



RESEARCH ARTICLE

Synergistic impact of zinc, iron, and nitrogen on nutrient availability and soil health for sustainable mustard (*Brassica juncea* L.) cultivation

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Abstract

The rising incidence of chronic diseases, such as cancer, osteoporosis, and cardiovascular disorders, is associated with micronutrient deficiencies. Significant shortages in iron, folate, zinc, iodine, and vitamin A lead to several health complications, highlighting the necessity for comprehensive preventive measures. Agronomic biofortification, especially by delivering micronutrients via foliar spraying, offers a viable method to improve crop nutritional value and alleviate malnutrition. This research investigated *Brassica juncea* L. (Indian mustard), an important oilseed crop in India, analyzing the impact of several fertilization treatments on soil and plant nutrient dynamics at Lovely Professional University during the winter season of 2022-2023. The field trial used a randomized block design with eight treatments, including control and combinations of urea, FeSO₄, and ZnSO₄. Soil tests indicated that foliar sprays enhanced nutrient availability, especially nitrogen, phosphorous, potassium, iron, and zinc, improving soil health and crop nutrient absorption. The findings demonstrated that integrating urea with micronutrient sprays optimized plant nutrient concentration and absorption in *Brassica juncea*, enhancing crop performance. Maximum soil pH, EC, organic carbon, and nutrient availability (N, P, K, S, Fe, Zn) were observed with RDF combined with 1 % urea, 0.5 % ZnSO₄·7H₂O, and 0.5 % FeSO₄·7H₂O foliar sprays at 45 DAS. Nutrient uptake and biofortification efficiency indices were significantly enhanced under these treatments, promoting plant growth and productivity. The control group showed the lowest values across all parameters. The results highlight the promise of agronomic biofortification in mitigating micronutrient deficits and enhancing food security, especially in areas dependent on staple crops for sustenance. This study promotes incorporating micronutrient management measures in agricultural practices to improve sustainable development and public health outcomes.

Keywords

biofortification; micro-nutrients; sustainability; soil health

Introduction

The increase in numerous chronic diseases such as cancer, osteoporosis, cardiovascular disorders, compromised immunity, and cognitive impairments is attributable to micronutrient shortages and deficiencies (1). Nutritional deficits in humans and inadequacies are widespread in the United States and other developing nations. The deficiency of any metabolic system component adversely impacts individuals and societies, resulting in

diminished health, reduced productivity, decreased educational attainment, and lower income potential (2). Iron, folate, zinc, iodine, and vitamin A are prevalent micronutrient deficiencies globally, all of which lead to cognitive impairment, stunted growth, prenatal difficulties, and increased morbidity and death (3). Preventing micronutrient deficits is essential and can be accomplished by supplementation and dietary strategies.

Brassica juncea L. is an essential oilseed crop that supplies vital nutrients to humans. In India, Rabi season crops are currently ranked as the world's third most significant oilseed crop in production and acreage. In India during 2021–22, mustard covered 29.17 m ha of the area and contributed 37.70 million tonnes to world production. Rapeseed and mustard were grown on 43.9 thousand hectares, producing 69.3 thousand tonnes during 2021–22 in the Punjab State. The paramount factors of Indian mustard are its oil yield and quality, predominantly influenced by the mineral fertilization of the plants (4). The intensity of cropping rises with the adoption of high-yielding cultivars and diminishes with the application of micronutrient-deficient fertilizers. Numerous oilseed crops' yields and nutrient contents have diminished due to micronutrient deficiencies in Indian soils, making them unsuitable for human consumption.

N is a crucial nutrient for cellular division, growth, photosynthesis, and protein synthesis. It also establishes a basis for enhancing yield, nutrient buildup, and the quality of oilseed crops, especially those from India (6). The application of nitrogen significantly influences the response of mustard crops (7). Among all biofortification approaches, foliar treatment is the most effective method for enhancing the micronutrient content in crops, as it delivers nutrients to the leaves during optimal growth stages (8–10). The crop output has markedly improved due to the nitrogen provided by the foliar spray (12–13). Over 50 % of the global population experiences shortages in zinc and iron due to their dependence on cereal crops, namely rice, maize, and wheat, for sustenance (14). Agronomic biofortification is the foremost agricultural strategy to enhance grain iron and zinc content in the fight against malnutrition. This is achieved by directly applying micronutrients

to the crop leaves or soil. (15–16). It can be readily adopted, proving more sustainable and economical than alternative strategies, including genetic engineering (17–19).

Materials and Methods

Preliminary Information

In the Rabi season of 2022–2023, this field experiment was carried out at Lovely Professional University's experimental field in Phagwara, Punjab. The agricultural area is located in the Northern Plain region, with coordinates at Latitude 31° N and Longitude 75.25° E. All intercultural activities followed the standards established by Punjab Agricultural University (PAU), Ludhiana, to ensure optimal growth (20). Weather data over the entire study duration referenced in Fig. 1. The experiment was conducted on sandy, loamy soil, consisting of 24 plots, each measuring 5 × 5 m, with a row spacing of 30 × 30 cm. Before the experiment, soil testing was done to evaluate its essential properties. The GHM-503 variety was sown on Nov 22, 2022. The experiment laid out in a Randomized Block Design (RBD) with eight treatments, each replicated 3 times: T₁: Control (recommended NPK only), T₂: RDF + 1 % Urea foliar spray at 45 DAS, T₃: RDF + 0.5 % FeSO₄·7 H₂O foliar spray at 45 DAS, T₄: RDF + 0.5 % ZnSO₄·7H₂O foliar spray at 45 DAS, T₅: RDF + 0.5 % FeSO₄·7H₂O + 0.5 % ZnSO₄·7H₂O foliar spray at 45 DAS, T₆: RDF + 1 % Urea foliar spray + 0.5 % FeSO₄·7 H₂O at 45 DAS, T₇: RDF + 1 % Urea foliar spray + 0.5 % ZnSO₄·7 H₂O at 45 DAS, T₈: RDF + 1 % Urea foliar spray + 0.5 % ZnSO₄ + 0.5 % FeSO₄ at 45 DAS. The soil was amended with Urea and Single Super Phosphate (SSP) as a basal application. At 45 days after sowing (DAS), the crop received Urea, FeSO₄, and ZnSO₄ through foliar treatment utilizing a backpack sprayer. Crop protection measures were executed as required using a knapsack sprayer. The soil conditions before and after sowing, together with nutrient concentrations and their uptake by the plants and seeds of the crops, have been recorded. Observations were conducted according to standard procedures, with average data recorded from five randomly selected plants in each plot to analyze the treatment effects.

