

Synchronized Nitrogen Application With Zinc and Boron Improved Productivity, Quality and Soil Dehydrogenase Activities in Indian Mustard (*Brassica Juncea* L.)

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Abstract

Background and Aims:

Imbalanced nitrogen (N) fertilization and inadequate micronutrient supply often limit nitrogen use efficiency, productivity, oil quality, and soil biological activity in Indian mustard (*Brassica juncea* L.). This study evaluated the effects of nitrogen scheduling combined with zinc (Zn) and boron (B) on productivity, oil quality, nutrient uptake, and soil dehydrogenase activity.

Methods

A two-year field experiment was conducted during 2020–21 and 2021–22 in a randomized block design with twelve treatments. Treatments included two nitrogen levels (120 and 90 kg ha⁻¹), with or without Zn (25 kg ha⁻¹) and B (10 kg ha⁻¹), and the recommended fertilizer dose (RDF) (120:60:40 kg ha⁻¹ N:P₂O₅:K₂O). Yield, quality parameters, nutrient uptake, and soil dehydrogenase activity were assessed.

Results

Compared with the RDF, split application of N at 30, 60, and 30 kg ha⁻¹ at 15, 30, and 45 days after sowing, combined with Zn and B, increased mean seed yield by 75.47%, stalk yield by 38.26%, oil yield by 89%, and protein yield by 79%. Nitrogen uptake increased by 40%, while Zn and B accumulation in seed was enhanced. Soil dehydrogenase activity increased by 10.5%, indicating improved soil biological health. Treatments receiving Zn and B showed better yield and quality than those without micronutrient supplementation.

Conclusions

Nitrogen scheduling based on crop demand, together with Zn and B application, improved productivity, nutrient uptake, oil quality, and soil biological activity in Indian mustard, demonstrating an effective strategy for sustainable nutrient management.

Highlights

Integrated N scheduling, Zn and B improved yield, quality, and soil health.

Synchronizing N supply with crop demand enhanced nutrient use efficiency.

Combined Zn and B application along with recommended dose of fertilizer increased seed nutrient accumulation.

Oil yield and protein yield improved substantially under this optimized nutrition.

Agronomic, quality, and biological responses were assessed simultaneously.

INTRODUCTION

Indian mustard (*Brassica juncea* L. Czern & Coss.), cultivated as an oilseed crop (38–40% oil content) during the winter season, thrives at relatively cool temperatures and adequate soil moisture, coupled with balanced nutrient application during its growth phase (Banerjee et al., 2010; Malik et al., 2024). Yield anomalies and poor quality of mustard are due to improper nutrient management of both major and micronutrients (Gupta et al., 2024; Tripathi et al., 2010).

Nitrogen plays a pivotal role in mustard production because of its essential contribution to chlorophyll synthesis and its integral presence in protoplasm, nucleic acids, and proteins. Additionally, among other physiological processes, the accumulation of nitrate in vacuoles is crucial for maintaining ionic balance and supporting osmoregulation (Burns et al., 2012). However, a major challenge in modern agriculture is that only 30–50% of the applied nitrogenous fertilizers are effectively utilized by crops, with significant losses occurring through runoff, leaching, denitrification, and volatilization (Gupta, 2005). These inefficiencies contribute to increased greenhouse gas (N_2O) concentrations in the atmosphere (Li et al., 2020; Aryal et al., 2022) and elevated NO_3^- levels in drinking water (Zhuang et al., 2023; Bisht et al., 2024).

Zinc is another critical nutrient that serves as a major component of various enzymes, including alcohol dehydrogenase, carbonic anhydrase, and Cu-Zn superoxide dismutase. It is also involved in protein synthesis, carbohydrate metabolism, membrane integrity, and indole acetic acid (Suganya et al., 2020). Boron is equally essential for the formation of organic complexes, root elongation, nucleic acid synthesis, cell wall synthesis, and tissue differentiation (Marschner, 2002). In Indian agriculture, boron is the second most significant micronutrient after zinc in terms of prevalence and deficiency (Sathya et al., 2009). The increasing reliance on high-analysis fertilizers, which often lack micronutrients, exacerbates Zn and B deficiency in soils and crops. Efficient nutrient management strategies, such as split applications of nitrogenous fertilizers timed with key crop demand stages, are crucial for enhancing crop growth and development, promoting soil enzymatic activities, and improving soil health (Sartaj et al., 2017).

Despite the recognized importance of N, Zn, and B, a significant research gap exists in understanding the integrated and synergistic effects of precision nitrogen scheduling alongside targeted Zn and B fertilization on Indian mustard. While the roles of individual nutrients have been established, the optimal interplay of these nutrients, particularly how their synchronized application influences not only yield but also critical quality parameters such as fatty acid and glucosinolate profiles, remains underexplored. Current practices often lead to suboptimal nutrient use efficiency and environmental burdens owing to generalized fertilization approaches and insufficient attention to micronutrient balance.

We hypothesized that a precisely scheduled split application of nitrogen, when combined with optimal zinc and boron fertilization, will significantly enhance nutrient use efficiency, improve soil health, and crucially, optimize the productivity and quality of Indian mustard by synchronizing nutrient availability with the crop's physiological demands.

Thus, the present study on the scheduling of nitrogen application coupled with Zn and B fertilization on Indian mustard was designed to suitably match the sigmoidal pattern of crop growth to enhance the productivity and quality of the oil.

MATERIALS AND METHODS

Site Description and Soil Type

The experiments were carried out at the N. E. Borlaug Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar (29° N, 79.29° E and at an altitude of 243.83 MSL), India, in the *rabi* (winter) season of 2020-21 and 2021-22. The initial soil fertility status of the experimental site is 0.38 dS m⁻¹ EC, 7.42 pH, 0.74% OC, 223 kg ha⁻¹ available N, 20 kg ha⁻¹ available P, 214 kg ha⁻¹ available K, 13.8 ppm S, 8.3 ppm Zn, and 11 ppm B.

Experimental Details

The experiment was laid out in a Randomized Block Design (RBD) with 12 treatments and replicated three times. The treatments details were as follows: N @30, 30, 30, 30 kg ha⁻¹ at 15, 30, 45, 60 days after sowing (DAS) respectively (T₁); N @30, 30, 30 kg ha⁻¹ at 15, 30, 45 DAS respectively (T₂); N @30, 60, 30 kg ha⁻¹ at 15, 30, 45 DAS respectively (T₃); N @30, 30, 30, 30 kg ha⁻¹ at 0, 15, 30, 45 DAS respectively (T₄); N @30, 30, 30 kg ha⁻¹ at 0, 15, 30 DAS respectively (T₅); T₁ + Zn + B (T₆); T₂ + Zn + B (T₇); T₃ + Zn + B (T₈); T₄ + Zn + B (T₉); T₅ + Zn + B (T₁₀); RDF (NPK) (T₁₁); RDF (NPK) + Zn + B (T₁₂).

Agronomic Practices

The land was prepared by plowing with a Mould Board plow, followed by the working of harrows and a rotavator. The gross area of each experimental plot was 21.5 m², and each plot had 17 rows. The variety used was NRCHB101, released by DRMR-Bharatpur, Rajasthan, and sowing was done with a seed rate of 5 kg ha⁻¹ and a row-to-row spacing of 30 cm. A plant-to-plant distance of 10 cm was maintained by thinning 15 DAS. In the treatment where the RDF (120:60:40 kg ha⁻¹ of N: P₂O₅: K₂O) was used, a half dose of nitrogen was applied along with full amounts of phosphorous, potassium, zinc, and boron were applied at the time of sowing as basal, and the remaining nitrogen was applied in two equal splits at 30 and 60 DAS. During the remaining treatments, fertilizers were applied as per the treatments. Urea, single superphosphate, and muriate of potash were used as N, P, and K sources, respectively. ZnSO₄ (25 kg ha⁻¹) and borax (10 kg ha⁻¹) were applied using ZnSO₄.7H₂O (21% Zn) and borax (11% B) respectively. Pendimethalin at 1 kg a.i. ha⁻¹ was applied as pre-emergence at 3 DAS to control early flushing of weeds. One-hand weeding was performed at 30 DAS to check weed growth. Irrigation was applied in the check

basin method as, and when required. Pests and diseases were managed according to the recommendations.

