 4.71% and 1.85% respectively when it is compared with its control (S0). The study showed a significant increase with 52.39%, 14.90% and 13.82% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the RWC was found in treatment S9, where the combined application of aqueous formulation of Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop. In the year (2022-23), at 30DAS, the main plot M2 shows maximum RWC as compared to M1 with values 0.46 (M2) and 0.44 (M1), respectively. A percentage increase of 2.34% was found in M1, where the crop was grown in reduced spacing (20*10). In subplots, a significant rise in RWC was observed in S7, i.e. 62.07 at 30DAS, whereas in S7, Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S7, followed by S8> S9> S6> S3> S4> S2, and the per cent values were 61.91%, 59.95%, 55.34%, 52.07%, 51.57%, 51.26% and 41.24% respectively. At 60DAS, main plot M2 shows maximum RWC compared to M1 with values 42.65 (M2) and 41.49 (M1), respectively. A percentage increase of 2.71% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S9 with a value of 52.06, where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar application. The per cent increase was found highest in S9, followed by S8> S3> S1> S2> S7> S6> S5, and the per cent values were 35.64%, 35.15%, 22.32%, 21.15%, 16.53%, 16.07%, 14.81% and 14.40% respectively when it is compared with its control (S0). At 90DAS, the main plot M2 shows maximum RWC compared to M1, with values of 60.28 (M2) and 58.67 (M1), respectively. A percentage increase of 2.67% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S2 with a value of

65.40, where Boron @ 1.5%) was applied to the crop as a foliar spray. The per cent increase was found highest in S2 followed by S6> S7> S3> S1> S4> S9> S8> S5, and the per cent values were 17.95%, 15.91%, 11.82%, 10.64%, 10.39%, 9.78%, 7.41%, 6.94% and 4.21% respectively when it is compared with its control (S0). The study showed a significant increase with 52.07%, 14.81% and 15.91% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the RWC was found in treatment S9, where the combined application of aqueous formulation of Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop. Relative water content shows the capacity of the plant to absorb and hold the water inside it. Application of boron increased the stability of leaf membranes, leaf RWC, and dry mass accumulation. Foliar boron application was more effective (Sayed 1998). Leaf RWC is linked to leaf elongation due to higher turgidity. Leaf RWC is one of the indicators for yield improvement under low water conditions. Increased RWC under S and B mixture might be because Boron and Cyt. are involved in stomatal regulation and help increase the growth and development of the crop. Giri et al. 2003 show a significant increase in relative water content under the application of micro and secondary nutrients. Relative water content is the ability of the plant to absorb and hold water to carry out various physiological and biochemical processes. The above study showed significant results in relative water content compared to its control.

Table 4.24 (a): Effect of spacing and nutrient on RWC (%) of the mustard crop during the rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	44.99	44.61	41.17	41.49	58.69	58.67
M2 (20×10)	46.07	46.46	42.43	42.65	60.08	60.28
C.D. at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.89	0.53	2.27	2.21	2.27	2.45
Nutrients foliar application						
S0-Control	23.35	23.64	33.24	33.51	54.79	53.66
S1-Boron @1%	25.77	26.20	42.28	42.50	59.67	59.88
S2-Sulphur @ 0.15%	39.87	40.23	39.87	40.14	65.27	65.40
S3-BAP @0.003%	48.48	48.81	42.72	43.14	59.76	60.05
S4-Boron @0.5% +Sulphur @0.25%	48.16	48.50	38.81	39.24	59.09	59.48
S5-Boron @ 1.5%+ Sulphur @0.075%	44.28	44.61	38.96	39.15	55.82	56.02
S6-Boron @ 0.5% + BAP (@0.0045%)	49.05	49.32	39.06	39.34	63.57	63.81
S7-Boron @ 1.5%+ BAP (@0.0015%)	65.17	62.07	39.71	39.93	60.64	60.85
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	58.55	59.03	51.43	51.67	57.50	57.66
S9-Sulphur @0.25%+ BAP (@0.0015%)	52.63	52.94	51.94	52.06	57.74	57.96
C.D. at p<0.05	9.52	8.05	10.20	10.20	10.42	10.38
SEM±	3.30	2.79	3.54	3.54	2.62	2.59
C.D. S×M at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	2.81	1.69	7.19	7.01	7.17	7.76
C.D. M×S at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	4.52	3.79	5.27	5.24	4.18	4.25

Where, C.D. represents critical difference, SE (m) represents standard error of mean and SE (d) represents the standard error of deviation.

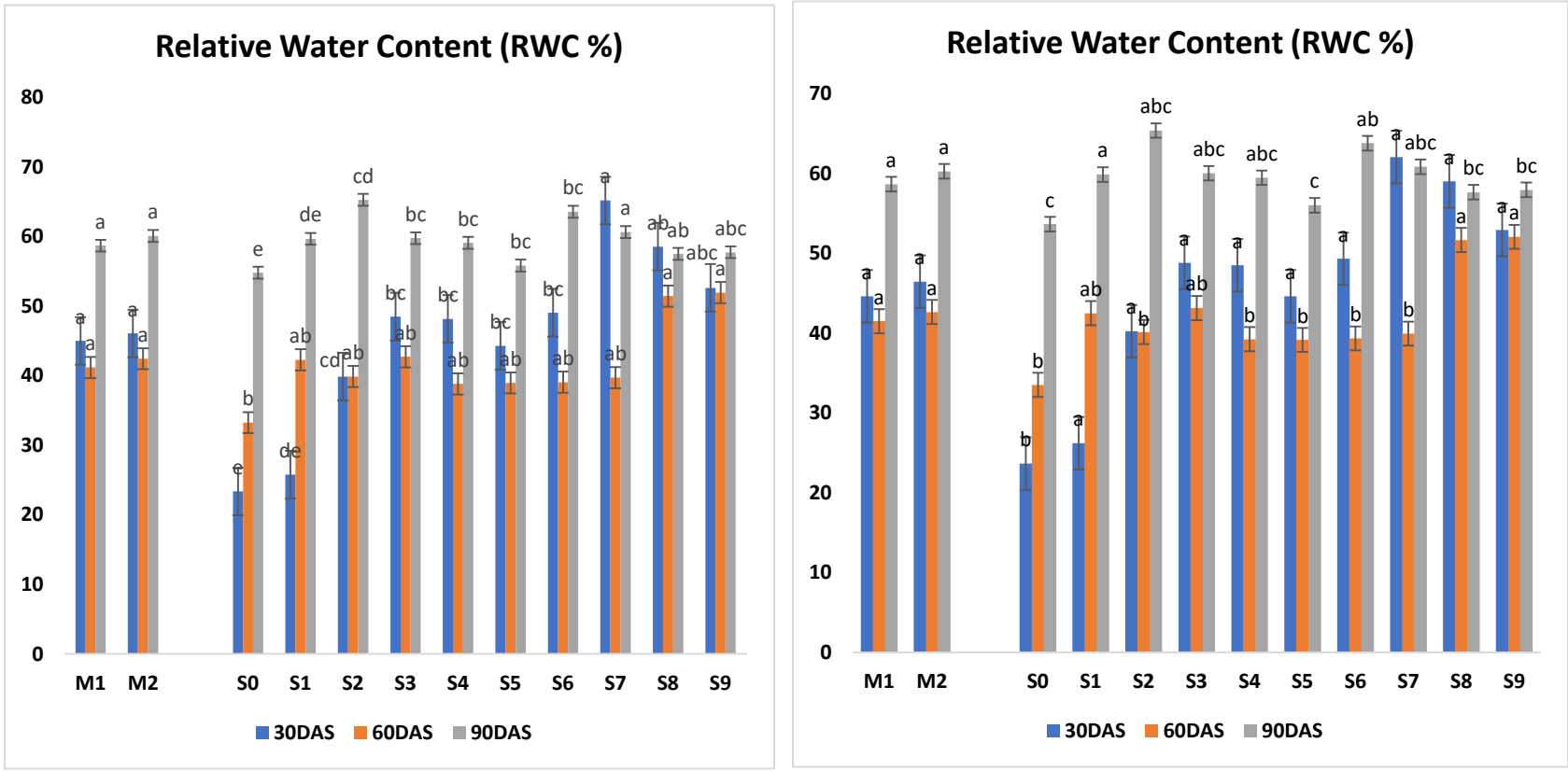


Fig-4.24 (a): Effect of spacing and nutrient on RWC of mustard crop during rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.3.17 Total Lipids (mg g^{-1} fresh weight)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in total lipids were observed at 30DAS, 60DAS, and 90DAS, as shown in Table 4.25 and Fig 4.25. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the total lipids in each treatment compared to control of both the spacings at 30, 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the total lipids was observed at 30, 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 30DAS, the main plot M2 shows maximum total lipids as compared to M1 with values 0.95 (M2) and 0.91 (M1), respectively. A percentage increase of 4.21% was found in M1, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total lipids was observed in S4, i.e. 1.51 at 30DAS, where in S4, Boron @ 0.5%+ Sulphur @0.25% was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S4, followed by S8> S5> S2> S1> S7> S3> S6, and the per cent values were 68.97%, 57.84%, 56.07%, 54.66%, 54.51%, 48.54%, 45.45% and 43.25% respectively. At 60DAS, the main plot M2 shows maximum lipid content compared to M1, with values of 1.34 (M2) and 1.31 (M1), respectively. A percentage increase of 2.23% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S7 with a value of 1.44, where Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop as a foliar application. The per cent increase was found highest in S7, followed by S4> S9> S5> S2> S8> S6> S1, and the per cent values were 22.22%, 21.40%, 20.56%, 19.23%, 17.94%, 17.84%, 15.15% and 14.61% respectively when it is compared with its control (S0). At 90DAS,

the main plot M1 shows maximum lipid content compared to M2, with 1.21 (M2) and 1.25 (M1) values, respectively. A percentage increase of 3.2% was found in M2, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S4 with a value of 1.50, where Boron @0.5% + sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S4, followed by S3> S5> S1> S2> S9> S8&S6, and the per cent values were 24.16%, 17.09%, 9.28%, 7.69%, 4.20%, 3.25%, 1.44% and 1.44% respectively when it is compared with its control (S0).

The study showed a significant increase with 43.25%, 15.15% and 1.44% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 30DAS and 90DAS, a significant increase in the lipid content was found in treatment S4, where the combined application of aqueous formulation of Boron @0.5% + sulphur @0.25% was applied to the crop.

In the year (2022-23), at 30DAS, main plot M1 shows maximum total lipids as compared to M2 with values 1.13 (M1) and 1.07 (M2), respectively. A percentage increase of 5.30% was found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase in total lipids was observed in S4, i.e. 1.59 at 30DAS, where in S4, Boron @ 0.5%+ Sulphur @0.25% was applied to the crop. Therefore, at 30 DAS, the percentage increase as compared to S0 was found to be highest in S4 followed by S8> S5> S2&S6> S1> S7> S3, and the per cent values were 45.91%, 31.56%, 24.22%, 21.69%, 21.69%, 20.98%, 10.41%, and 8.18% respectively. At 60DAS, the main plot M2 shows maximum lipid content compared to M1, with values of 1.36 (M2) and 1.33 (M1), respectively. A percentage increase of 2.20% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S7 with a value of 1.44, where Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop as a foliar application. The per cent increase was found highest in S7, followed by S4> S9> S5> S2> S8>

S6> S1, and the per cent values were 22.00%, 21.46%, 20.37%, 19.05%, 17.78%, 17.68%, 15.03% and 14.5% respectively when it is compared with its control (S0). At 90DAS, the main plot M1 shows maximum lipid content compared to M2, with 1.23 (M2) and 1.26 (M1) values, respectively. A percentage increase of 2.38% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S4 with a value of 1.52 where Boron @0.5% + sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S4, followed by S3> S5> S1> S2> S9> S6> S8 and the per cent values were 26.63%, 19.90%, 12.72%, 11.46%, 5.35%, 6.66%, 4.95% and 4.81% respectively when it is compared with its control (S0).

The study showed a significant increase with 21.69%, 15.03% and 1.17% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 30DAS and 90DAS, a significant increase in the lipid content was found in treatment S4, where the combined application of aqueous formulation of Boron @0.5% + sulphur @0.25% was applied to the crop. Chhokar, Vinod et al. (2008) reported that the lipid composition of mustard is the primary fatty acid of mustard seed, which was present in traces at the initial stages of plant growth. After that, it was absorbed to increase regularly with gradual accumulation of total lipids. Total lipid (fat) is a nutrient in many other foods, including mustard greens, which enhance the quality of mustard greens taken as leafy vegetables. The application of boron, sulphur, and cytokinin to the plant shows a significant increase in the lipid content compared to the controlled plot. This pathway undertakes the cytoplasmic synthesis of fatty acids from acetyl-CoA and malonyl-CoA. The degradation of carbohydrates and proteins produces acetyl-CoA. Central enzymes implicated are acetyl-CoA carboxylase and fatty acid synthase, which facilitate the synthesis of elongated fatty acids. Freshly produced fatty acids undergo esterification with glycerol to make triglycerides, serving as the main lipid's storage in plant cells. This biochemical

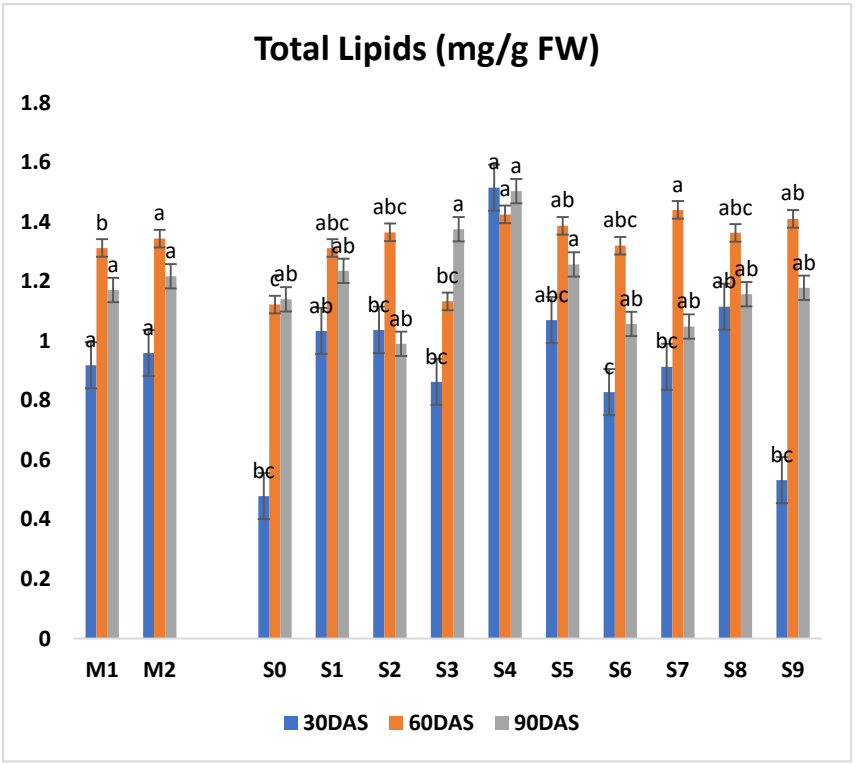
process occurs in the endoplasmic reticulum (ER) of the cytoplasm and the oil bodies, which are lipid droplets. Under stressful conditions or when energy is needed, stored triglycerides are enzymatically converted into free fatty acids and glycerol by lipolysis. These fuels are then used for energy generation or other metabolic activities. Cytokinins are phytohormones with a crucial regulatory function in controlling cellular division and differentiation. They exert their effect on lipid metabolism by regulating the expression of critical enzymes implicated in the synthesis and storage of lipids. The promotion of cell proliferation and the increase in substrate availability for lipid formation are mechanisms by which cytokinins can enhance lipid synthesis and storage. Sulphur is an essential constituent of amino acids, such as cysteine and coenzymes, that play a crucial role in lipid metabolism. Its impact on the availability of sulfur-containing compounds crucial for enzymatic activities affects lipid synthesis. Boron plays a role in cell wall biosynthesis and is essential for preserving the integrity and functionality of biological membranes, indirectly assisting in lipid metabolism. Boron, Sulphur, and Cytokinin together exert a synergistic effect on lipid metabolism. While cytokines promote cell division and expansion, resulting in increased lipid accumulation, sulphur and boron contribute to the synthesis of structural and functional components of lipid metabolism. This synergistic effect can enhance the overall lipid content by rationalizing lipids' synthesis, storage, and mobilization. The increased availability of Boron and Sulphur enhances the synthesis of essential lipid precursors and coenzymes. Boron exerts its control on lipid biosynthesis by modulating the metabolism of structural carbohydrates and components of the cell wall. Furthermore, Sulphur plays a role in the production of sulfur-containing amino acids and cofactors essential for synthesizing lipids. Cytokinins stimulate cellular division and expansion, augmenting the cellular surface area accessible for the deposition of lipids. Moreover, cytokinins control the expression of genes implicated in lipid biosynthesis, resulting in increased lipid synthesis. The closer the spacing, the more intense the competition for light, resulting in enhanced photosynthesis and a more abundant provision of carbohydrates for lipid preparation. The synergistic nutrient treatments enhance carbon allocation efficiency to lipid

storage, increasing overall lipid content. The higher lipid content at various growth phases can be ascribed to the dynamic interaction among lipid synthesis, storage, and mobilization. The application of nutrients during the early stages of growth enhances the accumulation of lipids, while the constant availability of nutrients promotes sustained lipid production throughout the entire growth cycle. The presence of sufficient lipid reserves is essential for the stress tolerance of plants. Augmented overall lipid content can bolster the plant's capacity to withstand environmental pressures by offering a readily accessible energy supply and preserving membrane integrity. Elevated total lipid content may suggest enhanced overall plant health and productivity. Augmented lipid concentrations enhance seed quality and yield, as lipids are vital for energy storage and cellular processes. It is possible to attribute the observed changes in total lipid content to the synergistic effects of Boron, Sulphur, and Cytokinin on lipid metabolism, further enhanced by optimum plant spacing. These findings justify using these nutrients to regulate lipid content, enhancing plant growth and productivity.

Table 4.25 (a): Effect of spacing and nutrients on Total Lipids (mg g^{-1} fresh weight) of mustard crop during the rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	30DAS		60DAS		90DAS	
Spacing						
M1 (30×10)	0.92	1.03	1.31	1.33	1.17	1.27
M2 (20×10)	0.96	1.08	1.34	1.37	1.22	1.23
C.D. at p<0.05	0.02	0.02	0.02	0.01	0.02	0.02
SEM±	0.00	0.00	0.00	0.00	0.00	0.02
Nutrients foliar application						
S0-Control	0.48	0.86	1.12	1.14	1.14	1.13
S1-Boron @1%	1.03	1.09	1.31	1.33	1.24	1.27
S2-Sulphur @ 0.15%	1.04	1.10	1.37	1.39	0.99	1.18
S3-BAP @0.003%	0.86	0.94	1.13	1.16	1.38	1.40
S4-Boron @0.5% +Sulphur @0.25%	1.52	1.59	1.43	1.45	1.50	1.53
S5-Boron @ 1.5%+ Sulphur @0.075%	1.07	1.14	1.39	1.41	1.26	1.28
S6-Boron @ 0.5% + BAP (@0.0045%)	0.83	1.10	1.32	1.34	1.06	1.18
S7-Boron @ 1.5%+ BAP (@0.0015%)	0.91	0.96	1.44	1.46	1.05	1.17
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	1.12	1.26	1.36	1.39	1.16	1.18
S9-Sulphur @0.25%+ BAP (@0.0015%)	0.53	1.02	1.41	1.43	1.18	1.20
C.D. at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.19	0.19	0.09	0.09	0.15	0.14
C.D. S×M at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.01	0.02	0.01	0.00	0.01	0.06
C.D. M×S at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.26	0.25	0.13	0.13	0.21	0.20

Where, C.D. represents critical difference, SE (m) represents standard error of mean and SE (d) represents the standard error of deviation.



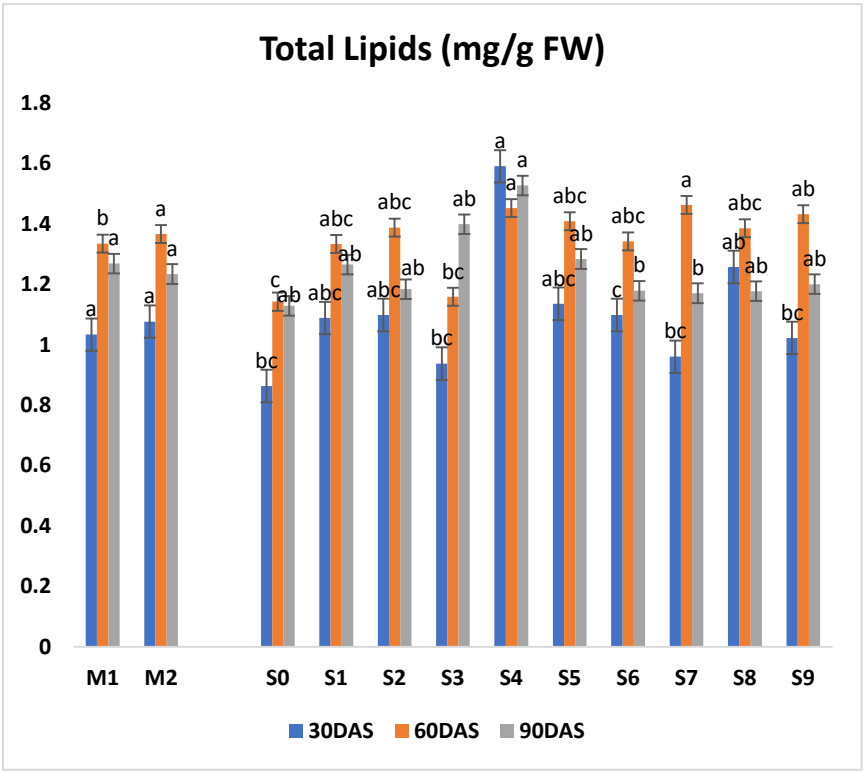


Fig-4.25 (a): Effect of spacing and nutrient on Total Lipids of mustard crop during rabi season of 2021-22

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.3.19 Membrane Stability Index (MSI) (%)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in membrane stability index (MSI) were observed at 60DAS and 90DAS, shown in Table 4.27 and Fig 4.27. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the membrane stability index in each treatment as compared to control of both the spacings at 60DAS and 90DAS. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the membrane stability index was observed at 60DAS and 90DAS in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), at 60DAS, the main plot M2 shows maximum MSI as compared to M1 with values of 24.56 (M2) and 23.54 (M1), respectively. A percentage increase of 4.15% was found in M1, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in membrane stability index was observed in S7, i.e. 35.70 at 60DAS, where in S7, Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop. Therefore, at 60 DAS, the percentage increase as compared to S0 was found to be highest in S7, followed by S5> S6> S3> S4> S8> S9> S1, and the per cent values were 66.58%, 64.51%, 62.03%, 57.37%, 54.98%, 46.73%, 44.99% and 26.68% respectively. At 90DAS, main plot M2 shows a maximum membrane stability index compared to M1 with values of 37.47 (M2) and 36.64 (M1), respectively. A percentage increase of 2.21% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S4 with a value of 43.24 where Boron @0.5% +Sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S4, followed by S7> S5> S1> S8> S6> S3> S2, and

the per cent values were 29.95%, 27.17%, 24.78%, 21.71%, 20.69%, 18.73%, 13.46% and 12.45% respectively when it is compared with its control (S0).

The study showed a significant increase with 62.03% and 18.73% cent values at 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS and 90DAS, a significant increase in the membrane stability index was found in treatment S7, where the combined application of aqueous formulation of Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop.

In the year (2022-23), at 60DAS, the main plot M2 shows maximum MSI as compared to M1 with values 31.10 (M2) and 30.56 (M1), respectively. A percentage increase of 1.73% was found in M1, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in membrane stability index was observed in S7, i.e. 33.58 at 60DAS, where in S7, Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop. Therefore, at 60 DAS, the percentage increase as compared to S0 was found to be highest in S7, followed by S6> S5> S9> S8> S4> S3> S1, and the per cent values were 22.10%, 20.51%, 20.37%, 19.84%, 19.52%, 13.01%, 12.35%, 10% respectively. At 90DAS, main plot M2 shows a maximum membrane stability index compared to M1 with values 36.67 (M2) and 34.55 (M1), respectively. A percentage increase of 5.78% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S5 with a value of 40.54 where Boron @ 1.5%+ Sulphur @0.075% was applied to the crop as a foliar spray. The per cent increase was found highest in S5, followed by S8> S7> S6> S1> S4> S9> S2 and the per cent values were 23.10%, 19.82%, 17.37%, 16.38%, 12.82%, 12.57%, 7.61% and 7.45% respectively when it is compared with its control (S0).

The study showed a significant increase with 20.51% and 16.38% cent values at 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. At 60DAS

and 90DAS, a significant increase in the membrane stability index was found in treatment S7, where the combined application of aqueous formulation of Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop. Cytokinin is a complex plant hormone involved in various plant growth and development processes as well as stress responses. Exogenous application of cytokinin up-regulates the expression of sulfur-responsive genes in leaves (Hirose et al. 2008). Along with cytokinin, applying nutrients like sulphur and boron enhances the growth and yield of the plant. Therefore, it is clear from the experiment that the application of sulphur and boron along with cytokinin shows better results in membrane stability index and membrane injury index than in controlled plots.

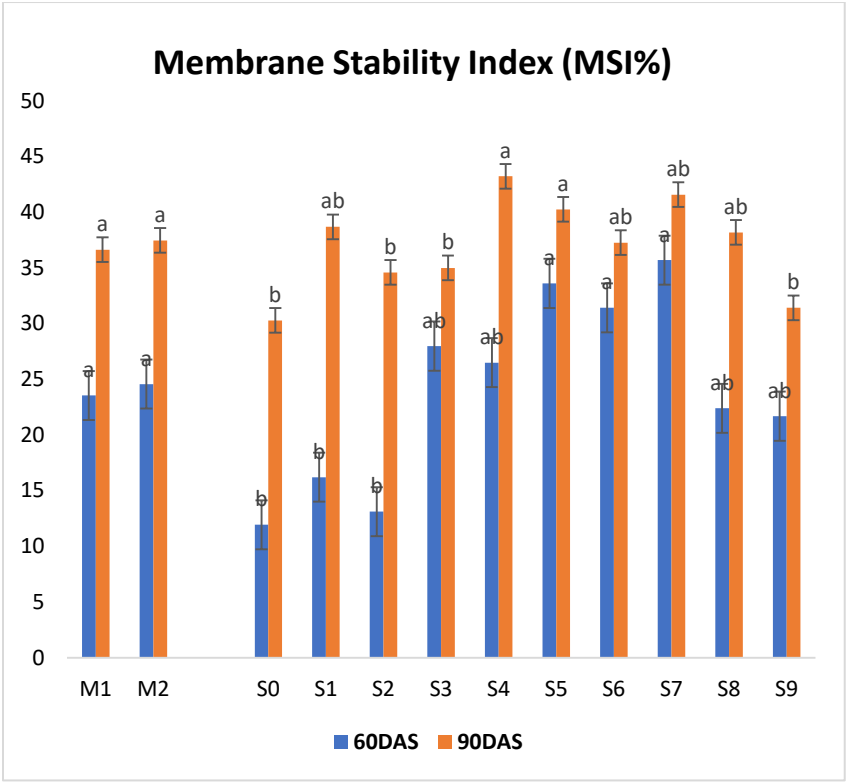
The Membrane Stability Index (MSI) is an essential physiological measure that indicates the structural fidelity of cell membranes when subjected to stressful circumstances. Gaining insight into the dynamic response of mustard crops to different nutrient concentrations and environmental conditions is crucial. The lipid bilayer, consisting of phospholipids, proteins, and sterols, is the main factor responsible for maintaining the stability of membranes at the cellular level. Under ideal circumstances, these components are arranged to preserve their fluidity and functionality, enabling effective absorption of nutrients, elimination of metabolic waste, and transmission of signals. An essential function of boron is to maintain the structural integrity of the cell wall and membrane. The process of cross-linking pectic polysaccharides in the cell wall indirectly contributes to the stabilization of the plasma membrane. Inadequate boron levels can impair membrane integrity, heightening vulnerability to leakage and oxidative stress. Sulphur is indispensable for producing vital amino acids (cysteine and methionine) and antioxidants such as glutathione. These components protect the membrane against reactive oxygen species (ROS)-induced oxidative damage. The cell's antioxidant capacity is enhanced by sufficient sulphur supply, which helps to stabilize the membrane. Membrane stability is influenced by cytokinin through the modulation of stress-responsive gene expression and enhancement of protective

protein synthesis, namely heat shock proteins (HSPs). These proteins inhibit the denaturation of membrane proteins when exposed to stress, preserving the integrity of the membrane. Under stressful circumstances, such as drought or nutrient shortage, reactive oxygen species (ROS) are excessively abundant. These highly reactive molecules can induce lipid peroxidation, impairing and destabilizing the membrane. By neutralizing reactive oxygen species (ROS), antioxidants produced from amino acids containing sulphur prevent membrane destabilization. Membrane lipids composed of unsaturated fatty acids are especially susceptible to reactive oxygen species (ROS) peroxidation. The outcome of this process is the production of malondialdehyde (MDA), which indicates oxidative stress and damage to the cell membrane. Applying nutrients that augment antioxidant synthesis or mitigate ROS generation can decrease MDA levels, elevating MSI. Experimental treatments including combined nutrients (e.g., Boron and Cytokinin or Sulphur and Cytokinin) exhibited greater Mean Serum Iron (MSI) levels than the control group. This phenomenon can be ascribed to multifaceted cellular processes. The concurrent administration of Boron and Cytokinin or Sulphur and Cytokinin probably led to a synergistic improvement in the integrity of the membrane. The structural function of boron and the regulatory function of cytokinin in gene expression and stress protein synthesis confer a dual protective effect on the membrane. Sulphur, which serves as a precursor for glutathione, enhances the antioxidant capacity through the reduction of ROS levels, so safeguarding the membrane against oxidative damage. Cytokinin enhances membrane stability by regulating stress-responsive pathways, increasing membrane stress index (MSI). Based on the lower MDA levels observed in treated plants, it is probable that the treatments effectively decreased lipid peroxidation. The reduction is crucial for preserving the fluidity and functionality of the membrane, which justifies the higher membrane stress index (MSI) in plants treated with nutrients. Justification for the observed increases in MSI can be attributed to the improved structural integrity, antioxidant defence, and decreased oxidative damage at the cellular level resulting from applying particular nutrient combinations in mustard crops.