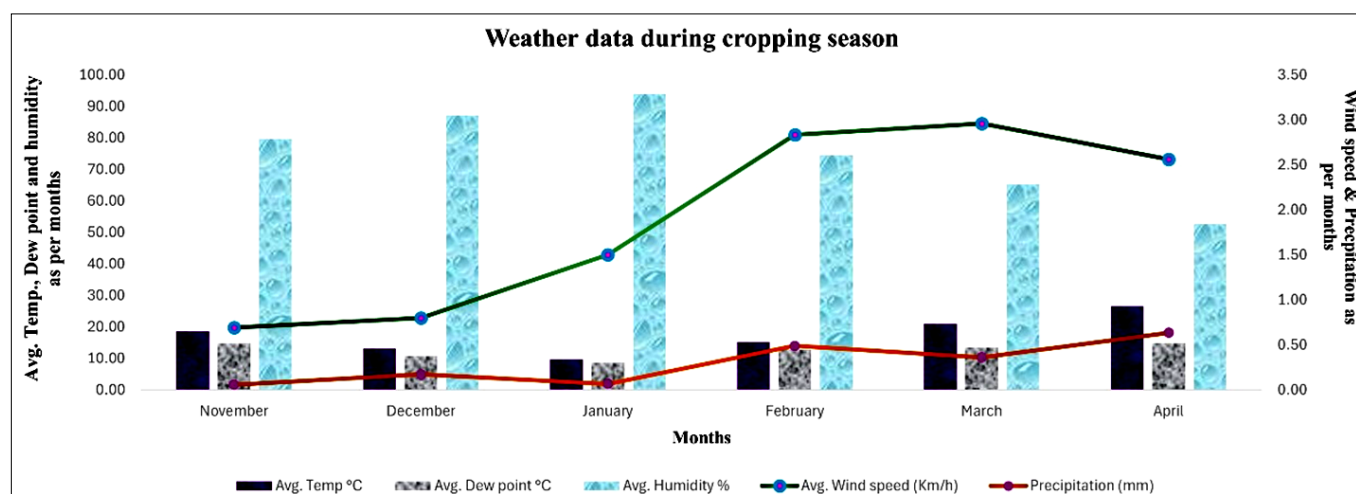


Fig. 1. Weather data (temperature °C, dew point °C, humidity %, wind speed km/hr and precipitation (mm) during cropping period from November 2022- April 2023.

Soil Parameters

Soil samples from each plot were obtained before and after crop seeding to ascertain the available nutrients and were subsequently dried at room temperature for 72 hrs. The soil's pH was measured with a pH meter, soil electrical conductivity (EC) was assessed using an EC meter, and organic carbon (OC) was determined (21). The Kjeldahl method was employed to ascertain the available nitrogen in the soil. The standard process described by (21) was employed to evaluate available phosphorus (P) and potassium (K). The amounts of phosphorus and potassium were assessed using a visible spectrophotometer and a flame photometer. The available sulfur was quantified using the Turbidimetric technique with a spectrophotometer.

Uptake and Concentration of Nutrients on plants and seed samples

To determine nutrient contents and the uptake by plant samples, shoot and grain samples were collected and desiccated in a hot air oven at 70°C for 24 to 48 hours. Upon drying, the materials were thoroughly crushed. The micro Kjeldahl method determined the nitrogen concentration in the plant and seed samples. The di-acid yellow colour method was utilized for the determination of phosphorus (P) and potassium (K) (21). A visible spectrophotometer and a flame photometer were employed to quantify the amounts of phosphorus, potassium, and sulfur.

Efficiency indices

The mobilization efficiency index (MEI) was calculated by using the following equation:

$$\frac{\text{Nutrient concentration in seed}}{\text{Nutrient concentration in plant}} \dots\dots(\text{Eqn.1})$$

The apparent recovery efficiency (ARE) is determined with equation 2 (22).

$$\text{ARE} = \frac{\text{Nut} - \text{Nuc}}{\text{Nutrient applied kg/ha}} \times 100 \dots\dots(\text{Eqn.2})$$

Statistical analysis

The data were examined utilizing analysis of variance (ANOVA) with SPSS version 20.0 statistical software (SPSS Inc.). The means were further analyzed using Duncan's multiple-range tests. A p-value below 0.05 was considered statistically significant, and the findings are shown as the mean value plus or minus the standard deviation (with a 95 % confidence range).

Results

Effect of fertilizer urea, FeSO₄, and ZnSO₄ on Soil parameters

The fundamental properties of the soil, including pH, electrical conductivity (EC), organic carbon (OC), and the availability of nitrogen (N), phosphorus (P), potassium (K), sulfur (S), iron (Fe), and zinc (Zn), were documented as follows: 7.5, 0.18 ds m⁻¹, 0.72 %, 196 kg ha⁻¹, 11.7 kg ha⁻¹,