Estimation of parameters

Seed Quality Parameters:

1. **Oil Content and Yield:** The oil content of the seeds was determined non-destructively using Fourier-transform near-infrared (FT-NIR) spectroscopy. This method allows for a rapid and accurate assessment. The oil yield was subsequently calculated by multiplying the oil content percentage by the seed yield (kg ha^{-1}).
2. **Protein Content and Yield:** Seed protein content was derived from the total nitrogen content of the seeds using the conversion factor: $\% \text{ Protein} = \% \text{ N (Total N in seeds)} \times 6.25$. The protein yield was then computed by multiplying the protein content percentage by the seed yield (kg ha^{-1}).
3. **Fatty Acid Composition:** The total saturated and unsaturated fatty acid compositions in the oil, including specific fatty acids, were estimated using FT-NIR spectroscopy.
4. **Glucosinolate Content:** Total glucosinolate content in seeds was determined using FT-NIR spectroscopy and expressed in $\mu\text{mol g}^{-1}$.

Note

For all parameters estimated using FT-NIR spectroscopy (oil content, fatty acid composition, protein content, and glucosinolate content), a rigorous calibration and validation process was undertaken, establishing a strong correlation with conventional wet lab methods. This ensures the reliability and accuracy of non-destructive FT-NIR analyses.

Plant Nutrient Content:

1. **Nitrogen Content:** Total nitrogen content in plant samples was determined using the modified micro-Kjeldahl method (Jackson, 1967) and expressed as a percentage of the dry weight.
2. **Phosphorus Content:** Phosphorus content was determined using the vanadomolybdo-phosphoric acid yellow color method in an acidic system. The intensity of the yellow color was measured using a UV-VIS spectrophotometer at 470 nm, as described by Chapman and Parker (1961).
3. **Potassium Content:** Potassium content in the digested plant material was estimated using a flame photometer.
4. **Sulfur (S) Content:** Sulfur content was determined using the barium chloride method and expressed as a percentage (%).
5. **Zinc Content:** Zinc content in plant samples was determined using an atomic absorption spectrophotometer (AAS) after digesting the plant samples with a diacid mixture (Lindsay and Norvell, 1978).
6. **Boron Content:** Boron content was determined colorimetrically using the Azomethine-H reagent, and the intensity of the color was measured using a spectrophotometer (Berger and Troug, 1939).

Soil Enzyme Activity:

Dehydrogenase Activity: Dehydrogenase activity in the soil was estimated using the 2-3-5-Triphenyl tetrazolium chloride (TTC) reduction technique described by Casida (1977). Soil samples for this analysis were collected from the experimental plots at the key stage (flowering stage). Samples were immediately transported to the laboratory, sieved to remove debris, and stored at 4°C prior to analysis to maintain enzyme integrity.

Statistical Analysis

The experimental data were analyzed using the analysis of variance technique (Gomez and Gomez, 1984), with SAS 9.4 to test the significance of the difference between any two means, regardless of the significance of the F-test. Tukey's honestly significant difference test (*Tukey's HSD*) at 5% level of significance was performed only when the ANOVA F-test was significant ($p \leq 0.05$) to compare the treatment mean effects.

RESULTS

Weather

The climate of the region is humid subtropical, with harsh winters and hot dry summers. During summer, the maximum temperature exceeds 40°C, whereas in winter, the minimum temperature is 3°C. The onset of the monsoon usually occurs in the third week of June and retreats in September. The mean RH remains around 80–90% from mid-June to end of February, steadily decreases to 50% by the first week of May, and remains as such until mid- June. The total amount of rainfall received in 2021 was 25.7 mm, whereas that in 2022 was 154.7 mm. During the study period, approximately 90–95% of total rainfall was received between January and February.

Seed, stalk and biological yields (kg ha^{-1})

The N application rates and scheduling coupled with Zn and B fertilization had significant impacts on mustard seed, stalk and biological yields during both years of the study (Table 1). Application of N at 30, 60, and 30 kg ha^{-1} at 15, 30, and 45 DAS, respectively, coupled with Zn and B (T_8) produced the highest mean seed and mean stalk yields, which were statistically at par with $T_{1, 2, 3, 6, 7, 9, 10 \text{ \& } 12}$ (mean seed yield) and $T_{1, 2, 3, 4, 6, 7, 9, 10 \text{ \& } 12}$ (mean stalk yield) during both the years of study. The increase in mean seed and stalk yields under T_8 were 64.6% and 38.26%, respectively, compared to T_{11} . Additionally, the benefit–cost ratio is provided in Supplementary Table 1 for a clearer economic comparison between RDF and T_8 . The biological yield followed a similar trend. The year-wise data for all yields are provided in Supplementary Table 2 for reference.

Table 1

Effect of nitrogen scheduling along with Zn and B on seed, stalk and biological yields (mean of both the years).

Treatment	Seed yield (t ha ⁻¹)	Stalk yield (t ha ⁻¹)	Biological yield (t ha ⁻¹)
	2 years mean	2 years mean	2 years mean
T ₁ : N @30, 30, 30, 30 kg ha ⁻¹ at 15, 30, 45, 60 DAS respectively	1.38 ^{abc}	5.40 ^{abc}	6.78 ^{abcd}
T ₂ : N @30, 30, 30 kg ha ⁻¹ at 15, 30, 45 DAS respectively	1.36 ^{abc}	5.14 ^{abc}	6.50 ^{abcd}
T ₃ : N @30, 60, 30 kg ha ⁻¹ at 15, 30, 45 DAS respectively	1.45 ^{abc}	4.73 ^{abc}	6.17 ^{bcd}
T ₄ : N @30, 30, 30, 30 kg ha ⁻¹ at 0, 15, 30, 45 DAS respectively	1.27 ^{bc}	5.03 ^{abc}	6.30 ^{abcd}
T ₅ : N @30, 30, 30 kg ha ⁻¹ at 0, 15, 30 DAS respectively	1.06 ^c	4.24 ^c	5.30 ^d
T ₆ : T ₁ + Zn + B	1.71 ^{ab}	6.15 ^{ab}	7.86 ^{ab}
T ₇ : T ₂ + Zn + B	1.59 ^{abc}	5.75 ^{abc}	7.34 ^{abc}
T ₈ : T ₃ + Zn + B	1.86 ^a	6.36 ^a	8.22 ^a
T ₉ : T ₄ + Zn + B	1.60 ^{abc}	5.66 ^{abc}	7.26 ^{abcd}
T ₁₀ : T ₅ + Zn + B	1.53 ^{abc}	5.82 ^{abc}	7.35 ^{abc}
T ₁₁ : RDF (NPK)	1.13 ^{bc}	4.60 ^{bc}	5.73 ^{cd}
T ₁₂ : RDF (NPK) + Zn + B	1.51 ^{abc}	6.19 ^{ab}	7.70 ^{abc}
CD (p = 0.05)	0.26	1.02	1.29
*ZnSO ₄ @25kg ha ⁻¹ ; B @10kg ha ⁻¹ and RDF (120:60:40 kg ha ⁻¹ of N: P2O ₅ : K ₂ O). Data in the column followed by different letters are significantly different at p ≤ 0.05 as analyzed by Tukey's honest significant difference (Tukey's HSD) test.			