Table 4.27 (a): Effect of spacing and nutrient on MSI (%) of mustard crop during rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23
	60DAS		90DAS	
Spacing				
M1 (30×10)	25.54	30.56	35.64	34.45
M2 (20×10)	26.56	31.1	36.47	36.67
C.D. at p<0.05	NS	NS	0.88	1.01
SEM±	1.77	0.39	0.13	0.15
Nutrients foliar application				
S0-Control	31.93	26.16	30.29	31.18
S1-Boron @1%	16.2	29.06	38.69	35.76
S2-Sulphur @ 0.15%	13.11	28.67	29.6	33.69
S3-BAP @0.003%	27.98	29.84	30.05	31.15
S4-Boron @0.5% +Sulphur @0.25%	26.5	30.07	43.24	35.66
S5-Boron @ 1.5%+ Sulphur @0.075%	33.62	32.85	40.27	40.54
S6-Boron @ 0.5% + BAP (@0.0045%)	31.42	32.91	37.27	37.29
S7-Boron @ 1.5%+ BAP (@0.0015%)	35.7	33.58	41.59	37.73
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	22.39	32.5	38.19	38.88
S9-Sulphur @0.25%+ BAP (@0.0015%)	21.68	32.63	31.42	33.75
C.D. at p<0.05	11.42	11.38	5.12	5.14
SEM±	3.97	1.86	3.36	1.78
C.D. S×M at p<0.05	NS	NS	NS	NS
SEM±	3.62	1.23	0.42	0.49
C.D. M×S at p<0.05	NS	NS	NS	NS
SEM±	2.84	2.53	4.87	2.40

Where, C.D. represents critical difference, SE (m) represents standard error of mean



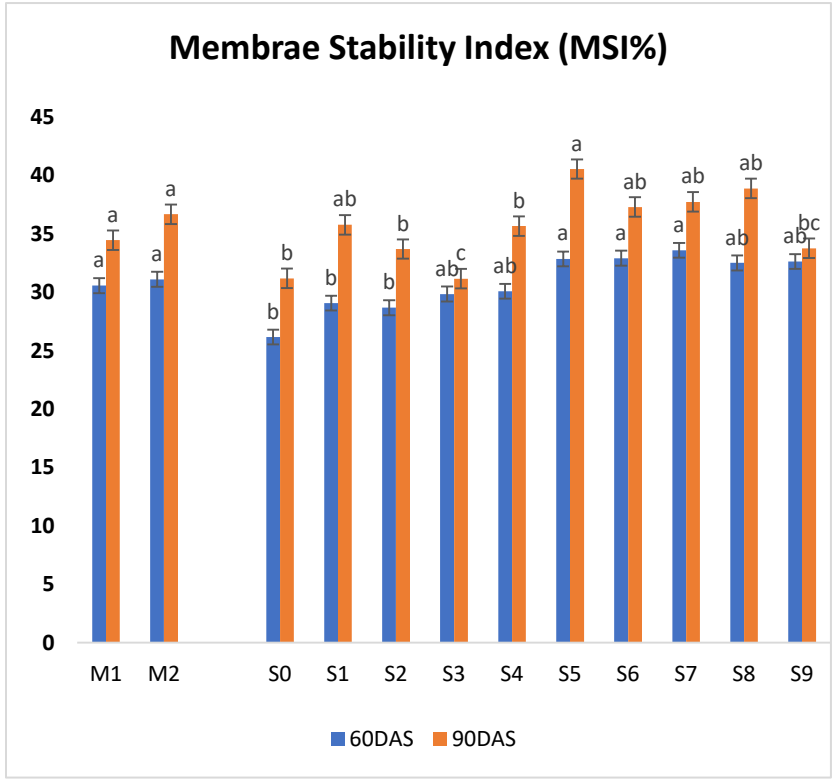


Fig-4.27 (a): Effect of spacing and nutrient on MSI of mustard crop during rabi season of 2021-22

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.4 Yield attributing parameters

4.4.1: No. of 1º branches (No. plant^{-1})

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in several primary branches per plant were observed at harvest, as shown in Table 4.28 and Fig 4.28. During this experiment on mustard crops, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the number of primary branches per plant in each treatment as compared to the control of both the spacings at harvest. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in the number of primary branches per plant was observed at harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22) main plot M1 (30*10) & M2 (20*10) shows same results. No significant difference was found between M1 and M2 at 90DAS of the crop. Significant results were observed in S6 with a value of 5.67 in subplots where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S6, followed by S1> S8> S2=S3=S5> S7>S9, and the per cent values were 5.82%, 3.95%, 3.50%, 3.48%, 3.48%, 3.48%, 2.90% and 0.06% respectively when it is compared with its control (S0).

The study shows a significant increase, with 5.82% per cent value, when comparing S6 and S0 (control). In treatment S6, the mustard crop was treated with a foliar application of Boron @ 0.5% + BAP (@0.0045%).

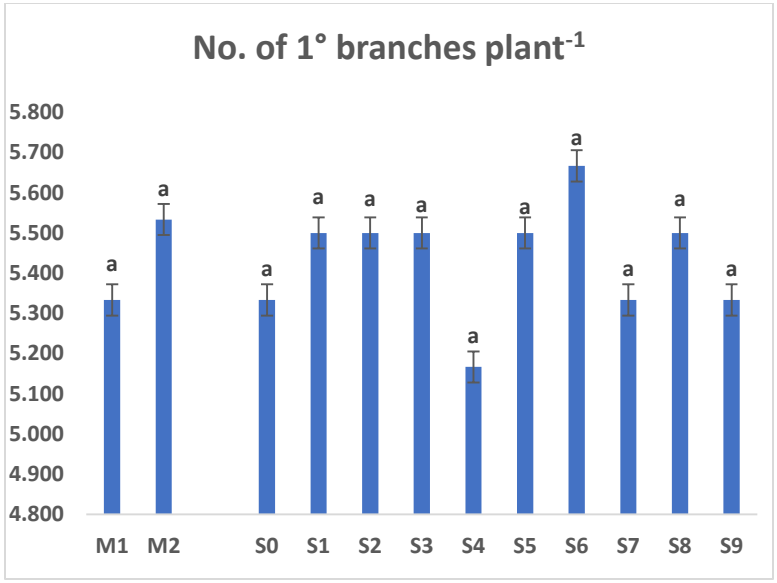
In the year (2022-23) main plot M2 shows the maximum no. of primary branches per plant compared to M1 with values 5.58 (M2) and 5.42 (M1), respectively. A percentage increase of 2.86% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots,

significant results were observed in S6 with a value of 5.72, where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S6 followed by S3> S8> S5> S4> S2> S1> S7&S9, and the per cent values were 6.81%, 4.13%, 3.96%, 3.87%, 3.70%, 3.17%, 3.02%, 1.20% and 1.20% respectively when it is compared with its control (S0).

The study showed a significant increase with 6.81% per cent value when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. The availability of nutrients in adequate amounts resulted in the formation of photosynthates, which promote metabolic activities, increase cell division, and ultimately increase the number of primary and secondary branches (Sharma et al., 2020). Similar results were found by Yadav et al. (2016).

The number of primary branches in mustard crops is critical to overall plant architecture and yield potential. The development and proliferation of these branches are regulated by a complex interplay of genetic, hormonal, and environmental factors that influence cell division, differentiation, and growth at the meristematic regions of the plant. At the cellular level, the initiation and growth of primary branches are primarily controlled by the activity of the shoot apical meristem (SAM) and the axillary meristems. These meristematic tissues are rich in undifferentiated cells that have the potential to divide and give rise to new organs, including leaves, flowers, and branches. Auxin, a plant hormone, plays a pivotal role in regulating the formation of primary branches. It is produced in the apical bud and is transported basipetally (downwards) through the plant. The distribution of auxin creates a gradient that inhibits the growth of axillary buds, a phenomenon known as apical dominance. However, when the auxin concentration decreases, or its transport is disrupted, axillary buds are released from dormancy and begin to form branches. Cytokinins are another class of hormones that counteract the effects of auxin by promoting cell division in the axillary meristems, leading to the initiation of primary

branches. Cytokinins activate genes associated with cell cycle progression and meristem activity, encouraging the outgrowth of axillary buds. The availability of nutrients, particularly Boron (B) and Sulphur (S), can significantly influence the branching pattern. Boron is essential for cell wall formation and stabilization, supporting newly formed branches' structural integrity. On the other hand, Sulphur is a vital component of amino acids and proteins, contributing to overall cellular metabolism and energy transfer processes. Environmental conditions, such as light intensity and spacing, also affect the number of primary branches. Adequate light and optimal spacing reduce competition among plants, enhancing the allocation of resources to axillary bud growth. The experimental results indicate that nutrient treatments, particularly the combined application of Boron, Sulphur, and Cytokinin, significantly increased the primary branches in mustard crops. This can be justified based on the following mechanisms: The exogenous application of Cytokinin likely stimulated the axillary meristems, leading to increased cell division and outgrowth of primary branches. Cytokinins may have modulated the expression of genes involved in branching, such as the *SHOOT MERISTEMLESS* (STM) and *CYTOKININ OXIDASE* (CKX) genes, promoting sustained meristem activity. Boron and Sulphur contributed to better nutrient assimilation and utilization, providing the necessary building blocks for cellular structures in the developing branches. Boron's role in stabilizing cell walls and Sulphur's involvement in protein synthesis and enzymatic functions supported robust branch formation. The nutrient treatments likely altered the hormonal balance between auxin and cytokinin, reducing apical dominance and allowing more axillary buds to break dormancy and develop into primary branches. The experimental design, which included optimal spacing, ensured that plants received adequate light and resources, further promoting the growth of primary branches by reducing competition and improving photosynthetic efficiency. Overall, the increase in the number of primary branches observed under specific nutrient treatments can be attributed to the synergistic effects of hormonal regulation, improved nutrient availability, and favourable environmental conditions, all of which contributed to enhanced cellular processes driving branch initiation and growth.



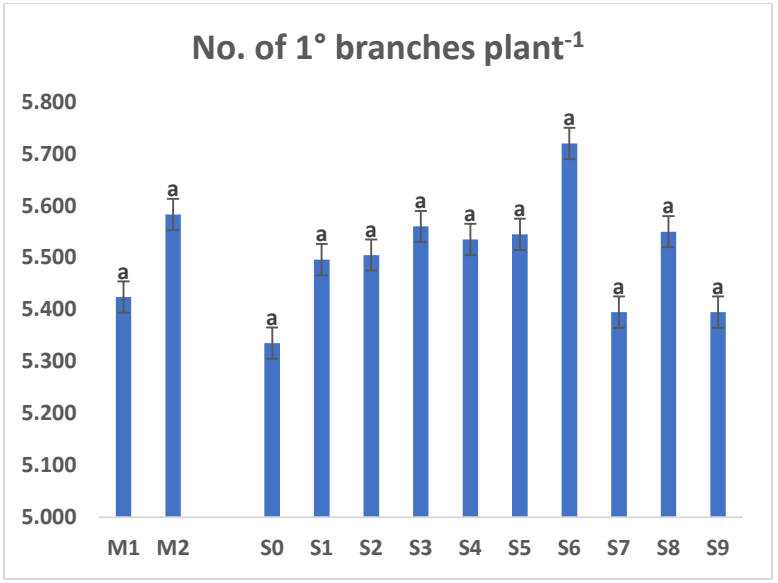


Fig-4.28 (a): Effect of spacing and nutrient on Primary branches of mustard crop during rabi season of 2021-22

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.4.2: No. of 2º branches (No. plant^{-1})

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in the number of secondary branches per plant were observed at harvest, as shown in Table 4.28 and Fig 4.29. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the number of secondary branches per plant in each treatment as compared to the control of both the spacings. Thus, the pattern of percentage increase in the number of secondary branches per plant was observed at harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), main plot M2 shows the maximum no. of secondary branches per plant as compared to M1 with values of 19.8 (M2) and 18.36 (M1), respectively. A percentage increase of 7.27% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S8 with value 24, where Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S8 followed by S9> S6> S7> S5=S2=S3> S4> S1, and the per cent values were 38.20%, 28.24%, 25.85%, 23.29%, 21.94%, 21.94%, 21.94%, 16.05% and 14.44% respectively when it is compared with its control (S0). The study showed a significant increase with 25.85% cent value when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. A significant increase in the no. of secondary branches per plant was found in treatment S8, where the combined application of aqueous formulation of Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop. In the year (2022-23), main plot M2 shows the maximum number of secondary branches per plant compared to M1 with values of 21.42 (M2) and 19.97 (M1), respectively. A percentage increase of 6.76% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S8 with a value of 23.50, where Sulphur @

0.075%+ BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S8 followed by S9> S3> S6> S7> S2> S5> S4> S1, and the per cent values were 37.76%, 30.16%, 26.12%, 26.04%, 24.26%, 22.86%, 21.59%, 19.94% and 18.29% respectively when it is compared with its control (S0). The study showed a significant increase with 26.04% value when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. A significant increase in the no. of secondary branches per plant was found in treatment S8, where the combined application of aqueous formulation of Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop. The availability of nutrients in an adequate amount resulted in the formation of photosynthates, which promote metabolic activities, increase cell division, and, therefore, increase the number of primary and secondary branches (Sharma et al. 2020). Developing secondary branches in mustard crops is intricate and controlled by genetic, hormonal, and environmental mechanisms. The initiation and subsequent development of secondary branches at the cellular level are influenced mainly by the interaction of auxin, cytokinins, and other growth regulators and the plant's efficient resource allocation. An indispensable plant hormone, synthesized in the apical meristems (growing tips), is vital in promoting apical dominance by inhibiting the growth of lateral buds, including those that would eventually develop into secondary branches. The optimal distribution and concentration of auxin in the plant are crucial. Insufficient levels of auxin in some areas of the plant, especially in the lateral buds, can trigger the activation of these buds and the subsequent development of secondary branches. Cytokinins stimulate cellular division and facilitate the development of lateral buds. A higher ratio of cytokinin to auxin in lateral buds can counteract the dominance of the apex, resulting in the initiation and development of secondary branches. In mustard cultivation, cytokinin (BAP) in treatments has probably increased the number of secondary branches by promoting the growth of these lateral buds. Adequate provision and distribution of crucial nutrients such as Boron and Sulphur are imperative for the proliferation and maturation of secondary branches. Boron is

instrumental in cell wall formation and the maintenance of the plasma membrane, both of which are vital for developing new growth sites, including secondary branches. Sulphur plays a role in synthesizing amino acids and proteins essential for cellular growth and division. The provision of sufficient nutrients, particularly in treatments that include Boron and Sulphur, guarantees that the plant has the necessary fundamental components to sustain the development of secondary branches. Moreover, the combination of Boron and cytokines in therapies has the potential to augment cell division and elongation processes, thereby facilitating secondary branching. Furthermore, the development of secondary branches is significantly influenced by environmental factors, including spacing. Plants cultivated with narrower spacing (denser planting) may encounter increased competition for light, nutrients, and water, inhibiting the growth of secondary branches. On the other hand, a greater spacing (e.g., 30x10) decreases competition, giving each plant more significant access to resources and making it possible to establish a more significant number of secondary branches. The measured variation in secondary branch numbers under different spacing conditions in the experiment can be ascribed to disparities in resource allocation and the plants' capacity to react to hormonal signals that stimulate lateral bud development. The increased number of secondary branches in treatments that involve the combined application of Boron, Sulphur, and Cytokinin can be explained by the improved hormonal activity and nutrient availability resulting from these treatments. The collective impact of these components stimulates the development of lateral buds, resulting in an increased quantity of secondary branches. The increased branching can enhance the plant's structural integrity, which may result in a more significant number of flowering sites and, therefore, a higher yield potential in mustard crops. The variation in branch numbers observed among different treatments and spacing conditions emphasizes the need to optimize hormonal and nutrient management to maximize branching and enhance overall crop performance.

Table 4.28 (a): Effect of spacing and nutrients on primary and secondary branches (No. plant^{-1}) of the mustard crop during the rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23
	1° branches plant^{-1}		2° branches plant^{-1}	
Spacing				
M1 (30×10)	5.33	5.48	18.37	19.97
M2 (20×10)	5.53	5.58	19.80	21.43
C.D. at p<0.05	NS	0.15	NS	NS
SEM±	0.10	0.02	0.51	0.52
Nutrients foliar application				
S0-Control	5.34	5.34	14.83	15.79
S1-Boron @1%	5.56	5.50	17.33	19.33
S2-Sulphur @ 0.15%	5.53	5.51	19.00	20.47
S3-BAP @0.003%	5.53	5.56	19.00	21.38
S4-Boron @0.5% +Sulphur @0.25%	5.34	5.54	17.67	19.73
S5-Boron @ 1.5%+ Sulphur @0.075%	5.53	5.55	19.00	20.14
S6-Boron @ 0.5% + BAP (@0.0045%)	5.67	5.72	20.00	21.35
S7-Boron @ 1.5%+ BAP (@0.0015%)	5.50	5.40	19.33	20.85
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	5.53	5.55	24.00	25.37
S9-Sulphur @0.25%+ BAP (@0.0015%)	5.34	5.40	20.67	22.61
C.D. at p<0.05	NS	NS	3.41	3.42
SEM±	0.25	0.26	1.18	1.19
C.D. S×M at p<0.05	NS	NS	NS	NS
SEM±	0.34	0.07	1.63	1.66
C.D. M×S at p<0.05	NS	NS	NS	NS
SEM±	0.36	0.36	1.67	1.68

Where, C.D. represents critical difference, SE (m) represents standard error of mean.

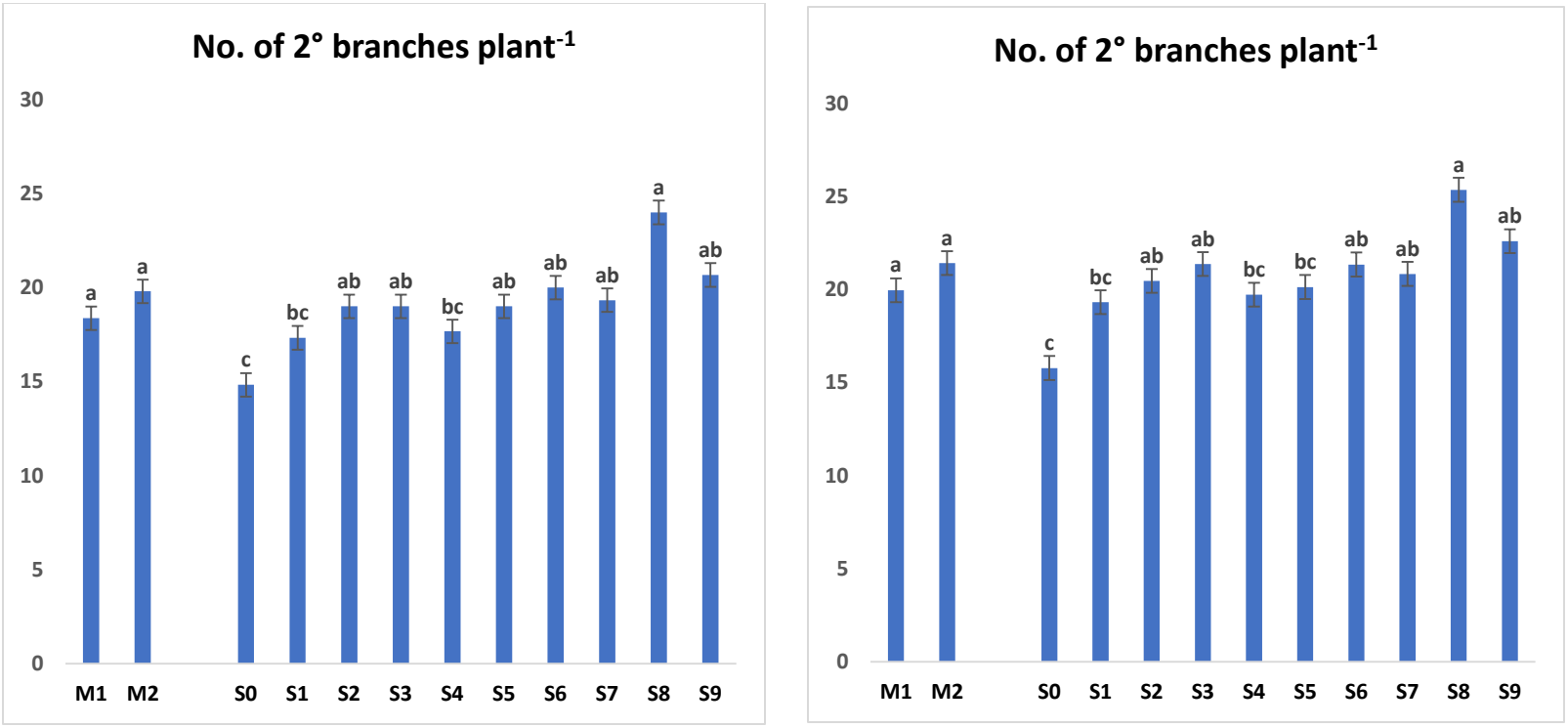


Fig-4.29 (a): Effect of spacing and nutrients on Secondary branches of the mustard crop during rabi season of 2021-22 and 2022-23

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.4.3: Number of siliquae (No. plant^{-1})

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in siliquae per plant were observed at harvest, as shown in Table 4.30, Fig 4.30. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the number of siliquae per plant in each treatment as compared to the control of both the spacings. The percentage increase was calculated by comparing all the treatments with the power and the spacings. Thus, the pattern of percentage increase in siliquae was observed at harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), main plot M2 shows a maximum no. of siliquae per plant compared to M1 with values 289.36 (M2) and 280.06 (M1), respectively. A percentage increase of 3.21% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S9 with a value of 346.33 where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent increase was found highest in S9, followed by S8> S6> S3> S7> S2> S1> S5> S4, and the per cent values were 49.80%, 47.95%, 44.34%, 42.63%, 41.86%, 39.14%, 36.32%, 35.37% and 30.74% respectively when it is compared with its control (S0).

The study showed a significant increase with 44.34% value when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. A significant increase in the number of siliquae per plant was found in treatment S9, where the combined application of an aqueous formulation of Sulphur @ 0.25%+ BAP (@0.0015%) was applied to the crop.

In the year (2022-23), main plot M2 shows a maximum no. of siliquae per plant compared to M1 with values 291.64 (M2) and 282.29 (M1), respectively. A percentage increase of 3.20% was

found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S9 with a value of 348.89 where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent increase was found highest in S9 followed by S8> S6> S3> S7> S2> S1> S5> S4, and the per cent values were 49.84%, 47.93%, 44.33%, 42.64%, 41.91%, 39.21%, 36.68%, 35.50% and 30.89% respectively when it is compared with its control (S0).

The study showed a significant increase with 44.33% values when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. A significant increase in the no. of siliquae per plant was found in treatment S9 where the combined application of aqueous formulation of Sulphur @ 0.25%+ BAP (@0.0015%) was applied to the crop. An increase in the number of siliquae per plant with the addition of S and B might be due to an increase in S and B concentration in the plant, which is favourable in the translocation of photosynthates. The seeds per siliqua increased significantly by applying sulphur and boron either singly or in combination. The positive response could be due to the increased absorption of sulphur, boron, and cytokinin from the leaf canopy in the formation of reproductive structure. Similar results have also been reported by Budhar et al. (2003) and Kumar et al. (2002).

The intricate interactions between nutrient availability, hormonal regulation, and environmental factors primarily influence the number of siliquae per plant in mustard crops. Understanding the cellular mechanisms behind this phenomenon helps justify the observed variations in siliqua production. Nutrients such as boron, sulphur, and cytokinin are crucial in determining the amount of siliqua per plant. Boron is essential for cell wall synthesis, and membrane stability is critical for developing reproductive organs. An adequate boron supply ensures proper pollen tube growth and fertilisation, increasing the amount of siliqua. Sulphur synthesises amino acids and proteins vital for flower and fruit sets. It also plays a role in the formation of chlorophyll, enhancing

photosynthetic activity and providing the necessary energy for siliqua formation. Cytokinins are plant hormones that regulate cell division and differentiation. In mustard crops, cytokines promote the growth of axillary buds, which can develop into siliqua-bearing branches. Applying Cytokinins can stimulate the formation of more flowers, subsequently increasing the number of siliqua. Additionally, cytokines can delay senescence, ensuring that the reproductive structures remain functional for a longer period, which further contributes to siliqua production. At the cellular level, the availability of Boron influences the integrity of cell walls and the formation of pectin, which are necessary for cell expansion and division in reproductive tissues. Through its role in protein synthesis, Sulphur ensures that the cells involved in flower and siliqua formation are adequately supplied with the necessary building blocks. Cytokinins promote cytokinesis, increasing the number of cells that can differentiate into reproductive organs. The combined effect of these nutrients ensures that the plants have a higher potential to form siliqua, provided that other environmental factors, such as light and water, are optimal. In the experimental results, treatments with combined applications of Boron, Sulphur, and Cytokinin consistently showed increased siliqua per plant compared to the control. This can be attributed to the synergistic effects of these nutrients on cellular processes that govern flower and fruit sets. The enhanced nutrient availability justifies the increased siliquae, which supports the cellular mechanisms responsible for reproductive organ development. The number of siliquae per plant directly results from the cellular processes influenced by nutrient availability and hormonal regulation. The observed increase in siliqua number in treatments with combined nutrient applications is supported by the role of Boron, Sulphur, and cytokines in promoting cell division, expansion, and differentiation in reproductive tissues. This justifies the experimental findings and underscores the importance of balanced nutrient management in mustard crop production.

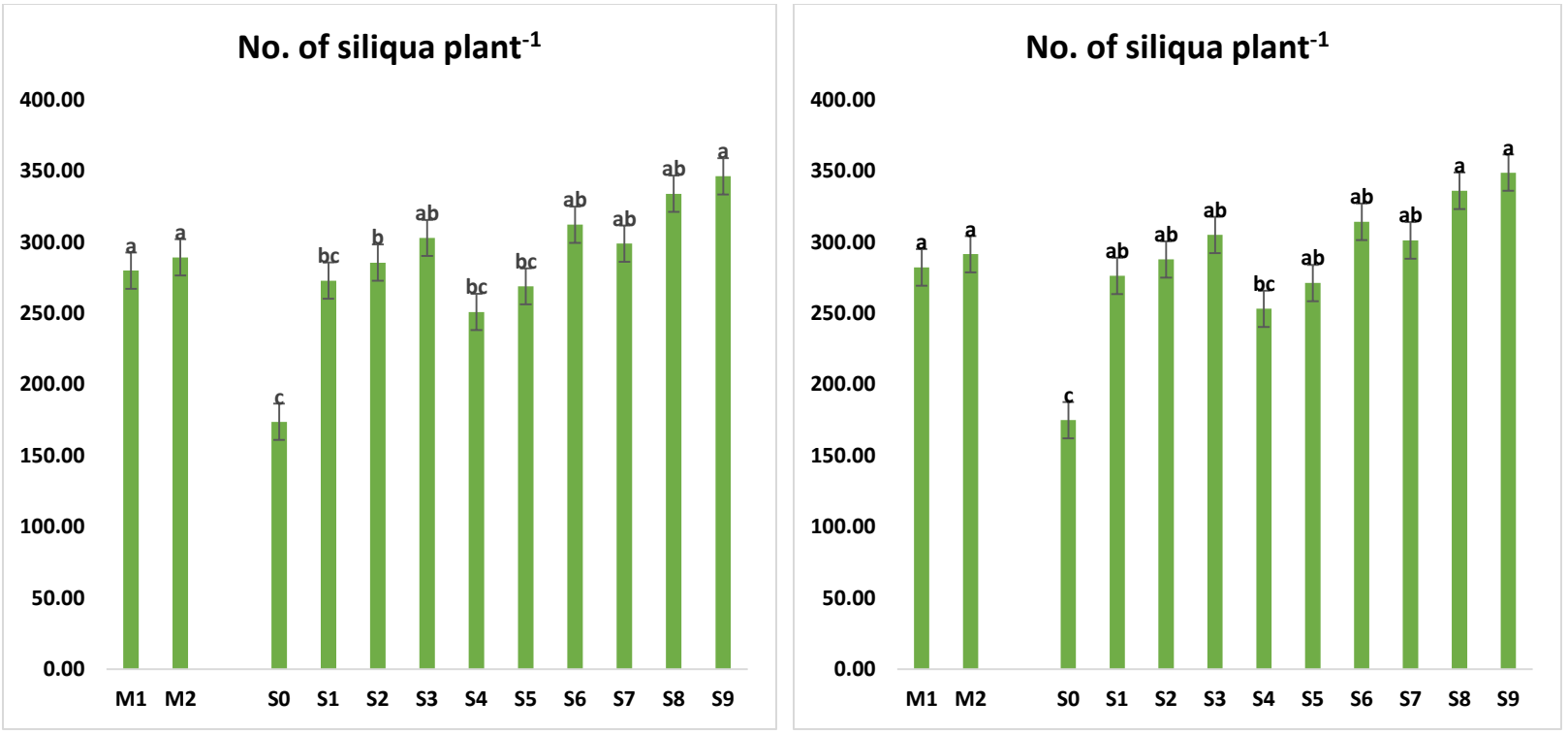


Fig-4.30 (a): Effect of spacing and nutrient on No. of siliquae per plant of the mustard crop during rabi season of 2021-22

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.4.4: Siliqua length (cm)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in siliquae length per plant were observed at harvest, shown in Table 4.30 and Fig 4.31. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the length of siliquae per plant in each treatment as compared to the control of both the spacings. The percentage increase was calculated by comparing all the treatments with the power and the spacings. Thus, the pattern of percentage increase in the length of siliquae was observed at harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), main plot M2 shows the maximum length of siliquae per plant as compared to M1 with values of 8.71 (M2) and 8.37 (M1), respectively. A percentage increase of 3.90% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S8 with a value of 9cm where Sulphur @0.075%+ BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S8, followed by S7> S6> S2> S9> S5>S4> S1> S3, and the per cent values were 17.22%, 16.13%, 15.97%, 14.69%, 14.36%, 14.20%, 13.03%, 10.77% and 9.33% respectively when it is compared with its control (S0). The study showed a significant increase with 17.22% value when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. A significant increase in the length of siliquae per plant was found in treatment S8, where the combined application of aqueous formulation of Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop.

In the year (2022-23), the main plot M2 shows the maximum length of siliquae per plant compared to M1, with values of 8.80 (M2) and 8.43 (M1), respectively. A percentage increase of

4.20% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S8 with a value of 8.8cm where Sulphur @0.075%+ BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S8, followed by S6> S7> S9> S2> S5> S4> S1> S3, and the per cent values were 16.58%, 15.69%, 15.45%, 14.73%, 14.49%, 14.39%, 13.11%, 11.52% and 9.39% respectively when it is compared with its control (S0). The study showed a significant increase with 18.45% and 15.69% cent values when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. A significant increase in the length of siliquae per plant was found in treatment S8, where the combined application of aqueous formulation of Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop. An increase in the length of siliqua could be explained by using a balanced nutrient supply and plant growth hormone, which enhance cell division and photosynthesis and are later converted into reproductive phases. Similar findings were also recorded by Kumar et al. (2000) and Akter et al. (2007). From the experiment, it was clear that the application of micronutrients and plant growth hormones plays a significant role in the growth and yield-attributing characteristics of the mustard crop. Siliqua length in mustard crops is influenced by various physiological and biochemical processes at the cellular level. The primary factors contributing to siliqua length include cell division, cell elongation, and the regulation of hormonal balance within the plant tissues, particularly in the siliqua. The formation of siliqua begins with the differentiation of floral organs, where the ovary develops into a siliqua after fertilization. During this phase, active cell division occurs within the ovary walls and along the length of the developing siliqua. The cell division rate determines the siliqua's initial size, while subsequent cell elongation contributes to the increase in its length. Cell elongation is driven by the synthesis and deposition of cell wall materials, primarily cellulose, hemicellulose, and pectins, which allow the cells to expand longitudinally. This expansion is facilitated by enzymes like expansins, which loosen the cell wall structure, making it more pliable for elongation. Auxins play a critical role in

promoting cell elongation. They stimulate the activity of proton pumps in the plasma membrane, leading to acidification of the cell wall, which activates enzymes that facilitate cell wall loosening and elongation. The localised concentration of auxins in the developing siliqua promotes differential growth, contributing to its elongation. Cytokinins, in synergy with auxins, enhance cell division and influence the final size of the siliqua. They also modulate gene expression in cell cycle regulation, further promoting cell division and expansion. Gibberellins (GA) are another group of hormones that significantly influence siliqua length by promoting cell division and elongation. GA stimulates the synthesis of enzymes like α-amylase, which mobilize starch reserves in developing seeds. This indirectly supports Siliqua's growth by providing the necessary energy and building blocks. These hormones act as growth regulators, with ethylene typically inhibiting elongation and ABA being involved in stress responses that can affect siliqua growth under adverse conditions. Adequate availability of nutrients, particularly boron, sulphur, and other micronutrients, is essential for the proper development of siliqua. Boron, for instance, is crucial for cell wall integrity and membrane function, both of which are vital for cell elongation. Sulphur is involved in synthesizing amino acids and proteins, which are necessary for the growth and development of the siliqua. The combined application of nutrients such as boron and sulphur, along with growth hormones like cytokinins, enhances the overall growth environment for the siliqua, leading to an increase in its length. This is evident in treatments where foliar applications of these nutrients result in significantly longer siliqua than controls. The observed increase in siliqua length in mustard crops treated with combinations of boron, sulphur, and cytokinins can be attributed to the synergistic effects of these factors on cellular processes. The enhanced cell division and elongation, driven by optimal hormonal regulation and nutrient availability, directly contribute to the increased length of siliqua. This reflects the importance of balanced nutrient management and hormonal application and highlights the potential for targeted agronomic practices to improve crop yield and quality through morphological enhancements such as siliqua length.