15.1 kg ha⁻¹, and 210 kg ha⁻¹, respectively. Soil pH influences the accessibility of vital nutrients to plants. The pH scale quantifies the acidity or alkalinity of the soil, often ranging from 0 to 14, with 7 denoting neutralities. The activity of beneficial soil bacteria is affected by pH, which influences soil health and plant growth. The maximum soil pH of 7.93 was observed with RDF + 1 % Urea Foliar Spray + 0.5 % ZnSO₄·7H₂O + 0.5 % FeSO₄·7H₂O at 45 days after sowing (DAS), whereas the second highest pH of 7.80 was recorded with RDF + 0.5 % FeSO₄·7H₂O + 0.5 % ZnSO₄·7H₂O foliar spray at 45 DAS. The minimum recorded value is 6.93 with control. The assessment of electrical conductivity in soil is crucial for nutrient availability. It signifies excessively high or low electrical conductivity, which may restrict plant nutrient absorption. Comprehending the electrical conductivity of soil aids in the management of irrigation operations, particularly in regions with variable water quality. The maximum electrical conductivity recorded was 0.24 dSm⁻¹ using RDF. At 45 days after sowing (DAS), a foliar spray of 1 % urea, 0.5 % ZnSO₄·7H₂O, and 0.5 % FeSO₄·7H₂O yielded the maximum conductivity at 0.22 dSm⁻¹, followed by the combination of the recommended dose of fertilizers (RDF) with 0.5 % FeSO₄·7H₂O and 0.5 % ZnSO₄. The maximum OC of 0.65 % was observed using RDF. 1 % Urea Foliar Spray combined with 0.5 % ZnSO₄·7H₂O and 0.5 % FeSO₄·7H₂O at 45 days after sowing (DAS) yielded the second highest concentration of 0.640 % when applied with recommended dose of fertilizers (RDF) with 0.5 % FeSO₄·7H₂O and 0.5 % ZnSO₄·7H₂O foliar spray at 45 DAS. The lowest OC of 0.45 % was observed in the Control group. Nitrogen availability impacts microbial activity in the soil, thus influencing the nutrient cycle. An optimal nitrogen concentration enhances overall soil fertility and health. The maximum nitrogen yield (477.26 kg ha⁻¹) was seen with RDF combined with a 1 % urea foliar spray, 0.5 % ZnSO₄·7H₂O, and 0.5 % FeSO₄·7H₂O at 45 days after sowing (DAS), whereas the second greatest yield (442.0 kg ha⁻¹) was achieved with RDF, 0.5 % ZnSO₄·7H₂O, and 0.5 % FeSO₄·7H₂O at 45 DAS. The minimum recorded nitrogen level was 396.12 kg ha⁻¹ in the Control group. Phosphorus is crucial for robust root development and overall plant vitality. It is an essential plant nutrient, facilitating energy transfer, photosynthesis, and root formation. The maximum phosphorus (21.27 kg ha⁻¹) was seen with RDF combined with 1 % urea foliar spray, 0.5 % ZnSO₄·7H₂O, and 0.5 % FeSO₄·7H₂O at 45 days after sowing (DAS), while the second highest (18.88 kg ha⁻¹) was achieved with RDF, 0.5 % ZnSO₄·7H₂O, and 0.5 % FeSO₄·7H₂O at 45 DAS. The minimum phosphorus (12.49 kg ha⁻¹) was observed in the Control plot. Potassium is an essential nutrient for plant development, influencing water control, photosynthesis, and enzyme activation. The maximum potassium level (295.46 kg ha⁻¹) was observed with RDF combined with 1 % urea foliar spray and 0.5 % ZnSO₄·7H₂O and 0.5 % FeSO₄·7H₂O at 45 DAS. The second highest level (256.46 kg ha⁻¹) was recorded with RDF in conjunction with 0.5 % FeSO₄·7H₂O and 0.5 % ZnSO₄·7H₂O foliar spray at 45 DAS. The minimum available potassium (227.62 kg ha⁻¹) corresponds to the Control treatment. Sulfur is essential for protein synthe-

sis, enzymatic activity, and chlorophyll production. It is crucial to the metabolic processes of plants. The maximum recorded S was 28.19 kg ha⁻¹, achieved with RDF + 1 % Urea Foliar Spray at 45 DAS, together with 0.5 % ZnSO₄·7H₂O and 0.5 % FeSO₄·7H₂O, followed by the second highest. 22.44 kg ha⁻¹ with RDF + 0.5 % ZnSO₄·7H₂O + 0.5 % FeSO₄·7H₂O at 45 days after sowing. The minimum recorded S was 11.10 kg ha⁻¹ under the Control condition. Iron (Fe) is a vital element in soil, significantly influencing plant growth and development. Ensuring sufficient iron concentrations in soil is essential for good plant vitality. Monitoring soil conditions and regulating pH, organic matter, and nutrient balance can facilitate enough iron availability for crops. The maximum Fe concentration of 35.18 kg ha⁻¹ was recorded under RDF. 1 % Urea Foliar Spray at 45 DAS combined with 0.5 % ZnSO₄·7H₂O and 0.5 % FeSO₄·7H₂O resulted in a yield of 33.82 kg ha⁻¹, which is the second highest, alongside the recommended dose of fertilizers (RDF) with the same chemicals at 45 DAS. The minimum observed Fe was 20.45 kg ha⁻¹ with the Control treatment. Zinc is essential for optimal plant growth and development. Regulating soil pH, enhancing organic matter, and observing nutrient interactions are useful methods for sustaining sufficient zinc levels in the soil. The maximum available zinc is 11.97 kg ha⁻¹ with a recommended dose of fertilizers. 1 % Urea Foliar Spray at 45 days after sowing (DAS) combined with 0.5 % ZnSO₄·7H₂O and 0.5 % FeSO₄·7H₂O resulted in the second best of 11.50 kg ha⁻¹, alongside the recommended dose of fertilizers (RDF) with the same concentrations of ZnSO₄·7H₂O and FeSO₄·7H₂O at 45 DAS. The minimum available Zn is 3.07 kg ha⁻¹ with control, as shown in Table 1.

Effect of fertilizer Urea, FeSO₄, and ZnSO₄ on nutrient content (%) and nutrient uptake (kg ha⁻¹) by seed and plant of *Brassica juncea*

Applying zinc, iron, and urea can substantially improve Gobhi Sarson's nitrogen, phosphorus, potassium, and sulfur levels and absorption, hence fostering enhanced plant health and productivity. In plant nutrition, nitrogen, phosphorus, potassium, and sulfur are essential for enhancing growth and maximizing crop yield. Nitrogen is a crucial element of amino acids, the constituents of proteins, which are vital for numerous physiological functions. One is chlorophyll synthesis, the pigment that facilitates photosynthesis, enabling plants to transform sunlight into energy. Phosphorus is an essential constituent of ATP (adenosine triphosphate), the cellular energy currency, enabling energy transmission in metabolic activities. It fosters robust root development, facilitating plant establishment and enhancing nutrition and water absorption. Potassium promotes the transfer of other minerals and carbohydrates inside the plant, ensuring optimal distribution and utilization. It stimulates many enzymes in photosynthesis, respiration, and protein synthesis, facilitating critical metabolic operations. Sulphur can affect the absorption and metabolism of other nutrients, enhancing total nutritional efficiency. Numerous enzymes necessitate sulfur for their functionality, serving essential roles in metabolic activities such as photosynthesis and respiration. The highest nutrient absorption in *Brassica juncea* was observed under RDF. Application of 1 % Urea Foliar Spray, 0.5 % ZnSO₄·7H₂O, and 0.5 % FeSO₄·7H₂O at 45 days after sowing resulted in nutrient absorption of N (51.37 kg ha⁻¹), P (34.08 kg ha⁻¹), K (91.92 kg ha⁻¹), and S (30.14 kg ha⁻¹).