Oil content (%) and oil yield (kg ha⁻¹)

N application rates and scheduling coupled with Zn and B fertilization had significant impacts on mustard seed oil, protein content, and oil and protein yield during both years of the study (Table 2). The Treatment T₈ had the highest oil content (43.6, 42.9%), which was statistically at par with T₆, 7, 9, 10, & 12

but significantly higher than the other treatments in both the years of study. Similarly, T₈ recorded the highest oil yield (815.8 and 789.5 kg ha⁻¹) during both years of experimentation. The mean increase of both the years in oil content and oil yield under T₈ was 8% and 89%, respectively, over RDF (T₁₁).

Table 2

Effect of nitrogen scheduling along with Zn and B on oil content, oil yield, protein content and protein yield in seed during 2020-21 and 2021-22.

Treatment	2020-21				2021-22			
	Oil content (%)	Oil yield (kg ha ⁻¹)	Protein content (%)	Protein yield (kg ha ⁻¹)	Oil content (%)	Oil yield (kg ha ⁻¹)	Protein content (%)	Protein yield (kg ha ⁻¹)
T ₁ : N @30, 30, 30, 30 kg ha ⁻¹ at 15, 30, 45, 60 DAS respectively	40.3 ^b	565.3 ^f	18.13 ^{ab}	254.7 ^f	39.7 ^b	539.9 ^f	17.99 ^{ab}	244.7 ^f
T ₂ : N @30, 30, 30 kg ha ⁻¹ at 15, 30, 45 DAS respectively	40.1 ^b	551.8 ^f	16.38 ^c	226.2 ^g	39.6 ^b	534.9 ^f	16.26 ^c	220.2 ^g
T ₃ : N @30, 60, 30 kg ha ⁻¹ at 15, 30, 45 DAS respectively	40.2 ^b	587.7 ^e	18.38 ^a	267.8 ^{de}	39.8 ^b	570.7 ^e	18.23 ^a	260.3 ^{de}
T ₄ : N @30, 30, 30, 30 kg ha ⁻¹ at 0, 15, 30, 45 DAS respectively	40.5 ^b	519.7 ^g	18.00 ^{ab}	230.9 ^g	40.1 ^b	500.9 ^g	17.91 ^{ab}	223.5 ^g
T ₅ : N @30, 30, 30 kg ha ⁻¹ at 0, 15, 30 DAS respectively	40.7 ^b	438.6 ^h	16.31 ^c	175.6 ⁱ	40.3 ^b	422.2 ^h	16.14 ^c	168.9 ⁱ
T ₆ : T ₁ + Zn + B	43.4 ^a	750.2 ^b	18.19 ^{ab}	314.0 ^b	42.8 ^a	724.3 ^b	18.06 ^{ab}	305.1 ^b
T ₇ : T ₂ + Zn + B	43.5 ^a	697.7 ^c	16.56 ^c	265.5 ^{ef}	42.8 ^a	675.4 ^c	16.43 ^c	258.4 ^e
*Zn @25kg ha ⁻¹ ; B @10kg ha ⁻¹ and RDF (120:60:40 kg ha ⁻¹ of N: P2O5: K2O). Data in the column followed by different letters are significantly different at p ≤ 0.05 as analyzed by Tukey's honest significant difference (Tukey's HSD) test.								

Treatment	2020-21				2021-22			
	Oil content (%)	Oil yield (kg ha ⁻¹)	Protein content (%)	Protein yield (kg ha ⁻¹)	Oil content (%)	Oil yield (kg ha ⁻¹)	Protein content (%)	Protein yield (kg ha ⁻¹)
T ₈ : T ₃ + Zn + B	43.6 ^a	815.8 ^a	18.38 ^a	344.8 ^a	42.9 ^a	789.5 ^a	18.28 ^a	337.8 ^a
T ₉ : T ₄ + Zn + B	43.2 ^a	699.4 ^c	18.25 ^a	295.4 ^c	42.5 ^a	673.1 ^c	18.04 ^{ab}	285.6 ^c
T ₁₀ : T ₅ + Zn + B	43.5 ^a	671.3 ^d	16.81 ^{bc}	260.0 ^{ef}	42.9 ^a	647.1 ^d	16.66 ^{bc}	251.5 ^{ef}
T ₁₁ : RDF (NPK)	40.4 ^b	433.2 ^h	18.06 ^{ab}	193.8 ^h	39.7 ^b	416.4 ^h	17.88 ^{ab}	187.4 ^h
T ₁₂ : RDF (NPK) + Zn + B	43.2 ^a	658.2 ^d	18.31 ^a	278.8 ^d	42.7 ^a	636.9 ^d	18.15 ^a	270.8 ^d
CD (p = 0.05)	1.5	104.5	0.68	44.4	1.5	100.5	0.67	44.0
*Zn @25kg ha ⁻¹ ; B @10kg ha ⁻¹ and RDF (120:60:40 kg ha ⁻¹ of N: P2O5: K2O). Data in the column followed by different letters are significantly different at p ≤ 0.05 as analyzed by Tukey's honest significant difference (Tukey's HSD) test.								

Protein content in seed (%) and protein yield (kg ha⁻¹)

T₈ had highest (18.38, 18.28%) protein content, which was statistically at par to T₁, 3, 4, 6, 9, 11 & 12 during both the years of study. T₅ had lowest (16.31, 16.14%) protein content. T₈ also recorded the highest protein yield (344.8 and 337.8 kg ha⁻¹) during both years of the study, whereas T₅ recorded the lowest protein yield (175.6 and 168.9 kg ha⁻¹) (Table 2). The mean increase of both the years in protein content and protein yield under T₈ were 2% and 79%, respectively, compared to RDF (T₁₁). There was no significant effect of year × treatment interaction on both protein content and yield.

Fatty acid composition in seeds (%)

Saturated fatty acid composition

The composition of saturated fatty acids (*i.e.*, palmitic acid and stearic acid) in seeds was not significantly influenced by the different treatments during either year of investigation. Palmitic (do not have food value) acid (16:0) content in seed ranged from 3.1% to 3.26% whereas, stearic acid (18:0) content in seed ranged from 0.36% to 0.43%. The total saturated fatty acid content was less than 7%, which is considered as a safe limit for human consumption point of view (Tables 3a and 3b).

Table 3

a. Effect of nitrogen scheduling along with Zn and B on fatty acid composition (%) of Indian mustard during 2020-21.

