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Effect of nutrient strength and *Trichoderma* bio-priming on the nutritional composition of hydroponically grown mustard microgreens

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Abstract
Microgreens are nutrient-dense functional foods whose composition can be modulated by cultivation practices. The present study evaluated the combined effects of hydroponic nutrient strength and *Trichoderma* seed biopriming on the nutritional and mineral composition of mustard (*Brassica juncea* L.) microgreens. Three hydroponic nutrient regimes, pure water, half-strength, and full-strength nutrient solution, were tested in combination with three strains of *Trichoderma viride*. The results revealed significant ($p \leq 0.05$) improvements in proximate composition, including crude fiber, carbohydrate, protein, fat, and calorific value, with increasing nutrient strength. Full-strength nutrient solution (N3) yielded the highest protein (8.47%), fat (0.98%), and calorific content (29.24 J 100 g^{-1}). Among biopriming treatments, *Trichoderma* strain T2 showed a notable increase in protein (7.32%) and mineral uptake compared to T1 and T3. The interaction of N3 × T2 recorded superior values for protein (8.92%), fat (1.04%), and potassium (0.62%), along with enhanced calcium (0.190%) and zinc (57.5 ppm). These findings suggest that both nutrient strength and *Trichoderma* biopriming act synergistically to enhance the nutritional quality of mustard microgreens. Optimizing these practices may provide a sustainable strategy to produce microgreens with improved health-promoting properties.

Keywords: Mustard microgreens, hydroponics, nutrient strength, *Trichoderma* biopriming, nutritional quality

Introduction
With rising challenges in nutritional and food security, there is an escalating demand for accessible, nutrient-dense, and sustainable dietary sources. Microgreens, tiny edible seedlings harvested between the sprout and baby-green stages, have emerged as functional foods rich in vitamins, minerals, antioxidants, and phytochemicals, offering superior nutritional density compared to mature greens and sprouts (Lone *et al*., 2024; Khan *et al*., 2025b) [23, 18]. Recent review (Khan *et al*., 2024; Khan *et al*., 2025b) [16, 18] emphasizes microgreens as high-value functional foods that can help combat malnutrition, especially due to their rapid, resource-efficient production and minimal cultivation requirements (Bhaswant *et al*., 2023) [4]. Due to their concentrated levels of vitamins, minerals, and bioactive compounds, microgreens are increasingly promoted as functional foods with potential health benefits (Khan *et al*., 2025c) [39]. Microgreens are increasingly recognized for their nutritional density and their role in sustainable food systems, with organic cultivation practices demonstrating significant health and environmental benefits (Khan *et al*., 2025a) [17].

Hydroponic systems, soilless methods of cultivation, offer precise control over growth conditions, efficient water and nutrient use, and clean, pesticide-free produce. These advantages make hydroponics especially suitable for microgreen production (Fernandez, 2020; Rajendran *et al*., 2024) [9, 27]. Among the critical factors in hydroponics is nutrient solution strength, which directly influences biomass production and nutrient uptake, yet its optimal levels for mustard microgreens remain under-investigated.

Seed biopriming with beneficial microorganisms like *Trichoderma* spp. is recognized for enhancing seed germination, seedling vigor, nutrient absorption, and resistance to stress. In soil

or conventional systems, *Trichoderma* has been shown to improve plant growth (e.g., pea, wheat), offer biocontrol benefits, and enhance nutrient bioavailability (Singh *et al*., 2016; Ali *et al*., 2022) [32, 1]. Its application in hydroponic systems has also shown promising results: for instance, *Trichoderma asperellum* induced significant improvements in growth, nutrient uptake (e.g., phosphorus and calcium), and biomass of hydroponically grown spinach (Hernández-Huerta *et al*., 2025) [12]. Seed priming with beneficial microbes such as Trichoderma or PGPR has been reported to enhance germination efficiency, seedling vigour, and stress tolerance (Hussain *et al*., 2025) [13], thereby laying the foundation for improved growth and nutritional quality in later stages.