Table 1. Effect of Zn, Fe and N on available pH, EC, organic carbon, nitrogen, phosphorus, potassium, sulphur, iron and zinc (kg ha⁻¹) of soil

Treatments	pH	EC (dSm ⁻¹)	Organic carbon (%)	Nitrogen (kg ha ⁻¹)	Phosphorus (kg ha ⁻¹)	Potassium (kg ha ⁻¹)	Sulphur (kg ha ⁻¹)	Iron (kg ha ⁻¹)	Zinc (kg ha ⁻¹)
Control (recommended NPK only)	6.93 ^d ±0.12	0.11 ^f ±0.01	0.45 ^f ±0.03	396.12 ^f ±1.99	12.49 ^f ±0.36	227.62 ^f ±0.60	11.10 ^g ±0.20	20.45 ^f ±0.28	3.07 ^c ±0.21
RDF+1 % Urea foliar spray at 45DAS	7.03 ^d ±0.06	0.13 ^{ef} ±0.01	0.50 ^e ±0.01	401.81 ^f ±1.81	14.16 ^f ±0.52	237.36 ^e ±1.98	15.21 ^f ±0.23	22.87 ^e ±0.89	3.19 ^c ±0.15
RDF + 0.5 % FeSO ₄ · 7 H ₂ O foliar spray at 45 DAS	7.23 ^c ±0.06	0.14 ^{def} ±0.02	0.53 ^d ±0.02	409.45 ^e ±1.69	15.10 ^{ef} ±0.39	237.67 ^e ±0.74	18.22 ^e ±0.06	28.69 ^d ±0.82	3.49 ^c ±0.17
RDF + 0.5 % ZnSO ₄ · 7H ₂ O foliar spray at 45 DAS	7.23 ^c ±0.06	0.15 ^{de} ±0.01	0.55 ^{cd} ±0.01	413.37 ^e ±1.95	15.67 ^{de} ±0.27	243.07 ^d ±2.02	20.11 ^d ±0.26	29.55 ^{cd} ±0.69	3.58 ^c ±0.13
RDF + 0.5 % FeSO ₄ · 7 H ₂ O + 0.5 % ZnSO ₄ · 7H ₂ O foliar spray at 45 DAS	7.80 ^a ±0.10	0.22 ^{ab} ±0.02	0.64 ^a ±0.02	442.0 ^b ±3.35	18.88 ^b ±0.71	256.46 ^b ±0.82	22.44 ^b ±0.23	33.82 ^a ±1.45	11.50 ^a ±1.01
RDF + 1 % Urea Foliar spray + 0.5 % FeSO ₄ · 7H ₂ O at 45 DAS	7.47 ^b ±0.12	0.16 ^{cd} ±0.02	0.58 ^{bc} ±0.01	420.96 ^d ±2.18	16.76 ^{cd} ±0.48	247.32 ^c ±2.31	21.73 ^c ±0.22	30.88 ^{bc} ±0.61	7.07 ^b ±0.51
RDF + 1 % Urea foliar spray + 0.5 % ZnSO ₄ · 7H ₂ O at 45 DAS	7.50 ^b ±0.10	0.19 ^{bc} ±0.02	0.61 ^{ab} ±0.01	428.13 ^c ±4.62	17.99 ^{bc} ±0.93	249.59 ^c ±0.68	22.40 ^b ±0.11	31.36 ^b ±0.36	10.83 ^a ±0.70
RDF +1 % Urea Foliar Spray 45 DAS + 0.5 % ZnSO ₄ · 7H ₂ O + 0.5 % FeSO ₄ · 7H ₂ O at 45DAS	7.93 ^a ±0.15	0.24 ^a ±0.03	0.65 ^a ±0.01	477.26 ^a ±4.86	21.27 ^a ±0.82	295.46 ^a ±0.69	28.19 ^a ±0.17	35.18 ^a ±1.65	11.97 ^a ±1.07

In contrast, the control exhibited the lowest nutrient uptake with N (14.64 kg ha^{-1}), P (4.62 kg ha^{-1}), K (16.72 kg ha^{-1}), and S (9.17 kg ha^{-1}). The maximum overall nutrient absorption in *Brassica juncea* grain was observed under RDF. 1 % Urea Foliar Spray at 45 Days After Sowing, along with 0.5 % Zinc Sulfate Heptahydrate and 0.5 % Iron Sulfate. At 45 DAS, the treatment with $7\text{H}_2\text{O}$ exhibited nutrient uptake of nitrogen (N) at 94.82 kg ha^{-1} , phosphorus (P) at 91.40 kg ha^{-1} , potassium (K) at 26.71 kg ha^{-1} , and sulfur (S) at 13.69 kg ha^{-1} . In contrast, the control group showed the lowest nutrient uptake, with N at 16.45 kg ha^{-1} , P at 17.93 kg ha^{-1} , K at 12.16 kg ha^{-1} , and S at 15.15 kg ha^{-1} . The nutritional content of the *Brassica juncea* plant was analyzed under RDF conditions. +1 % Urea Foliar + 0.5 % Zinc Sulfate Heptahydrate + 0.5 % $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ at 45DAS with N (0.91 %), P (0.61 %), K (1.64 %), S (0.54 %), succeeded by RDF + 0.5 % $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ + 0.5 % ZnSO_4 . At 45 days after sowing, a $7\text{H}_2\text{O}$ foliar spray resulted in nitrogen (0.78 %), phosphorus (0.47 %), potassium (1.53 %), and sulfur (0.49 %). In contrast, the control exhibited the lowest levels of nitrogen (0.32 %), phosphorus (0.10 %), potassium (0.36 %), and sulfur (0.21 %). The highest total amount of N (3.31 %), P (3.19 %), K (0.93 %), and S (0.48 %) in *Brassica juncea* grain was observed under RDF. Application of 1 % urea foliar spray, 0.5 % $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.5 % $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ at 45 days after sowing (DAS), followed by recommended dose of fertilizers (RDF) combined with 0.5 % $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and 0.5 % $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ foliar spray at the same interval, includ-

ing nitrogen (3.20 %), phosphorus (2.78 %), potassium (0.88 %), and sulfur (0.44 %). Conversely, the minimum nutrient content was noted in the control group, with N (0.82 %), P (0.90 %), K (0.61 %), and S (0.26 %), as illustrated in Tables 2 & 3.