Treatment	Saturated fatty acids		Unsaturated fatty acids				Glucosinolate (μ moles/g)
	Palmitic (16:0)	Stearic (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Linolenic acid (18:3)	Erucic acid (22:1)	
T ₁ : N @30, 30, 30 kg ha ⁻¹ at 15, 30, 45, 60 DAS respectively	3.13 ^a	0.38 ^a	8.08 ^a	18.08 ^a	10.18 ^a	39.12 ^a	103.1 ^a
T ₂ : N @30, 30, 30 kg ha ⁻¹ at 15, 30, 45 DAS respectively	3.14 ^a	0.37 ^a	8.10 ^a	18.09 ^a	10.21 ^a	39.23 ^a	103.7 ^a
T ₃ : N @30, 60, 30 kg ha ⁻¹ at 15, 30, 45 DAS respectively	3.25 ^a	0.41 ^a	8.11 ^a	18.12 ^a	10.22 ^a	39.24 ^a	104.7 ^a
T ₄ : N @30, 30, 30, 30 kg ha ⁻¹ at 0, 15, 30, 45 DAS respectively	3.17 ^a	0.43 ^a	8.06 ^a	18.11 ^a	10.21 ^a	39.17 ^a	104.1 ^a
T ₅ : N @30, 30, 30 kg ha ⁻¹ at 0, 15, 30 DAS respectively	3.18 ^a	0.37 ^a	8.12 ^a	18.13 ^a	10.24 ^a	39.14 ^a	103.9 ^a
T ₆ : T ₁ + Zn + B	3.21 ^a	0.37 ^a	8.11 ^a	18.10 ^a	10.19 ^a	39.21 ^a	104.0 ^a
T ₇ : T ₂ + Zn + B	3.19 ^a	0.43 ^a	8.09 ^a	18.13 ^a	10.21 ^a	39.21 ^a	104.3 ^a
T ₈ : T ₃ + Zn + B	3.20 ^a	0.43 ^a	8.14 ^a	18.14 ^a	10.23 ^a	39.25 ^a	104.4 ^a
T ₉ : T ₄ + Zn + B	3.26 ^a	0.43 ^a	8.13 ^a	18.12 ^a	10.20 ^a	39.19 ^a	103.8 ^a
T ₁₀ : T ₅ + Zn + B	3.18 ^a	0.43 ^a	8.10 ^a	18.08 ^a	10.23 ^a	39.20 ^a	104.6 ^a
*Zn @25kg ha ⁻¹ ; B @10kg ha ⁻¹ and RDF (120:60:40 kg ha ⁻¹ of N: P2O5: K2O). Data in the column followed by different letters are significantly different at $p \leq 0.05$ as analyzed by Tukey's honest significant difference (Tukey's HSD) test.							

Treatment	Saturated fatty acids		Unsaturated fatty acids				Glucosinolate (μmoles/g)
	Palmitic (16:0)	Stearic (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Linolenic acid (18:3)	Erucic acid (22:1)	
T ₁₁ : RDF (NPK)	3.12 ^a	0.39 ^a	8.12 ^a	18.07 ^a	10.18 ^a	39.14 ^a	103.5 ^a
T ₁₂ : RDF (NPK) + Zn + B	3.17 ^a	0.43 ^a	8.13 ^a	18.11 ^a	10.21 ^a	39.19 ^a	104.1 ^a
CD (p = 0.05)	NS	NS	NS	NS	NS	NS	NS
*Zn @25kg ha ⁻¹ ; B @10kg ha ⁻¹ and RDF (120:60:40 kg ha ⁻¹ of N: P2O5: K2O). Data in the column followed by different letters are significantly different at p ≤ 0.05 as analyzed by Tukey's honest significant difference (Tukey's HSD) test							

Table 3

b. Effect of nitrogen scheduling along with Zn and B on fatty acid composition (%) of Indian mustard during 2021-22

Treatment	Saturated fatty acids		Unsaturated fatty acids				Glucosinolate (μ moles g^{-1})
	Palmitic (16:0)	Stearic (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Linolenic acid (18:3)	Erucic acid (22:1)	
T ₁ : N @30, 30, 30 kg ha ⁻¹ at 15, 30, 45, 60 DAS respectively	3.12 ^a	0.37 ^a	8.07 ^a	18.06 ^a	10.13 ^a	39.10 ^a	102.8 ^a
T ₂ : N @30, 30, 30 kg ha ⁻¹ at 15, 30, 45 DAS respectively	3.13 ^a	0.36 ^a	8.09 ^a	18.11 ^a	10.18 ^a	39.22 ^a	102.8 ^a
T ₃ : N @30, 60, 30 kg ha ⁻¹ at 15, 30, 45 DAS respectively	3.24 ^a	0.39 ^a	8.10 ^a	18.08 ^a	10.19 ^a	39.23 ^a	104.0 ^a
T ₄ : N @30, 30, 30 kg ha ⁻¹ at 0, 15, 30, 45 DAS respectively	3.16 ^a	0.42 ^a	8.04 ^a	18.09 ^a	10.18 ^a	39.15 ^a	103.7 ^a
T ₅ : N @30, 30, 30 kg ha ⁻¹ at 0, 15, 30 DAS respectively	3.17 ^a	0.36 ^a	8.09 ^a	18.11 ^a	10.21 ^a	39.46 ^a	103.6 ^a
T ₆ : T ₁ + Zn + B	3.25 ^a	0.42 ^a	8.13 ^a	18.08 ^a	10.15 ^a	39.19 ^a	102.7 ^a
T ₇ : T ₂ + Zn + B	3.18 ^a	0.42 ^a	8.08 ^a	18.12 ^a	10.17 ^a	39.22 ^a	104.2 ^a
T ₈ : T ₃ + Zn + B	3.20 ^a	0.36 ^a	8.09 ^a	18.12 ^a	10.21 ^a	39.19 ^a	104.0 ^a
T ₉ : T ₄ + Zn + B	3.19 ^a	0.41 ^a	8.11 ^a	18.10 ^a	10.17 ^a	39.17 ^a	103.4 ^a
T ₁₀ : T ₅ + Zn + B	3.16 ^a	0.42 ^a	7.74 ^a	18.06 ^a	10.19 ^a	39.19 ^a	104.4 ^a
*Zn @25kg ha ⁻¹ ; B @10kg ha ⁻¹ and RDF (120:60:40 kg ha ⁻¹ of N: P2O5: K2O). Data in the column followed by different letters are significantly different at $p \leq 0.05$ as analyzed by Tukey's honest significant difference (Tukey's HSD) test.							

Treatment	Saturated fatty acids		Unsaturated fatty acids				Glucosinolate (μ moles g^{-1})
	Palmitic (16:0)	Stearic (18:0)	Oleic acid (18:1)	Linoleic acid (18:2)	Linolenic acid (18:3)	Erucic acid (22:1)	
T ₁₁ : RDF (NPK)	3.10 ^a	0.38 ^a	8.10 ^a	18.04 ^a	10.15 ^a	39.11 ^a	103.2 ^a
T ₁₂ : RDF (NPK) + Zn + B	3.16 ^a	0.42 ^a	8.11 ^a	18.09 ^a	10.18 ^a	39.17 ^a	103.8 ^a
C D (P = 0.05)	NS	NS	NS	NS	NS	NS	NS
*Zn @25kg ha ⁻¹ ; B @10kg ha ⁻¹ and RDF (120:60:40 kg ha ⁻¹ of N: P2O5: K2O). Data in the column followed by different letters are significantly different at $p \leq 0.05$ as analyzed by Tukey's honest significant difference (Tukey's HSD) test.							

Unsaturated fatty acid composition

The composition of unsaturated fatty acid (*i.e.*, oleic, linoleic, linolenic, and erucic acids) in seeds was not significantly influenced by the different treatments during either year of the study. In both years, the maximum amounts of linoleic and linolenic acids were observed in the T₈ treatment, whereas the minimum amounts were observed in T₁₁ (Table 3a and 3b).

Glucosinolate content in seed (μ moles/g)

The glucosinolate content in seeds was not significantly influenced by the treatments; however, the maximum and minimum glucosinolate contents were recorded with T₁₀ (104.7, 104.4) and T₁ (103.1, 102.1) treatments, respectively, during both years of the study (Table 3a and b).

N, P, K, and S contents in seed (%)

The N content in the seeds varied significantly among the treatments (Fig. 1a and 1b). T₈ recorded the highest (2.94 and 2.92%) per cent of seed nitrogen during both the years, which was statistically at par with T_{1, 3, 4, 6, 9, 11, & 12}. The minimum (2.61 and 2.58%) was observed in T₅ in both the years. T₈ was 1.7 and 2.1% more than (RDF) T₁₁ in both the years, respectively. However, there were no significant differences in the P, K, and S contents of seeds during either of the year.