However, the interactive influence of hydroponic nutrient strength and *Trichoderma* biopriming on mustard microgreens' nutritional and mineral composition has not been systematically investigated. Understanding this interaction is vital for developing protocols to consistently produce mustard microgreens with enhanced nutritive quality.

Most existing microgreen studies focus on species variety, antioxidant profiles, and yield parameters. There is a notable absence of research exploring how different hydroponic nutrient strengths, combined with microbial seed biopriming (with *Trichoderma*), affect the proximate and mineral composition of mustard microgreens. Therefore, the present study aimed to evaluate the combined effects of hydroponic nutrient strength and *Trichoderma* seed biopriming on the nutritional and mineral composition of mustard microgreens.

Materials and Methods

The experiment was conducted during 2023-24 at the Division of Basic Sciences & Humanities, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar. The study involve three nutrient concentrations (pure water, half strength of hydroponic nutrient solution and full strength of hydroponic nutrient solution) and three Trichoderma Strains (*Trichoderma viride* smppsap1, *Trichoderma viride* smppsap2 and *Trichoderma viride* smppsap3). Pure cultures of *Trichoderma viride* strains (smppsap1, smppsap2, smppsap3) were grown on PDA for 7-10 days at 25 ± 2°C, and spores were harvested by flooding the plates with sterile distilled water containing 0.01% Tween-80. The suspension was filtered through sterile cheesecloth and adjusted to 1×10^7 conidia mL^{-1} using a haemocytometer. For biopriming 100 g of mustard seeds, free from chemical treatments, were soaked in 700 mL spore suspension (1:7 w/v) at 22-25°C for 6 h with intermittent agitation, *following protocols similar to seed priming studies with PGPR under stress conditions (Hussain et al., 2025b)* [14]. The soaked seeds were incubated on moist sterile filter paper for 18 h in darkness, shade-dried to original moisture, and used for microgreen production.

The experiment was laid out in a factorial completely randomized design (CRD) with three replications. Plastic trays of uniform size (200 sq. inch) were filled with coco peat to a depth of 2.0 cm and lightly moistened prior to sowing. Bioprimed mustard seeds (*Brassica juncea* L.) were evenly broadcast over the medium at the specified seed densities (20 g/200 $inch^2$), gently pressed to ensure proper contact with growing medium, and lightly misted with water. After sowing, the trays were covered with a dark breathable cloth to simulate soil darkness and kept under blackout conditions for 2-3 days (48-72 hours) until most seeds germinated. The surrounding temperature was maintained at 25±2°C, and humidity was kept within 70-80% by light misting when necessary. Once the hypocotyls emerged and the cotyledons began to unfold, the blackout cover was removed. In the laboratory, the trays were maintained under controlled conditions with artificial fluorescent light (150-200 µmol m^{-2} s^{-1} PAR) for a 12 h photoperiod, at a temperature of 25±2 °C and relative humidity of 70-80%. Irrigation was done twice daily with fine mist spraying to maintain adequate moisture without waterlogging. Crops were allowed to grow until the appearance of the first true leaf, at which stage they were harvested by cutting the stems just above the medium surface using sterilized scissors.

The biochemical composition of the samples was analyzed following standard procedures. Crude fiber content (g 100 g^{-1}) was determined by the acid-alkali digestion method as outlined in AOAC (2016) [3], which involves sequential treatment of the dried and ground samples with 1.25% sulfuric acid and 1.25% sodium hydroxide, followed by incineration to calculate crude fiber residue. Total carbohydrates (g 100 g^{-1}) were quantified using the phenol-sulfuric acid method described by Dubois *et al*. (1956) [8]. Crude fat (%) was estimated by Soxhlet extraction using petroleum ether (boiling range 40-60 °C) as the solvent, following AOAC (2016) [3]. Protein content (%) was measured by the Kjeldahl method, in which total nitrogen was estimated and converted to protein using a factor of 6.25 (Jackson, 1973) [15].