Effect of biofortification on efficiency indices

Alongside the prescribed fertilizer dosages (RDF), Table 4 shows information on nutrient availability (ARE-N, ARE-P, ARE-S) and nutrient efficiency indices (MEI-N, MEI-P, MEI-S) for various treatments in an experiment employing diverse foliar sprays. The treatment exhibiting the most significant overall nutrient availability is RDF combined with 1 % urea, iron, and zinc. It is especially efficacious for nitrogen (ARE-N=51.15) and sulfur (ARE-S=36.21), although phosphorus (ARE-P=1.32) exhibits markedly low levels. The levels of accessible nitrogen (ARE-N=2.56), phosphorus (ARE-P=9.00), and sulfur (ARE-S=1.24) are relatively low in the control group (NPK alone). The optimal nitrogen efficiency is achieved with RDF combined with 1 % urea and 0.5 % FeSO_4 (MEI-N=4.76). Reduced phosphorus efficiency (5.23) is observed with RDF + 1 % Urea + Fe + Zn, which suggests greater phosphorus availability rather than effective utilization. The Sulphur efficiency of the control treatment is 1.24, whereas the other treatments typically have lower efficiency, ranging from 0.79 to 1.07, suggesting that diminished efficiency correlates with increased availability. The correlation between variables in a two-dimensional

Table 2. Effect of Zn, Fe and urea on total nitrogen, phosphorus, potassium, sulphur uptake (kg ha^{-1}) on *Brassica juncea* seed and plant

Treatments	Nitrogen uptake by plant	Nitrogen uptake by seed	Phosphorus Uptake by plant	Phosphorus uptake by seed	Potassium uptake by plant	Potassium uptake by seed	Sulphur uptake by plant	Sulphur Uptake by seed
Control (Recommended NPK only)	$14.64^e \pm 5.29$	$16.45^f \pm 2.25$	$4.62^f \pm 0.48$	$17.93^g \pm 1.19$	$16.72^g \pm 1.79$	$12.16^e \pm 1.73$	$9.71^f \pm 1.42$	$5.15^f \pm 1.03$
RDF+1 % Urea foliar spray at 45DAS	$20.18^{de} \pm 5.35$	$31.03^e \pm 5.71$	$5.18^f \pm 0.48$	$19.84^g \pm 1.33$	$23.99^f \pm 1.46$	$14.04^{de} \pm 1.50$	$12.54^e \pm 0.93$	$6.27^{ef} \pm 1.26$
RDF + 0.5 % $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ foliar spray at 45 DAS	$25.00^{cde} \pm 6.48$	$45.82^d \pm 10.46$	$7.82^e \pm 0.66$	$24.28^f \pm 1.15$	$36.09^e \pm 1.56$	$15.88^{cd} \pm 0.82$	$18.52^d \pm 1.31$	$7.34^{de} \pm 0.72$
RDF + 0.5 % $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ foliar spray at 45 DAS	$30.26^{bcd} \pm 6.67$	$63.92^c \pm 10.49$	$12.63^d \pm 0.71$	$28.00^e \pm 1.39$	$44.99^d \pm 1.89$	$18.36^c \pm 0.93$	$20.34^{cd} \pm 1.75$	$9.14^{cd} \pm 0.56$
RDF + 0.5 % $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ + 0.5 % $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ foliar spray at 45 DAS	$41.43^{ab} \pm 6.57$	$90.86^{ab} \pm 3.88$	$24.87^b \pm 2.07$	$79.04^b \pm 2.48$	$81.09^b \pm 1.59$	$22.09^b \pm 1.24$	$26.14^c \pm 0.19$	$12.62^{ab} \pm 1.45$
RDF + 1 % Urea Foliar spray + 0.5 % $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ at 45 DAS	$31.21^{bcd} \pm 1.81$	$78.91^b \pm 12.42$	$17.92^c \pm 0.24$	$41.06^d \pm 1.14$	$46.88^d \pm 2.28$	$20.04^{bc} \pm 0.89$	$21.45^b \pm 1.48$	$10.59^{bc} \pm 0.77$
RDF + 1 % Urea foliar spray + 0.5 % $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ at 45 DAS	$34.59^{bc} \pm 1.46$	$87.89^{ab} \pm 1.21$	$18.90^c \pm 0.41$	$56.48^c \pm 1.44$	$75.96^c \pm 1.50$	$22.18^b \pm 1.52$	$24.89^b \pm 0.51$	$11.65^{ab} \pm 1.22$
RDF +1 % Urea Foliar Spray 45 DAS + 0.5 % $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ + 0.5 % $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ at 45DAS	$51.37^a \pm 9.80$	$94.82^a \pm 3.29$	$34.08^a \pm 3.14$	$91.40^a \pm 3.50$	$91.92^a \pm 3.85$	$26.71^a \pm 2.60$	$30.14^a \pm 1.60$	$13.69^a \pm 1.60$

Table 3. Effect of Zn, Fe and urea on total Nitrogen, Phosphorus, Potassium, and Sulphur content (%) in *Brassica juncea* plant and seed