Zn and B contents in seed (%)

During both years, the treatments varied significantly with respect to Zn content in the seeds (Fig. 2). T₇ had the highest (55.57 and 55.24 ppm) seed zinc content during both years, which was statistically at par with T_{6-10&12}. T₇ was 37.4 and 37.1% more than (RDF) T₁₁ in both the years, respectively. Similarly, different treatments had differential effects on the B content of seeds. T₇ recorded the highest (13.24 and 12.95 ppm) seed B content during both years, which was statistically at par with T_{6-10 & 12}. T₇ was 343.5 and 328.5% more than (RDF) T₁₁ in both the years, respectively.

Dehydrogenase activity ($\mu\text{g TPF g}^{-1} \text{ h}^{-1}$)

The schedules of nitrogen coupled with Zn and B fertilization significantly affected dehydrogenase activity at 60 DAS (flowering stage) in both years. At 60 DAS, T_{12} showed the highest (45.7, 45.1) dehydrogenase activity (Table 4), which was statistically similar to that of $T_{1, 6 \& 8}$ and $T_{6, 8 \& 11}$ respectively, in both years. The increases in T_{12} in both the years were 27.3 and 3.4%, respectively, over RDF (T_{11}).

Table 4
Effect of nitrogen scheduling along with Zn and B on dehydrogenase activity at flowering stage during 2020-21 and 2021-22

Treatment	Dehydrogenase activity ($\mu\text{g TPF g}^{-1} \text{ h}^{-1}$)	
	2020-21	2021-22
Flowering stage	Flowering stage	
T ₁ : N @30, 30, 30, 30 kg ha ⁻¹ at 15, 30, 45, 60 DAS respectively	44.4 ^a	38.7 ^{bc}
T ₂ : N @30, 30, 30 kg ha ⁻¹ at 15, 30, 45 DAS respectively	36.3 ^{bc}	35.3 ^c
T ₃ : N @30, 60, 30 kg ha ⁻¹ at 15, 30, 45 DAS respectively	38.9 ^{bc}	36.3 ^{bc}
T ₄ : N @30, 30, 30, 30 kg ha ⁻¹ at 0, 15, 30, 45 DAS respectively	39.5 ^{bc}	35.8 ^{bc}
T ₅ : N @30, 30, 30 kg ha ⁻¹ at 0, 15, 30 DAS respectively	36.8 ^{bc}	38.4 ^{bc}
T ₆ : T ₁ + Zn + B	44.1 ^a	43.4 ^a
T ₇ : T ₂ + Zn + B	37.0 ^{bc}	39.3 ^b
T ₈ : T ₃ + Zn + B	44.1 ^a	43.8 ^a
T ₉ : T ₄ + Zn + B	39.7 ^b	36.3 ^{bc}
T ₁₀ : T ₅ + Zn + B	39.0 ^{bc}	38.3 ^{bc}
T ₁₁ : RDF (NPK)	35.9 ^c	43.6 ^a
T ₁₂ : RDF (NPK) + Zn + B	45.7 ^a	45.1 ^a
CD (p = 0.05)	4.4	4.4
*Zn @25kg ha ⁻¹ ; B @10kg ha ⁻¹ and RDF (120:60:40 kg ha ⁻¹ of N: P2O5: K2O). Data in the column followed by different letters are significantly different and similar letters are non-significantly different at p ≤ 0.05 as analyzed by Tukey's honest significant difference (Tukey's HSD) test.		

DISCUSSION

Seed and stalk yield

The higher seed yields in $T_{8,6 \& 9}$ treatments are the result of a greater number of branches per plant and other growth parameters (Bhatnagar et al., 2019). Highest stalk ($6.38, 6.34 \text{ t ha}^{-1}$) was obtained in T_8 in both the years (Pandey et al. 2018; Singh et al. 2023). Nitrogen scheduling at T_8 (15, 30, and 45 DAS at 30, 60, and 30 kg ha^{-1}) matched the crop's critical growth stages, reducing losses (leaching, volatilization, and denitrification), and ensuring a continuous nitrogen supply for vegetative development (basal application). The first and second top dressings supported branching and grain filling, thereby improving photosynthesis, biomass production, and seed yield. Fageria et al. (2008) reported that staged nitrogen application enhanced the nitrogen recovery and yield. T_8 also optimized source-sink dynamics by promoting vegetative growth (source) and efficient partitioning of photosynthates to reproductive organs (sink), leading to a higher seed yield with support from Zn and B fertilization. Improved vegetative growth resulted in higher aboveground biomass, reflected in the higher stalk yield of 6.38 t ha^{-1} . Pandey et al. (2018) and Sarvadamana and Shukla (2022) highlighted that balanced nitrogen and micronutrient management during the growing season is critical for achieving higher stalk and grain yields.

Oil content in seed (%) and oil yield

The significant effects of nutrient treatment on seed oil content and yield observed in this study highlight the importance of balanced nutrient management. The highest oil content and yield recorded under T_8 (8.73–8.55% higher oil content and 88.27–89.58% higher oil yield compared to T_2 and T_{11} , respectively) could be attributed to the synergistic effects of N, Zn, and B. Although the increase in nitrogen dose leads to decrease in oil content of oilseed crops, in this experiment various doses and schedules of nitrogen inconsistently affected oil content and oil yield, which agrees with the findings of Duval (2015). In addition, the effects of Zn and boron were prominent on oil content and oil yield, as they positively affected the oil content and oil yield. The increase in oil content and yield could be the result of improved activation of enzymes *viz.*, such as cysteine disulphydrase, dihydropeptilase, and glycyl-glycine dipeptidase, in plants. Thus, the addition of zinc might have activated the enzymes responsible for producing oil, resulting in higher oil content and yield (Bhadauria et al., 2012; Sahito et al., 2014). Furthermore, Singh et al. (2017) reported that the application of boron increased seed production and oil percentage because boron promotes pollen formation and enhances pollen viability.

Protein content in seed (%) and protein yield (kg ha^{-1})

The significant effect of the imposed treatments on seed protein content and yield highlights the importance of balanced nutrient application in enhancing crop quality and productivity. Among the treatments, T_8 had the highest protein content (18.38%, 18.23%) during both the years. Nitrogen, the major component of proteins, plays a vital role in determining the protein content and yield. The most probable reason for the maximum protein content and yield in T_8 is that N was applied at 15, 30, and 45 DAS in three splits at the rates of 30, 60, and 30 kg ha^{-1} respectively, which matched the sigmoidal pattern of crop growth (Karthik et al., 2022), thereby resulting in maximum fertilizer use efficiency and protein yield (Allen and Morgan, 1972; Liu et al., 2018). Nitrogen is essential for producing enzymes, such

as nitrate reductase and glutamine synthetase, which govern nitrogen assimilation and protein synthesis. By applying nitrogen in the splits, enzymatic activity likely remained active for a longer period, allowing higher protein accumulation in the grains (Hakeem et al., 2012).

Fatty Acid Composition in Seeds (%)

Although no statistically significant differences were observed across the treatments, the trends in fatty acid composition provided insights into the metabolic pathways.

Saturated Fatty Acids

Higher levels of saturated fatty acids were generally observed with N treatment at 30, 60, and 30 kg ha⁻¹ at 15, 30, and 45 DAS, respectively. This can be attributed to the surplus nitrogen at 30 DAS increasing metabolic activity and lipid precursor accumulation, favoring the synthesis of primary saturated fatty acids such as palmitic and stearic acids (Cheema et al., 2010).