The calorific value (J 100 g^{-1}) of the samples was calculated using Atwater's physiological fuel value system, based on the values of protein, fat, and carbohydrate (Merrill and Watt, 1973) [24]. Sodium and potassium were determined by flame photometry after wet digestion of samples with nitric-perchloric acid mixture (Chapman and Pratt, 1961) [5]. Sulphur (%) was analyzed turbidimetrically using the barium chloride method as described by Chesnin and Yien (1951) [6]. Phosphorus (%) was estimated colorimetrically by the vanadomolybdate yellow color method (Jackson, 1973) [15]. Calcium (%) and magnesium (%) were determined by versenate (EDTA) titration method following wet digestion with tri-acid mixture (HNO_3:H_2SO_4:$HClO_4$ in 9:1:4 ratio) as per Lindsay and Norvell (1978) [22]. Iron (ppm) and zinc (ppm) were measured by atomic absorption spectrophotometry (AAS) after di-acid digestion of the powdered samples (Lindsay and Norvell, 1978) [22].

The experimental data were subjected to statistical analysis using analysis of variance (ANOVA) appropriate to the design of the experiment. Treatment means were compared using the least significant difference (LSD) test at 5% probability level to determine statistical significance, following the procedure outlined by Gomez and Gomez (1984) [11].

Results and Discussion

Effect of nutrient strength and *Trichoderma* strains on crude fiber, carbohydrate, fat, protein, and calorific content of mustard microgreens

The proximate composition of mustard (*Brassica juncea* L.) microgreens was significantly influenced by both hydroponic nutrient strength and *Trichoderma* biopriming, as shown in Table 1. Nutrient solution strength exhibited a strong positive effect on crude fiber, carbohydrate, protein, fat, and calorific value, with full-strength nutrient solution (N3) consistently recording the highest values across all parameters. This indicates that increasing nutrient availability in hydroponics directly enhanced biomass accumulation and nutrient assimilation.

Crude fiber content ranged from 1.93 g 100 g^{-1} under pure water (N1) to 2.14 g 100 g^{-1} in full-strength nutrient solution (N3). Carbohydrate content showed a similar trend, increasing from 3.06 g 100 g^{-1} (N1) to 3.20 g 100 g^{-1} (N3). Fiber and

carbohydrate accumulation in microgreens are closely linked to photosynthetic efficiency and structural growth, which are enhanced by adequate nutrient supply (Kyriacou *et al*., 2016) [21]. Comparable findings were reported by Xiao *et al*. (2016) [37], who observed that higher nutrient concentrations promoted carbohydrate accumulation in several Brassica microgreens. Protein and fat content showed significant improvement with increasing nutrient strength. Protein levels rose from 5.61% under N1 to 8.47% under N3, while fat increased from 0.76% to 0.98%. The interaction effect of N3 × T2 was particularly remarkable, with protein and fat values peaking at 8.92% and 1.04%, respectively. These results reflect the critical role of nitrogen in protein biosynthesis and lipid metabolism (Ghoora *et al*., 2020) [10]. Nutrient enrichment promotes amino acid synthesis, which subsequently supports protein accumulation in rapidly growing tissues like microgreens. Calorific content followed the same pattern, with N3 recording 29.24 J 100 g^{-1} compared to 26.62 J 100 g^{-1} under N1. This suggests that hydroponic nutrient strength not only boosts proximate composition but also enhances the energy density of mustard microgreens. These findings corroborate reports by Kyriacou *et al*. (2021) [20], who found that enriched hydroponic solutions significantly improved the calorific content of leafy microgreens.

Among the three *Trichoderma viride* strains, T2 consistently outperformed T1 and T3. Biopriming with T2 improved protein (7.32%), fat (0.89%), and crude fiber (2.09 g 100 g^{-1}) contents. This enhancement may be attributed to the well-established role of *Trichoderma* in promoting root exudation, solubilizing nutrients, and stimulating phytohormone activity (Yadav *et al*., 2022) [38]. In microgreens, where growth is rapid and resource-dependent, microbial biopriming likely accelerates nutrient mobilization, resulting in improved nutritional density. Similar effects of *Trichoderma* biopriming were reported in pea and spinach, where enhanced protein and carbohydrate accumulation were observed (Shukla *et al*., 2016; Saharan & Mehta, 2018) [31, 29].