Treatments	Nitrogen (%) in plant	Nitrogen (%) in seed	Phosphorus (%) in seed	Phosphorus (%) in seed	Potassium (%) in seed	Potassium (%) in seed	Sulphur (%) in seed	Sulphur (%) in seed
Control (Recommended NPK only)	0.32 ^e ± 0.12	0.82 ^e ± 0.09	0.10 ^f ± 0.01	0.90 ^g ± 0.02	0.36 ^f ± 0.04	0.61 ^e ± 0.05	0.21 ^d ± 0.03	0.26 ^d ± 0.04
RDF+1 % Urea foliar spray at 45DAS	0.43 ^{de} ± 0.12	1.42 ^d ± 0.20	0.11 ^f ± 0.01	0.91 ^g ± 0.02	0.51 ^e ± 0.03	0.64 ^d ± 0.03	0.27 ^c ± 0.02	0.29 ^c ± 0.05
RDF + 0.5 % FeSO ₄ · 7 H ₂ O foliar spray at 45 DAS	0.52 ^{cde} ± 0.14	1.94 ^c ± 0.48	0.16 ^e ± 0.02	1.02 ^f ± 0.05	0.75 ^d ± 0.03	0.67 ^{cde} ± 0.04	0.39 ^b ± 0.03	0.31 ^b ± 0.04
RDF + 0.5 % ZnSO ₄ · 7H ₂ O foliar spray at 45 DAS	0.62 ^{bcd} ± 0.15	2.52 ^b ± 0.46	0.26 ^d ± 0.02	1.10 ^e ± 0.08	0.91 ^c ± 0.03	0.72 ^{bcd} ± 0.04	0.41 ^b ± 0.04	0.36 ^b ± 0.03
RDF + 0.5 % FeSO ₄ · 7 H ₂ O + 0.5 % ZnSO ₄ · 7H ₂ O foliar spray at 45 DAS	0.78 ^{ab} ± 0.15	3.20 ^a ± 0.10	0.47 ^b ± 0.05	2.78 ^b ± 0.02	1.53 ^b ± 0.05	0.80 ^b ± 0.03	0.49 ^a ± 0.02	0.44 ^a ± 0.04
RDF + 1 % Urea Foliar spray + 0.5 % FeSO ₄ · 7H ₂ O at 45 DAS	0.62 ^{bcd} ± 0.06	2.95 ^{ab} ± 0.42	0.35 ^c ± 0.02	1.54 ^d ± 0.02	0.92 ^c ± 0.02	0.75 ^{bc} ± 0.02	0.42 ^b ± 0.04	0.40 ^b ± 0.03
RDF + 1 % Urea foliar spray + 0.5 % ZnSO ₄ · 7H ₂ O at 45 DAS	0.67 ^{bc} ± 0.06	3.17 ^a ± 0.09	0.37 ^c ± 0.02	2.04 ^c ± 0.01	1.47 ^b ± 0.05	0.78 ^b ± 0.02	0.48 ^a ± 0.03	0.42 ^a ± 0.04
RDF +1 % Urea Foliar Spray 45 DAS + 0.5 % ZnSO ₄ · 7H ₂ O + 0.5 % FeSO ₄ · 7H ₂ O at 45DAS	0.91 ^a ± 0.17	3.31 ^a ± 0.06	0.61 ^a ± 0.05	3.19 ^a ± 0.02	1.64 ^a ± 0.06	0.93 ^a ± 0.05	0.54 ^a ± 0.03	0.48 ^a ± 0.04

Table 4: Effect biofortification on efficiency indices (Apparent recovery efficiency, mobilization efficiency index (MEI) of mustard

Treatments	ARE-N (%)	ARE-P (%)	ARE-S (%)	MEI-N (%)	MEI-P (%)	MEI-S (%)
Control (recommended NPK only)	-	-	-	2.56 ^d ± 0.24	9.00 ^a ± 0.56	1.24 ^a ± 0.006
RDF+1 % Urea foliar spray at 45DAS	8.94 ^b ± 1.00	1.32 ^f ± 1.21	4.93 ^g ± 0.36	3.30 ^{bc} ± 0.14	8.27 ^{ab} ± 0.92	1.07 ^{ab} ± 0.009
RDF + 0.5 % FeSO ₄ · 7 H ₂ O foliar spray at 45 DAS	17.6 ^g ± 0.67	5.10 ^e ± 1.24	13.75 ^f ± 0.24	3.73 ^c ± 0.25	6.38 ^c ± 0.53	0.79 ^b ± 0.016
RDF + 0.5 % ZnSO ₄ · 7H ₂ O foliar spray at 45 DAS	28.04 ^f ± 0.49	9.66 ^d ± 0.83	18.27 ^e ± 0.85	4.06 ^{ab} ± 0.22	4.23 ^e ± 0.73	0.88 ^b ± 0.009
RDF + 0.5 % FeSO ₄ · 7 H ₂ O + 0.5 % ZnSO ₄ · 7H ₂ O foliar spray at 45 DAS	44.97 ^b ± 0.58	43.50 ^a ± 0.87	29.87 ^b ± 0.50	4.10 ^{ab} ± 0.09	5.91 ^d ± 0.71	0.90 ^b ± 0.029
RDF + 1 % Urea Foliar spray + 0.5 % FeSO ₄ · 7H ₂ O at 45 DAS	35.124 ^d ± 0.42	19.48 ^c ± 1.02	21.47 ^d ± 0.50	4.76 ^a ± 0.31	4.40 ^e ± 1.07	0.95 ^b ± 0.049
RDF + 1 % Urea foliar spray + 0.5 % ZnSO ₄ · 7H ₂ O at 45 DAS	40.61 ^c ± 0.25	28.25 ^b ± 0.23	27.1 ^{bc} ± 0.08	4.73 ^a ± 0.08	5.51 ^d ± 0.76	0.83 ^b ± 0.070
RDF +1 % Urea Foliar Spray 45 DAS + 0.5 % ZnSO ₄ · 7H ₂ O + 0.5 % FeSO ₄ · 7H ₂ O at 45DAS	51.15 ^a ± 0.67	1.320 ^f ± 1.22	36.21 ^a ± 0.62	3.64 ^c ± 0.34	5.23 ^d ± 0.95	0.8 ^{ab} ± 0.004

space represented by PC1 and PC2 (Principal Component 1 and Principal Component 2) is illustrated in a biplot derived from a Principal Component Analysis (PCA). The horizontal axis represents PC1. The vertical axis represents PC2. The first two principal components account for the majority of the variance in the data. The magnitude and orientation of these vectors indicate the extent of influence each variable exerts on the respective principal component. ARE-N, ARE-P, and ARE-S are aligned with the negative aspects of PC1 and PC2, resulting in their collective detrimental impact on PC1. MEI-P's positioning on the positive side of PC1 signifies its substantial enhancement of PC1. MEI-S significantly contributes to the variation in PC2, aligning predominantly in its negative direction (Fig. 2).