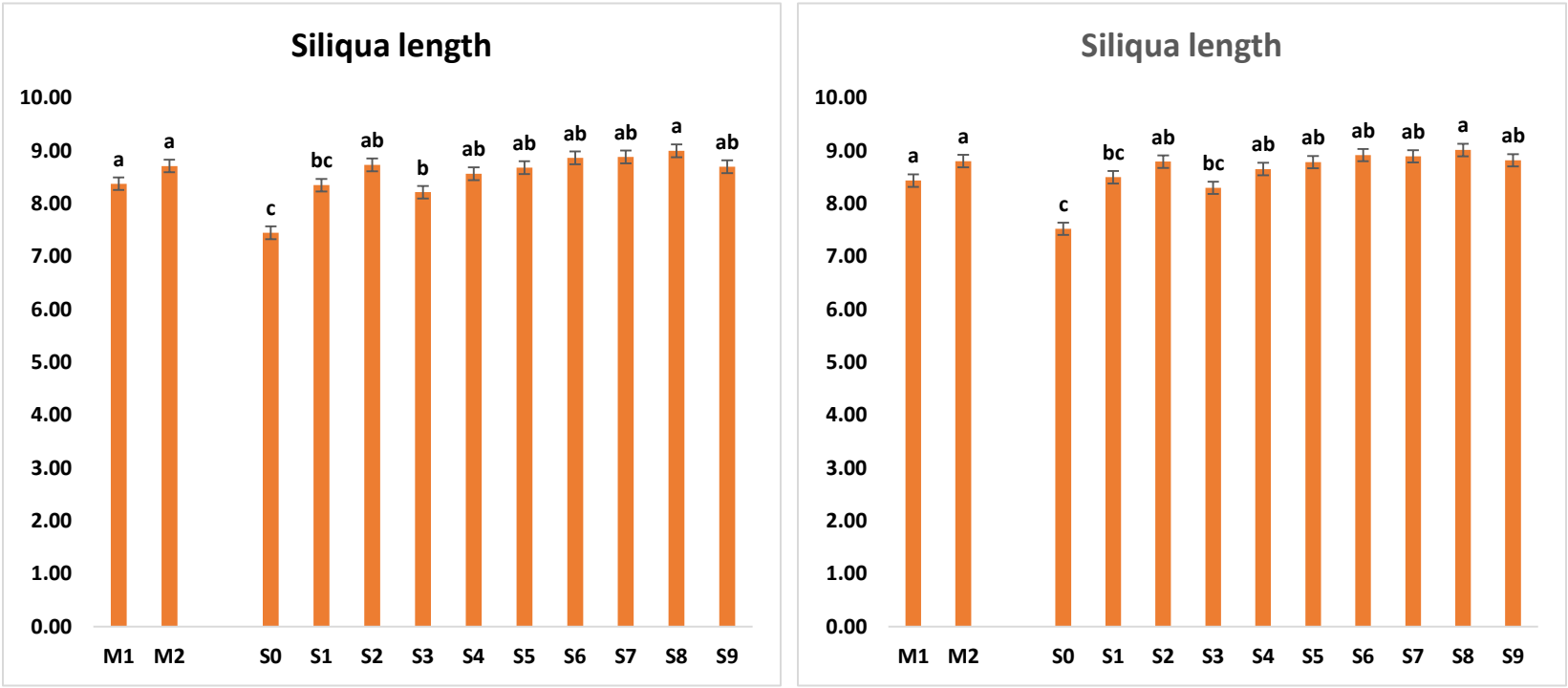


Fig-4.31 (a): Effect of spacing and nutrient on Siliqua length per plant of mustard crop during rabi season of 2021-22 and 2022-23.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.4.5: No. of seeds siliqua^{-1} (No. siliqua^{-1})

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in no. of seeds per siliqua were observed at harvest, as shown in Table 4.30, Fig 4.32. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the no. of seeds per siliqua in each treatment as compared to the control of both the spacings. The percentage increase was calculated by comparing all the treatments with the power and the spacings. Thus, the pattern of percentage increase in the no. of seeds per siliqua was observed at harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), main plot M2 shows the maximum number of seeds per siliqua compared to M1 with values of 21.11 (M2) and 20.53 (M1), respectively. A percentage increase of 2.74% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S8 with a value of 22.84, where Sulphur @0.075%+ BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S8, followed by S7> S9> S3> S6> S2> S1> S5> S4, and the per cent values were 31.62%, 31.02%, 30.03%, 26.09%, 25.95%, 25.91%, 25.03%, 23.48% and 22.78% respectively when it is compared with its control (S0).

The study showed a significant increase with 25.95% cent values when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. A significant increase in the number of seeds per siliqua was found in treatment S8, where the combined application of an aqueous formulation of Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop.

In the year (2022-23), main plot M2 shows the maximum number of seeds per siliqua compared to M1 with values of 22.14 (M2) and 21.52 (M1), respectively. A percentage increase of 2.80%

was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S9 with a value of 24.27, where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent increase was found highest in S9, followed by S8> S7> S3> S2> S6> S5> S1> S4, and the per cent values were 31.12%, 30.49%, 26.95%, 24.46%, 24.27%, 24.15%, 22.44%, 22.04% and 20.81% respectively when it is compared with its control (S0).

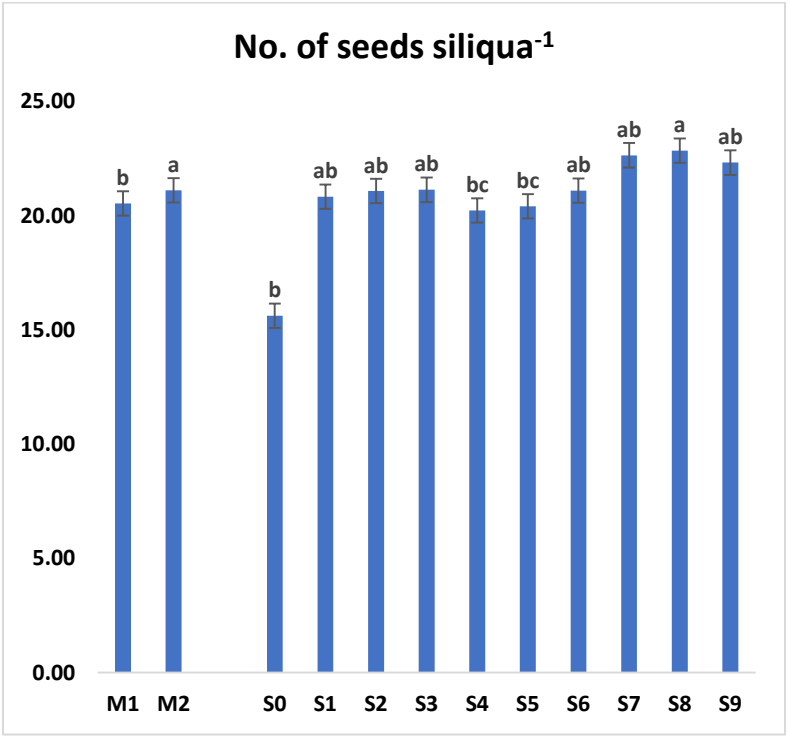
The study showed a significant increase with 24.15% cent values when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. A significant increase in the no. of seeds per siliqua was found in treatment S8, where the combined application of aqueous formulation of Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop. The optimum dose of boron significantly increased the number of seeds siliqua^{-1}. During the initial stages, the nutrient requirement increases to develop the grain-filling stages in the mustard plant. Thus, applying boron and sulphur helps in photosynthesis and translocation to sink. Kumar et al. (2000) and Jat et al. (2008) reported similar results. Thus, it is proved from the experiment that the application of micronutrients along with plant growth hormones is involved in increased yield and production. In treatment T9, the combined application of sulphur and cytokinin at its recommended dose shows better results for the number of seeds per siliquae in the mustard crop. Thus, this treatment is better suited to grow a mustard crop with increased production and higher yield than controlled ones. The number of seeds per siliqua is a crucial determinant of yield in mustard crops, influenced by various cellular and physiological mechanisms that are affected by nutrient availability, particularly Boron, Sulphur, and Cytokinin. Boron is a vital micronutrient that plays a significant role in cell wall synthesis, membrane integrity, and reproductive development in plants. In mustard, Boron enhances the formation and function of pollen tubes during fertilization, ensuring successful fertilization of ovules. The fertilization process directly influences the

number of seeds per siliqua. Adequate Boron levels lead to improved pollen viability and pollen tube growth, increasing the chances of fertilizing more ovules. This results in a higher number of seeds per siliqua. Sulphur is essential for synthesizing certain amino acids (like cysteine and methionine), vitamins, and coenzymes critical for plant growth and development. It also forms glucosinolates, secondary metabolites in mustard that contribute to plant defence and growth. Sulphur's role in protein synthesis and enzymatic activity supports plant health and reproductive success. An adequate supply of Sulphur enhances the plant's ability to produce healthy and viable seeds, increasing the number of seeds per siliqua. Cytokinins are plant hormones that promote cell division and differentiation. In reproductive development, cytokinins are vital in regulating ovule development, embryo formation, and seed setting. By promoting cell division in the ovary and developing seeds, cytokinins ensure that more ovules are fertilized and develop into seeds. The combined application of Cytokinin with Boron or Sulphur enhances these effects, significantly increasing the number of seeds per siliqua. The combined application of boron, sulphur, and cytokinin has synergistically affected the number of seeds per siliqua. Boron ensures successful fertilization by promoting pollen tube growth, Sulphur supports the synthesis of essential compounds for seed development, and Cytokinin enhances cell division and seed formation. Together, these nutrients optimize the cellular processes in the seed setting, leading to more seeds per siliqua. The experiment's results demonstrated that treatments with combined applications of these nutrients significantly increased the number of seeds per siliqua compared to the control. This increase can be attributed to the enhanced cellular processes facilitated by the availability of Boron, Sulphur, and Cytokinin, which create a more favourable environment for seed development.

Table 4.30 (a): Effect of spacing and nutrients on the Number of Siliqua (No. plant^{-1}), siliqua length (cm) and number of seeds per siliqua (No. siliqua^{-1}) of the mustard crop during the rabi season of 2021-22 and 2022-23.

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	No. of siliqua plant^{-1}		Siliqua length		No. of seeds siliqua^{-1}	
Spacing						
M1 (30×10)	280.07	282.30	8.38	8.44	20.53	21.52
M2 (20×10)	289.37	291.64	8.71	8.81	21.11	22.14
C.D. at p<0.05	NS	NS	NS	NS	0.28	0.26
SEM±	10.18	10.31	0.08	0.08	0.04	0.12
Nutrients foliar application						
S0-Control	173.83	175.01	7.45	7.53	15.62	16.72
S1-Boron @1%	273.00	276.38	8.35	8.50	20.83	21.44
S2-Sulphur @ 0.15%	285.67	287.91	8.73	8.80	21.08	22.08
S3-BAP @0.003%	303.00	305.13	8.22	8.30	21.13	22.13
S4-Boron @0.5% +Sulphur @0.25%	251.00	253.24	8.57	8.66	20.23	21.11
S5-Boron @ 1.5%+ Sulphur @0.075%	269.00	271.33	8.68	8.79	20.41	21.56
S6-Boron @ 0.5% + BAP (@0.0045%)	312.33	314.39	8.87	8.92	21.09	22.04
S7-Boron @ 1.5%+ BAP (@0.0015%)	299.00	301.30	8.88	8.90	22.64	22.89
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	334.00	336.14	9.00	9.02	22.84	24.05
S9-Sulphur @0.25%+ BAP (@0.0015%)	346.33	348.89	8.70	8.82	22.32	24.27
C.D. at p<0.05	91.08	91.81	0.54	0.58	2.47	2.48
SEM±	31.62	31.88	0.18	0.20	0.84	0.87
C.D. S×M at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	32.19	32.62	0.14	0.36	0.12	0.37
C.D. M×S at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	43.63	44.00	0.15	0.16	0.16	0.16

Where, C.D. represents critical difference, SE (m) represents standard error of mean.



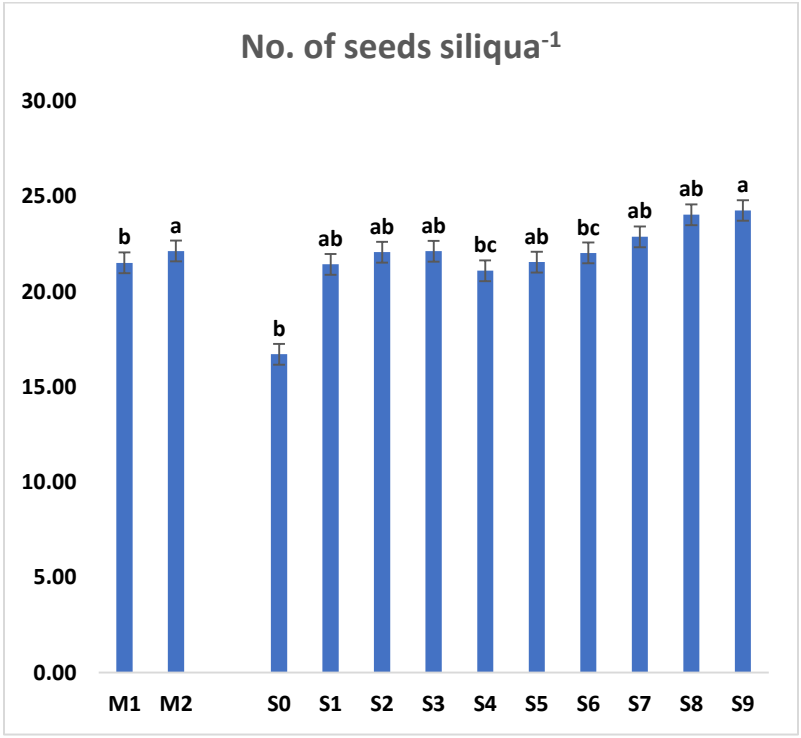


Fig-4.32 (a): Effect of spacing and nutrient on Number of Seeds per Siliqua of mustard crop during rabi season of 2021-22

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.4.6: Test weight (g)

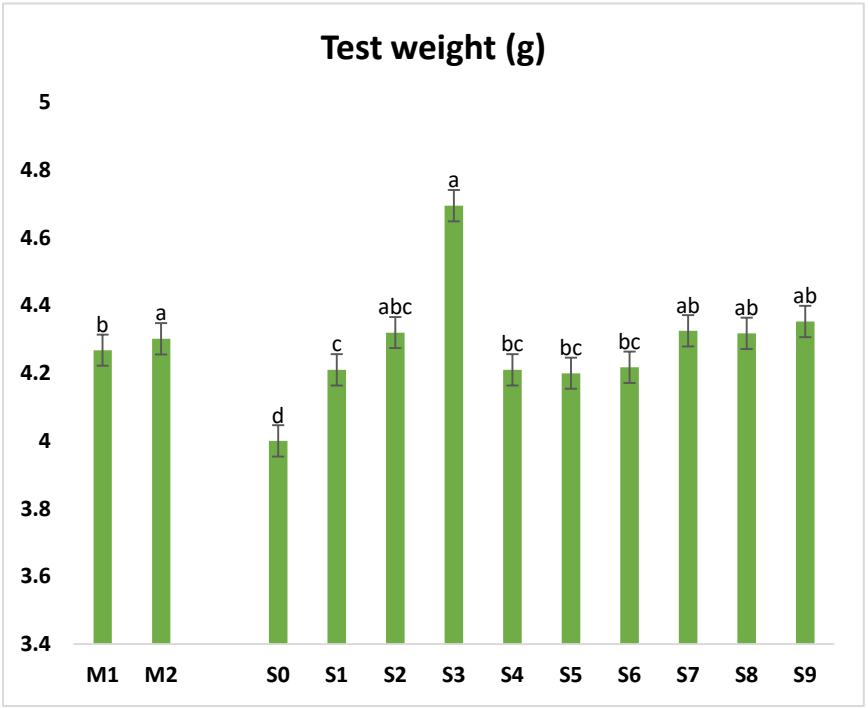
In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in test weight (1000 seed weight) observed after harvest were shown in Table 4.33, Fig 4.33. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the test weight in each treatment compared to the control of both spacings. The percentage increase was calculated by comparing all the treatments with the power and the spacings. Thus, a percentage increase in the test weight pattern was observed after harvesting for two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22) the main plot M2 shows maximum test weight as compared to M1 with values of 4.30g (M2) and 4.26g (M1), respectively. A percentage increase of 0.93% was found in M2, where the crop was grown in reduced spacing (20*10). Significant results were observed in S3 with a value of 4.76 in subplots where BAP @0.003% was applied to the crop as a foliar spray. The per cent increase was found highest in S3 followed by S9> S7> S2> S8> S6> S4&S1> S5, and the per cent values were 14.80%, 8.09%, 7.51%, 7.40%, 7.35%, 5.15%, 4.98%, 4.98% and 4.76% respectively when it is compared with its control (S0).

In the year (2022-23) the main plot M2 shows maximum test weight as compared to M1 with values 4.26g (M2) and 4.23g (M1), respectively. A percentage increase of 0.70% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S9 with a value of 4.34, where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent increase was found highest in S9 followed by S7> S8> S3> S2> S4> S5&S6> S1, and the per cent values were 7.99%, 7.46%, 7.24%, 7.19%, 6.75%, 5.88%, 5.15%, 5.15%, 5.04% respectively when it is compared with its control (S0).

The study showed a significant increase with 5.15% per cent values in both the years when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. In 2021-22 and 2022-23, a significant increase in the test weight was found in treatment S9, where the combined application of aqueous formulation of Sulphur @ 0.25%+ BAP (@0.0015%) was applied to the crop. Applying cytokinin promotes shooting and thus increases the number of branches, ultimately leading to higher yield and bold seed production per plot. Similar results were found by (Sharma et al. 2020). Applying plant growth hormone, boron, and sulphur induces a green colour, thus promoting photosynthesis in the mustard leaves. These nutrients help translocate photosynthate from source to sink, hence improving yield.

An essential factor indicating the general seed quality and yield potential is the test weight of mustard seeds. The cellular processes responsible for test weight encompass various physiological and biochemical processes during seed development and maturation. These processes encompass the absorption of nutrients, the filling of seeds, and the control of metabolic pathways that govern the sequestration of storage substances such as proteins, lipids, and carbohydrates. Assimilates, primarily sucrose, generated in leaves are conveyed to the seeds through the phloem during seed filling. The concentration gradient of sugars between the source (leaves) and sink (developing seeds) drives this process. The efficiency of this chemical reaction is affected by the plant's photosynthetic capacity, which is improved by sufficient availability of nutrients, particularly Boron and Sulphur. Boron is essential for the synthesis and stability of cell walls. It enhances the movement of sugars across cell membranes, effectively loading phloem and guaranteeing a consistent provision of nutrients to the growing seeds. Sulphur is indispensable for the biosynthesis of amino acids such as methionine and cysteine, which serve as fundamental protein components. Adequate sulphur levels guarantee a more excellent protein content in seeds, increasing seed weight. The synthesis of storage proteins, a significant element of seed dry

weight, depends on the availability of nitrogen and sulphur. Cytokinins affect protein synthesis by stimulating the cell process of division and differentiation, generating additional seed cells that can store proteins. The oil content in mustard seeds is substantial and contributes to the test weight. The regulation of enzymes involved in lipid biosynthesis, such as acetyl-CoA carboxylase, is contingent upon the botanical nutrient condition. Compounds containing sulfur are also implicated in producing glutathione, a protective agent against oxidative damage during seed maturation, ensuring lipid storage levels and quality preservation. Cytokinins, namely BAP (Benzylaminopurine), promote embryonic cell division and growth in developing seeds. In addition, they postpone the process of senescence, so enabling extended absorption of nutrients and biosynthesis of storage compounds. This hormonal regulation results in the production of larger and heavier seeds, so contributing to an increased test weight. Combining boron, sulphur, and cytokinin's synergistic effect promotes the optimization of seed filling's metabolic processes. By enhancing the efficiency of nutrient use, this synergy results in a more significant accumulation of storage compounds and, as a result, higher test weight. The substantial rise in test weight resulting from applying Boron, Sulphur, and Cytokinin treatments can be ascribed to the improved cellular processes described earlier. The presence of Boron guarantees adequate transportation of nutrients, while Sulphur facilitates the production of proteins and lipids. Cytokinins stimulate cellular proliferation and postpone the inevitable ageing process, enabling prolonged seed-filling durations. Collectively, these elements contribute to the observed rise in seed mass, as evidenced by the elevated test weight of mustard seeds in the treated plants. This result underscores the significance of a well-balanced nutrient regimen in attaining ideal seed development and maximizing yield potential.



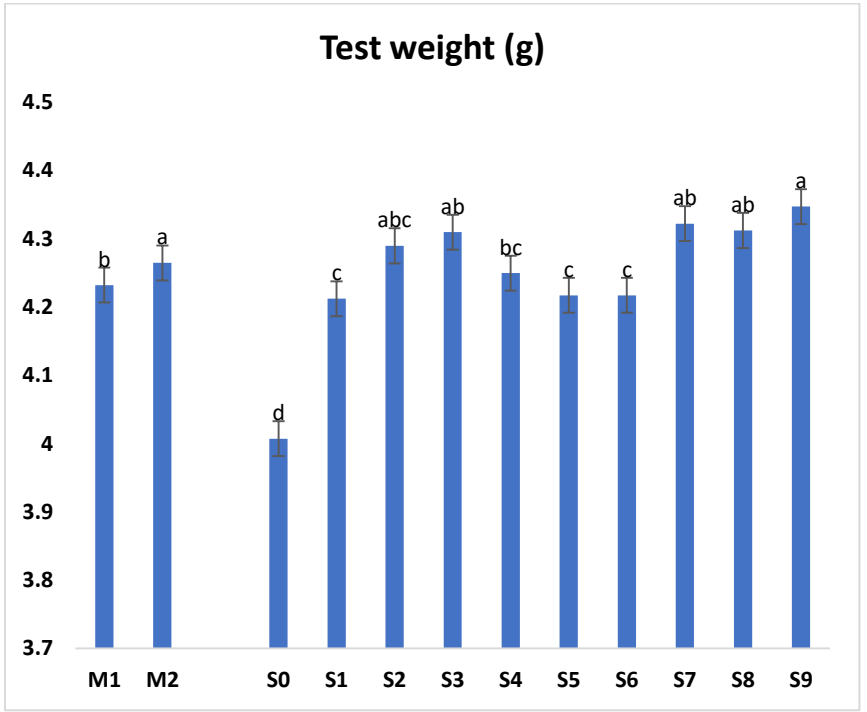


Fig-4.33 (a): Effect of spacing and nutrient on test weight of mustard crop during rabi season of 2021-22

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.4.7: Seed yield (q ha^{-1})

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in seed yield observed after harvest are shown in Table 4.34, Fig 4.34. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the seed yield in each treatment compared to the control of both spacings. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in seed yield was observed after harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22) the main plot M2 shows higher seed yield as compared to M1 with values of 24.73 q ha-1 (M2) and 24.03 q ha-1 (M1), respectively. A percentage increase of 2.83% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S8 with a value of 26.17 q ha-1 where Sulphur @0.07%+ BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S8, followed by S7> S9> S6> S3> S2> S4=S5> S1, and the per cent values were 21.66%, 20.63%, 19.06%, 18.00%, 1.43%, 15.74%, 14.58%, 14.58% and 13.97% respectively when it is compared with its control (S0).

In the year (2022-23) the main plot M2 shows maximum seed yield as compared to M1 with values 25.89 q ha-1 (M2) and 25.15 q ha-1 (M1), respectively. A percentage increase of 2.85% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S8 with a value of 27.00 q ha-1 where Sulphur @0.075%+ BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S8, followed by S7> S6> S9> S3> S2> S4> S5> S1, and the per cent values were 19.07%,

17.70%, 17.67%, 17.10%, 16.73%, 15.60%, 12.63%, 12.06% and 12.03% respectively when it is compared with its control (S0).

The study shows a significant increase with 22.00% and 22.77% per cent values in 1st and 2nd yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. In 2021-22 and 2022-23, a significant increase in the seed yield was found in treatment S8 where the combined application of aqueous formulation of Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop. Sharma found the same results, S et al. (2020). The increase in seed yield under an adequate supply of boron and sulphur is mainly due to the combined effect of nutrients and plant growth hormones. As the growth of mustard in T3 increased, it ultimately resulted in an increased yield of mustard. Suresh et al. (2002) and Raut et al. (2003) reported the enhancement of seed yield in mustard due to sulphur application. This improvement might be due to the translocation of photosynthates, which leads to higher seed and stover yields.

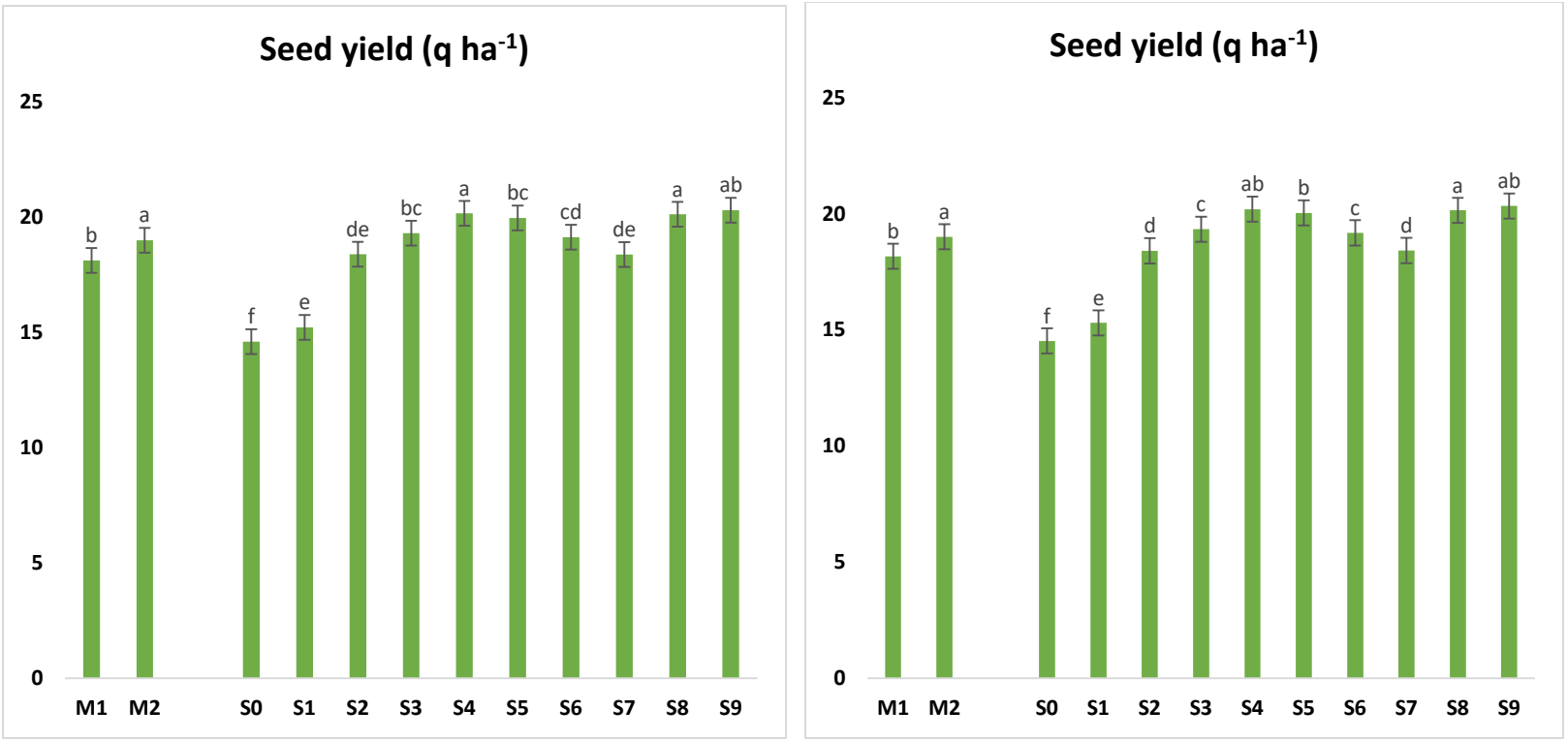


Fig-4.34 (a): Effect of spacing and nutrients on seed yield of the mustard crop during rabi season of 2021-22.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.4.8: Biological yield (q ha^{-1})

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in biological yield observed after harvest are shown in Table 4.35, Fig 4.35. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the biological yield in each treatment compared to the control of both spacings. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the percentage increase in biological yield pattern was observed after harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22) the main plot M2 shows higher biological yield as compared to M1 with values of 50.11 q ha-1 (M2) and 49.03q ha-1 (M1), respectively. A percentage increase of 2.15% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S3 with a value of 52.73q ha-1 where Boron @0.5% + sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S3, followed by S8> S4> S6> S9> S5> S7> S2> S1, and the per cent values were 23.09%, 22.51%, 22.36%, 22.04%, 21.35%, 21.4%, 18.60%, 17.46% and 9.70% respectively when it is compared with its control (S0).

In the year (2022-23) the main plot M2 shows higher biological yield as compared to M1 with values of 51.32q ha-1 (M2) and 50.23 q ha-1 (M1), respectively. A percentage increase of 2.12% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S3 with a value of 54.17q ha-1 where Boron @0.5% +Sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S3, followed by S8> S4> S6> S9> S5> S7> S2> S1, and the per cent values were 22.35%, 20.92%, 21.10%,

21.57%, 20.08%, 19.97%, 16.84%, 17.07% and 8.48% respectively when it is compared with its control (S0).

The study shows a significant increase with 29.68% and 29.66% per cent values in 1st and 2nd yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. In 2021-22 and 2022-23, a significant increase in the biological yield was found in treatment S4, where the combined application of aqueous formulation of Boron @0.5% + sulphur @0.25% was applied to the crop. Sharma et al. (2020). This improvement might be due to the translocation of photosynthates, which leads to higher seed and stover yields. Chatterjee et al. (1985) reported that applying borax increased the seed yield of mustard over control. This may be due to the role of boron in fertility improvement and the translocation of photosynthates to sink. These results closely conform to those of Chander et al. (2010). Biological yield also increases due to increased plant height and the number of branches.