Unsaturated Fatty Acids

Unsaturated fatty acids, including oleic, linoleic, linolenic, and erucic acids, are important for nutritional quality. Despite non-significant differences, treatments with higher nitrogen (N @ 30, 60, and 30 kg ha⁻¹ at 15, 30, and 45 DAS) tended to show higher unsaturated fatty acid levels than the N @ 30, 30, 30, and 30 kg ha⁻¹ treatments. This may be linked to the higher nitrogen peak (60 kg ha⁻¹) at 30 DAS, a critical stage for lipid metabolism, which potentially enhances photosynthetic efficiency and desaturase enzyme activity, which converts saturated into unsaturated fatty acids (Ghafoor et al., 2022). Conversely, a steady and low N supply might limit these desaturation processes.

Glucosinolate Content

Although different treatments did not significantly affect glucosinolate content, their presence is relevant for both human and animal consumption. Moderate levels of glucosinolates are recognized for their potential anti-carcinogenic properties and role in plant defense mechanisms, whereas high levels are undesirable in animal feed because of their anti-nutritional factors (Fahey et al., 2001).

Nitrogen contents in seed

The trends in nitrogen content were consistent across the two-year study period (2020-21 and 2021-22), demonstrating the reliability and robustness of the effects of nitrogen application rates and treatments on seed nitrogen. The significant variation in seed nitrogen content across treatments underscores the critical role of nitrogen fertilization in determining seed quality. Nitrogen is a primary nutrient that is essential for protein synthesis and seed development. Increased N content in seeds is directly correlated with higher protein levels, which are crucial for human and animal nutrition. Similar findings have been reported in previous studies, in which higher nitrogen application rates led to improved seed N (Roul et al., 2006) and protein contents (Good and Beatty, 2011; Sinclair and Rufty, 2012). The study showed that applying 120 kg N/ha at 30, 60, and 30 kg ha⁻¹ at 15, 30, and 45 DAS, respectively, coupled with Zn and B

supplementation, was optimal for achieving a higher seed nitrogen content. This aligns with other studies indicating that higher nitrogen doses typically enhance nitrogen uptake and accumulation in seeds (Peoples and Craswell, 1992). However, care must be taken to avoid excessive nitrogen application, as it could lead to environmental concerns such as nitrate leaching and greenhouse gas emissions (Robertson and Vitousek, 2009).

Phosphorous, Potassium and Sulphur contents in seed

The treatments had non-significant effects on P, K, and S contents in seeds in both years. As all treatments were applied with an equal dose of P, K, and S, the uptake and subsequent accumulation of these nutrients were also relatively uniform among the treatments in both periods. Although slight numerical variations were observed in the nutrient content in both years, the P% content in seeds ranged from 0.67 (T₃; 2021-22) to 0.76 (T₁₀; 2020-21), K% from 0.79 (T₂, 7; 2021-22) to 0.89 (T₁₀; 2020-21) and S% from 0.42 (T₂; 2021-22) to 0.54 (T₄; 2020-21).

Zinc contents in seed

Zinc is an essential micronutrient that plays a vital role in plant physiological processes including enzyme function, protein synthesis, and hormonal regulation. In seeds, adequate Zn content is crucial for human and animal nutrition, helping to combat Zn deficiency in the diet (Alloway, 2008). The application of 25 kg ZnSO₄ ha⁻¹, as reflected in treatments T₆₋₁₀ & 12 ensured optimal Zn enrichment of seeds. Treatments without Zn application resulted in significantly lower Zn content although the initial amount was high, indicating that the natural availability of Zn in the soil was insufficient to meet the seed requirements. This aligns with previous studies reporting that soil test Zn may not fully reflect Zn availability to plants, as Zn solubility and mobility are strongly influenced by soil pH, organic matter content, and mineral interactions that control Zn adsorption/desorption dynamics (Saleem et al., 2022). However, the difference between treatments receiving Zn and those that were devoid of Zn application was clear, emphasizing the substantial impact of Zn supplementation. Similar findings have been reported, wherein a lack of Zn application resulted in lower Zn content in seeds, even under favorable growing conditions (Hafeez et al., 2013). Hence, the application of ZnSO₄ could enhance the content of soluble Zn⁺⁺ ions in the soil solution, thereby facilitating greater uptake of Zn by the crop and resulting in a higher Zn content in seeds (Mankar et al., 2004).

Boron contents in seed

Boron is essential for several physiological and biochemical processes in plants, including cell wall formation, pollen tube growth, and seed and grain development. A sufficient supply of boron is necessary for proper seed formation and enrichment in plants. The significant differences observed in seed boron content across the treatments validated earlier research on the importance of boron fertilization in improving seed quality (Gupta, 1993; Marschner, 2011). The highest boron content in seeds (13.24 and 12.95 ppm) was observed in treatments with 10 kg borax ha⁻¹. This shows the efficacy

of borax (a common boron fertilizer) in meeting the boron requirements of the crop and biofortifying seeds with sufficient levels of this micronutrient. These findings align with those of previous studies reported that borax application is an effective method for improving boron uptake and accumulation in seeds (Ahmad et al., 2012). With the application of borax, the content of soluble H_3BO_3 could have increased in the soil solution, which facilitated greater uptake of B by the crop, resulting in a higher B content in the seeds (Mohapatra & Dixit, 2010).

Dehydrogenase activity

The schedules of N coupled with Zn and B fertilization exerted a significant influence on dehydrogenase activity, which was most pronounced at the flowering stage (60 DAS). During the crop growth cycle, dehydrogenase activity followed a temporal trend, increasing up to the flowering stage, and declining thereafter. At the flowering stage, the dehydrogenase activity peaked with T_{12} recording the highest values (45.7 and 45.1), which was statistically at par with treatments $T_{1,6, \& 8}$. This surge in enzymatic activity at the flowering stage can be attributed to the specific nitrogen fertilization schedules: The superior performance of these treatments is likely due to these treatments receiving a split dose of 30 kg N ha^{-1} specifically at 60 DAS, whereas other treatments did not receive nitrogen at this critical stage. Higher nitrogen doses facilitated root exudate secretion. These exudates serve as excellent nutritional sources for rhizospheric bacteria, actinomycetes, and fungi. The resulting increase in the microbial population directly enhances dehydrogenase activity, as noted by Dick (1994). The subsequent decrease in activity after the flowering stage (post-60 DAS) and the non-significant effect observed at 90 DAS can be attributed to the depletion of hydrolyzed urea and senescence of the microbial population. These observations regarding peak activity at flowering and the influence of nitrogen splitting are consistent with the findings of Patil et al. (2019).

CONCLUSION

The current study highlights the importance of adopting nitrogen application schedules that align with the physiological demands of the crop to achieve optimal productivity and quality in Indian mustard. The application of nitrogen at intervals of 30, 60, and 30 kg ha^{-1} at 15, 30, and 45 DAS, respectively, coupled with Zn and B supplementation, significantly enhanced the yields, oil content, protein content, oil and protein yields, and seed nutrient content. Moreover, the incorporation of micronutrients (Zn and B) improved the nutritional value of the seeds, played a vital role in increasing oil yield and quality, and contributed to nutrient biofortification of the crop. This study also demonstrated the potential of these management practices to improve soil dehydrogenase activity, thereby promoting soil health. Therefore, adopting synchronized nutrient management strategies that align with crop demand and incorporate both macro- and micronutrients is crucial for achieving sustainable agricultural practices and improving crop productivity.

Declarations

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DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable requests.