Correlation matrix

The association matrix for the factors linked to nutritional efficiency indices (MEI-N, MEI-P, and MEI-S) and nutrient availability (ARE-N, ARE-P, and ARE-S) is shown in Fig.1. The degree and direction of the linear link between two variables are indicated by the Pearson correlation coefficient, which is represented by each value. The presence of

positive correlations among nutrient availability (ARE-N, ARE-P, and ARE-S) suggests that these nutrients—especially sulphur and nitrogen—are commonly absorbed simultaneously. Increased availability may result in lower nutrient efficiency, most likely due to luxury consumption, in which plants take in more nutrients than they require. This is suggested by negative correlations found between availability (ARE-N, ARE-S) and efficiency (MEI-P, MEI-S). Nutrient efficiencies have trade-offs, such as those between phosphorus and nitrogen, meaning that maximizing the efficiency of one nutrient may decrease the efficiency of another, as indicated in Fig. 3.

Discussion

Effect of fertilizer urea, FeSO₄, and ZnSO₄ on Soil parameters

Compared to the control, the highest soil properties were observed at 45DAS using RDF +1 % Urea Foliar Spray + 0.5 % ZnSO₄ · 7H₂O + 0.5 % FeSO₄ · 7H₂O. This is due to the advantageous effects of foliar application of Iron, Zinc, and

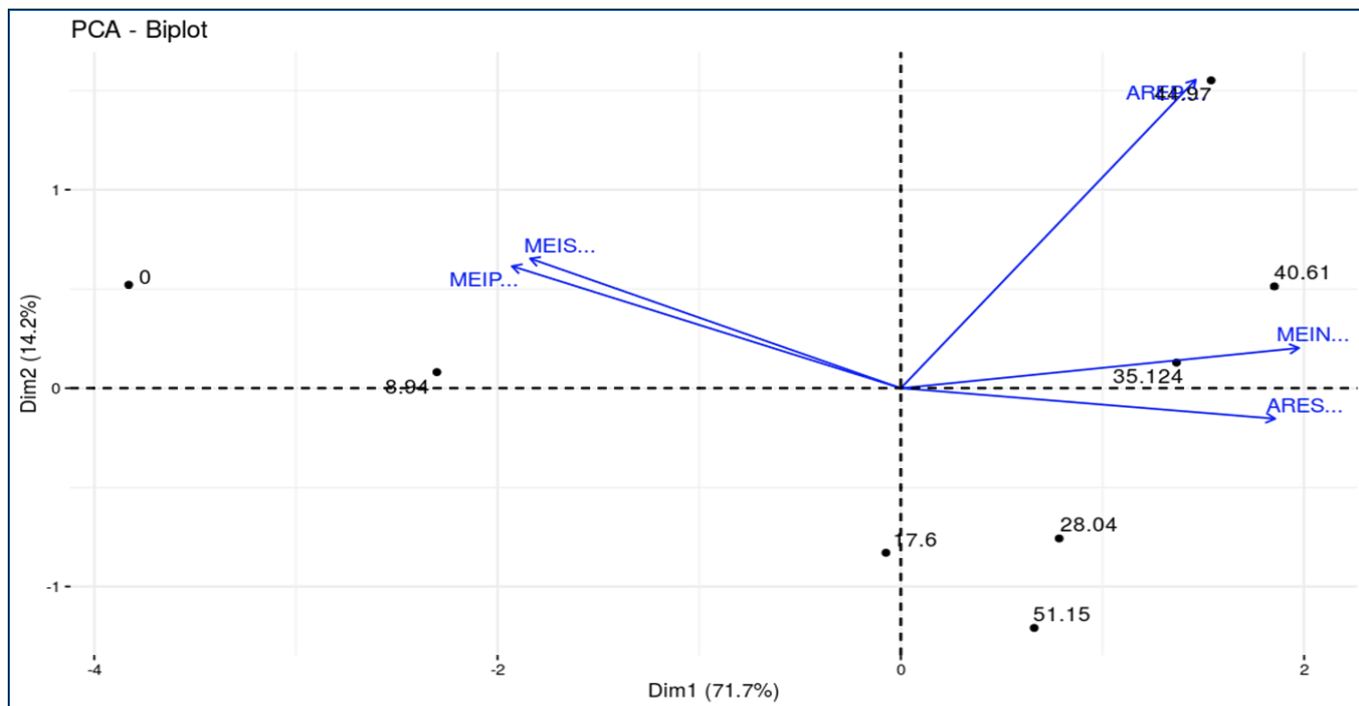


Fig. 2. This image is a biplot from a Principal Component Analysis (PCA), showing the relationship between variables in a two-dimensional space defined by PC1 and PC2 (Principal Component 1 and Principal Component 2). **PC1**, with a strong negative association, explains the variance related to nitrogen, phosphorus and sulphur availability (ARE-N, ARE-P and ARE-S). MEI-P has a strong positive association with PC1. **PC2** explains the variance associated with MEI-S, which has a significant negative contribution to this component.