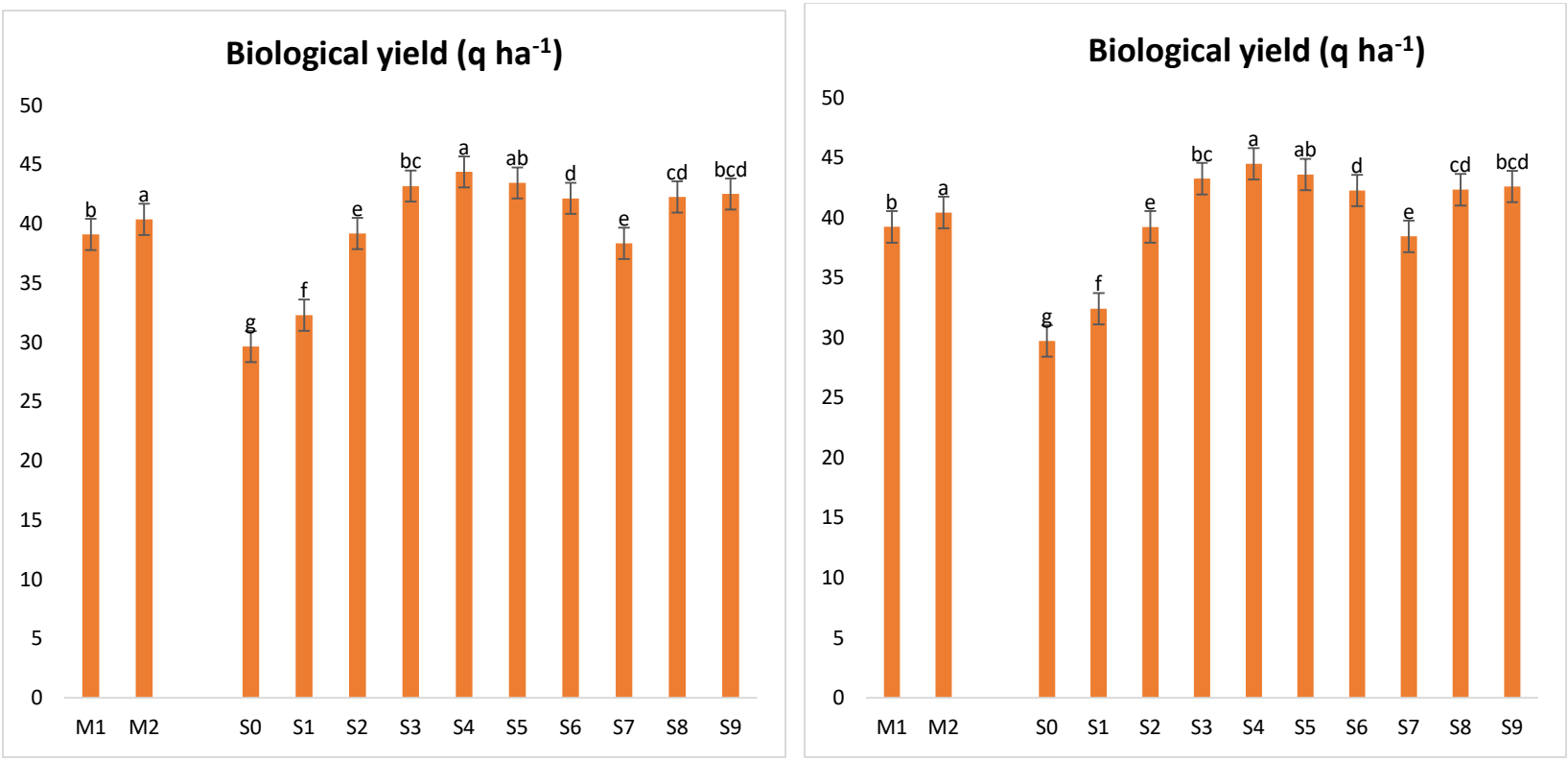


Fig-4.35 (a): Effect of spacing and nutrient on biological yield of mustard crop during rabi season of 2021-22.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

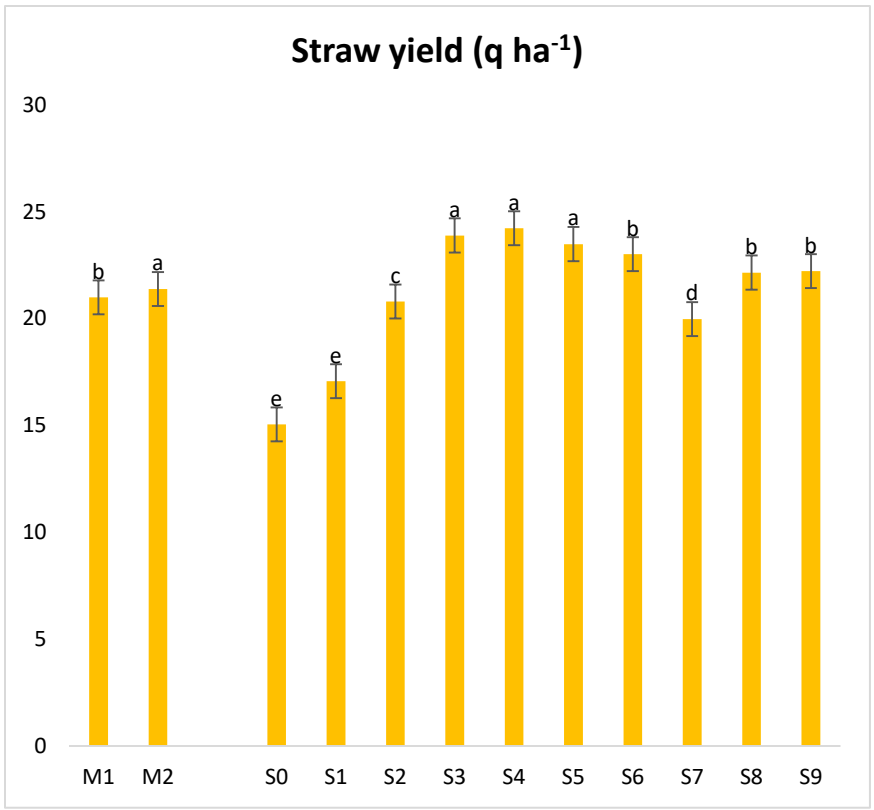
4.4.9: Straw yield (q ha^{-1})

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in straw yield observed after harvest are shown in Table 4.36, Fig 4.36. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the straw yield in each treatment compared to the control of both the spacings. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in straw yield was observed after harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), the main plot M2 shows a higher stover yield than M1, with values of 25.38q ha-1 (M2) and 25.00q ha-1 (M1), respectively. A percentage increase of 1.49% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S4 with a value of 28.23q ha-1 where Boron @0.5% +Sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S4, followed by S3> S5> S6> S9> S8> S2> S7> S1, and the per cent values were 37.89%, 37.02%, 35.93%, 34.62%, 32.2%, 32.08%, 2.64%, 24.68% and 11.85% respectively when it is compared with its control (S0).

In the year (2022-23) the main plot M2 shows higher straw yield as compared to M1 with values 25.43q ha-1 (M2) and 25.08 q ha-1 (M1), respectively. A percentage increase of 1.37% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S4 with a value of 28.30q ha-1 where Boron @0.5% +Sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S4, followed by S3> S5> S6> S9> S8> S2> S7> S1, and the per cent values were 37.40%, 36.43%, 35.44%,

34.12%, 31.70%, 31.45%, 26.98%, 24.06% and 11.13% respectively when it is compared with its control (S0).

The study shows a significant increase with 34.62% and 34.12% per cent values in 1st and 2nd yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. In 2021-22 and 2022-23, a significant increase in the straw yield was found in treatment S4, where the combined application of aqueous formulation of Boron @0.5% + sulphur @0.25% was applied to the crop. Sharma et al., 2020, found similar results that applying micronutrients and plant growth hormones increases the stover yield. This improvement might be due to the translocation of photosynthates, which leads to higher seed and straw yields. This may be due to the role of boron in fertility improvement and the translocation of photosynthates to sink. These results closely conform to those of Chander et al. (2010). Straw yield also increases due to increased plant height and number of branches.



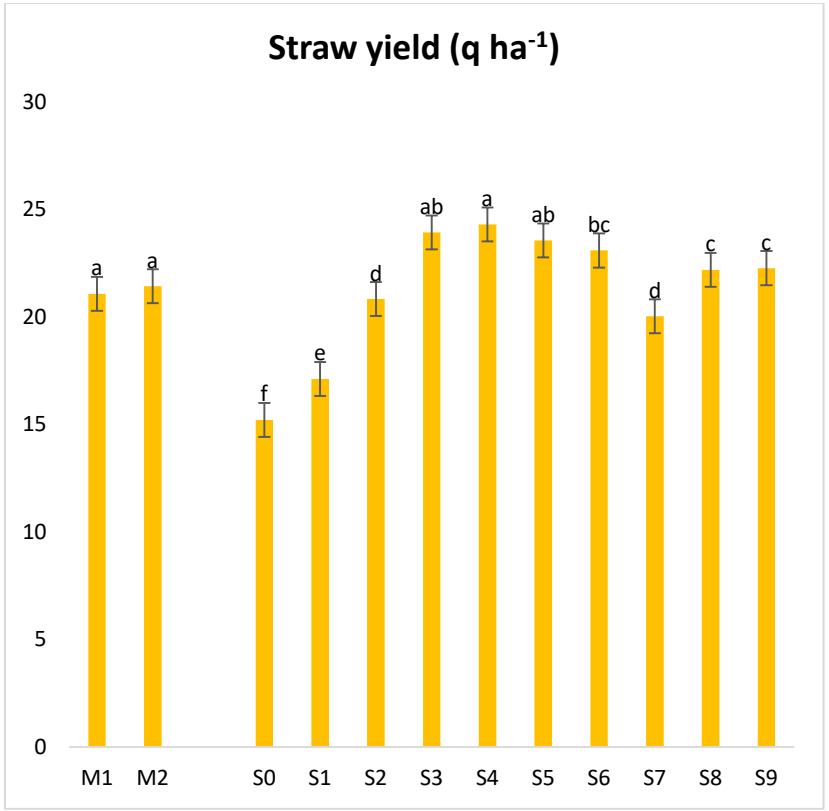


Fig-4.36 (a): Effect of spacing and nutrient on straw yield of mustard crop during rabi season of 2021-22.

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

Table-4.33 (a): Effect of spacing and nutrient on seed yield, biological yield and straw yield (q ha^{-1}) of mustard crop during rabi season of 2021-22 and 2022-23

Treatments	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
	Seed yield (q ha^{-1})		Biological yield (q ha^{-1})		Straw yield (q ha^{-1})	
Spacing						
M1 (30×10)	24.03	25.15	49.03	50.23	25.00	25.08
M2 (20×10)	24.73	25.89	50.11	51.32	25.38	25.43
C.D. at p<0.05	0.13	0.14	0.17	0.38	0.46	0.09
SEM±	0.03	0.03	0.04	0.09	0.11	0.02
Nutrients foliar application						
S0-Control	20.50	21.85	40.55	42.06	20.05	20.21
S1-Boron @1%	23.83	24.84	44.91	45.96	21.08	21.12
S2-Sulphur @ 0.15%	24.33	25.89	49.13	50.72	24.80	24.83
S3-BAP @0.003%	24.83	26.24	52.73	54.17	27.90	27.93
S4-Boron @0.5% +Sulphur @0.25%	24.00	25.01	52.23	53.31	28.23	28.30
S5-Boron @ 1.5%+ Sulphur @0.075%	24.00	25.00	51.49	52.56	27.49	27.56
S6-Boron @ 0.5% + BAP (@0.0045%)	25.00	26.54	52.02	53.63	27.02	27.09
S7-Boron @ 1.5%+ BAP (@0.0015%)	25.83	26.55	49.82	50.58	23.98	24.03
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	26.17	27.00	52.33	53.19	26.16	26.19
S9-Sulphur @0.25%+ BAP (@0.0015%)	25.33	26.36	51.56	52.63	26.22	26.27
C.D. at p<0.05	0.25	0.16	0.23	0.20	0.51	0.14
SEM±	0.08	0.05	0.08	0.06	0.17	0.05
C.D. S×M at p<0.05	0.44	0.28	0.40	0.34	0.89	0.25
SEM±	0.15	0.09	0.14	0.12	0.30	0.08
C.D. M×S at p<0.05	0.42	0.29	0.40	0.49	0.93	0.25
SEM±	0.14	0.09	0.13	0.14	0.30	0.08

Where, C.D. represents critical difference, SE (m) represents standard error of mean

Table 4.33 (b): Interaction effect of spacing and nutrient on seed yield of mustard crop during rabi season 2021-22 and 2022-23.

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	19.67	23.00	24.00	24.00	23.67	24.00	25.33	25.67	26.00	25.00	24.03
M2	21.34	24.67	24.67	25.67	24.33	24.00	24.67	26.00	26.33	25.67	24.73
Mean B	20.50	23.83	24.33	24.83	24.00	24.00	25.00	25.83	26.17	25.33	24.38
C.D. S×M at p<0.05						0.44					
SEM±						0.15					
C.D. M×S at p<0.05						0.42					
SEM±						0.14					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	20.67	24.02	26.12	26.21	24.67	24.66	26.43	26.43	26.34	26.03	25.15
M2	23.02	25.66	25.66	26.26	25.34	25.34	26.64	26.66	27.66	26.68	25.89
Mean B	21.85	24.84	25.89	26.24	25.01	25.00	26.54	26.55	27.00	26.36	25.53
C.D. S×M at p<0.05						0.28					
SEM±						0.09					
C.D. M×S at p<0.05						0.29					
SEM±						0.09					

Table 4.33 (c): Interaction effect of spacing and nutrient on biological yield of mustard crop during rabi season 2021-22 and 2022-23.

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	39.37	44.30	48.75	51.65	51.69	51.30	51.93	49.37	51.95	51.05	49.03
M2	41.74	45.52	49.52	53.81	52.78	51.69	52.11	50.27	52.70	52.06	50.11
Mean B	40.56	44.91	49.13	52.73	52.23	51.49	52.02	49.82	52.33	51.56	49.57
C.D. S×M at p<0.05						0.40					
SEM±						0.14					
C.D. M×S at p<0.05						0.40					
SEM±						0.13					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	40.49	45.37	50.90	53.89	52.79	52.08	53.15	50.21	52.32	52.15	50.34
M2	43.62	46.54	50.54	54.44	53.82	53.04	54.10	50.94	54.06	53.10	51.42
Mean B	42.06	45.96	50.72	54.17	53.31	52.56	53.63	50.58	53.19	52.63	50.88
C.D. S×M at p<0.05						0.34					
SEM±						0.12					
C.D. M×S at p<0.05						0.49					
SEM±						0.14					

Table 4.33 (d): Interaction effect of spacing and nutrient on straw yield of mustard crop during rabi season 2021-22 and 2022-23.

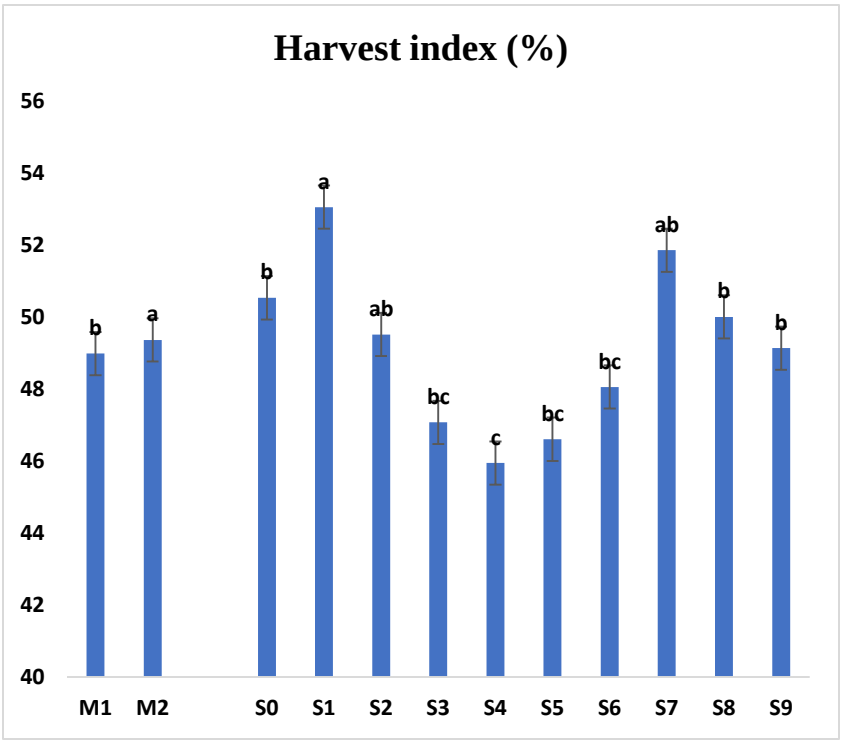
2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	19.70	21.30	24.75	27.65	28.02	27.30	26.60	23.70	25.95	26.05	25.10
M2	20.40	20.85	24.85	28.15	28.45	27.69	27.44	24.27	26.37	26.40	25.48
Mean B	20.05	21.08	24.80	27.90	28.23	27.49	27.02	23.98	26.16	26.22	25.29
C.D. S×M at p<0.05						0.89					
SEM±						0.30					
C.D. M×S at p<0.05						0.93					
SEM±						0.30					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	19.82	21.35	24.78	27.68	28.12	27.42	26.72	23.78	25.98	26.12	25.18
M2	20.60	20.88	24.88	28.18	28.48	27.70	27.46	24.28	26.40	26.42	25.53
Mean B	20.21	21.12	24.83	27.93	28.30	27.56	27.09	24.03	26.19	26.27	25.35
C.D. S×M at p<0.05						0.25					
SEM±						0.08					
C.D. M×S at p<0.05						0.25					
SEM±						0.08					

4.4.10: Harvest index (%)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in stover yield observed after harvest are shown in Table 4.37, Fig 4.37. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the stover yield in each treatment compared to the control of both the spacings. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in stover yield was observed after harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22) the main plot M2 shows the maximum harvest index as compared to M1 with values of 55.74 (M2) and 55.79 (M1), respectively. A percentage increase of 0.08% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S1, where boron @1% was applied as foliar spray. Besides this, significant results were observed in S7 followed by S0 and S8. In S7, boron+cytokinin @ 1.5% and 0.0015% respectively was applied to the crop as a foliar spray.

In the year (2022-23) the main plot M2 shows a maximum harvesting index as compared to M1 with values of 50.48 (M2) and 50.07 (M1), respectively. A percentage increase of 0.81% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S1, where boron @1% was applied as foliar spray. Besides this, significant results were observed in S7 followed by S0 and S8. In S7, boron+cytokinin @ 1.5% and 0.0015% respectively was applied to the crop as a foliar spray.



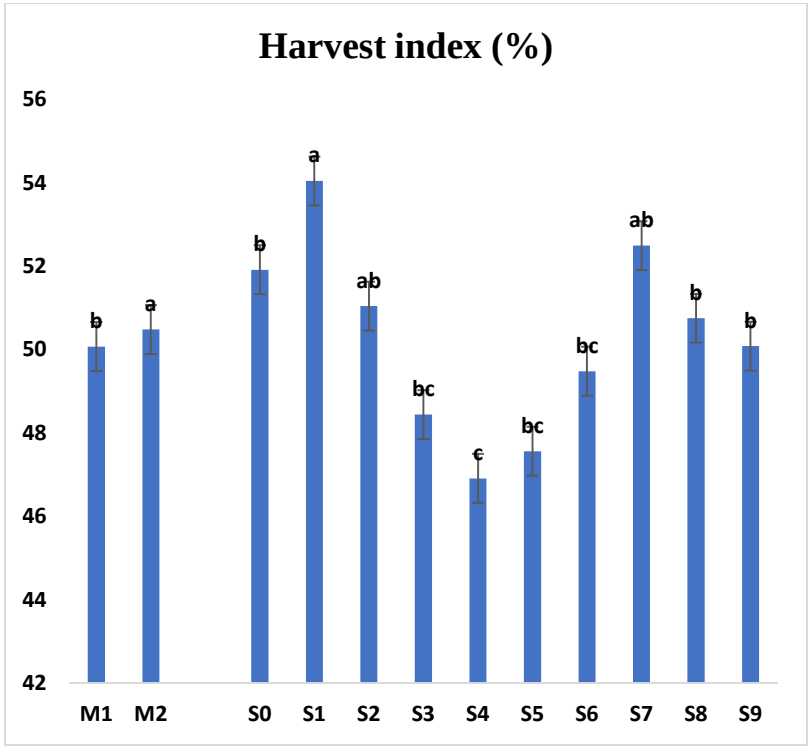


Fig-37 (a): Interaction effect of spacing and nutrient on Harvesting index of mustard crop during rabi season of 2021-22

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

Table-34 (a): Effect of spacing and nutrient on Harvesting index and test weight of mustard crop during rabi season of 2021-22 and 2022-23

Treatments	Harvesting index (%)		Test weight (g)	
	2021-22	2022-23	2021-22	2022-23
Spacing				
M1 (30×10)	55.74	50.07	4.27	4.23
M2 (20×10)	55.79	50.48	4.30	4.27
C.D. at p<0.05	0.52	1.18	0.03	0.04
SE(m)	0.13	0.30	0.00	0.00
Nutrients foliar application				
S0-Control	50.54	51.91	4.00	4.01
S1-Boron @1%	53.06	54.04	4.21	4.21
S2-Sulphur @ 0.15%	49.52	51.04	4.32	4.29
S3-BAP @0.003%	47.08	48.44	4.70	4.31
S4-Boron @0.5% +Sulphur @0.25%	45.95	46.91	4.21	4.25
S5-Boron @ 1.5%+ Sulphur @0.075%	46.61	47.56	4.20	4.22
S6-Boron @ 0.5% + BAP (@0.0045%)	48.06	49.48	4.22	4.22
S7-Boron @ 1.5%+ BAP (@0.0015%)	51.86	52.49	4.33	4.32
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	50.01	50.75	4.32	4.31
S9-Sulphur @0.25%+ BAP (@0.0015%)	49.14	50.08	4.35	4.35
C.D. at p<0.05	0.47	1.32	0.23	0.24
SEM±	0.16	0.45	0.08	0.07
C.D. S×M at p<0.05	0.82	2.29	NS	NS
SEM±	0.28	0.79	0.00	0.01
C.D. M×S at p<0.05	0.91	2.38	NS	NS
SEM±	0.29	0.78	0.10	0.10

Where C.D. represents the critical difference, SE (m) represents the standard error of the mean

Table 4.34 (b): Interaction effect of spacing and nutrient on harvest index of mustard crop during rabi season 2021-22 and 2022-23.

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	56.50	64.47	56.09	51.53	49.50	51.09	56.00	61.57	56.61	54.05	55.74
M2	59.11	66.62	57.13	53.36	49.56	49.63	52.13	60.09	56.08	54.25	55.79
Mean B	57.81	65.54	56.61	52.45	49.53	50.36	54.06	60.83	56.34	54.15	
C.D. S×M at p<0.05						0.82					
SEM±						0.28					
C.D. M×S at p<0.05						0.91					
SEM±						0.29					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	59.05	67.01	61.07	56.09	51.49	52.15	58.07	63.13	57.17	56.08	58.13
M2	60.78	69.12	58.19	54.25	51.55	52.20	56.17	61.24	59.00	56.28	57.88
Mean B	59.92	68.06	59.63	55.17	51.52	52.17	57.12	62.18	58.09	56.18	
C.D. S×M at p<0.05						2.29					
SEM±						0.79					
C.D. M×S at p<0.05						2.38					
SEM±						0.78					

4.4.11: Oil content (%)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in oil content observed after harvest are shown in Table 4.38, Fig 4.38. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the oil content in each treatment compared to the control of both spacings. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in oil content was observed after harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), the main plot M2 shows higher oil content than M1, with values of 23.83% (M2) and 23.53% (M1), respectively. A percentage increase of 1.25% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S9 with a value of 27.84%, where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent increase was found highest in S9, followed by S5> S3> S6> S2> S1> S7> S8> S4, and the per cent values were 20.68%, 24.94%, 24.18%, 23.67%, 23.61%, 22.84%, 22.72%, 22.66% and 22.26% respectively when it is compared with its control (S0).

In the year (2021-22), the main plot M2 shows higher oil content than M1, with values of 23.80% (M2) and 23.66% (M1), respectively. A percentage increase of 0.58% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S9 with a value of 27.51%, where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent increase was found highest in S9, followed by S3> S5> S2> S6> S1> S8> S7> S4, and the per cent values were 20.26%, 11.72%, 11.26%, 7.68%, 7.00%, 5.06%, 4.45%, 4.31% and 0.13% respectively when it is compared with its control (S0).

The study shows a significant increase with 6.72% and 7.00% per cent values in 1st and 2nd yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. In 2021-22 and 2022-23, a significant increase in the oil content was found in treatment S9 where the combined application of aqueous formulation of Sulphur @ 0.25%+ BAP (@0.0015%) was applied to the crop. Singh, R., Singh, Y., & Singh, S. (2017) show that seed protein and oil content are important parameters that govern the quality of mustard. The oil content of mustard seeds was significantly increased with sulphur and boron application. The oil content of the seed was highest under micronutrient application. The oil content was, however, lower with a recommended dose of fertilizers. These results agree with those reported by Jaiswal et al. (2015) and Singh et al. (2017).

The oil content in mustard seeds is a crucial agricultural characteristic affected by multicellular and biochemical mechanisms. The plastids and the endoplasmic reticulum (ER) within the seed cells are the central locations for oil biosynthesis in mustard. The process initiates with the production of fatty acids in the plastids, which are subsequently transported to the endoplasmic reticulum (ER) for additional elongation and desaturation, ultimately resulting in the synthesis of triacylglycerols (TAGs), the primary constituent of mustard oil. The fatty acid synthesis pathway begins in the plastids by converting acetyl-CoA to malonyl-CoA by acetyl-CoA carboxylase (ACCase). Subsequently, a series of condensation, reduction, dehydration, and successive reduction processes occur, forming saturated fatty acids, predominantly palmitic acid. Iterative chain elongation leads to the synthesis of oleic acid, the precursor for linoleic and linolenic acids. These processes are precisely controlled by the presence of substrates (acetyl-CoA and NADPH), the activity of enzymes, and environmental parameters such as temperature and nutrient availability. Specifically, sulfur is a crucial constituent of cysteine, which serves as a precursor for glutathione and coenzyme A, vital for producing fatty acids. Following synthesis in the

plastids, the fatty acids are conveyed to the endoplasmic reticulum (ER), where they undergo additional elongation and desaturation. Oleoyl-ACP desaturase enzymes catalyse the conversion of oleic acid into polyunsaturated fatty acids, namely linoleic and linolenic esters. Boron is essential for preserving the structural integrity and optimal functioning of the endoplasmic reticulum (ER), which enables the effective breakdown of fatty acids. Moreover, cytokinin impacts the expression of crucial genes implicated in the metabolism of fatty acids, influencing the final oil content. Oil biosynthesis culminates in forming Transferable Acid Groups (TAGs) from glycerol-3-phosphate and acyl-CoAs. This process is facilitated by enzymes such as glycerol-3-phosphate acyltransferase (GPAT), lysophosphatidic acid acyltransferase (LPAAT), and diacylglycerol acyltransferase (DGAT). Furthermore, these TAGs are stored in oil bodies within the seed cells. The availability of nutrients, namely sulphur and boron, dramatically affects the enzymatic activity. Optimization of TAG formation and accumulation is facilitated by the participation of sulfur in coenzyme synthesis, the preservation of redox equilibrium, and the contribution of boron to membrane stability. Experiments have demonstrated that applying sulphur, boron, and cytokinin can increase the oil content in mustard by increasing the activity of key enzymes responsible for fatty acid production and TAG formation. Sulphur facilitates the production of co-factors essential for fatty acid metabolism, while boron maintains the structural integrity of the cellular organelles engaged in oil biosynthesis. In contrast, Cytokinin regulates the expression of genes associated with oil biosynthesis, resulting in heightened accumulation of TAG. The increase in oil content observed under specific nutrient treatments can be ascribed to the improved efficiency of the cellular machinery responsible for oil synthesis. It is probable that the combination of these nutrients results in synergistic effects, so the conditions for achieving maximum oil accumulation in mustard seeds should be optimized. The elucidation of this mechanism establishes a solid basis for the observed fluctuations in oil content under various nutrient treatments. It supports the need to achieve balanced fertilization in managing mustard crops.