The interaction between nutrient strength and *Trichoderma* priming revealed a synergistic impact. The N3 × T2 treatment combination yielded the maximum values for protein, fat, and calorific content, highlighting the complementary role of optimal nutrient supply and microbial stimulation. Such synergism has been previously documented by Colla *et al*. (2015) [7], who demonstrated that microbial inoculants combined with nutrient optimization significantly improved growth and nutritional quality of leafy vegetables.

Effect of nutrient strength and *Trichoderma* strains on sodium, potassium, sulphur, and phosphorus content of mustard microgreens

Sodium concentration in mustard microgreens was significantly affected by hydroponic nutrient strength. Sodium levels ranged from 53.66 ppm (N1) to 57.00 ppm (N3), with the highest values recorded under full-strength nutrient solution (N3). Among the interactions, N3 × T2 exhibited the maximum sodium accumulation (57.05 ppm). Although sodium is not an essential nutrient for most plants, it can contribute to osmotic adjustment and substitute for potassium in certain metabolic processes under controlled conditions (Subbarao *et al*., 2003) [33]. The slight increase observed here may reflect enhanced ion uptake under higher nutrient availability. These results are in agreement with Kyriacou *et al*. (2016) [21], who reported that nutrient enrichment increases sodium absorption in Brassica microgreens, albeit at non-toxic levels.

Potassium, a vital macronutrient for enzyme activation, stomatal regulation, and protein synthesis, showed marked increases with nutrient strength. Potassium content rose from 0.57% (N1) to 0.60% (N3). *Trichoderma* biopriming, particularly T2, enhanced potassium uptake, with the N3 × T2 combination recording the highest value (0.62%). This is consistent with the role of *Trichoderma* in producing organic acids and enzymes that mobilize potassium from nutrient sources (Altomare *et al*., 1999) [2]. Similar synergistic effects of microbial inoculation on potassium uptake in leafy greens were documented by Colla *et al*. (2015) [7], indicating that microbial priming combined with nutrient optimization improves cation assimilation and plant tissue enrichment.

Sulphur content increased slightly with nutrient enrichment, from 0.86% under N1 to 0.88% under N3. The effect of *Trichoderma* strains was not as pronounced, though T2 treatments generally maintained higher sulphur content than T1. Sulphur is a key element in glucosinolate biosynthesis, which contributes to the pungency and health-promoting properties of mustard microgreens (Verkerk *et al*., 2009) [34]. Adequate sulphur supply in hydroponic systems is known to enhance secondary metabolite pathways, including sulphur-rich amino acids like methionine and cysteine (Sánchez-Rodríguez *et al*., 2011). The relatively small differences in sulphur levels observed here suggest that mustard microgreens are efficient accumulators of sulphur, with hydroponic solutions supplying sufficient amounts even at lower strengths.

Phosphorus content was positively influenced by nutrient strength, with values ranging from 0.195% (N1) to 0.198% (N3). *Trichoderma* strain T2 again proved most effective, with N3 × T2 recording the highest phosphorus content (0.199%). The improved uptake can be attributed to phosphate solubilization by *Trichoderma* through organic acid exudation and enzymatic activity (Richardson & Simpson, 2011) [28]. Phosphorus plays a central role in ATP synthesis, nucleic acids, and energy transfer, which are critical during the rapid growth phase of microgreens. Comparable findings were reported by Yadav *et al*. (2022) [38], where microbial inoculants enhanced phosphorus bioavailability in leafy vegetables under controlled cultivation.

Effect of nutrient strength and *Trichoderma* strains on calcium, magnesium, iron, and zinc content of mustard microgreens

Calcium concentrations in mustard microgreens increased progressively with nutrient strength, ranging from 0.174% under pure water (N1) to 0.188% in full-strength nutrient solution (N3). *Trichoderma* strains improved calcium assimilation, with T2 showing the best performance (0.184%). The N3 × T2 interaction yielded the highest calcium content (0.190%). Calcium is critical for cell wall stability and membrane integrity, and higher accumulation under enriched nutrient regimes reflects increased uptake and transport in hydroponic systems (White & Broadley, 2003) [36]. Previous studies on Brassica microgreens reported that optimized nutrient solutions enhance calcium biofortification, improving both nutritional value and postharvest quality (Kyriacou *et al*., 2016) [21].