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Figures

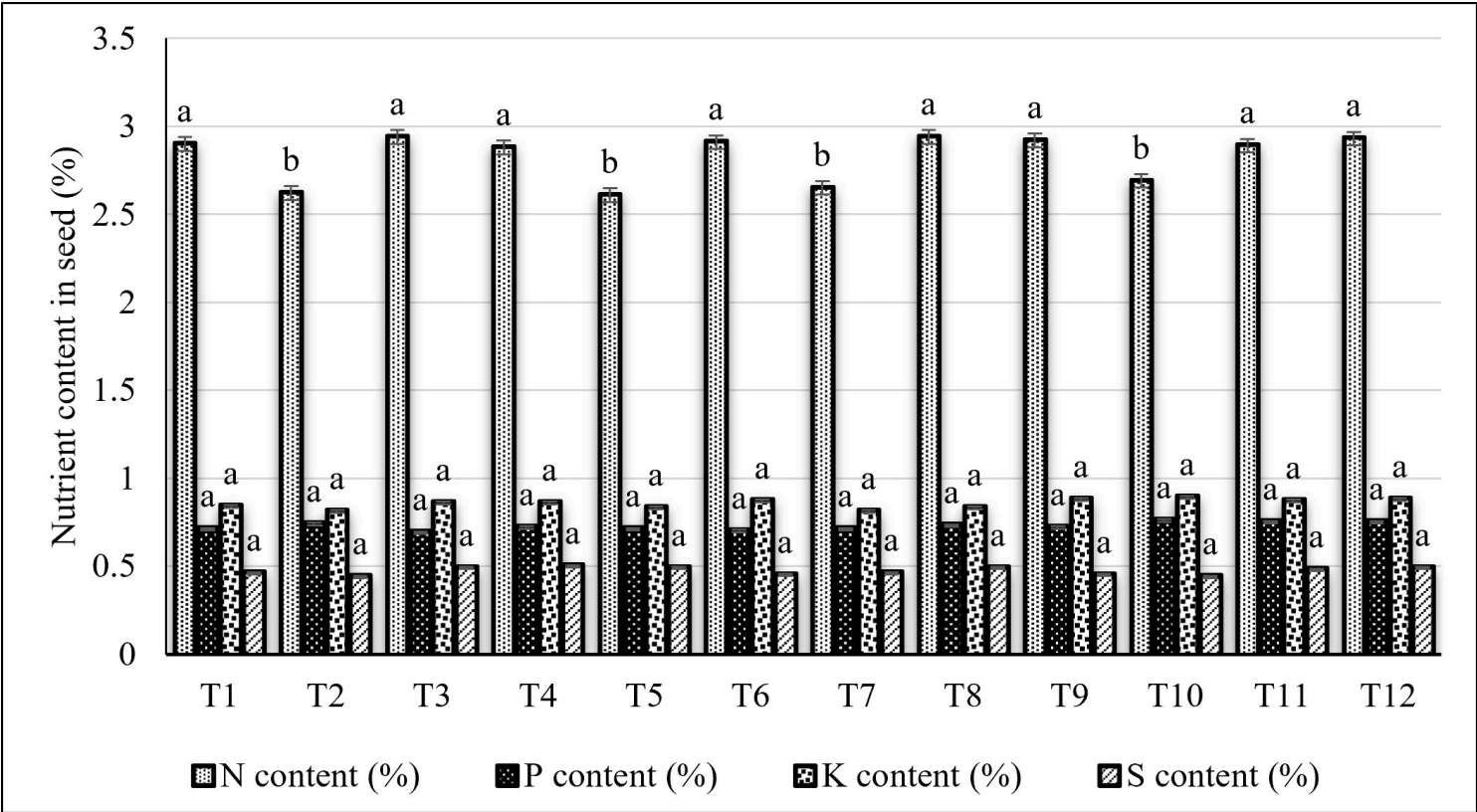


Figure 1

Fig. 1a Effect of nitrogen scheduling along with Zn and B on N, P, K & S content in seed at harvest during 2020-21

(#T1: N @30, 30, 30, 30 kg ha⁻¹ at 15, 30, 45, 60 DAS respectively; T2: N @30, 30, 30 kg ha⁻¹ at 15, 30, 45 DAS respectively; T3: N @30, 60, 30 kg ha⁻¹ at 15, 30,45 DAS respectively; T4: N @30, 30, 30, 30 kg ha⁻¹ at 0, 15, 30, 45 DAS respectively; T5: N @30, 30, 30 kg ha⁻¹ at 0, 15, 30 DAS respectively; T6: T1 + Zn + B; T7: T2 + Zn + B; T8: T3 + Zn + B; T9: T4 + Zn + B; T10: T5 + Zn + B; T11: RDF (NPK); and T12: RDF (NPK) + Zn + B, *Zn @25kg ha⁻¹; B @10kg ha⁻¹ and RDF (120:60:40 kg ha⁻¹ of N: P2O5: K2O). Data in the graph followed by different letters are significantly different at $p \leq 0.05$ as analyzed by Tukey's honest significant difference (Tukey's HSD) test). Error bars represent $\pm$ standard error of the mean.