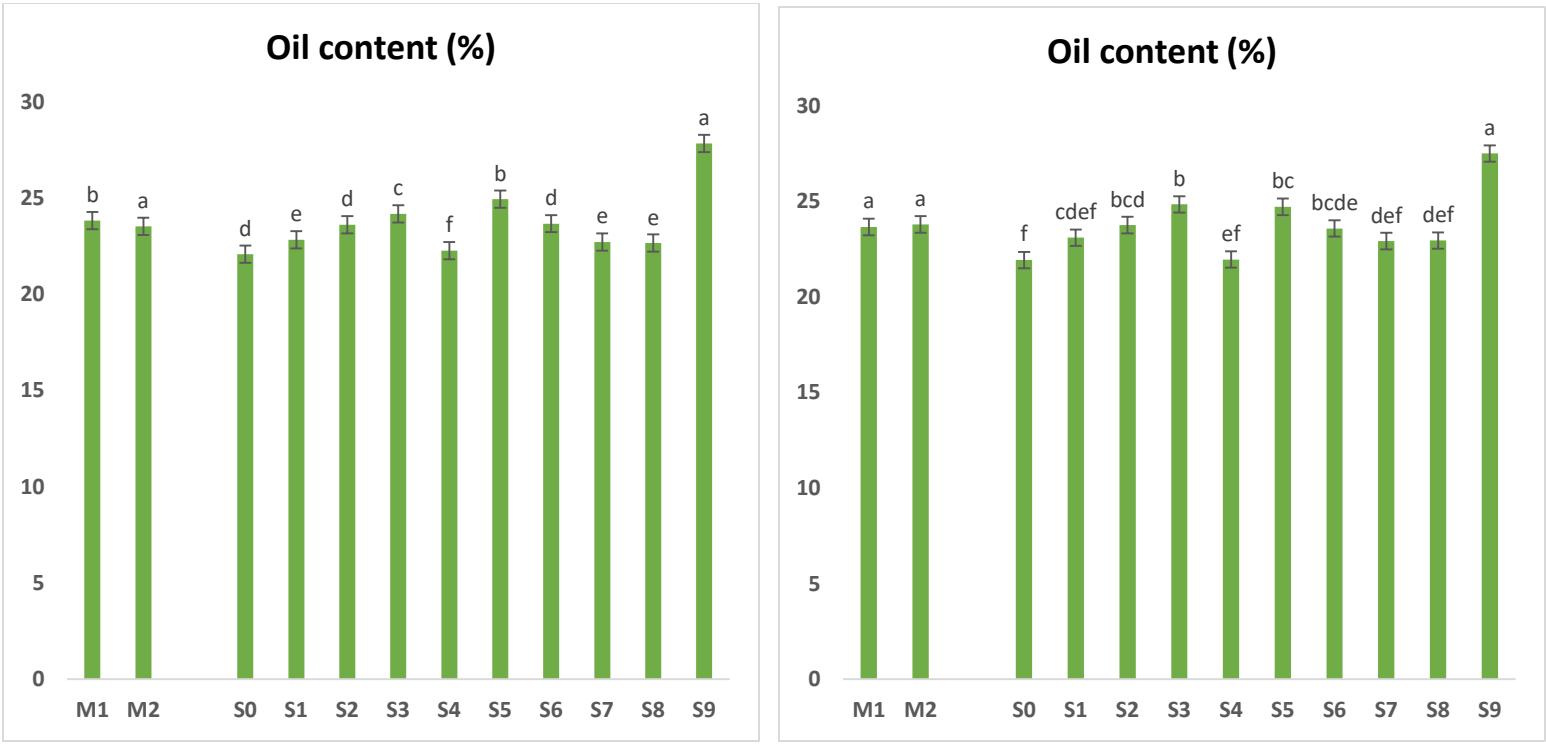


Fig-38 (a): Interaction effect of spacing and nutrient on oil content of mustard crop during rabi season of 2021-22

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.4.13: Oil cake weight (g/100g seeds)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in oil cake weight observed after harvest are shown in Table 4.38, Fig 4.38. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the oil cake weight in each treatment compared to the control of both spacings. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. In the year (2021-22), the main plot M2 shows a higher oil cake weight than M1, with values of 79.29g (M2) and 78.22g (M1), respectively. A percentage increase of 1.34% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S4 with a value of 81.68g where Boron @0.5% +Sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S4, followed by S7> S8> S3> S6> S2> S1> S5> S9, and the per cent values were 8.75%, 8.11%, 7.70%, 6.59%, 6.19%, 5.82%, 4.04%, 3.26% and 2.47% respectively when it is compared with its control (S0). In the year (2021-22), the main plot M2 shows a higher oil cake weight than M1, with values of 80.05g (M2) and 78.72g (M1), respectively. A percentage increase of 1.66% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S4 with a value of 82.30g where Boron @0.5% +Sulphur @0.25% was applied to the crop as a foliar spray. The per cent increase was found highest in S4, followed by S7> S8> S3> S6> S2> S5> S1> S9, and the per cent values were 9.64%, 8.85%, 8.53%, 7.48%, 7.42%, 6.4%, 5.29%, 4.80% and 3.92% respectively when it is compared with its control (S0). The study shows a significant increase with 6.19% and 7.42% per cent values in the 1^{st} and 2^{nd} yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. In 2021-22 and 2022-23, a significant increase in the oil cake weight

was found in treatment S8, where the combined application of an aqueous formulation of Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop. Khatun et al. (2015) shows the same results as its control. Compared to the control, the highest increase was found in treatment with the application of sulphur and cytokinin. The application of cytokinin helps translocate photosynthates and sugars, increasing the oil content and weight of the mustard crop. Oilcake is generally used to feed animals and as manure for better production. The predominant factor influencing the oil content in mustard seeds is the buildup of triacylglycerols (TAGs) in the developing seeds, which serve as the principal lipid storage form. This process is rigorously controlled at the cellular level, encompassing many crucial biochemical pathways and cellular mechanisms. Fatty acid biosynthesis takes place in the plastids of mustard seed cells. The rate-limiting step in fatty acid synthesis is the carboxylation of acetyl-CoA to malonyl-CoA by acetyl-CoA carboxylase. The malonyl-CoA is subsequently utilized by fatty acid synthase (FAS) to extend the carbon chain, producing long-chain fatty acids, namely oleic acid, linoleic acid, and linolenic acid. These fatty acids undergo additional modification after being transported to the endoplasmic reticulum (ER). Within the endoplasmic reticulum (ER), fatty acids undergo esterification to glycerol-3-phosphate, forming triglyceride galactomers (TAGs). This process is facilitated by a sequence of enzymatic reactions mediated by glycerol-3-phosphate acyltransferase (GPAT), lysophosphatidic acid acyltransferase (LPAAT), and diacylglycerol acyltransferase (DGAT). Tags serve as the primary means of storing oil in mustard seeds. Following synthesis, TAGs are enclosed into lipid droplets inside the seed cells. These lipid droplets are enveloped by a phospholipid monolayer containing particular proteins, such as oleosins, which stabilize the droplets and prevent them from merging. A direct correlation exists between the size and quantity of lipid droplets internal to the cells and the oil content in the seeds. Upregulation of critical genes involved in fatty acid and triglyceride biosynthesis, including FAD2, FAD3, and DGAT1, occurs during the seed-filling stage. The regulation of these genes by transcription factors such as WRINKLED1 (WRI1) influences oil accumulation. Specific

hormone signals, namely abscisic acid (ABA) and auxins regulate the expression of genes related to lipid metabolism. ABA, for instance, increases oil accumulation by increasing the expression of genes related to transporter-associated glycogen (TAG) biosynthesis. The oil concentration in mustard seeds is a crucial characteristic that impacts both the economic worth of the crop and its nutritional value. A more excellent oil content is preferable for mustard cultivars cultivated for oil extraction. The cellular processes described above demonstrate the integration of intricate metabolic networks, genetic control, and hormonal signalling in the production and storage of oil in mustard seeds. In agricultural practices, several elements, including the availability of nutrients, the spacing between crops, and the use of growth regulators (such as cytokines), can impact these cellular processes. The combination of Boron and Sulphur with Cytokinin has been demonstrated to augment the production of fatty acids and TAGs, increasing oil content. Boron is crucial for maintaining cell membranes' structural and operational integrity, promoting practical synthesis and assembly of lipids. In lipid metabolism, sulphur is a constituent of specific amino acids and coenzymes, whereas cytokinin indirectly influences cell division and growth, facilitating increased oil accumulation. Analysing the cellular processes that control the oil content in mustard seeds allows for precise agricultural interventions to maximize oil production. By altering the nutrient levels and growth conditions, improving the metabolic pathways concerned with lipid synthesis is possible, resulting in increased oil content in mustard seeds.

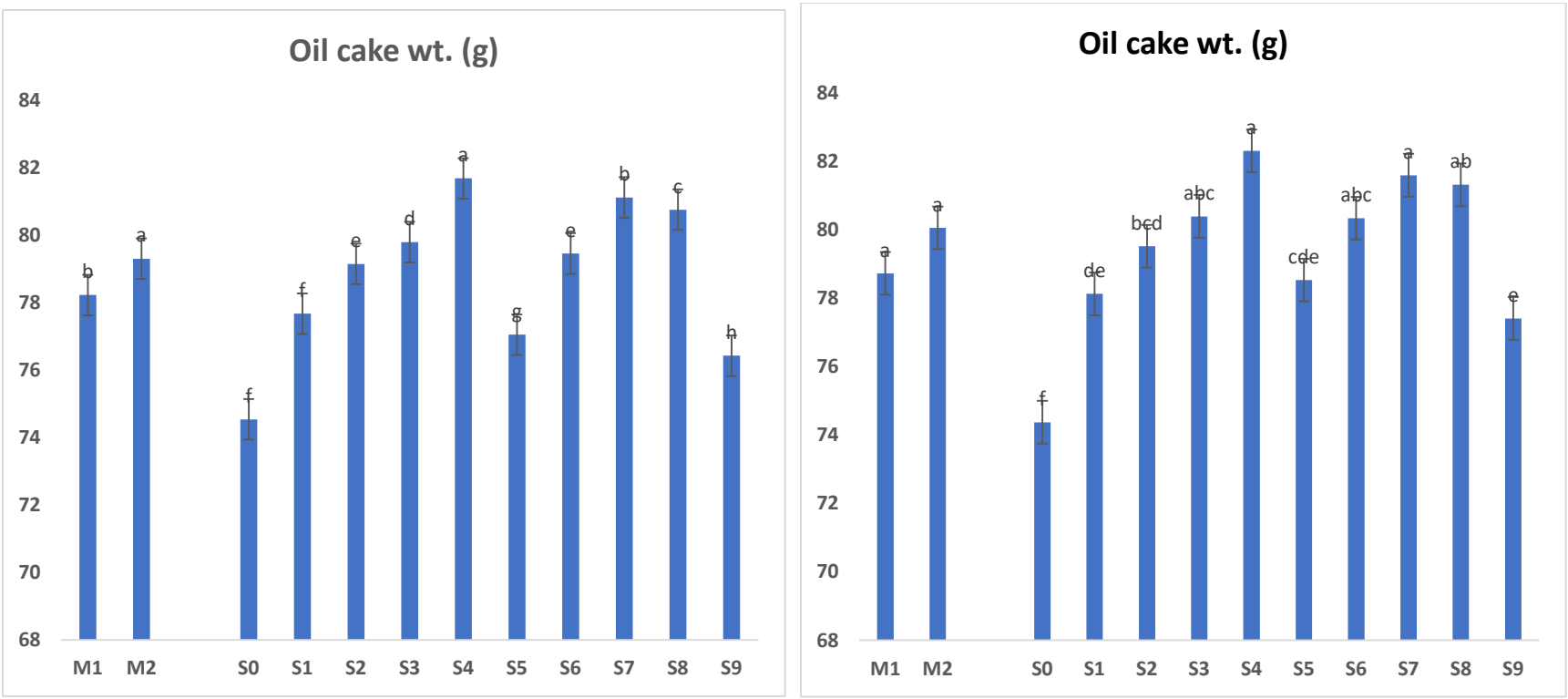


Fig-38 (a): Interaction effect of spacing and nutrient on oil cake weight of mustard crop during rabi season of 2022-23

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

Table-35 (a): Effect of spacing and nutrient on oil content and oil cake weight of mustard crop during rabi season of 2021-22 and 2022-23

Treatments	Oil content (%)		Oil cake weight	
	2021-22	2022-23	2021-22	2022-23
Spacing				
M1 (30×10)	23.83	23.67	78.22	78.72
M2 (20×10)	23.53	23.81	79.30	80.05
C.D. at p<0.05	NS	NS	NS	NS
SE(m)	0.70	0.31	0.06	0.20
Nutrient foliar spray				
S0-Control	22.08	21.94	74.54	74.37
S1-Boron @1%	22.84	23.11	77.67	78.13
S2-Sulphur @ 0.15%	23.61	23.77	79.14	79.52
S3-BAP @0.003%	24.18	24.86	79.79	80.39
S4-Boron @0.5% +Sulphur @0.25%	22.26	21.97	81.68	82.31
S5-Boron @ 1.5%+ Sulphur @0.075%	24.94	24.73	77.05	78.53
S6-Boron @ 0.5% + BAP (@0.0045%)	23.67	23.59	79.45	80.34
S7-Boron @ 1.5%+ BAP (@0.0015%)	22.72	22.93	81.11	81.59
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	22.67	22.96	80.75	81.31
S9-Sulphur @0.25%+ BAP (@0.0015%)	27.84	27.52	76.42	77.41
C.D. at p<0.05	2.42	1.64	2.55	2.01
SEM±	0.80	0.55	0.85	0.67
C.D. S×M at p<0.05	NS	4.12	NS	NS
SEM±	2.23	0.99	0.20	0.64
C.D. M×S at p<0.05	NS	4.24	NS	NS
SEM±	1.29	0.80	1.14	0.92

Where, C.D. represents critical difference, SE (m) represents standard error of mean

Table 4.35 (b): Interaction effect of spacing and nutrient on oil content and oil cake weight of mustard crop during rabi season 2021-22 and 2022-23.

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	20.66	22.6	23.665	26.88	22.215	24.08	24.23	21.18	23.995	27.165	23.667
M2	23.22	23.62	23.87	22.83	21.725	25.37	22.955	24.68	21.93	27.87	23.807
Mean B	21.94	23.11	23.768	24.855	21.97	24.725	23.593	22.93	22.963	27.518	
C.D. S×M at p<0.05						4.12					
SEM±						0.99					
C.D. M×S at p<0.05						4.24					
SEM±						0.80					

4.5 Oil Quality Parameters

4.5.1: Acid Value (mg KOH /g)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied on oil quality parameters in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in the acid value of oil observed after harvest are shown in Table 4.39, Fig 4.39. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the acid value in each treatment compared to the control of both spacings. The percentage increase and decrease were calculated by comparing all the treatments with the control and comparing both the spacings together. A significant decrease was found when the primary and sub-treatment values were compared. In the year (2021-22) main plot M1 shows a higher acid value as compared to M2 with values of 0.28 (M1) and 0.25(M2), respectively. A percentage decrease of 10.71% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S7 with a value of 0.21, where Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent decrease was found highest in S7, followed by S4> S2> S1> S6> S8> S9> S5> S3, and the per cent values were -64.62%, -63.85%, -62.16%, -42.85%, -36.73%, -30.32%, -30.01%, -17.93% and -17.72% respectively when it is compared with its control (S0).

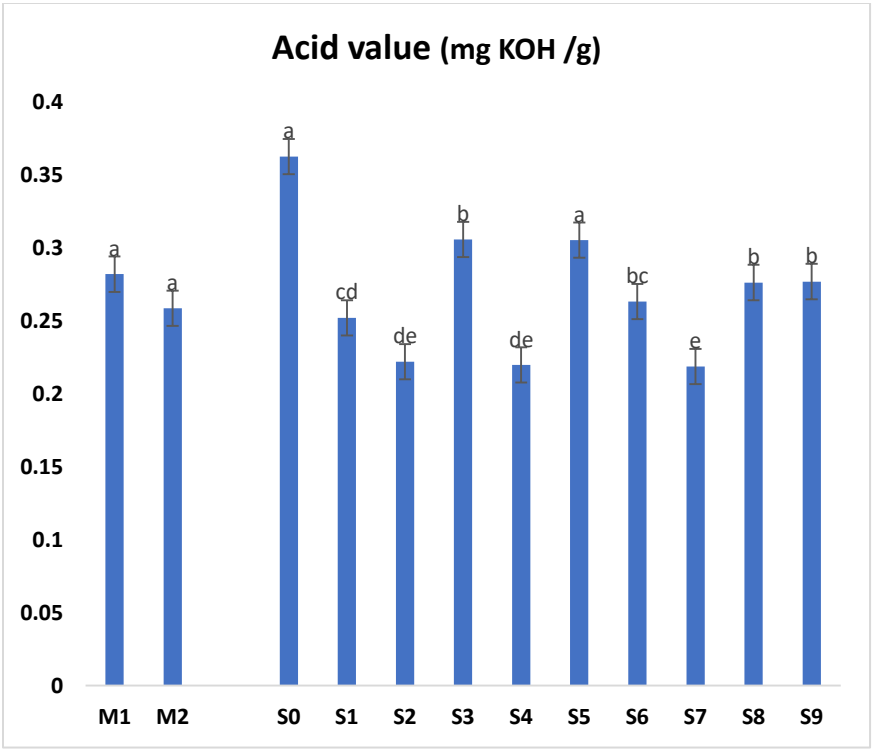
In the year (2022-23) main plot M1 shows a higher acid value as compared to M2 with values of 0.27 (M1) and 0.26 (M2), respectively. A percentage decrease of 3.70% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S7 with a value of 0.21, where Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The per cent decrease was found highest in S7, followed by S4> S2> S1> S6> S8> S9> S3, and the per cent values were -50.34%, -49.49%, -46.34%, -32.93%, -23.71%, -19.02%, -18.65% and -12.43% respectively when it is compared with its control (S0).

The study shows a significant decrease with -36.73% and -23.71% per cent values in 1st and 2nd yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. In 2021-22 and 2022-23, a significant decrease in the acid value of oil was found in treatment S7, where the combined application of aqueous formulation of Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop. The acid value of oil may be used as a measure of quality. However, the acid value of the oil must not be too high, as this denotes an excessively high content of free fatty acids, which causes the oil to turn sour. Discolouration may also occur. Mustard oil should have an acid value of at most 6.1% (Wendlinger et al., 2014). Applying sulphur, boron and cytokinin increases the plant's growth and development, which significantly involves transporting food material to the source to sink part and increase the oil quality.

The acid value of mustard oil is an important quality indicator that precisely measures the oil's concentration of free fatty acids (FFAs). It serves as a measure of the oil's level of rancidity and resistance to oxidation. Optimisation of oil quality during extraction and processing requires a comprehensive understanding of the cellular processes that influence the acid value. In mustard seeds, lipases are enzymes that facilitate the breakdown of triglycerides into glycerol and free fatty acids by hydrolysis. Enhancement of lipase activity can occur during seed storage or processing, resulting in elevated concentrations of free fatty acids. The presence of moisture, temperature, and mechanical damage can frequently stimulate this enzymatic activity, facilitating the degradation of triglycerides and increasing the oil's acidity level. Regulatory factors for lipase activity include enzyme concentration, substrate availability, and environmental conditions. Managerial control of these parameters during oil extraction and processing can effectively reduce the production of free fatty acids. The increased acid value of mustard oil is mainly attributed to oxidative degradation. Unsaturated fatty acids undergo oxidation, resulting in the formation of peroxides and free fatty acids. Iterative exposure to oxygen, light, and heat speeds

up this process. Lipid peroxidation can be further intensified in the presence of pro-oxidant metals and other pollutants. The inherent antioxidant properties of the oil, such as tocopherols and phenolic compounds, contribute to the prevention of oxidative deterioration. Nevertheless, their efficacy can be reduced with time or when subjected to processing conditions, resulting in higher acid values. The seed quality employed during the extraction process can affect the acidity coefficient of mustard oil. Seed maturity, storage conditions, and exposure to environmental stress can influence the degradation susceptibility of the initial lipid content. Inadequate management and storage of mustard seeds can result in elevated moisture levels and microbial activities, stimulating lipase activity and oxidative breakdown, ultimately increasing the extracted oil's acidity. The acid value can be influenced by the technique employed for oil extraction, such as mechanical pressing or solvent extraction. Mechanical compression may result in residual quantities of free fatty acids in the oil, whereas solvent extraction techniques usually include refining procedures that can decrease acidity. The purification procedures, such as degumming, neutralisation, and bleaching, are specifically engineered to eliminate contaminants, including free fatty acids. The final oil product may exhibit elevated acid values due to inefficient refining. The acidity value is a direct indicator of the quality and freshness of the oil. A more excellent acid value signifies elevated levels of free fatty acids, a characteristic frequently linked to the occurrence of rancidity and consequent decrease in shelf-life. Monitoring the acid value facilitates the assessment of soil degradation and the assurance of product quality. High acid values are typically undesirable for consumers since they can impact the oil's taste, aroma, and general acceptability. Maintaining a low acid value is essential for ensuring compliance with quality standards and satisfying customer expectations. Increased levels of free fatty acids can affect the nutritional composition of the oil, possibly diminishing its health advantages. Furthermore, elevated acid levels may suggest the existence of potentially detrimental oxidation byproducts, jeopardising the safety of food. Oils with elevated acid levels often need further refining to enhance quality, leading to higher production input costs. Producers can improve the

economic feasibility of mustard oil production by strategically managing the acidity level through efficient processing and storage methods. Compliance with food safety regulations and quality assurance programs requires strict adherence to regulatory standards for acid value. This guarantees that the oil complies with industry standards and is suitable for consumption under safe conditions. The aggregate acidity of mustard oil is determined by enzymatic hydrolysis, oxidative degradation, seed quality, and processing methods. Understanding these cellular processes is crucial for managing oil quality, guaranteeing consumer satisfaction, and upholding economic and regulatory standards.



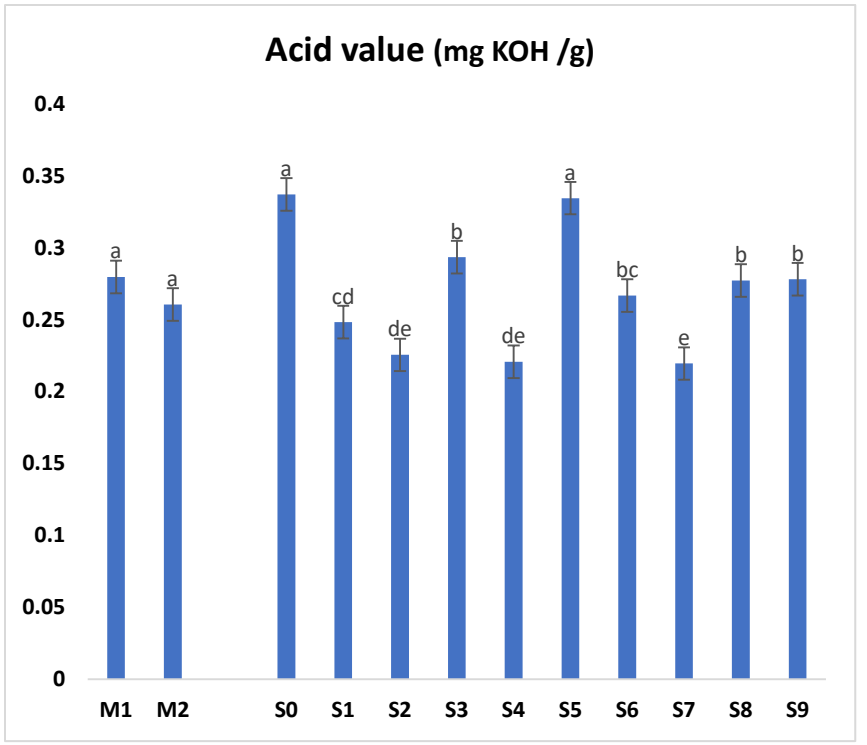


Fig-39 (a): Interaction effect of spacing and nutrient on acid value of mustard oil during rabi season of 2021-22

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.5.2: Peroxide Value (milli eq. iodine/g of oil)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in the peroxide value of mustard oil observed after harvest are shown in Table 4.40 and Fig 4.40. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the peroxide value of oil in each treatment compared to the control of both spacings. Thus, the pattern of percentage increase in peroxide value was observed after harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22) there was no significant difference found in main plots M1 (30*10) and M2 (20*10). M1 and M2 main plots show the peroxide value of 2.16 in mustard oil. In subplots, significant results were observed in S1 with a value of 2.45, where Boron @1% was applied to the crop as a foliar spray. The per cent increase was found highest in S1 followed by S8> S9=S3> S7= S2> S6> S5> S4, and the per cent values were 33.87%, 33.19%, 32.5%, 32.5%, 26.36%, 23.36%, 25.51%, 22.85% and 1.81% respectively when it is compared with its control (S0). In the year (2021-22) main plot M1 shows a higher peroxide value as compared to M2 with values of 2.28 (M1) and 2.20 (M2), respectively. A percentage increase of 3.50% was found in M1, where the crop was grown in spacing (30*10). Significant results were observed in S1 with a value of 2.55 in subplots where Boron @1% was applied to the crop as a foliar spray. The per cent increase was found highest in S1 followed by S3> S8> S9> S7> S2> S6> S5> S4, and the per cent values were 32.54%, 31.88%, 31.20, 30.50%, 26.02%, 25.21%, 21.81%, 16.09% and 3.09% respectively when it is compared with its control (S0). The study shows a significant increase with 25.51% and 21.81% per cent values in 1st and 2nd yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop.

In 2021-22 and 2022-23, a significant increase in the peroxide value of oil was found in treatment S1 where the aqueous formulation of Boron @ 1% was applied to the crop. Bardhan et al. (2014) show that the peroxide value is a common indicator of lipid oxidation, but its use is limited to the early stages of oxidation. Peroxides are primary products of lipid oxidation and play a central role in the auto-oxidation of lipids. They are decomposed into carbonyls and other compounds. This index accounts for hydroperoxides, labile intermediate compounds that decompose into several secondary oxidation products. The parameter, peroxide value, measures the edible oil system's total peroxide and hydroperoxide content. Peroxide value shows the quality character of the mustard oil. It is an important parameter to check the quality of the mustard oil. The peroxide value (PV) of an oil, such as mustard oil, measures its oxidation level and is used to determine the extent of rancidity. It indicates the amount of peroxides and hydroperoxides present in the oil, which are primary oxidation products formed during the breakdown of fatty acids. The peroxide value is crucial for assessing the freshness and quality of the oil. The oxidation of mustard oil begins with the formation of free radicals. Various factors, including light, heat, and exposure to air can generate these radicals. The free radicals react with the unsaturated fatty acids present in the oil, forming peroxides. This initial step is often catalysed by metals such as iron or copper, which can act as pro-oxidants. Once peroxides are formed, they react with additional unsaturated fatty acids, forming secondary oxidation products. This process propagates the oxidation chain reaction, increasing the peroxide value. Mustard oil includes the oxidation of linoleic and oleic acids, which are present in significant amounts. The oxidation reaction eventually terminates when the free radicals are neutralised by antioxidants in the oil or other means. However, the presence of antioxidants does not entirely prevent oxidation but delays its progress. Mustard oil contains natural antioxidants such as tocopherols (vitamin E) and polyphenols. These antioxidants can scavenge free radicals and inhibit peroxide formation, thus helping maintain a lower peroxide value. In some cases, synthetic antioxidants may be added to the oil to enhance its stability and shelf life. These antioxidants interrupt the oxidation chain reaction and protect the oil from rapid

degradation. A high peroxide indicates that the oil has undergone significant oxidation, compromising its quality and freshness. Measuring the peroxide value helps assess the degree of spoilage and ensure that the oil meets quality standards. The peroxide value is used to estimate the shelf life of mustard oil. An increasing peroxide value over time signals that the oil is becoming rancid and may no longer be suitable for consumption or use. Rancid oils can produce harmful compounds that may affect health. The peroxide value provides an early indication of rancidity, helping to prevent the consumption of degraded oil that could potentially be harmful. High peroxide values can decrease the oil's nutritional quality, including the loss of beneficial components such as essential fatty acids and vitamins. Monitoring peroxide levels helps in maintaining the nutritional integrity of the oil. Regulatory bodies often set maximum permissible peroxide values for edible oils. Measuring and reporting the peroxide value ensures compliance with these regulations and helps maintain the oil's marketability. Understanding the peroxide value helps optimise processing and storage conditions to minimise oxidation. This includes controlling temperature, exposure to light, and pro-oxidant presence during oil processing and storage. The peroxide value of mustard oil is a critical parameter for assessing the extent of oxidation and ensuring the oil's quality, safety, and nutritional value. By understanding the cellular mechanisms involved in lipid oxidation and the role of antioxidants, producers can implement effective measures to maintain low peroxide values and extend the shelf life of mustard oil.

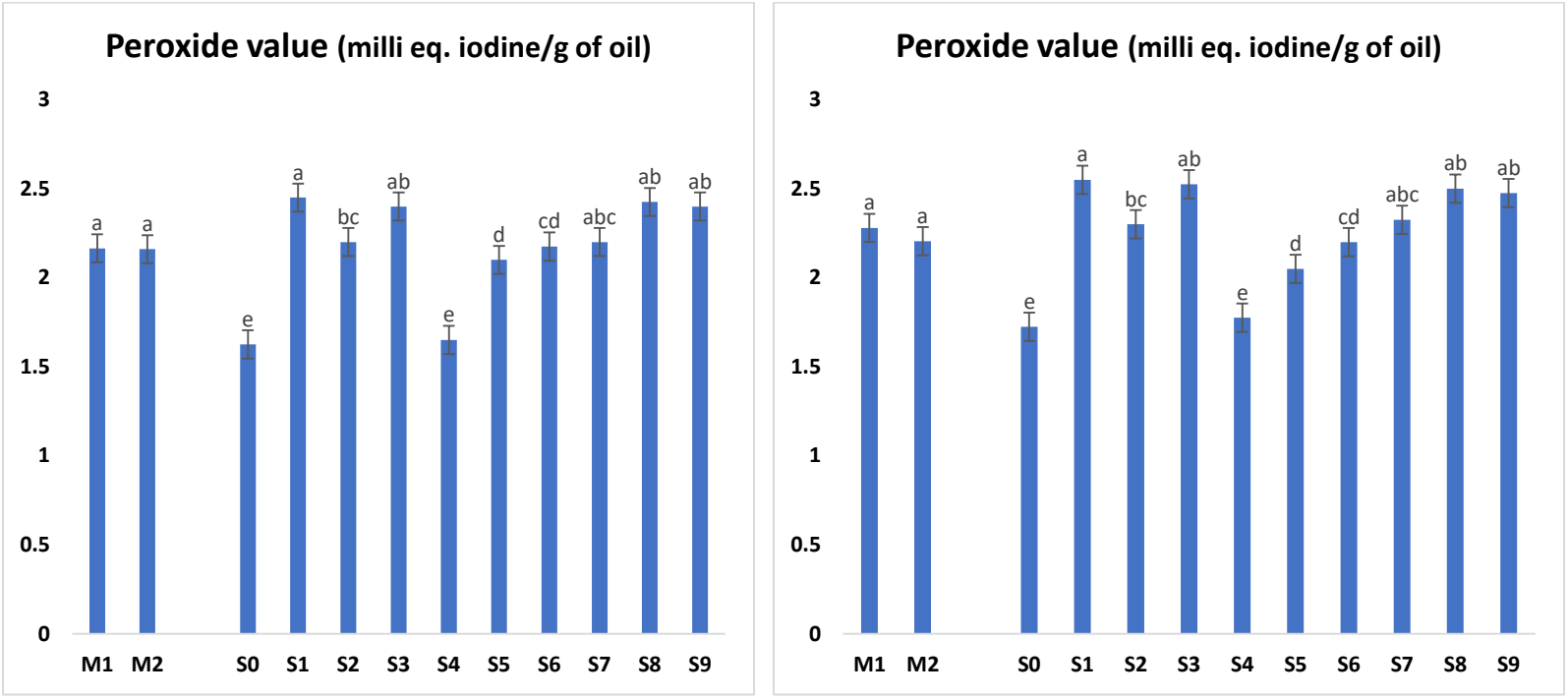


Fig-40 (a): Interaction effect of spacing and nutrient on peroxide value of mustard oil during rabi season of 2021-22

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.5.3: P-Anisidine Value (g/100 ml isooctane)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in the ranitidine value of mustard oil observed after harvest are shown in Table 4.41 and Fig 4.41. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the ranitidine value of oil in each treatment compared to the control of both spacings. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in ranitidine value was observed after harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), the main plot M2 shows a higher p-anisidine value than M1, with values of 2.05 (M2) and 2.03 (M1), respectively. A percentage increase of 0.97% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S2 with a value of 2.82, where Sulphur @ 0.15% was applied to the crop as a foliar spray. The per cent increase was found highest in S2 followed by S3> S1> S4> S5> S6> S8> S9> S7, and the per cent values were 58.19%, 54.31%, 54.28%, 54.07%, 51.34%, 35.52%, 25.38%, 25.07% and 8.56% respectively when it is compared with its control (S0).

In the year (2022-23), the main plot M2 shows a higher p-anisidine value than M2, with values of 1.89 (M2) and 1.84 (M2), respectively. A percentage increase of 2.64% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S2 with a value of 2.82, where Sulphur @ 0.15% was applied to the crop as a foliar spray. The per cent increase was found highest in S2, followed by S1> S3> S4> S5> S6> S8> S9> S7, and the per cent values were 52.19%, 47.84%, 47.76%, 47.52%, 44.72%, 26.56%, 15.17%, 14.98% and 6.78% respectively when it is compared with its control (S0).

The study shows a significant increase with 35.52% and 26.56% per cent values in 1st and 2nd yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. In 2021-22 and 2022-23, a significant increase in the ranitidine value of oil was found in treatment S2 where the application of aqueous formulation of sulphur @ 0.15% was applied to the crop. This finding could be explained by the fact that low constant primary oxidised compounds (hydroperoxide) decomposed to form aldehyde compounds. An increase in peroxide value and P-anisidine value results in oil oxidation. Its peroxide and para-anisidine value governs the shelf stability of the oil. It was found that the rate of oxidation in mustard oil is very high. There is a correlation between peroxide value and P- anisidine value, as reported by Ghosh et al. (2012). Similar results were found by Chen Jet al. (2020). Applying nutrients such as sulphur and boron improves oxidation and thus helps increase the oil's shelf life.