Magnesium, the central atom in chlorophyll and a cofactor in many enzymatic reactions, also showed significant increases with nutrient enrichment. Levels rose from 0.139% (N1) to 0.149% (N3). Among *Trichoderma* treatments, T2 again improved magnesium content (0.145%) over T1 (0.141%). The N3 × T2 combination was superior (0.151%). These results emphasize the role of balanced nutrient supply in enhancing photosynthetic efficiency through chlorophyll synthesis. Similar

observations were reported by Pannico *et al*. (2020) [26], who found that microgreens cultivated under nutrient-rich hydroponics had higher chlorophyll and magnesium content, translating into improved photosynthetic activity and nutritional density.

Iron concentration ranged from 95.2 ppm (N1) to 97.5 ppm (N3). *Trichoderma* biopriming enhanced iron uptake, with T2 again performing best (97.0 ppm). The maximum iron content was observed under N3 × T2 (98.3 ppm). Iron is a vital micronutrient for respiration and chlorophyll biosynthesis, and its deficiency is a widespread nutritional problem globally. *Trichoderma* spp. have been documented to produce siderophores that enhance iron solubility and uptake in plants (Vinale *et al*., 2008) [35]. In spinach, *Trichoderma asperellum* application improved Fe accumulation under hydroponic conditions (Nguyen *et al*., 2023) [25], supporting the findings of this study. Thus, combining hydroponic optimization with microbial priming offers a sustainable strategy for iron biofortification of microgreens.

Zinc, an essential micronutrient for protein metabolism and auxin biosynthesis, showed significant improvement under nutrient enrichment. Zinc content increased from 53.1 ppm (N1) to 57.0 ppm (N3), with T2 outperforming other strains (55.4 ppm). The N3 × T2 treatment recorded the highest zinc accumulation (57.5 ppm). Zinc uptake efficiency is often constrained in plants due to low bioavailability; however, hydroponic cultivation ensures greater solubility, and microbial inoculants like *Trichoderma* enhance further mobilization (Richardson & Simpson, 2011) [28]. These findings align with Khan *et al*. (2025) [16], who highlighted the potential of microgreens as biofortified foods addressing micronutrient malnutrition, particularly in zinc-deficient diets.

Table 1: Effect of nutrient strength and Trichoderma strains on crude fiber and carbohydrate content of mustard microgreens

Treatment	Crude fiber (g 100 g^{-1})	Carbohydrate (g 100 g^{-1})	Fat (%)	Protein (%)	Calorie (J 100 g^{-1})
Main Effect of hydroponic nutrient (N)					
Pure water (N1)	1.93	3.06	0.76	5.61	26.62
Half-strength nutrient (N2)	2.07	3.10	0.84	7.33	28.06
Full-strength nutrient (N3)	2.14	3.20	0.98	8.47	29.24
C.D. ($p \leq 0.05$)	0.02	0.01	0.02	0.08	0.06
Trichoderma strains biopriming (T)					
Trichoderma viride strains 1 (T1)	2.00	3.10	0.83	6.37	27.60
Trichoderma viride strains 1 (T2)	2.09	3.13	0.89	7.32	27.98
Trichoderma viride strains 1 (T3)	2.05	3.12	0.87	7.72	28.12
C.D. ($p \leq 0.05$)	0.02	0.01	0.02	0.08	0.06
Interaction effects (N × T)					
N1 × T1	1.91	3.05	0.71	5.26	26.56
N2 × T1	2.05	3.12	0.85	6.23	27.51
N3 × T1	2.05	3.15	0.94	7.62	28.73
N1 × T2	1.96	3.06	0.78	5.47	26.57
N2 × T2	2.08	3.08	0.85	7.59	28.03
N3 × T2	2.23	3.27	1.04	8.92	29.98
N1 × T3	1.93	3.08	0.81	6.09	26.73
N2 × T3	2.08	3.12	0.82	8.19	28.65
N3 × T3	2.15	3.18	0.98	8.89	29.00
C.D. ($p \leq 0.05$)	0.03	0.02	0.03	0.15	0.10