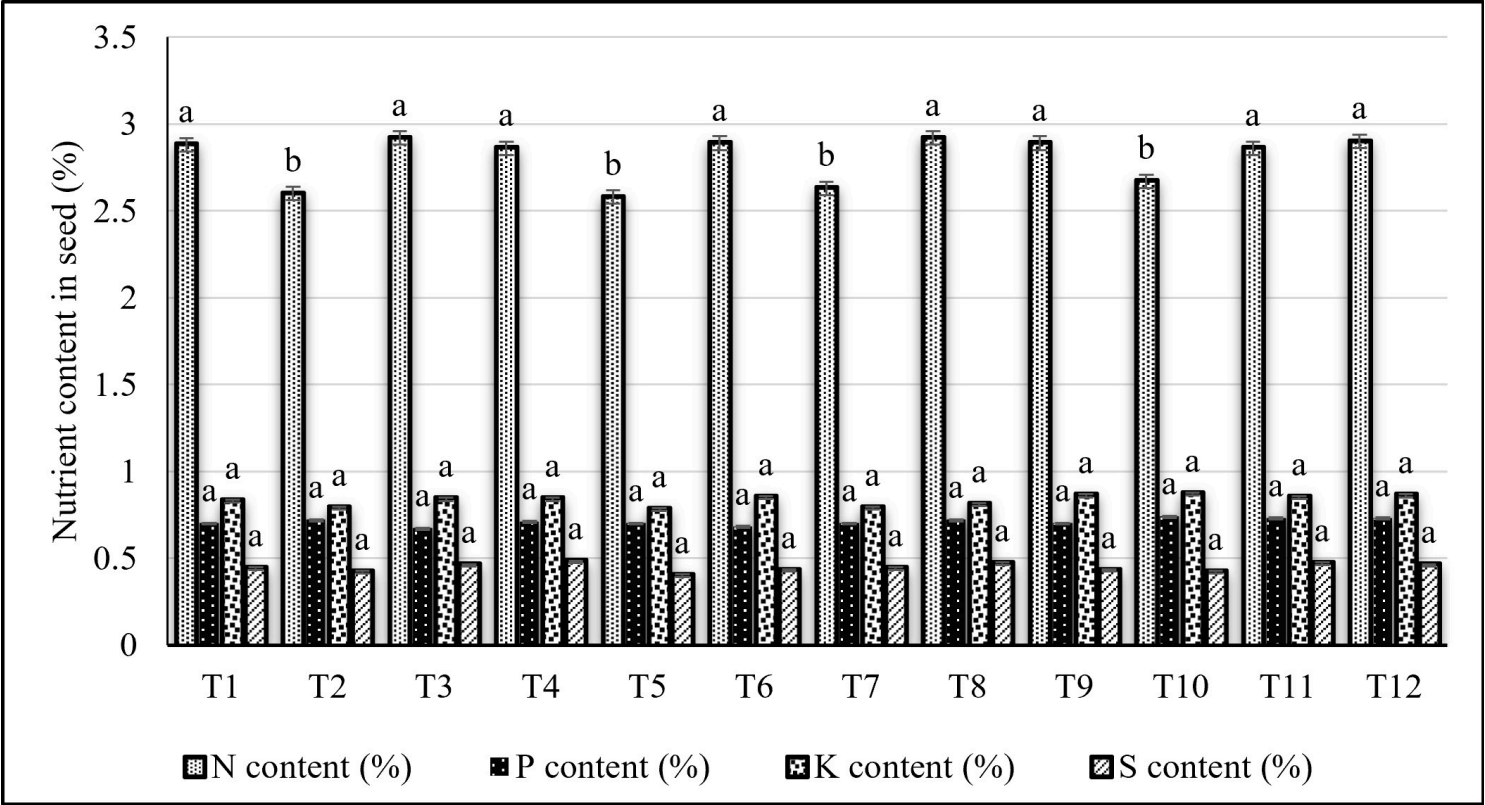


Figure 2

Fig. 1b Effect of nitrogen scheduling along with Zn and B on N, P, K & S content in seed at harvest during 2021-22

(#T1: N @30, 30, 30, 30 kg ha⁻¹ at 15, 30, 45, 60 DAS respectively; T2: N @30, 30, 30 kg ha⁻¹ at 15, 30, 45 DAS respectively; T3: N @30, 60, 30 kg ha⁻¹ at 15, 30,45 DAS respectively; T4: N @30, 30, 30, 30 kg ha⁻¹ at 0, 15, 30, 45 DAS respectively; T5: N @30, 30, 30 kg ha⁻¹ at 0, 15, 30 DAS respectively; T6: T1 + Zn + B; T7: T2 + Zn + B; T8: T3 + Zn + B; T9: T4 + Zn + B; T10: T5 + Zn + B; T11: RDF (NPK); and T12: RDF (NPK) + Zn + B, *Zn @25kg ha⁻¹; B @10kg ha⁻¹ and RDF (120:60:40 kg ha⁻¹ of N: P2O5: K2O). Data in the graph followed by different letters are significantly different at $p \leq 0.05$ as analyzed by Tukey's honest significant difference (Tukey's HSD) test). Error bars represent $\pm$ standard error of the mean.

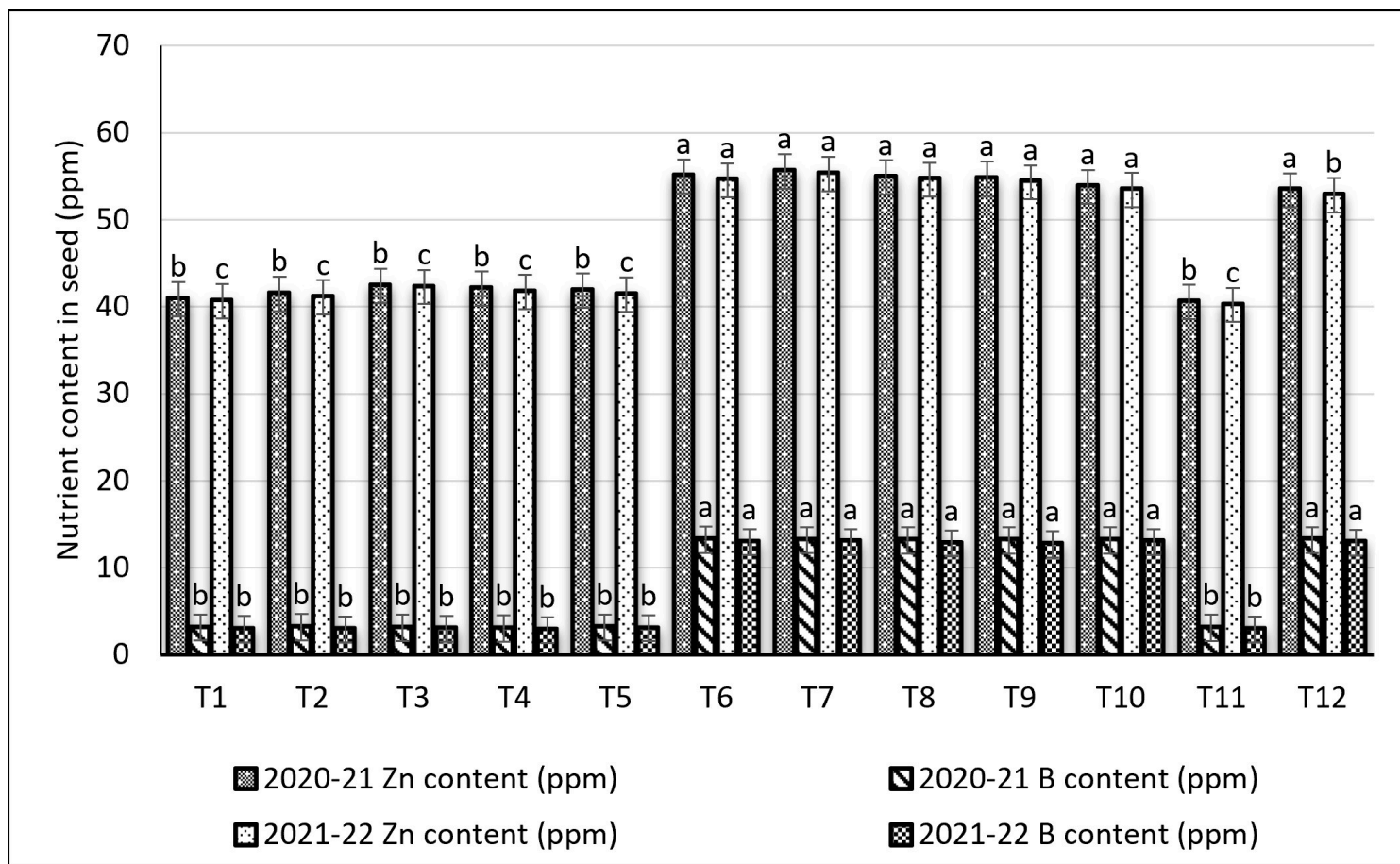


Figure 3

Fig. 2 Effect of nitrogen scheduling along with Zn and B on Zn and B content in seed at harvest during 2020-21 and 2021-22

(#T1: N @30, 30, 30, 30 kg ha⁻¹ at 15, 30, 45, 60 DAS respectively; T2: N @30, 30, 30 kg ha⁻¹ at 15, 30, 45 DAS respectively; T3: N @30, 60, 30 kg ha⁻¹ at 15, 30, 45 DAS respectively; T4: N @30, 30, 30, 30 kg ha⁻¹ at 0, 15, 30, 45 DAS respectively; T5: N @30, 30, 30 kg ha⁻¹ at 0, 15, 30 DAS respectively; T6: T1 + Zn + B; T7: T2 + Zn + B; T8: T3 + Zn + B; T9: T4 + Zn + B; T10: T5 + Zn + B; T11: RDF (NPK); and T12: RDF (NPK) + Zn + B, *Zn @25kg ha⁻¹; B @10kg ha⁻¹ and RDF (120:60:40 kg ha⁻¹ of N: P2O5: K2O). Data in the graph followed by different letters are significantly different at $p \leq 0.05$ as analyzed by Tukey's honest significant difference (Tukey's HSD) test). Error bars represent $\pm$ standard error of the mean.

Supplementary Files

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