The ranitidine value is used to assess the oxidative stability of oils and fats, mainly focusing on the number of secondary oxidation products, such as aldehydes, that form during lipid oxidation. This value is critical for evaluating the quality and shelf-life of oils, including mustard oil. The p-anisidine value is calculated based on the reaction of p-anisidine with aldehydes, which produces a coloured complex that can be quantified spectrophotometrically. Lipid oxidation in mustard seeds is primarily driven by the action of lipoxygenases (LOX) and other oxidative enzymes. These enzymes catalyse the formation of hydroperoxides from unsaturated fatty acids in the oil. The oxidation process involves forming lipid hydroperoxides, which subsequently decompose into secondary oxidation products, including aldehydes and ketones. Hydroperoxides decompose, producing volatile aldehydes, such as (E)-2-alkenyl and (E)-2,4-alkadienal. These aldehydes are responsible for the characteristic off-flavours and odours in oxidized oils. P-anisidine reacts specifically with these aldehydes, forming a coloured complex that can be measured to determine the p-anisidine value. In the p-anisidine test, p-anisidine reacts

with the aldehydes in the oil sample. This reaction forms a coloured compound that absorbs light at a specific wavelength. The intensity of the colour, measured using a spectrophotometer, correlates with the concentration of aldehydes and, consequently, the extent of oxidation. The ranitidine value quantitatively measures the extent of oxidation in mustard oil, which is crucial for quality control. High p-anisidine values indicate advanced oxidation and potential rancidity, affecting the oil's flavour, aroma, and nutritional quality. By assessing the p-anisidine value, manufacturers can estimate the shelf-life of mustard oil. Oils with lower p-anisidine values are considered fresher and more stable, while higher values indicate deterioration and reduced shelf-life. Oxidized oils can lose their nutritional value due to the breakdown of essential fatty acids and the formation of potentially harmful compounds. Additionally, the sensory properties of the oil, such as taste and odour, can be adversely affected by oxidation. The ranitidine value helps monitor these changes and ensure the oil meets quality standards. Many food safety and quality standards require the assessment of oxidative stability in oils. The ranitidine value is a standardized method that aligns with international regulations and guidelines for oil quality. Consuming oxidized oils can pose health risks due to the formation of toxic compounds. Monitoring the p-anisidine value helps prevent the consumption of mustard oil that may have undergone excessive oxidation, thus safeguarding consumer health. The ranitidine value is critical for evaluating mustard oil's oxidative stability and quality. It reflects the amount of secondary oxidation products, particularly aldehydes, indicative of oil deterioration. By assessing the p-anisidine value, one can monitor oil quality, determine shelf-life, ensure compliance with regulatory standards, and prevent potential health risks associated with oxidized oils. The cellular mechanisms underlying this process involve the enzymatic oxidation of lipids and the subsequent formation of reactive aldehydes, which are effectively quantified using the ranitidine test.

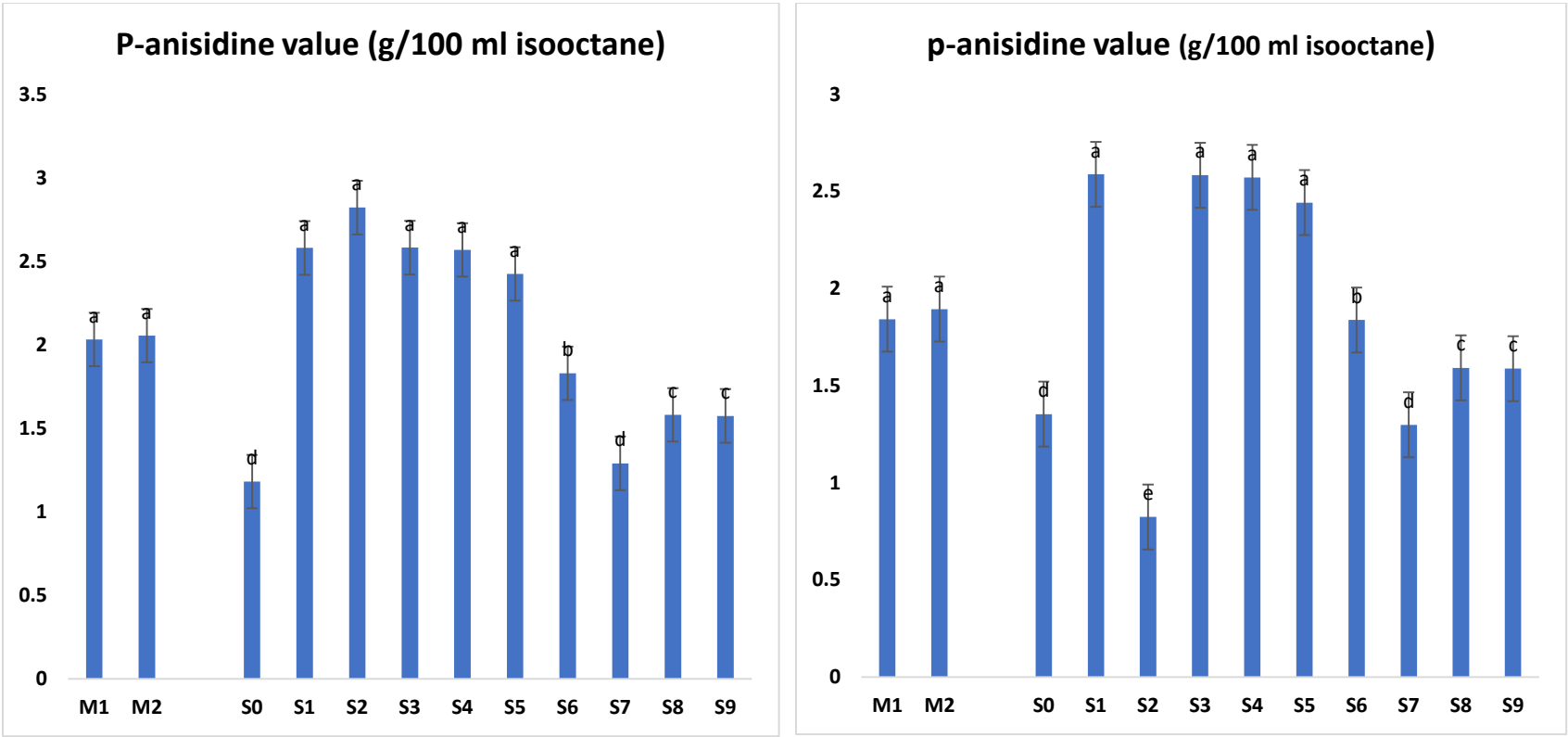


Fig-41 (a): Interaction effect of spacing and nutrient on p-anisidine value of mustard oil during rabi season of 2021-22

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

Table-36 (a): Effect of spacing and nutrient on acid value, peroxide value and p-anisidine value of mustard oil during rabi season of 2021-22 and 2022-23

Treatments	Acid value		Peroxide value		p-Anisidine value	
	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
Spacing						
M1 (30×10)	0.28	0.28	2.17	2.28	2.03	1.84
M2 (20×10)	0.26	0.26	2.16	2.21	2.06	1.89
C.D. at p<0.05	NS	NS	NS	NS	NS	NS
SE(m)	0.00	0.00	0.06	0.03	0.02	0.02
Nutrient foliar spray						
S0-Control	0.36	0.34	1.63	1.73	1.18	1.35
S1-Boron @1%	0.25	0.25	2.45	2.55	2.58	2.59
S2-Sulphur @ 0.15%	0.22	0.23	2.20	2.30	2.82	2.82
S3-BAP @0.003%	0.31	0.29	2.40	2.53	2.58	2.58
S4-Boron @0.5% +Sulphur @0.25%	0.22	0.22	1.65	1.78	2.57	2.57
S5-Boron @ 1.5%+ Sulphur @0.075%	0.31	0.33	2.10	2.05	2.43	2.44
S6-Boron @ 0.5% + BAP (@0.0045%)	0.26	0.27	2.18	2.20	1.83	1.84
S7-Boron @ 1.5%+ BAP (@0.0015%)	0.22	0.22	2.20	2.33	1.29	1.44
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	0.28	0.28	2.43	2.50	1.58	1.59
S9-Sulphur @0.25%+ BAP (@0.0015%)	0.28	0.28	2.40	2.48	1.58	1.59
C.D. at p<0.05	0.03	0.02	0.34	0.23	0.14	0.15
SEM±	0.01	0.00	0.11	0.07	0.04	0.05
C.D. S×M at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.01	0.01	0.21	0.12	0.04	0.06
C.D. M×S at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.01	0.01	0.16	0.11	0.05	0.07

Where, C.D. represents critical difference, SE (m) represents standard error of mean.

4.5.4: Iodine Value (g/100g oil)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in the iodine value of mustard oil observed after harvest are shown in Table 4.42 and Fig 4.42. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the iodine value of oil in each treatment compared to control of both the spacings. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in iodine value was observed after harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), the main plot M2 shows a higher iodine value than M1, with values of 7.12 (M2) and 7.10 (M1), respectively. A percentage increase of 0.28% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S8 with a value of 7.86, where Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S8 followed by S9> S5> S4=S7> S6> S2> S3> S1, and the per cent values were 19.36%, 18.30%, 18.04%, 13.80%, 13.80%, 11.97%, 6.57%, 1.49% and 0.10% respectively when it is compared with its control (S0).

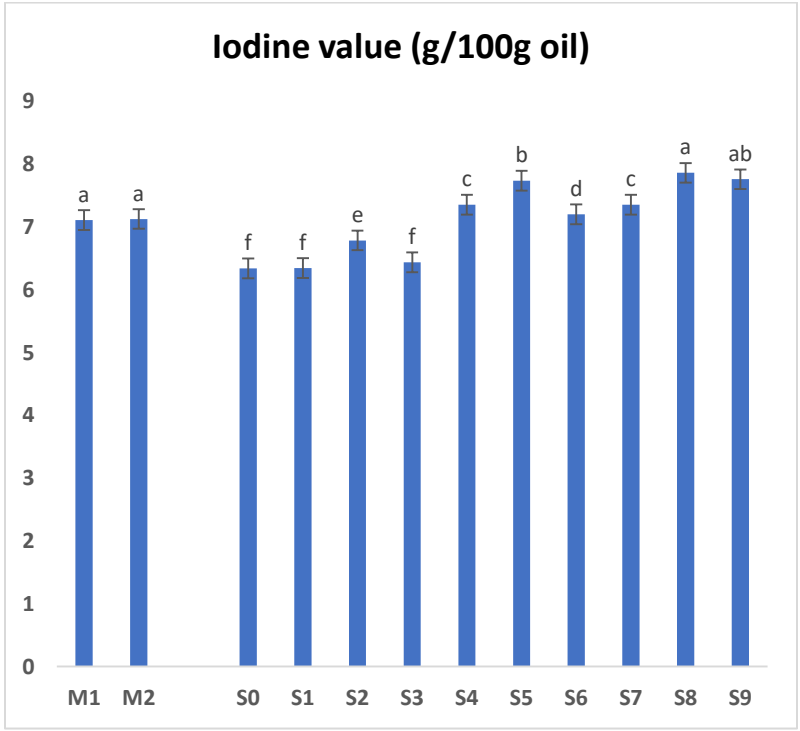
In the year (2021-22) main plot M1 shows a higher iodine value as compared to M2 with values of 7.11 (M1) and 7.10 (M2), respectively. A percentage increase of 0.14% was found in M1, where the crop was grown in spacing (30*10). In subplots, significant results were observed in S8 with a value of 7.86, where Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop as a foliar spray. The per cent increase was found highest in S8 followed by S9> S5> S7> S4> S6> S2> S3> S1, and the per cent values were 19.43%, 18.38%, 18.14%, 13.92%, 13.92%, 12.05%, 5.17%, 0.67% and 0.43% respectively when it is compared with its control (S0).

The study shows a significant increase with 11.97% and 12.05% per cent values in the 1st and 2nd yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. In 2021-22 and 2022-23, a significant increase in the iodine value of oil was found in treatment S8, where the application of aqueous formulation of Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop. The iodine value (IV) measures the degree of unsaturation in a fat or vegetable oil. It determines the stability of oils to oxidation and allows the overall unsaturation of the fat to be determined qualitatively (AOCS, 1993; Asuquo et al., 2012). It was observed that measured iodine values for Mustard oils are 8.10 g. The low iodine values may have contributed to its greater oxidative storage stability. The oxidative and chemical changes in oils during storage are characterised by an increase in free fatty acid contents and a decrease in the total unsaturation of oils (Perkin, 1992).

The Iodine Value (IV) of mustard oil measures the degree of unsaturation of the fatty acids present in the oil. It reflects the number of double bonds in the fatty acid chains that react with iodine. Understanding the cellular mechanisms that influence the iodine value involves examining the biosynthesis of fatty acids in mustard plants and the biochemical processes affecting oil composition. In mustard plants, fatty acids are synthesized in the plastids of seed cells. The process begins with the conversion of acetyl-CoA to malonyl-CoA via acetyl-CoA carboxylase. The fatty acid synthase complex then catalyses chain elongation, adding two carbon units to the growing fatty acid chain. The fatty acids are primarily synthesized as saturated or monounsaturated chains, but desaturation occurs to form polyunsaturated fatty acids. The formation of double bonds in fatty acids is catalyzed by desaturase enzymes, which are crucial for determining the iodine value. These enzymes, such as stearoyl-CoA desaturase (SCD) and oleoyl-CoA desaturase, introduce double bonds into the fatty acid chains, converting saturated fatty acids into unsaturated forms. The level of desaturase activity and the availability of substrates

influence the extent of unsaturation and, consequently, the iodine value of the oil. Once synthesised, fatty acids are esterified into triglycerides and stored in oil bodies within the seed cells. The stored oil is mobilized for germination during seed maturation and under certain environmental conditions. The iodine value of the oil can be affected by changes in the composition of fatty acids during storage and mobilization processes. The iodine value is also influenced by genetic factors that determine the expression of desaturase enzymes and the overall fatty acid profile. Additionally, environmental conditions such as temperature and nutrient availability can affect the activity of desaturase enzymes and the composition of fatty acids in mustard seeds. The iodine value serves as a key indicator of the degree of unsaturation in mustard oil. Higher iodine values correspond to higher levels of unsaturated fatty acids with multiple double bonds. This is important because unsaturated fatty acids generally have better nutritional profiles and health benefits than saturated fatty acids. The iodine value is crucial for assessing the quality and stability of mustard oil. Oils with higher iodine values are more prone to oxidation, leading to rancidity and decreased nutritional quality. Therefore, monitoring the iodine value helps in evaluating the shelf-life and stability of mustard oil. Mustard oil with a high iodine value contains a higher proportion of polyunsaturated fatty acids essential for various physiological functions, including cardiovascular health and cellular membrane fluidity. The iodine value provides insight into the oil's potential health benefits and its suitability for dietary use. Understanding the iodine value helps in optimising agronomic practices and oil processing. For example, selecting mustard varieties with desirable fatty acid profiles and adjusting processing conditions can help achieve the target iodine value for specific applications, whether for culinary or industrial purposes. The iodine value is used in regulatory standards and quality control for edible oils. Ensuring that mustard oil meets specific iodine value criteria helps maintain product consistency and meet consumer expectations. The iodine value of mustard oil reflects the extent of unsaturation of its fatty acids, influenced by enzymatic desaturation during fatty acid biosynthesis. This value is essential for assessing the oil's nutritional quality, stability, and

suitability for various applications. Understanding the cellular mechanisms and justifications for iodine value helps optimise oil production and processing to achieve desired quality standards.



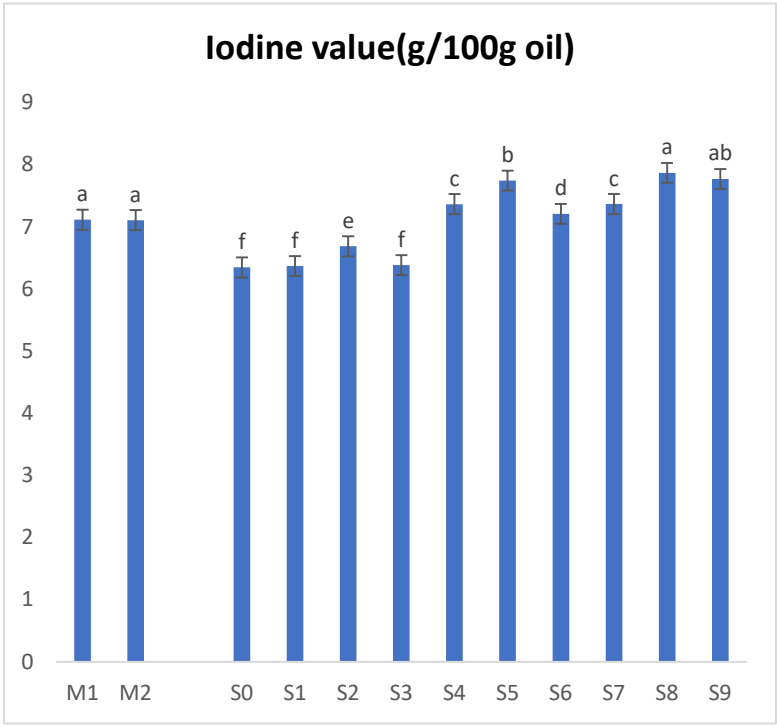


Fig-42 (a): Interaction effect of spacing and nutrient on iodine value of mustard oil during rabi season of 2021-22

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.5.5: Saponification Value (mg KOH/g oil)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in the saponification value of mustard oil observed after harvest are shown in Table 4.43, Fig 4.43. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the saponification value of oil in each treatment compared to the control of both spacings. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in saponification value was observed after harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22) the main plot M2 shows a higher saponification value as compared to M1 with values of 3.48 (M2) and 3.44 (M1), respectively. A percentage increase of 1.14% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S2 with a value of 5.84, where sulphur @0.15% was applied to the crop as a foliar spray. The per cent increase was found highest in S2, followed by S6> S7> S5> S3> S4> S9> S8> S1, and the per cent values were 66.65%, 63.02%, 53.21%, 50.28%, 37.72%, 37.69%, 21.97%, 21.18% and 12.93% respectively when it is compared with its control (S0).

In the year (2022-23), the main plot M2 shows a higher saponification value than M1, with values of 3.49 (M2) and 3.42 (M1), respectively. A percentage increase of 2.00% was found in M2,

where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S2 with a value of 5.84, where sulphur @0.15% was applied to the crop as a foliar spray. The per cent increase was found highest in S2, followed by S6> S7> S5> S4> S3> S9> S8> S1, and the per cent values were 66.58%, 62.87%, 53.09%, 47.72%, 37.78%, 37.64%, 21.90%, 21.19% and 12.82% respectively when it is compared with its control (S0).

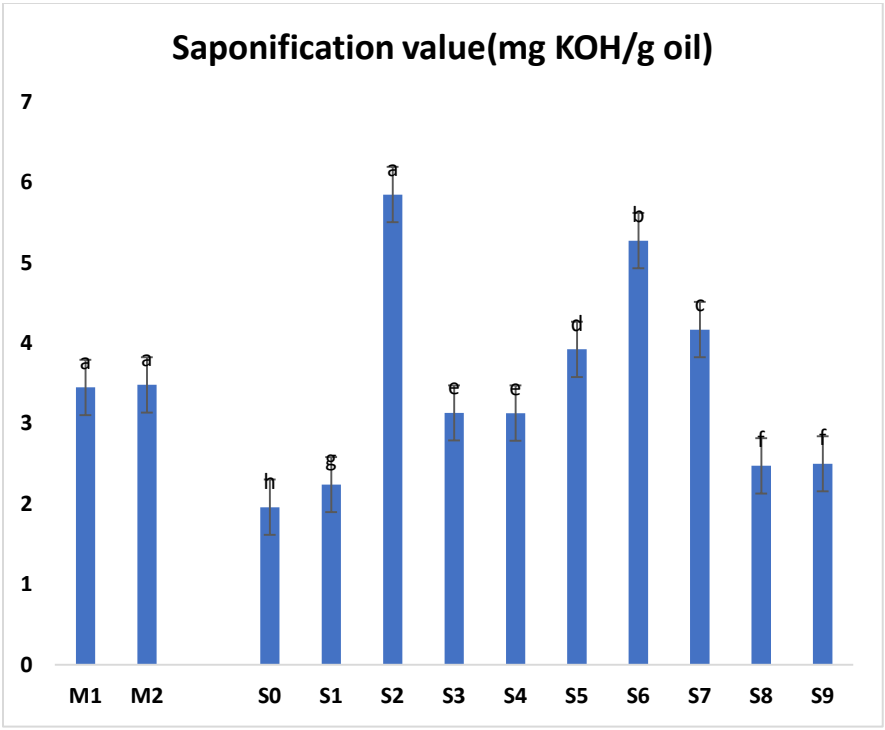
The study shows a significant increase with 63.02% and 62.87% per cent values in 1st and 2nd yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. In 2021-22 and 2022-23, a significant increase in the saponification value of oil was found in treatment S2, where an aqueous formulation of sulphur @0.15% was applied to the crop. The saponification value (SV) is an index of the oil sample's average molecular mass of fatty acid. The SV value obtained for the oil samples in the lower saponification values suggests that the mean molecular ten weight of fatty acids is lower or that the number of ester bonds is less. This might imply that the fat molecules were not intact with each other (Denniston et al., 2004). Applying plant hormones and micronutrients shows better results in saponification value than the control.

The saponification value of an oil or fat measures the base (usually potassium hydroxide) required to saponify a given quantity of oil or fat. It is a vital indicator of the oil's free fatty acid content. It is crucial to understand its chemical characteristics, such as its usability in soap making and other industrial applications. The saponification process involves the hydrolysis of triglycerides (fats and oils) into glycerol and fatty acids when treated with a strong base. In mustard oil, the triglycerides consist of various fatty acids, including oleic, linoleic, and erucic

acids. The saponification value is influenced by the composition and chain length of these fatty acids. Oils with a higher proportion of short-chain fatty acids typically have higher saponification values. These fatty acids react more readily with the base, requiring less base quantity for saponification. Conversely, oils with longer chain fatty acids have lower saponification values because they need more bases to react fully. The saponification value reflects the molecular structure of triglycerides in mustard oil. Triglycerides are composed of three fatty acid molecules esterified to a glycerol backbone. The saponification degree is directly proportional to the number of ester bonds in the triglycerides. Each ester bond reacts with one base molecule to produce one molecule of soap and one molecule of glycerol. The triglycerides are hydrolysed during saponification into their constituent fatty acids and glycerol. The saponification value quantifies the base needed to convert the total triglyceride content into fatty acids, indicating the oil's total ester content. Mustard oil also contains unsaponifiable matter, which includes compounds such as sterols, tocopherols, and other lipids that do not participate in the saponification reaction. These components contribute to the oil's overall chemical composition but do not affect the saponification value directly. The saponification value thus indicates the oil's triglyceride content relative to its unsaponifiable fraction. The saponification value of mustard oil is compared with other vegetable oils to assess its relative fatty acid composition and potential applications. Mustard oil typically has a moderate saponification value, indicating its balanced fatty acid profile. This value helps determine its suitability for various industrial applications, including soap manufacturing, where specific saponification values are desirable for optimal product quality. In soap making, a higher saponification value indicates that the oil contains a higher proportion of fatty acids that can be saponified, which is desirable for producing soap with better lathering properties and stability. Monitoring the saponification value helps in quality control by ensuring the consistency of the oil's fatty acid composition, which is crucial for maintaining the desired properties of the end products. The saponification value is critical for evaluating mustard oil's quality and suitability for various applications. It provides valuable insights into the oil's

fatty acid composition and triglyceride content, directly impacting its usability in industrial processes. By understanding the saponification value, manufacturers can tailor the oil's application to meet specific requirements, such as in soap production, where a certain level of saponification is necessary to achieve optimal results.

The cellular mechanism underlying the saponification value involves the hydrolysis of triglycerides into fatty acids and glycerol in the presence of a base. This process is influenced by the triglycerides' molecular structure and the presence of unsaponifiable matter. The saponification value of mustard oil reflects its fatty acid content and is essential for determining its suitability for various industrial applications.



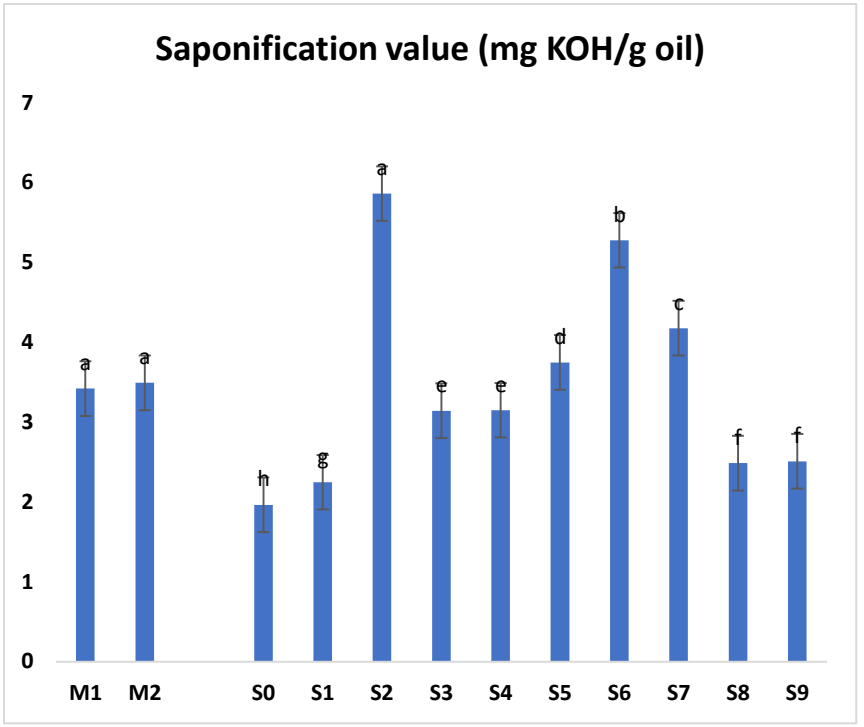


Fig-43 (a): Interaction effect of spacing and nutrient on saponification value of mustard oil during rabi season of 2021-22 and 2022-23

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.5.6: Totox Value (mg/g)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in the totox value of mustard oil observed after harvest are shown in Table 4.44, Fig 4.44. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the totox value of oil in each treatment compared to the control of both spacings. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in totox value was observed after harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22) the main plot M2 shows a higher totox value as compared to M1 with values of 5.60 (M2) and 5.58 (M1), respectively. A percentage increase of 0.35% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S3 with a value of 7.28, where BAP @0.003% was applied to the crop as a foliar spray. The per cent increase was found highest in S3, followed by S1> S9> S8> S6> S4> S7> S2> S5, and the per cent values were 55.23%, 55.22%, 49.23%, 48.09%, 45.020%, 42.49%, 40.62%, 33.78% and 4.88% respectively when it is compared with its control (S0).

In the year (2022-23) the main plot M2 shows a higher totox value as compared to M1 with values of 5.62 (M2) and 5.59 (M1), respectively. A percentage increase of 0.53% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S3 with a value of 7.28, where BAP @0.003% was applied to the crop as a foliar spray. The per cent increase was found highest in S3, followed by S1> S9> S8> S6> S4> S7> S2> S5 and the per cent values were 53.75%, 53.41%, 47.58%, 46.41%, 43.15%, 40.62%, 38.71%, 31.67% and 2.31% respectively when it is compared with its control (S0).

The study shows a significant increase with 45.02% and 43.15% per cent values in 1st and 2nd yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. In 2021-22 and 2022-23, a significant increase in the totox value of oil was found in treatment S3, where an aqueous formulation of BAP@0.003% was applied to the crop. Totox value measures both antioxidants in oils. However, soybean oil had the highest hydroperoxides and breakdown products. It provided a higher amount of linoleic acid than others, and mustard oil had a better estimation of the progressive oxidative deterioration of the highest erucic acid than other oils studied in this of oils (Velo-Gala, et al. 2014)

The Totox (Total Oxidation) value is a critical indicator of the oxidative stability of edible oils, including mustard oil. It is a composite measure that reflects the total oxidation level in the oil, encompassing both primary oxidation products (peroxides) and secondary oxidation products (like aldehydes and ketones). High Totox values indicate advanced oxidation and degradation of the oil, which can lead to off-flavours, reduced nutritional quality, and potential health risks. The oxidation of mustard oil is primarily driven by the breakdown of its lipid components, which include triglycerides and fatty acids. The cellular mechanisms underlying the oxidation process involve several vital steps. Oxidative stress in mustard oil begins with the initiation of lipid peroxidation, where reactive oxygen species (ROS) such as hydroxyl radicals (•OH), superoxide anions ($O_2^{\bullet-}$), and hydrogen peroxide (H_2O_2) react with the unsaturated fatty acids in the oil. This reaction produces lipid hydroperoxides (LOOH), the primary oxidation products. Lipid hydroperoxides are unstable and decompose to form a range of secondary oxidation products, including aldehydes, ketones, and other volatile compounds. This stage is marked by the propagation of the oxidation process, where further oxidation of primary products generates additional free radicals and reactive intermediates, contributing to an increased Totox value. The oxidation process is eventually terminated by antioxidants, which neutralise free radicals and

stabilise the lipid molecules. Natural antioxidants such as tocopherols (vitamin E) and phenolic compounds in mustard oil significantly mitigate oxidative damage. However, their effectiveness can be overwhelmed by prolonged exposure to oxygen, light, and heat. Several factors affect the Totox value of mustard oil, reflecting the degree of lipid oxidation and the extent of oxidative stress. Mustard oil contains many unsaturated fatty acids, mainly oleic and linoleic acids. Unsaturated fatty acids are more susceptible to oxidation than saturated fatty acids, leading to higher Totox values. Exposure to air, light, and heat accelerates the oxidation process. Inadequate storage conditions can lead to increased formation of lipid peroxides and secondary oxidation products, thus elevating the Totox value. The natural antioxidant content in mustard oil can help reduce the Totox value by inhibiting the formation of oxidation products. However, processing and storage conditions can diminish or affect the antioxidant capacity. The Totox value is a quality indicator for mustard oil, ensuring it meets safety and quality standards. High Totox values indicate advanced oxidation, adversely affecting the oil's sensory qualities and nutritional value. The Totox value provides insights into the shelf life of mustard oil. Oils with high Totox values will likely have shorter shelf life due to accelerated degradation. Monitoring Totox helps predict the oil's stability and ensure it remains within acceptable quality ranges. The oxidation products in mustard oil, particularly aldehydes and ketones, can have health implications if consumed in significant quantities. Elevated Totox values may indicate higher levels of potentially harmful oxidation products, posing health risks.

The Totox value is a crucial measure of mustard oil's oxidative stability, reflecting the cumulative effect of lipid oxidation processes. Understanding the cellular mechanisms of lipid peroxidation and the factors influencing the Totox value helps manage mustard oil's quality and shelf life. Monitoring Totox values ensures that mustard oil remains safe, nutritious, and high-quality.