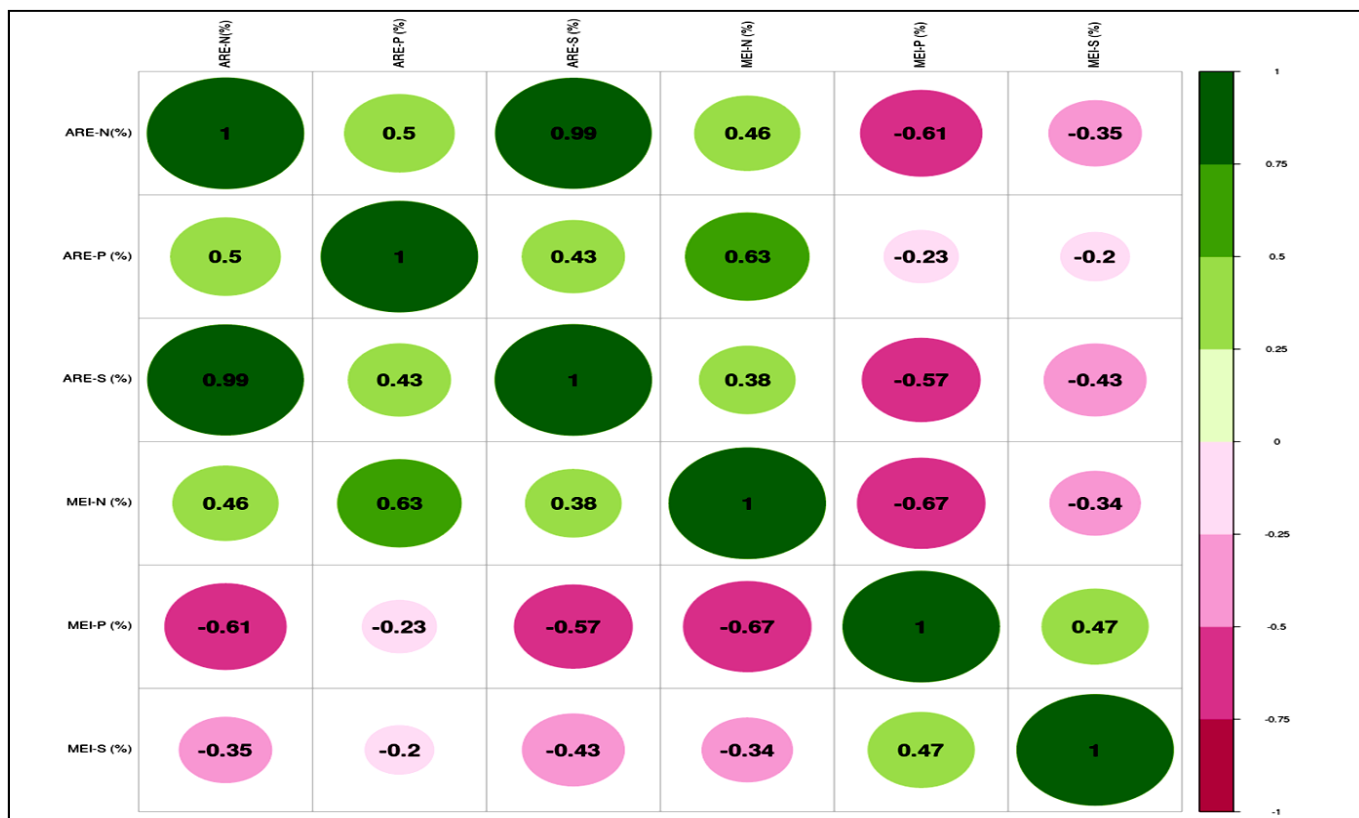


Fig.3. Correlogram and correlation matrix between efficiency indices.

Nitrogen, which improves the mineralization of both its nutrients and those native to the soil. It creates favourable conditions for microbial and chemical processes, enhancing the soil's available nutrient reservoir (7,8,23). Not all nutrients are assimilated by plants; the surplus nutrients remain in the soil, improving its nutrient profile post-harvest. The balanced fertilization of macro and micronutrients creates optimal conditions for microbial and chemical activities, resulting in enhanced nutrient availability in

the soil. Incorporating micronutrients such as Fe and Zn likely improved nutrient activation by mobilizing native nutrients, thereby increasing their accessibility.

Effect of fertilizer urea, FeSO_4 , and ZnSO_4 on nutrient content and uptake of *Brassica juncea*

The optimal nutrient absorption of *Brassica juncea* plants and grains was recorded under RDF + 1 % Urea Foliar Spray + 0.5 % $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ + 0.5 % $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ at 45 days

after sowing, in comparison to the control group. The principal cause of the elevated nutrient consumption of nitrogen, phosphorus, potassium, and sulfur was increased yield, nutrient concentration, nutrient transport, and absorption. Consequently, the augmented administration of Zn, Fe, and N in appropriate amounts may have facilitated the efficient absorption and distribution of nutrients throughout different plant sections, thereby improving nutrient uptake via the broad growth of the root system (10,11,13,25). The increased concentration of these nutrients in the root zone and enhanced cellular metabolic activity may have resulted in the synthesis and accumulation of additional nutrients in other plant tissues. The highest nutrient content in *Brassica juncea* plants and grains was recorded under RDF + 1 % Urea Foliar Spray + 0.5 % $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ + 0.5 % $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ at 45 days after sowing, in comparison to the control group. These results may be associated with nitrogen's role in synthesizing the Zn and Fe regulatory protein, enhancing nutrient translocation in crops. Furthermore, the increased application rates of micronutrients and urea have enhanced the plant's nitrogen, phosphorus, potassium, and sulfur concentrations (26). Applying N, Fe, and Zn fertilizers 45 days after sowing enhanced root growth, resulting in increased nutrient uptake by root tips and subsequent transportation to the phloem in the plant (22,19,27).

Effect of biofortification on efficiency indices

Nitrogen availability is greatly increased by adding micro-nutrient foliar sprays, especially ZnSO_4 and combining ZnSO_4 + FeSO_4 . The combination of urea, zinc, and iron sprays showed the maximum nitrogen availability (ARE-N=51.15). This shows that applying zinc and iron directly to the leaves of plants enhances their ability to absorb nitrogen or mobilize it, perhaps due to better root growth and physiological processes (28). Phosphorus availability was significantly increased by treatments comprising FeSO_4 + ZnSO_4 , especially in the RDF + 0.5 % FeSO_4 + 0.5 % ZnSO_4 treatment (ARE-P=43.50). The phosphorus availability decreased when urea was supplied along with FeSO_4 and ZnSO_4 , indicating connections between nitrogen dynamics and phosphorus intake. The RDF + 1 % Urea + 0.5 % Fe + 0.5 % Zn treatment has the maximum Sulphur availability (ARE-S=36.21). The FeSO_4 foliar sprays, which may give the plant more sulphur, probably cause the increased Sulphur availability (28-29).

Conclusion

The research demonstrates that effective nutrient management can mitigate the adverse effects of soil nutrient deficiencies on crop quality and productivity. This, in turn, improves health outcomes for individuals relying on these crops for nourishment. Agronomic biofortification via foliar treatment is a successful and sustainable approach that enhances nutritional content, promoting food security and increasing public health. Given the ongoing nutritional challenges the world population faces, utilizing crops such as *Brassica juncea* through improved fertilization techniques may be crucial in mitigating these urgent health concerns.

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Authors' contributions

All the authors contributed equally in writing the manuscript, analysing data, and formatting the final draft.

Compliance with ethical standards

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Ethical issues: None

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