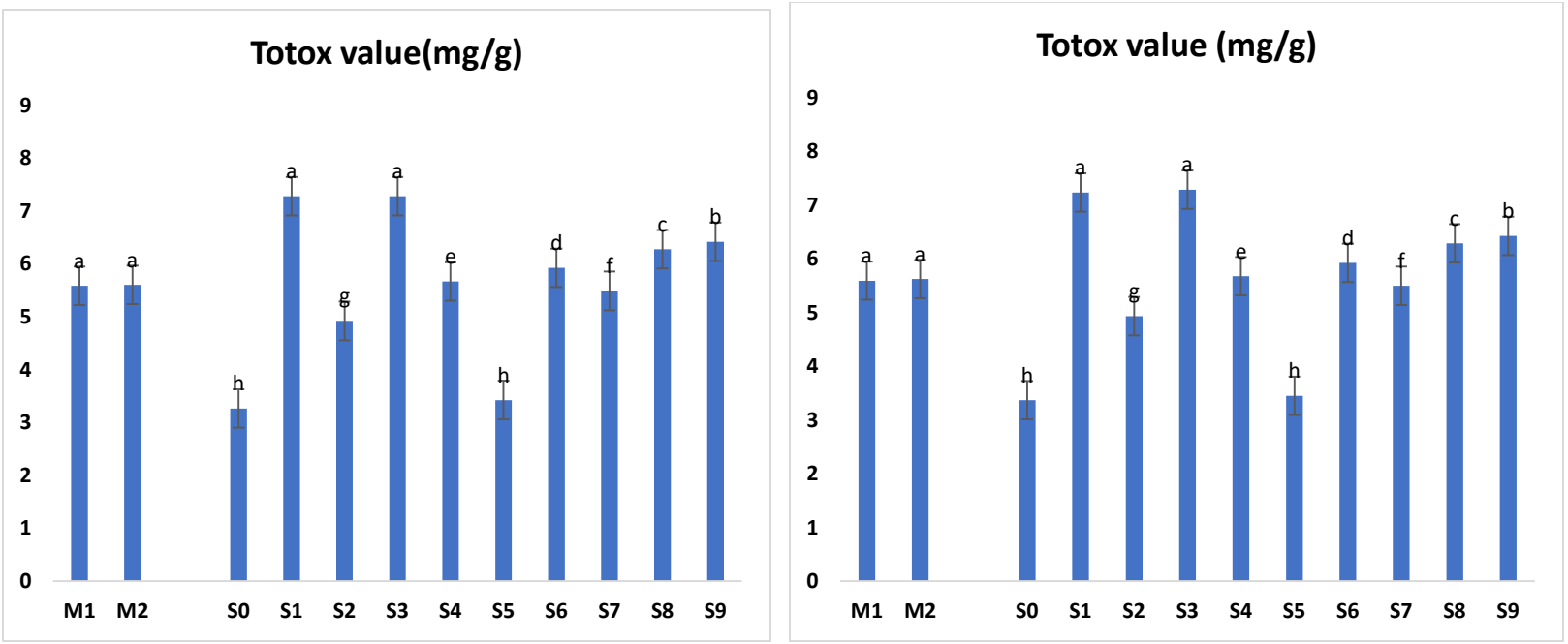


Fig-44 (a): Interaction effect of spacing and nutrient on totox value of mustard oil during rabi season of 2021-22

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

Table-37 (a): Effect of spacing and nutrient on Iodine value, saponification value and totox value of mustard oil during rabi season of 2021-22 and 2022-23

Treatments	Iodine value		Saponification value		Totox value	
	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
Spacing						
M1 (30×10)	7.11	7.11	3.45	3.42	5.59	5.59
M2 (20×10)	7.13	7.11	3.48	3.49	5.61	5.62
C.D. at p<0.05	NS	NS	NS	NS	NS	NS
SE(m)	0.00	0.00	0.00	0.02	0.00	0.00
Nutrients foliar spray						
S0-Control	6.34	6.35	1.96	1.96	3.27	3.37
S1-Boron @1%	6.35	6.37	2.24	2.25	7.28	7.23
S2-Sulphur @ 0.15%	6.79	6.69	5.85	5.87	4.92	4.93
S3-BAP @0.003%	6.44	6.38	3.13	3.14	7.28	7.29
S4-Boron @0.5% +Sulphur @0.25%	7.36	7.37	3.13	3.15	5.67	5.68
S5-Boron @ 1.5%+ Sulphur @0.075%	7.74	7.75	3.92	3.75	3.43	3.45
S6-Boron @ 0.5% + BAP (@0.0045%)	7.20	7.21	5.27	5.28	5.93	5.93
S7-Boron @ 1.5%+ BAP (@0.0015%)	7.36	7.37	4.17	4.18	5.49	5.50
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	7.86	7.87	2.47	2.49	6.28	6.29
S9-Sulphur @0.25%+ BAP (@0.0015%)	7.76	7.77	2.50	2.51	6.42	6.43
C.D. at p<0.05	0.03	0.11	0.00	0.17	0.03	0.12
SEM±	0.01	0.03	0.00	0.05	0.01	0.04
C.D. S×M at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.02	0.02	0.00	0.09	0.02	0.02
C.D. M×S at p<0.05	NS	NS	NS	NS	NS	NS
SEM±	0.01	0.05	0.00	0.08	0.04	0.05

Where, C.D. represents critical difference, SE (m) represents standard error of mean and SE (d) represents the standard error of deviation.

5.7: Oil Density (kg/m^3)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in the density of mustard oil observed after harvest are shown in Table 4.45 and Fig 4.45. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the oil density of oil in each treatment compared to the control of both spacings. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in density of mustard oil was observed after harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22) main plot M1 shows higher density as compared to M2 with values 878.4 (M1) and 877.7 (M2), respectively. A percentage increase of 0.07% was found in M1, where the crop was grown in spacing (30*10). In subplots, it was found that the density of oil in all the treatments was almost the same, and no such difference was found in the treatments compared to its control. The density of oil is higher than that of water. A slight decrease in the density with values 869.25 was found in treatment S1=S2=S3=S7=S8=S9. The percentage decrease observed was -2.32%, -2.32%, -2.32%, -2.32%, -2.32%, -2.32%, -2.15% -1.94% and -1.86%, respectively, when it is compared with its control.

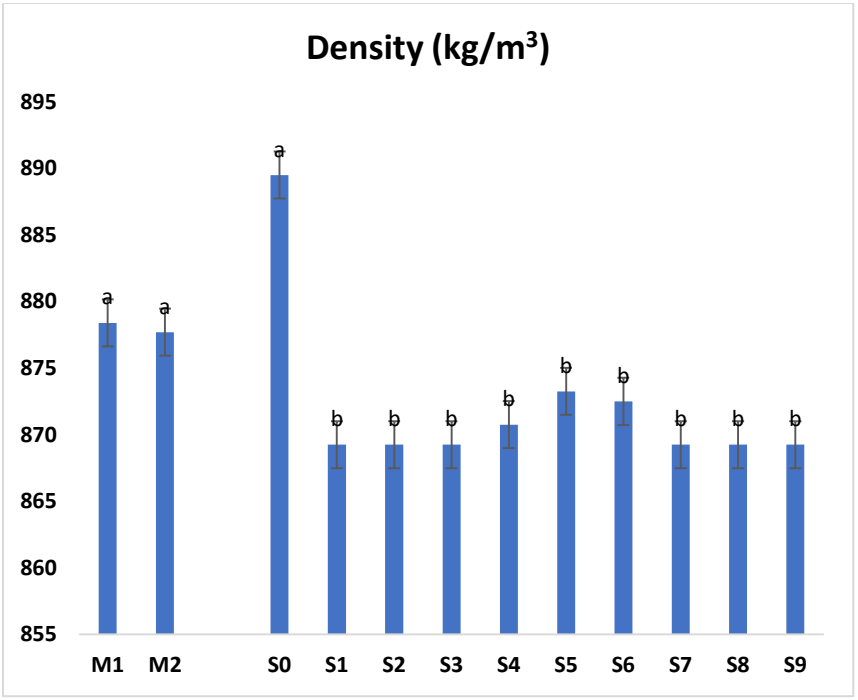
In the year (2022-23) main plot M1 shows higher density as compared to M2 with values 878.9 (M1) and 876.85 (M2), respectively. A percentage increase of 0.23% was found in M1, where the crop was grown in spacing (30*10). In subplots, it was found that the density of oil in all the treatments was almost the same, and no such difference was found in the treatments compared to its control. The density of oil is higher than that of water. A slight decrease in the density with values 870 was found in treatment S1=S2=S3=S7=S8=S9. The percentage decrease observed was

-2.30%, -2.18%, -2.18%, -2.18%, -2.18%, -2.15%, -2.15%, -2.15%, -2.03% respectively when it is compared with its control.

The study shows a significant decrease with -1.94% and -2.40% per cent values in 1st and 2nd yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. Birker et al (1987) show that the relative density of mustard oil should be 0,9100 - 0,9210g/ml. The density of mustard oil should be greater than that of water. The application of micronutrients and plant growth hormones plays a role in enhancing the quality of mustard oil.

The oil density in mustard seeds is a critical trait influencing mustard oil's quality and commercial value. This trait is determined by a complex interplay of genetic, biochemical, and physiological factors at the cellular level. Understanding these mechanisms provides insight into optimizing oil density and improving mustard crops' seed quality. The biosynthesis of oil in mustard seeds primarily involves the synthesis of fatty acids in the plastids. Acetyl-CoA is carboxylated to form malonyl-CoA, which is then elongated through a series of enzymatic reactions to produce fatty acids. Key enzymes involved in this process include acetyl-CoA carboxylase and fatty acid synthase. Fatty acids are esterified with glycerol to form triacylglycerols (TAGs), the primary storage form of oil in seeds. This process occurs in the endoplasmic reticulum, where enzymes such as diacylglycerol acyltransferase (DGAT) and phospholipid In mature mustard seeds, TAGs are stored in specialized organelles called oil bodies or lipid bodies. A phospholipid monolayer surrounds these oil bodies and contains proteins such as oleosins that stabilize the oil bodies and prevent coalescence. During seed development, the accumulation of oil is tightly regulated. In the early stages, the seeds focus on growth and cell division, while later stages are dedicated to accumulating storage compounds, including oil. Hormonal signals and gene expression changes regulate the transition from cell division to oil accumulation. Several genes are involved in controlling oil content and density in mustard seeds. For instance, genes encoding fatty acid

desaturases, which influence the fatty acid composition of the oil, and transcription factors such as WRINKLED1 (WRI1), which regulate the expression of oil biosynthesis genes, play a role in determining oil density. Nutrient availability, mainly the carbon and nitrogen supply, affects oil biosynthesis. Adequate availability of these nutrients supports optimal oil production. For instance, sulfur is essential for the synthesis of sulfur-containing amino acids, which are components of critical enzymes in oil biosynthesis. Environmental factors such as temperature and light intensity can influence oil accumulation. High temperatures during seed development can increase oil density, while inadequate light can reduce oil accumulation. These factors affect enzyme activities and metabolic pathways involved in oil biosynthesis. High oil density is desirable as it enhances the quality and yield of mustard oil. Mustard oil with higher density is more economically valuable and has better processing efficiency. Additionally, higher oil content in seeds can lead to better oil extraction yields. Mustard oil with higher density often contains more beneficial fatty acids, such as unsaturated fatty acids, essential for human health. Optimizing oil density can, therefore, enhance the nutritional quality of the oil. The oil density in seeds affects their functional properties, including flavour, shelf-life, and stability. Seeds with higher oil density generally produce oil with better flavour profiles and stability during storage. Understanding the cellular mechanisms behind oil density allows targeted breeding programs to improve oil content. Breeders can develop mustard varieties with enhanced oil density by selecting favourable genetic traits and optimizing growth conditions. Higher oil density in mustard seeds can meet market demand for high-quality mustard oil. This can lead to increased profitability for farmers and a competitive edge in the market. The oil density in mustard crops is determined by complex cellular mechanisms involving oil biosynthesis, accumulation, and regulation. Genetic factors, nutrient availability, and environmental conditions are crucial in deciding oil density. Optimizing these factors can improve oil quality and yield, which is essential for agronomic and economic reasons.



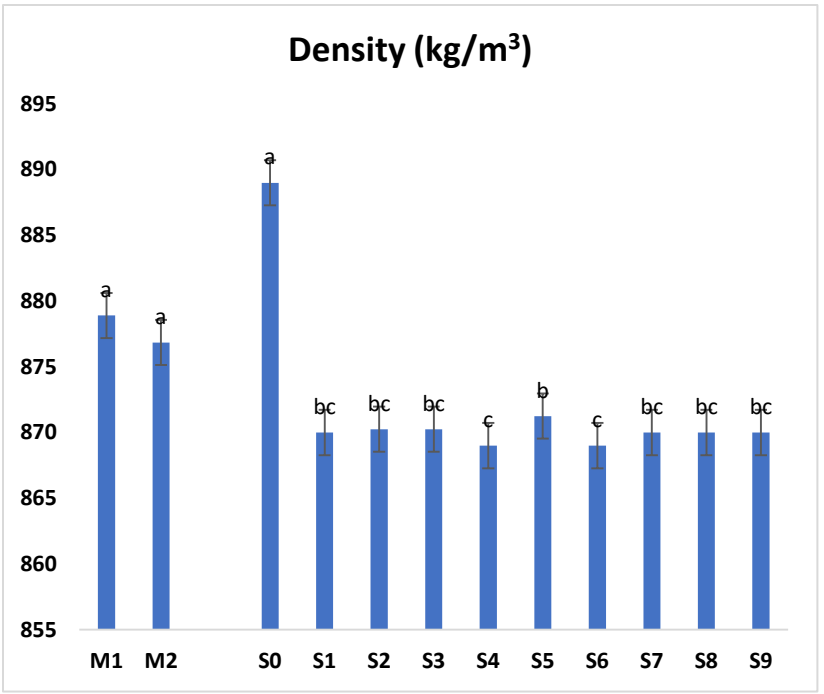


Fig-45 (a): Interaction effect of spacing and nutrients on the density of mustard oil during rabi season of 2021-22

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.5.8 Glucosinolates (µmol/g)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in glucosinolates observed after harvest are shown in Table 4.46, Fig 4.46. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the glucosinolates in each treatment compared to control of both the spacings. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in glucosinolates was observed after harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), the main plot M2 shows higher glucosinolates than M1, with values of 70.63 (M2) and 69.83 (M1), respectively. A percentage increase of 1.13% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S9 with a value of 71.69 where Sulphur @0.25%+ BAP (@0.0015% was applied to the crop as a foliar spray. The per cent increase was found highest in S9, followed by S8> S7> S2> S5> S4> S3> S6> S1, and the per cent values were 3.92%, 3.26%, 2.19%, 2.13%, 2.13%, 1.714%, 1.70%, 1.23% and 0.87% respectively when it is compared with its control (S0).

In the year (2021-22), the main plot M2 shows higher glucosinolates than M1, with values of 71.39(M2) and 70.61 (M1), respectively. A percentage increase of 1.09% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S9 with a value of 72.23 where Sulphur @0.25%+ BAP (@0.0015% was applied to the crop as a foliar spray. The per cent increase was found highest in S9 followed by S8> S2> S7> S5> S6> S3> S4> S1, and the per cent values were 3.54%, 2.82%, 2.41%, 2.39%, 2.04%, 1.49%, 1.36%, 1.26% and 1.19% respectively when it is compared with its control (S0).

The study shows a significant increase with 1.23% and 1.49% per cent values in the 1st and 2nd yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. In 2021-22 and 2022-23, a significant increase in the glucosinolates was found in treatment S9, where an aqueous formulation of Sulphur @0.25%+ BAP (@0.0015% was applied to the crop. Jaiswal et al. (2015) reported that Sulphur is a constituent of glucosinolate, which plays a vital role in the synthesis of mustard oil. The application of S might have favoured the synthesis of CoA and lipoic acid, resulting in increased oil content (Mathew and George 2013). The sulphur application improves glucosinolate content in the mustard crops, enhancing the quality of the mustard crop.

Glucosinolates are sulfur-containing compounds synthesised in the mustard plant (Brassica spp.) through a series of enzymatic reactions. The biosynthesis primarily occurs in the cells of the Brassicaceae family and is localised in specialised tissues such as the leaves, seeds, and stems. The biosynthesis begins with the amino acids' methionine, tryptophan, or phenylalanine, which are converted into corresponding precursor molecules. For instance, methionine is converted into S-methyl-L-cysteine, and tryptophan is converted into indole. The precursor molecules undergo a series of modifications, including side-chain elongation, which involves the addition of sulfur and other functional groups to form the core structure of glucosinolates. Specific transferases and other enzymes then modify the core structure to form the final glucosinolate compounds. These modifications include the addition of glucose molecules and other substituents to the core structure. Glucosinolates are stored in vacuoles within plant cells. They are typically present in a relatively stable and inactive form, which prevents their potential toxicity to the plant. During tissue damage or pathogen attack, glucosinolates are hydrolysed by the enzyme myrosinase. This reaction produces active compounds such as isothiocyanates, thiocyanates, and nitriles, which have various biological activities. The hydrolysis of glucosinolates by myrosinase produces isothiocyanates, which play a crucial role in

the plant's defence mechanism. These compounds are toxic to herbivores and have antimicrobial properties, protecting the plant from damage and infection. The glucosinolate hydrolysis products can also act as signalling molecules, inducing defence responses in neighbouring plants. Additionally, some isothiocyanates have been shown to have detoxifying effects on plant tissues. Glucosinolates and their hydrolysis products, particularly isothiocyanates, possess potent antioxidant properties. These compounds help neutralise free radicals and reduce oxidative stress, which benefits human health. Research has shown that isothiocyanates derived from glucosinolates have potential anti-cancer effects. They can induce apoptosis (programmed cell death) in cancer cells and inhibit tumour growth. Isothiocyanates have been found to possess anti-inflammatory properties, which can reduce chronic inflammation and associated diseases. The presence of glucosinolates in mustard oil contributes to pest resistance. The isothiocyanates produced from glucosinolate hydrolysis effectively fight various insect pests and pathogens, reducing the need for chemical pesticides. Mustard plants are often used in crop rotation systems because they suppress soil-borne pathogens and pests. The glucosinolates released into the soil can have biofumigant properties, helping to improve soil health and fertility. Glucosinolates and their hydrolysis products contribute to mustard oil's characteristic flavour and aroma. This is essential to mustard oil's culinary appeal and use in various cuisines. The health benefits and agricultural uses of glucosinolates enhance the economic value of mustard oil. It is considered a premium oil due to its multifunctional properties, which can be leveraged for higher market value and consumer demand. Glucosinolates in mustard oil play a crucial role in the plant's defence mechanism, contribute to health benefits, and enhance the oil's flavour and aroma. The cellular mechanism of glucosinolate biosynthesis and activation demonstrates their importance in protecting the plant and offering various benefits for human health and agriculture. Understanding these mechanisms and justifications helps us appreciate the multifaceted value of glucosinolates in mustard oil.

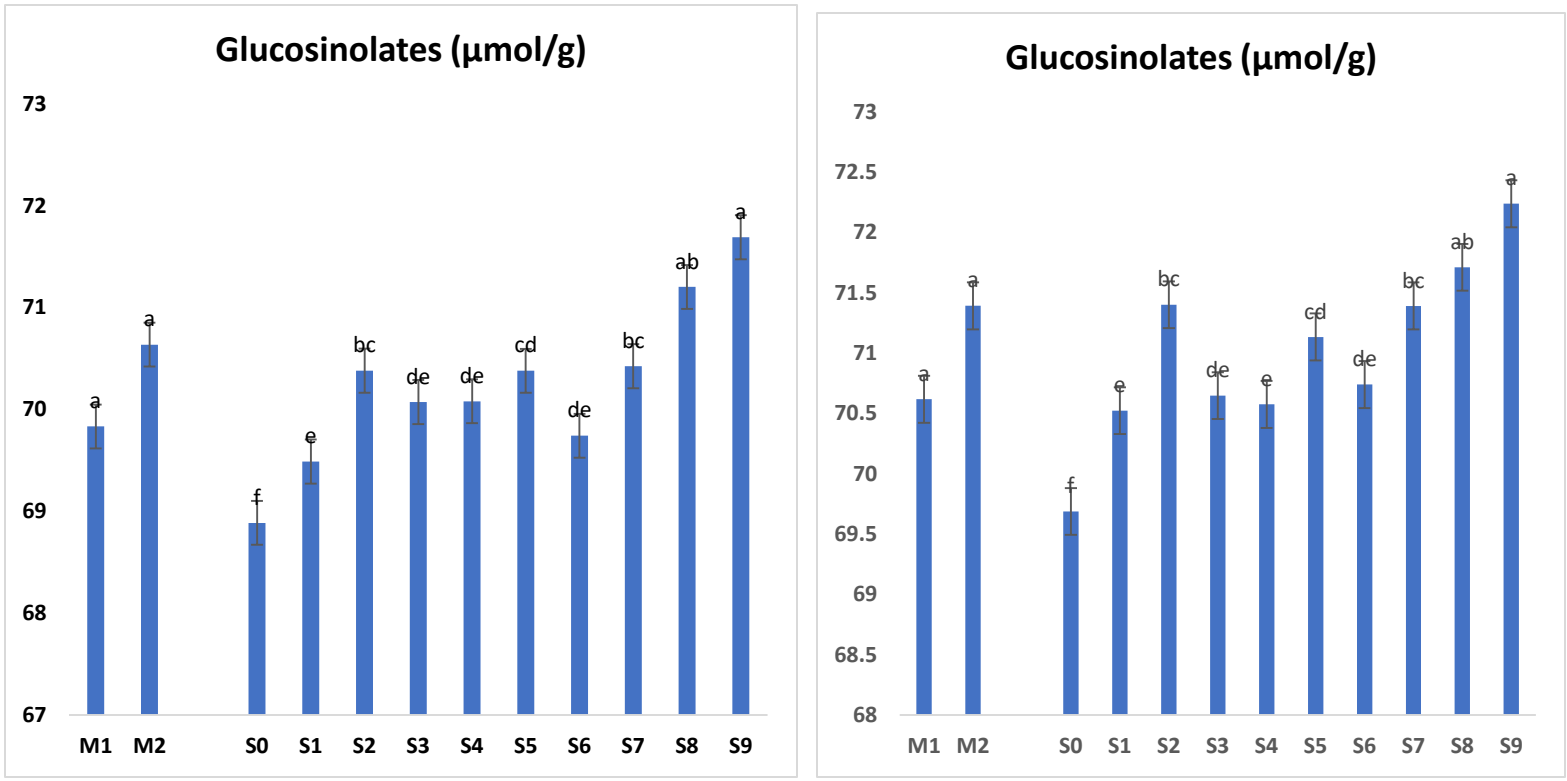


Fig-46 (a): Interaction effect of spacing and nutrient on glucosinolates of mustard oilcake during rabi season of 2021-22

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

4.5.9 Refractive Index (°Brix)

In this experiment, the combined and individual effect of Boron, Sulphur, and Cytokinin nutrients was studied in the NB-RIMUL-2019 (Nandi Bull) variety of mustard crops under two different spacings during the year 2021-22 and 2022-23. Changes in refractive index observed after harvest are shown in Table 4.47, Fig 4.47. During this experiment on the mustard crop, various treatments were applied in different doses at different stages of crop growth. It was found that there is a significant difference in the refractive index in each treatment compared to the control of both the spacings. The percentage increase was calculated by comparing all the treatments with the control and comparing both the spacings together. Thus, the pattern of percentage increase in refractive index was observed after harvest in two years. A significant increase was found by comparing the values of main and sub-treatments. In the year (2021-22), the main plot M2 shows a higher refractive index than M1, with values of 72.12 (M2) and 70.40 (M1), respectively. A percentage increase of 2.38% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S9 with a value of 71.7 where Sulphur @0.25%+ BAP (@0.0015% was applied to the crop as a foliar spray. The per cent increase was found highest in S9 followed by S7> S4> S6> S2= S3= S5 S8> S1, and the per cent values were 0.73%, 0.66%, 0.60%, 0.14%, 0.11%, 0.11%, 0.11%, 0.11%, 0.07% respectively when it is compared with its control (S0).

In the year (2021-22), the main plot M2 shows a higher refractive index than M1, with values of 72.38 (M2) and 71.33 (M1), respectively. A percentage increase of 1.45% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S9 with value 72, where Sulphur @0.25%+ BAP (@0.0015% was applied to the crop as a foliar spray. The per cent increase was found highest in S9 followed by S6=S3=S1> S2> S8> S7> S4> S1, and the per cent values were 0.83%, 0.72%, 0.72%, 0.72%, 0.69%, 0.66%, 0.62%, 0.59% and 0.72% respectively when it is compared with its control (S0).

The study shows a significant increase with 0.14% and 0.72% per cent values in the 1st and 2nd yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. In 2021-22 and 2022-23, a significant increase in the refractive index was found in treatment S9 where the application of aqueous formulation of Sulphur @0.25%+ BAP (@0.0015% was applied to the crop. The refractive index is used to detect rancidity in edible oil. Mustard oil should be between 1.4646 and 1.4662 (AOCS, 1993). However, in this study, we used a hand and a digital refractometer to measure the oil's refractive index; the values observed are shown in the table below. From this study, no significant difference was found in the refractive index. The Refractive index is almost similar in all treatments compared to the control.

Like any other substance, the refractive index (RI) of mustard oil measures how much the substance bends light as it passes through it. The molecular and cellular composition of the oil influences this optical property. Here's a detailed explanation of the cellular mechanisms and factors affecting the refractive index of mustard oil. Mustard oil primarily comprises triglycerides, esters formed from glycerol and fatty acids. The specific types and ratios of fatty acids (e.g., oleic acid, linoleic acid, erucic acid) influence the oil's density and refractive index. The presence of different fatty acids affects the oil's molecular structure and intermolecular interactions, affecting its refractive index. Fatty acids with varying saturation and chain length degrees contribute to the oil's overall optical properties. Minor components such as free fatty acids, phospholipids, and other impurities can also impact the refractive index. These components can alter the oil's molecular arrangement and affect light transmission. The type of intermolecular forces in the oil influences the refractive index. For instance, hydrogen bonding or van der Waals forces between triglyceride molecules can affect the refraction of light. The ability of the molecules in mustard oil to polarise in response to an electric field (or light) affects the oil’s refractive index. Molecules with higher polarizability tend to have higher refractive indices. The

density of mustard oil, determined by its molecular mass and structure, influences the refractive index. A higher density generally corresponds to a higher refractive index. How molecules are packed in the oil (e.g., crystalline or amorphous) affects light interaction. In liquid oils, the molecules are usually more disordered, leading to different refractive properties compared to solid fats. The refractive index of mustard oil is also temperature-dependent. As temperature increases, the oil's density decreases, leading to a decrease in the refractive index. The molecular movement becomes more pronounced at higher temperatures, which affects light bending. The refractive index is a critical parameter in quality control for edible oils. It helps identify the purity and authenticity of mustard oil. Deviations from the expected refractive index can indicate adulteration or the presence of impurities. The refractive index provides insights into mustard oil's molecular composition and structure. Understanding the refractive index helps characterise the oil's physical properties and behaviour under different conditions. Knowledge of the refractive index helps optimise the processing and formulation of mustard oil-based products. It can guide adjustments in extraction, refining, and blending processes to achieve desired properties. In scientific studies, the refractive index of mustard oil can be used to investigate interactions between oil and other substances. It is valuable in oil chemistry, food science, and material science studies. Measurement of the refractive index is often required to ensure that mustard oil products meet regulatory standards. Accurate and consistent refractive index values are essential for compliance with food safety regulations. The refractive index of mustard oil is determined by its molecular composition, molecular interactions, and density, among other factors. Understanding these mechanisms is crucial for various applications, including quality control, characterisation, and scientific research.

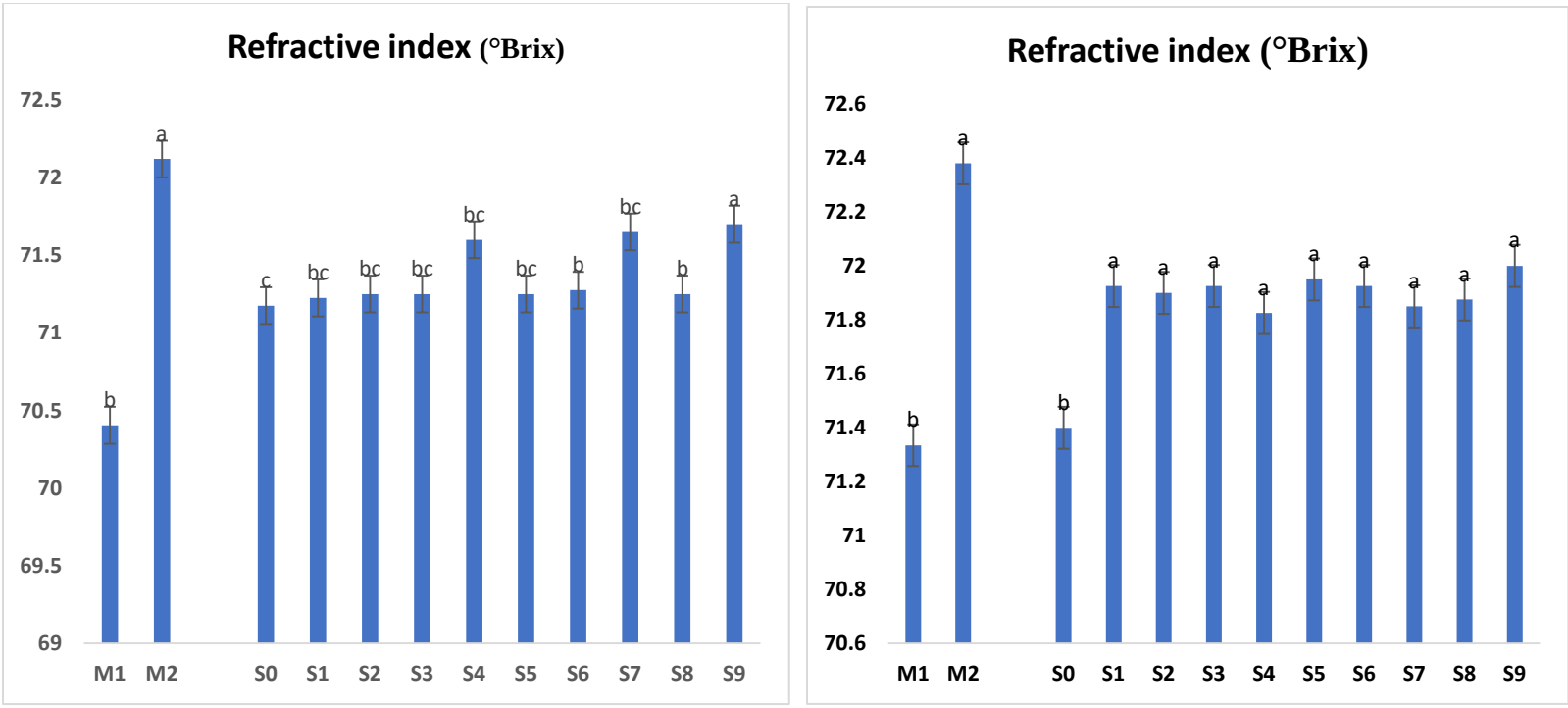


Fig-47 (a): Interaction effect of spacing and nutrients on the refractive index of mustard oil during the rabi season of 2021-22

Where M1 represents-30*10 (spacing) and M2represents 20*10 (spacing) whereas Sub plots shows- S_0 : Control, S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

Table-38 (a): Effect of spacing and nutrient on density, refractive index and glucosinolates of mustard oil during rabi season of 2021-22 and 2022-23

Treatments	Density		Glucosinolates		Refractive index	
	2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
M1 (30×10)	878.40	878.90	69.83	70.62	70.41	71.34
M2 (20×10)	877.70	876.85	70.64	71.39	72.12	72.38
C.D. at p<0.05	NS	NS	NS	NS	NS	NS
SE(m)	0.49	0.17	0.14	0.14	0.14	0.62
Nutrients foliar spray						
S0-Control	889.50	889.00	68.89	69.69	71.18	71.40
S1-Boron @1%	869.25	870.00	69.49	70.52	71.23	71.93
S2-Sulphur @ 0.15%	869.25	870.25	70.38	71.40	71.25	71.90
S3-BAP @0.003%	869.25	870.25	70.07	70.65	71.25	71.93
S4-Boron @0.5% +Sulphur @0.25%	870.75	869.00	70.08	70.58	71.60	71.83
S5-Boron @ 1.5%+ Sulphur @0.075%	873.25	871.25	70.38	71.13	71.25	71.95
S6-Boron @ 0.5% + BAP (@0.0045%)	872.50	869.00	69.74	70.74	71.28	71.93
S7-Boron @ 1.5%+ BAP (@0.0015%)	869.25	870.00	70.42	71.39	71.65	71.85
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	869.25	870.00	71.20	71.71	71.25	71.88
S9-Sulphur @0.25%+ BAP (@0.0015%)	869.25	870.00	71.69	72.24	71.70	72.00
C.D. at p<0.05	2.52	1.78	0.58	0.55	0.28	0.29
SEM±	0.84	0.59	0.19	0.18	0.11	0.09
C.D. S×M at p<0.05	6.44	0.55	NS	NS	NS	NS
SEM±	1.56	3.11	0.44	0.46	0.46	1.97
C.D. M×S at p<0.05	6.66	0.82	NS	NS	NS	NS
SEM±	1.23	3.02	0.29	0.29	0.21	0.63

Where, C.D. represents critical difference, SE (m) represents standard error of mean

Table 4.38 (b): Interaction effect of spacing and nutrient on Density of mustard oil during rabi season 2021-22 and 2022-23.

2021-22											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	953	869.5	869	869	868.5	869	877.5	869.5	869.5	869.5	878.4
M2	944	869	869.5	869.5	873	877.5	867.5	869	869	869	877.7
Mean B	948.5	869.25	869.25	869.25	870.75	873.25	872.5	869.25	869.25	869.25	
C.D. S×M at p<0.05						6.44					
SEM±						1.56					
C.D. M×S at p<0.05						6.66					
SEM±						1.23					

2022-23											
	S0	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**Mean A**
M1	954	870.5	870	870	869.5	874	869.5	870.5	870.5	870.5	878.9
M2	944	869.5	870.5	870.5	868.5	868.5	868.5	869.5	869.5	869.5	876.85
Mean B	949	870	870.25	870.25	869	871.25	869	870	870	870	
C.D. S×M at p<0.05						0.55					
SEM±						3.11					
C.D. M×S at p<0.05						0.82					
SEM±						3.02					

4.6 Economic analysis:

COST OF CULTIVATION (FIXED COST)

S. No.	Operation		Quantity/Duration	Cost per quantity/hour	Total
1	Land preparation	Tractor cost	3hr	500	1500
2	Layout preparation		4 labours	400 per day	1600
3	Seed		5kg/ha	500/kg	2500
4	Sowing and fertilizer application		4 labours	400 per day	1600
5	Fertilizer				
	Nitrogen	Urea	90kg	268per 50kg/bag	485
	Phosphorus	DAP	40kg	362 per 50kg/bag	290
	Potassium	MOP	40kg	872 per 50kg/bag	700
6	Labour for split dose		2 splits*1 labours per split (4)	400 per day	400
7	Intercultural operations				
	Hand weeding		4 labours	400 per day	1600
	Spraying	15 DAS	1 labour	400 per day	400
		45DAS	1 labour	400 per day	400
		75DAS	1 labour	400 per day	400
		115DAS	1 labour	400 per day	400
8	Plant protection chemicals	Insecticide	Thiomethoxam (50g/ha)	3000rupees/kg	150
		Fungicide	Metalaxyl+Mancozeb (620g/ha)	2000rupees/kg	1240
9	Irrigation for cropping season		3	1000 per time	3000
10	Harvesting, threshing and winnowing		10 labours*2days	400 per day	8000
11	Land lease and miscellaneous for cropping season				2000
12	**Total**				**26665**

COST OF CULTIVATION (VARIABLE COST)

S. No.	Treatment combination	Boron	Sulphur	BAP	Total cost
1	M1S0	0	0	0	0
2	M1S1	300	0	0	300
3	M1S2	0	1200	0	1200
4	M1S3	0	0	600	600
5	M1S4	150	1800	0	1950
6	M1S5	450	600	0	1050
7	M1S6	150	0	900	1050
8	M1S7	450	0	300	750
9	M1S8	0	600	900	1500
10	M1S9	0	1800	300	2100
11	M2S0	0	0	0	0
12	M2S1	300	0	0	300
13	M2S2	0	1200	0	1200
14	M2S3	0	0	600	600
15	M2S4	150	1800	0	1950
16	M2S5	450	600	0	1050
17	M2S6	150	0	900	1050
18	M2S7	450	0	300	750
19	M2S8	0	600	900	1500
20	M2S9	0	1800	300	2100

TOTAL COST (FIXED+VARIABLE)

S.No.	Treatment combination	Fixed cost	Variable cost	Total cost
1	M1S0	26665	0	26665
2	M1S1	26665	300	26965
3	M1S2	26665	1200	27865
4	M1S3	26665	600	27265
5	M1S4	26665	1950	28615
6	M1S5	26665	1050	27715
7	M1S6	26665	1050	27715
8	M1S7	26665	750	27415
9	M1S8	26665	1500	28165
10	M1S9	26665	2100	28765
11	M2S0	26665	0	26665
12	M2S1	26665	300	26965
13	M2S2	26665	1200	27865
14	M2S3	26665	600	27265
15	M2S4	26665	1950	28615
16	M2S5	26665	1050	27715
17	M2S6	26665	1050	27715
18	M2S7	26665	750	27415
19	M2S8	26665	1500	28165
20	M2S9	26665	2100	28765

Gross return= Mustard yield*MP of mustard (Rs. 4650 in 2021-22 and Rs. 5450 in 2022-23, average taken Rs. 5050 for both years)

1. Cost of Cultivation

The effect of Boron, Sulphur and cytokinin individual and their combinations on the cost of cultivation in Indian mustard at harvest is shown in (Table 4.39.). For calculating cost of cultivation, we have taken average of both the years. So, there was a significant difference in the cost of cultivation of Indian mustard. The highest cost of cultivation was found in treatment S9, i.e. Rs. 28765 ha^{-1}, where, Sulphur @ 0.25%+ BAP (@0.0015%) was applied in the plot. Total cost of cultivation in treatments S0, S1, S2, S3, S4, S5, S6, S7, S8 and S9 was Rs. 26665, 26965, 27865, 27265, 28615, 27715, 27715, 27415, 28165 and 28765 respectively.

2. Gross return

The effect of Boron, Sulphur and cytokinin individual and their combinations on gross return in Indian mustard at harvest is shown in (Table 4.39.). In 2022 and 2023 there was significant difference in gross return in Indian mustard. In 2022, in main plots, the highest gross return was found in M2, i.e. Rs. 124398 ha^{-1} where, mustard crop is grown at reduced spacing (20*10). In sub plots, the highest gross return was found in treatment S8 i.e. Rs. 132141.67 ha^{-1}, where, (Sulphur@0.075%+BAP@0.0045%) was applied to the crop. Total gross return in treatments S0, S1, S2, S3, S4, S5, S6, S7, S8 and S9 was Rs. 98475, 120358, 122883, 125408, 121200, 116150, 126250, 130458, 132141 and 127933 respectively. In 2023, in main plots, the highest gross return was found in M2, i.e. Rs. 130249 ha^{-1} where, mustard crop is grown at reduced spacing (20*10). In sub plots, the highest gross return was found in treatment S8 i.e. Rs. 136350 ha^{-1}, where, (Sulphur@0.075%+BAP@0.0045%) was applied to the crop. Total gross return in treatments S0, S1, S2, S3, S4, S5, S6, S7, S8 and S9 was Rs. 105267, 125442, 130744, 132486, 126275, 126250, 134001, 134052, 136350 and 133092 respectively.

3. Net return

The effect of Boron, Sulphur and cytokinin individual and their combinations on Net return in Indian mustard at harvest is shown in (Table 4.39.). In 2022 and 2023 there was significant difference in Net return in Indian mustard. In 2022, in main plots, the highest Net return was found in M2 i.e., Rs. 96683 ha^{-1} where, mustard crop is grown at reduced spacing (20*10). In sub plots, the higher net return was found in treatment S8 i.e. Rs. 103976.67 ha^{-1}, where, (Sulphur@0.075%+BAP@0.0045%) was applied to the crop. Total gross return in treatments S0, S1, S2, S3, S4, S5, S6, S7, S8 and S9 was Rs.71810, 93393, 95018, 98143, 92585, 88435, 98535, 103043, 103976 and 99168 respectively. In 2023, in main plots, the highest net return was found in M2, i.e. Rs. 102534.60 ha^{-1} where, mustard crop is grown at reduced spacing (20*10). In sub plots, the highest gross return was found in treatment S8 i.e. Rs. 108185 ha^{-1}, where,

(Sulphur@0.075%+BAP@0.0045%) was applied to the crop. Total net return in treatments S0, S1, S2, S3, S4, S5, S6, S7, S8 and S9 was Rs. 78602, 98477, 102879, 105221, 97660, 98535, 106286, 106637, 108185 and 104327 respectively.

4. B:C

The effect of Boron, Sulphur and cytokinin individual and their combinations on B:C ratio in Indian mustard at harvest is shown in (Table 4.39.). In 2022 and 2023 there was significant difference in B:C ratio in Indian mustard. In 2022, in main plots, the highest B:C ratio was found in M2 i.e., 3.49 where, mustard crop is grown at reduced spacing (20*10). In sub plots, the higher B:C was found in treatment S7 i.e. 3.76, where, (Boron@1.5%+BAP@0.0015%) was applied to the crop. Total B:C in treatments S0, S1, S2, S3, S4, S5, S6, S7, S8 and S9 was 2.69, 3.46, 3.41, 3.60, 3.24, 3.19, 3.56, 3.76, 3.69 and 3.45 respectively. In 2023, in main plots, the highest net return was found in M2, i.e. 3.70 where, mustard crop is grown at reduced spacing (20*10). In sub plots, the highest B:C was found in treatment S7 i.e. 3.89, where, (Boron@1.5%+BAP@0.0015%) was applied to the crop. Total B:C in treatments S0, S1, S2, S3, S4, S5, S6, S7, S8 and S9 was2.95, 3.65, 3.69, 3.86, 3.41, 3.56, 3.83, 3.89, 3.84 and 3.63 respectively.

Table-4.39: Economic analysis

Treatments	Cost of cultivation (Rs. ha^{-1}) 2021-22 & 2022-23	Gross return (Rs. ha^{-1})		Net return (Rs. ha^{-1})		B:C	
		2021-22	2022-23	2021-22	2022-23	2021-22	2022-23
Spacing							
M1 (30×10)	27715.00	119853.33	126542.90	92138.33	98827.90	3.32	3.56
M2 (20×10)	27925.00	124398.33	130249.60	96683.33	102534.60	3.49	3.70
Nutrients foliar application							
S0-Control	26665.00	98475.00	105267.25	71810.00	78602.25	2.69	2.95
S1-Boron @1%	26965.00	120358.33	125442.00	93393.33	98477.00	3.46	3.65
S2-Sulphur @ 0.15%	27865.00	122883.33	130744.50	95018.33	102879.50	3.41	3.69
S3-BAP @0.003%	27265.00	125408.33	132486.75	98143.33	105221.75	3.60	3.86
S4-Boron @0.5% +Sulphur @0.25%	28615.00	121200.00	126275.25	92585.00	97660.25	3.24	3.41
S5-Boron @ 1.5%+ Sulphur @0.075%	27715.00	116150.00	126250.00	88435.00	98535.00	3.19	3.56
S6-Boron @ 0.5% + BAP (@0.0045%)	27715.00	126250.00	134001.75	98535.00	106286.75	3.56	3.83
S7-Boron @ 1.5%+ BAP (@0.0015%)	27415.00	130458.33	134052.25	103043.33	106637.25	3.76	3.89
S8-Sulphur @ 0.075%+ BAP (@0.0045%)	28165.00	132141.67	136350.00	103976.67	108185.00	3.69	3.84
S9-Sulphur @0.25%+ BAP (@0.0015%)	28765.00	127933.33	133092.75	99168.33	104327.75	3.45	3.63

CHAPTER 5

SUMMARY AND CONCLUSION

The present research work entitled **"IMPACT OF BORON, SULPHUR, AND CYTOKININ IN INDIAN MUSTARD (*Brassica juncea* L.) UNDER SPATIAL DYNAMICS"** is conducted during the *Rabi* season of the year 2021-22 ad 2022-23 at Lovely Professional University field, Jalandhar, Punjab.

Mustard is a vital oilseed crop cultivated for its seeds, which are used to produce mustard oil, a popular cooking oil, and as a spice in various cuisines. It is a crop grown during the Rabi season in a temperate climate. As a cold-growing crop, it can also be grown in tropical or subtropical climates. A mustard crop may assimilate the most CO_2 at warmer temperatures and exhibit an excellent photosynthetic response. It is a crop for the fantastic season that grows well in regions that receive rain. Because mustard leaves and stems are a good source of nutrients and minerals, they can be fed to cattle. The young plants' leaves, which can be consumed as a leafy vegetable in the human diet, are abundant in sulphur and other mineral nutrition. Mustard can be grown as a trap crop for various insect pests. In addition to being high in protein and essential elements like calcium and omega-3 fatty acids, mustard seeds have a 30–40% oil content. In addition to being used for oil production, mustard greens are a popular leafy food and are prized for improving soil when used as a cover crop. Mustard is a significant and adaptable crop worldwide due to its versatility and concise growing cycle. These days, beekeeping activities are more suited to the mustard crop. Because mustard oil offers a variety of quality characteristics, it may be utilised for a wide range of industrial applications. Mustard is a common oilseed crop that exhibits a high demand for fertilizers, such as boron and sulphur. This is so because the production of sulphur amino acids and glucosinolates requires both B and S. Both nutrients are well-received by oilseed rape, according to several study reports; however, the B and S fertilisation strategy has the reverse effect. The most effective method to provide B to plants is soil amendment; seed and

band placement may be detrimental, and foliar treatment can be highly efficient in delivering B, particularly if deficiencies are identified during the growth season. Chlorophyll levels, crop yield, specific physiological indicators, and plant biometric or yield features are significantly correlated. The effects of B, S, and cytokinin are investigated here to determine the growth and yield of the mustard crop.

The present study was carried out to estimate the Impact of B, S, and BAP on the growth and yield-attributing characteristics of mustard crop variety NB-RIMUL-2019 (Nandi Bull) at 30, 60, 90, and 120 DAS under spatial dynamics.

The experiment was laid out using the Split Plot Design. The total area required for the experiment was approx. 1200 $m^{2.}$ The experiment was conducted with 20 treatments and three replications; thus, the total number of plots was 60. All the treatments were arranged with randomisation (unbiased) in the plots. Each subplot size was 5m × 3 m = 15 sq. m. The exogenous application of B, S, and Cyt. was applied by selecting the best concentration in earlier studies. The concentrations applied were B @1%, S @0.15%, and BAP@0.003%as a foliar spray for fifteen days after sowing. The various observations were taken at four stages, such as 30 DAS, 60 DAS, 90 DAS, and 120 DAS in all the treatments. The detailed plan of treatments are:- Main plots: M1-30*10 (spacing) and M2-20*10 (spacing)

Sub plots are:- S_0 : Control (30*10), S1: Boron @1%, S2: Sulphur @ 0.15%, S3: BAP @0.003%, S4: Boron @0.5% +Sulphur @0.25%, S5: Boron @ 1.5%+ Sulphur @0.075%, S6: Boron @ 0.5% + BAP (@0.0045%, S7: Boron @ 1.5%+ BAP (@0.0015%, S8: Sulphur @ 0.075%+ BAP (@0.0045%, S9: Sulphur @0.25%+ BAP (@0.0015%)

S10: Control (20*10), S11: Boron @1%, S12: Sulphur @ 0.15%, S13: BAP @0.003%, S14: Boron @0.5% +Sulphur @0.25%, S15: Boron @ 1.5%+ Sulphur @0.075%, S16: Boron @ 0.5%

+ BAP (@0.0045%, S17: Boron @ 1.5%+ BAP (@0.0015%, S18: Sulphur @ 0.075%+ BAP (@0.0045%, S19: Sulphur @0.25%+ BAP (@0.0015%)

Essential nutrients to the crop were applied at the time of sowing and as a top dressing. 2-3 weeding was carried out, and two irrigations were provided for good growth and production. The salient findings of the investigation are summarised below:

1. The study provides evidence based on observations that the main plot M2 shows better plant height than M1, with per cent values of 3.87%, 10.89%, 1.92%, and 1.64% at 30, 60, 90, and 120DAS, respectively.

2. The highest number of leaves per plant was observed in main plot M2 at different day intervals, with 5.53%, 0.77%, and 0.66% increases compared to M1.

3. It is evident that main plot M1 shows the maximum leaf area compared to M2, with 9.77%, 1.78%, 1.41%, and 1.64% increases at 30, 60, 90, and 120DAS, respectively.

4. Main plot M1 shows the maximum stem diameter compared to M2, with a per cent increase of 28.26%, 11.62%, 3.35%, and 3.89% at 30, 60, 90, and 120DAS, respectively. In subplots, significant results were observed in S6, where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray.

5. Study shows that main plot M2 shows a maximum leaf area index compared to M1 with a per cent increase of 31.25%, 31.89%, 31.88% and 34.25% at 30, 60, 90 and 120DAS, respectively. In subplots, significant results were observed in S4 where Boron @0.5% + sulphur @0.25% was applied to the crop as a foliar spray compared to its control.

6. 6. The result observed is that main plot M1 shows maximum NAR compared to M2, with values of 7.40%, 6.89%, and 6.89% at 30-60DAS and 60-90DAS, respectively.

Treatment S6 shows a maximum increase, where the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop.

7. A significant increase in the relative growth rate was observed in main plot M1 as compared to M2, with a per cent increase of 7.40%, 6.89%, and 6.89% at 30-60DAS and 60-90DAS, respectively.

8. Based on the observations, main plot M1 shows the maximum crop growth rate compared to M2, with per cent values of 1.63%, 5.88%, and 60-90DAS, respectively.

9. The significant increase in chlorophyll 'a' was observed in main plot M1 as compared to M2 with percent values 4.42%, 0.68%, and 11.29% at 30, 60 and 90DAS. The study showed a significant increase with 35.82%, 1.38% and 17.77% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S8 and S0 (control). In treatment S8, the foliar application of Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the mustard crop.

10. It is observed that in chlorophyll 'b', the percentage increase of 10.58%, 3.38% and 16.27% was found at 30, 60 and 90DAS, respectively, in M1, where the crop was grown in spacing (30*10). Treatment S8 showed better results when the foliar application of Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the mustard crop.

11. A significant increase in chlorophyll 'a:b' was observed in main plot M2 compared to M1 with a per cent increase of 6.94%, 7.74% and 3.56% at 30, 60 and 90DAS, respectively.

12. It is observed that main plot M2 shows maximum chlorophyll 'a:b' content as compared to M1 with a per cent increase of 0.49% and 3.56% at 60 and 90DAS, respectively. In subplots, significant results were observed in S6, where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray.

13. It is evident that the main plot M2 shows maximum chlorophyll ‘a+b’ as compared to M1 with values 2.03 (M2) and 2.02 (M1), respectively. A percentage increase of 0.49% was found in M2, where the crop was grown in reduced spacing (20*10).

14. The main plot M1 shows maximum carotenoids compared to M2, with a percent increase of 13.92%, 15.18%, and 17.14% at 30, 60, and 90DAS, respectively. In the subplots, a significant increase was found in S2, with a value of 3.84, where sulphur @ 0.15% was applied to the crop as a foliar application.

15. The study showed that main plot M2 shows the maximum chlorophyll index compared to M1, with a percent increase of 10.21%, 2.80%, and 1.37% at 30, 60, and 90DAS, respectively. In subplots, significant results were observed in S6, with a value of 45.48, where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar spray.

16. It has been observed that the main plot M2 shows maximum total soluble sugars (TSS) as compared to M1, with values of 1.43 (M2) and 1.15 (M1), respectively. A percentage increase of 19.58% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total soluble sugars (TSS) was observed in S9, i.e. 1.63 at 30DAS, where in S9, Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop.

17. M2 shows maximum total starch compared to M1, with a per cent increase of 4.86%, 2.70%, and 2.0% at 30, 60, and 90DAS, respectively. At 60DAS and 90DAS, the total starch content was significantly increased in treatment S5, where the combined application of boron and sulphur was applied to the crop. The aqueous application of Boron @ 1.5%+ Sulphur @0.075% is applied to the crop compared to its control (S0).

18. The study showed that the main plot M2 shows the maximum total soluble protein (TSP) compared to M1, with a percentage increase of 1.49%, 4.28%, and 1.65% at 30, 60, and

90DAS, respectively, in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total soluble protein (TSP) was observed in S8, i.e. 53.87 at 30DAS, where in S8, Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop.

19. The observations show that the main plot M2 shows maximum total phenols compared to M1, with a percentage increase of 0.92%, 0.24%, and 1.24% at 30, 60, and 90DAS, respectively, in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total phenols was observed in S9, where sulphur @0.25%+ BAP (@0.0015%) was applied to the crop in S9.

20. The main plot M2 shows the maximum total Flavonols compared to M1, with a percentage increase of 6.36%, 8.77%, and 12.03% at 30, 60, and 90DAS, respectively, found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total Flavonols was observed in S6, i.e. 7.20 at 30DAS, whereas in S6, Boron @ 0.5% + BAP (@0.0045%) was applied to the crop.

21. A significant increase in total Flavonoids was found in main plot M2 as compared to M1, with percentage increases of 7.23%, 8.27%, and 0.26% at 30, 60, and 90DAS, respectively, in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in total Flavonoids was observed in S7, i.e. 15.06 at 30DAS, where in S7, Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop.

22. The study shows a significant increase, with 11.29%, 8.65%, and 13.77% per cent values at 30DAS, 60DAS, and 90DAS when comparing S6 and S0 (control). In treatment S6, the mustard crop was treated with a foliar application of Boron @ 0.5% + BAP (@0.0045%).

23. From the experiment, the main plot M2 shows maximum amino acid content compared to M1, with a percentage increase of 7.69% and 20% at 60 and 90DAS found in M2, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase was found in S6, where Boron @ 0.5% + BAP (@0.0045%) was applied to the crop as a foliar application.

24. It is observed from the study that the main plot M1 shows maximum ascorbic acid content as compared to M2, with percentage increases of 4.54%, 8% and 2.22% found in M1, where the crop was grown in spacing (30*10). In subplots, a significant increase in ascorbic acid content was observed in S4, where in S4, Boron @0.5% +Sulphur @0.25% was applied to the crop.

25. Evidence-based observations show that the main plot M2 shows maximum RWC as compared to M1, with a percentage increase of 2.34%, 2.96%, and 2.31% at 30, 60, and 90DAS, respectively, found in M1, where the crop was grown in reduced spacing (20*10). In subplots, a significant increase in RWC was observed in S7, where Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop in S7.

26. The study shows a significant increase with 21.69%, 15.03% and 1.17% per cent values at 30DAS, 60DAS and 90DAS when a comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop.

27. The experiment shows a significant increase, with 32.17%, 46.55%, and 1.44% per cent values at 30DAS, 60DAS, and 90DAS compared to S0 (control). In treatment S6, the mustard crop was treated with a foliar application of Boron @ 0.5% + BAP (@0.0045%).

28. The main plot M2 shows maximum MSI compared to M1, with a percentage increase of 1.73% and 5.78% at 60 and 90DAS, respectively, found in M1, where the crop was

grown in reduced spacing (20*10). In subplots, a significant increase in membrane stability index was observed in S7, i.e. 33.58 at 60DAS, whereas in S7, Boron @ 1.5%+ BAP (@0.0015%) was applied to the crop.

29. The study shows a significant increase, with 5.94% and 5.82% cent values at 90DAS and 120DAS when comparing S6 and S0 (control). In treatment S6, the mustard crop was treated with a foliar application of Boron @ 0.5% + BAP (@0.0045%).

30. The main plot M2 shows the maximum number of secondary branches per plant compared to M1, with a percentage increase of 11.89% and 7.27% at 90 and 120 DAS, respectively, in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S8, where Sulphur @ 0.075%+ BAP (@0.0045%) was applied to the crop as a foliar spray.

31. The study shows a significant increase, with 45.35% and 44.34% cent values at 90DAS and 120DAS when comparing S6 and S0 (control). In treatment S6, the mustard crop was treated with a foliar application of Boron @ 0.5% + BAP (@0.0045%).

32. The experiment shows a significant increase, with 18.00% and 17.22% cent values at 90DAS and 120DAS compared to S0 (control). In treatment S6, the mustard crop was treated with a foliar application of Boron @ 0.5% + BAP (@0.0045%).

33. The main plot M2 shows a maximum number of seeds per siliqua compared to M1, with a percentage increase of 3.39% and 2.74% at 90 and 120DAS, respectively, found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S8, where Sulphur @0.075%+ BAP (@0.0045%) was applied to the crop as a foliar spray.

34. The study shows a significant increase, with 5.15% cent values in both years when comparing S6 and S0 (control). In treatment S6, the mustard crop was treated with a foliar application of Boron @ 0.5% + BAP (@0.0045%).

35. The study shows a significant increase in economic yield, with 23.74% and 24.29%; biological yield, with 29.68% and 29.66%; and stover yield, with 34.62% and 34.12% per cent values in the first and second years, respectively.

36. From the study, it is observed that the main plot M2 shows higher oil content as compared to M1 with a percentage increase of 1.25%, 0.58% in 1^{st} and 2^{nd} yr. Respectively, it was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S9 with a value of 27.84%, where Sulphur @0.25%+ BAP (@0.0015%) was applied to the crop as a foliar spray.

37. The study shows a significant increase, with 6.19% and 7.42% per cent values in the first and second years, respectively, when comparisons were made between S6 and S0 (control). In treatment S6, the mustard crop was treated with a foliar application of Boron @ 0.5% + BAP (@0.0045%).

38. The study shows a significant decrease with -36.73% and -23.71% per cent values in 1^{st} and 2^{nd} yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. Main plot M1 shows a higher peroxide value than M2, with a percentage increase of 3.50% in M1, where the crop was grown in spacing (30*10). Significant results were observed in S1 with a value of 2.55 in subplots where Boron @1% was applied to the crop as a foliar spray. The study shows a significant increase with 35.52% and 26.56% per cent values in 1^{st} and 2^{nd} yr. respectively when comparison

was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop.

39. It is evidence based on the observations that the study shows a significant increase with 11.97% and 12.05% per cent values in 1st and 2nd yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop. Main plot M2 shows a higher saponification value than M1, with values of 3.48 (M2) and 3.44 (M1), respectively. A percentage increase of 1.14% was found in M2, where the crop was grown in reduced spacing (20*10). The study shows a significant increase in botox value with 45.02% and 43.15% per cent values in 1st and 2nd yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop.

40. In oil density, main plot M1 shows higher density than M2 with values 878.4 (M1) and 877.7 (M2), respectively. A percentage increase of 0.07% was found in M1, where the crop was grown in spacing (30*10). In glucosinolate content, M2 shows higher glucosinolates than M1, with values of 70.63 (M2) and 69.83 (M1), respectively. A percentage increase of 1.13% was found in M2, where the crop was grown in reduced spacing (20*10). In subplots, significant results were observed in S9 with a value of 71.69 where Sulphur @0.25%+ BAP (@0.0015% was applied to the crop as a foliar spray. The study shows a significant increase with 0.14% and 0.72% per cent values in the 1st and 2nd yr. respectively when comparison was made between S6 and S0 (control). In treatment S6, the foliar application of Boron @ 0.5% + BAP (@0.0045%) was applied to the mustard crop.

CONCLUSION

The results of the study conducted at the Agronomy Research Farm of Lovely Professional University, Phagwara (India), revealed that the combined application of boron, sulphur, and cytokinin significantly improved the growth, physiology, yield attributes, yield, and quality of the mustard crop grown under spatial dynamics. The mustard crop is a widely used oilseed crop showing an excellent need for fertilizers like sulfur and boron. The application of plant growth hormone-like cytokinin enhances the growth and yield production of the mustard crop.

- Boron, Sulphur and BAP treatments positively influence the growth, yield, and quality of Indian mustard in reduced spacing grown under open field conditions. The study provides evidence based on observations that the morphological parameters like plant height and no. of leaves show better results where Boron @0.5% + Sulphur @0.25% was applied to the crop in reduced spacing. Similarly, Leaf area and stem diameter show better results when grown in recommended spacing. The treatment with reduced spacing (20*10) shows a maximum number of primary and secondary branches, no. of siliquae, no. of seeds per siliqua compared to recommended spacing. Significant results were observed in subplots where Sulphur @0.025%+ BAP (@0.0015%) was applied to the crop as a foliar spray. The study shows a substantial increase in economic yield, with 23.74% and 24.29%; biological yield, with 29.68% and 29.66%; and stover yield, with 34.62% and 34.12% per cent values in the first and second years, respectively. When it comes to the quality parameters, higher oil content and oil quality parameters like acid value, peroxide value, saponification value, iodine value, and botox value were found in the treatments grown under reduced spacing where the combined application of sulphur and cytokinin was applied to the crop as a foliar spray at different days interval.
- The application of boron, sulfur, and BAP treatments positively influences the physiological parameters of Indian mustard in reduced spacing when grown under open field conditions. The

study shows that the main plot with reduced spacing shows a maximum leaf area index, NAR, RGR, and CGR compared to the recommended one. Significant results were observed in treatment where Boron @0.5% + sulphur @0.25% was applied to the crop as a foliar spray compared to its control.

- Boron, Sulphur and BAP treatments notably improve the biochemical responses of Indian mustard grown in open field conditions. From the research trial, it is observed that chlorophyll 'a' and 'b' shows higher results in recommended spacing, whereas chlorophyll 'a+b'and chlorophyll 'a:' shows better results in reduced spacing (20*10) where foliar application of Sulphur + BAP was applied to the mustard crop. Biochemical parameters like TSS, total starch, TSP, total phenols, total flavonols, total flavonoids, amino acids, ascorbic acids, and total lipids. RWC, dry matter accumulation shows better results when grown in limiting spacing (20*10). In subplots, the above parameters show better results when Sulphur + BAP is applied to the crop.
- The effect of Boron, Sulphur and cytokinin individual and their combinations on economic analysis in Indian mustard was observed at harvest. The research trial found that higher gross and net returns were observed in treatment with limiting spacing where Sulphur was combined with plant growth hormone cytokinin to the mustard crop. Higher B: C was observed in treatments where combined doses were used as a foliar spray.

The treated plots with limiting spacing showed a significant increase in growth, physiological, biochemical, yield and quality characteristics of the mustard crop compared to the recommended spacing and controlled plots. So, it is clear from the study that the application of plant growth hormone along with secondary and micronutrients greatly improves the yield and quality of the mustard crop. It should be adapted as a package of practices while growing mustard crops and will play a better role to increase profit to the farmer. Effective management of natural resources, integrated approach to plant-water, nutrient and pest management, and extension of rapeseed-mustard cultivation to newer areas under different cropping systems will play a key role in further increasing and stabilizing the productivity and production of rapeseed-mustard**.**

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