Table 2: Effect of nutrient strength and Trichoderma strains on sodium and potassium content of mustard microgreens

Treatment	Sodium (ppm)	Potassium (%)	Sulphur (%)	Phosphorus (%)
Main Effect of hydroponic nutrient (N)				
Pure water (N1)	53.66	0.57	0.86	0.195
Half-strength nutrient (N2)	54.33	0.58	0.87	0.197
Full-strength nutrient (N3)	57.00	0.60	0.88	0.198
C.D. ($p \leq 0.05$)	1.12	0.003	0.007	0.002
Trichoderma strains biopriming (T)				
Trichoderma viride strains 1 (T1)	53.87	0.57	0.86	0.195
Trichoderma viride strains 1 (T2)	56.01	0.59	0.88	0.198
Trichoderma viride strains 1 (T3)	55.11	0.58	0.87	0.197
C.D. ($p \leq 0.05$)	1.12	0.003	0.007	0.002
Interaction effects (N × T)				
N1 × T1	51.00	0.55	0.85	0.193
N2 × T1	53.66	0.57	0.87	0.197
N3 × T1	56.95	0.59	0.87	0.197
N1 × T2	55.00	0.57	0.88	0.197
N2 × T2	56.00	0.58	0.87	0.198
N3 × T2	57.05	0.62	0.89	0.199
N1 × T3	55.00	0.58	0.87	0.196
N2 × T3	53.33	0.58	0.87	0.196
N3 × T3	57.00	0.60	0.88	0.198
C.D. ($p \leq 0.05$)	1.95	0.006	0.001	0.005

Table 3: Effect of nutrient strength and Trichoderma strains on calcium and magnesium content of mustard microgreens

Treatment	Calcium (%)	Magnesium (%)	Iron (ppm)	Zinc (ppm)
Main Effect of hydroponic nutrient (N)				
Pure water (N1)	0.174	0.139	95.2	53.1
Half-strength nutrient (N2)	0.186	0.142	97.0	54.8
Full-strength nutrient (N3)	0.188	0.149	97.5	57.0
C.D. ($p \leq 0.05$)	0.11	0.0009	1.16	1.03
Trichoderma strains biopriming (T)				
Trichoderma viride strains 1 (T1)	0.181	0.141	96.0	54.1
Trichoderma viride strains 1 (T2)	0.184	0.145	97.0	55.4
Trichoderma viride strains 1 (T3)	0.183	0.144	96.7	55.3
C.D. ($p \leq 0.05$)	0.11	0.0009	1.16	1.03
Interaction effects (N × T)				
N1 × T1	0.173	0.135	95.1	52.3
N2 × T1	0.185	0.141	96.4	53.3
N3 × T1	0.187	0.149	96.5	56.5
N1 × T2	0.175	0.138	95.4	52.4
N2 × T2	0.187	0.146	97.4	56.3
N3 × T2	0.190	0.151	98.3	57.5
N1 × T3	0.176	0.144	95.2	54.5
N2 × T3	0.186	0.141	97.4	54.6
N3 × T3	0.188	0.149	97.7	57.0
C.D. ($p \leq 0.05$)	0.14	0.0017	1.23	1.78

Conclusion

The present study demonstrated that both hydroponic nutrient strength and *Trichoderma* biopriming significantly influence the nutritional and mineral composition of mustard microgreens. Full-strength nutrient solution consistently enhanced crude fiber, protein, fat, and calorific content, while also improving macro- and micro-mineral accumulation. Among the *Trichoderma* strains, T2 was most effective, showing synergistic effects with nutrient enrichment, particularly in protein, potassium, calcium, and zinc uptake. The findings highlight that integrating optimized hydroponic practices with microbial biostimulants can enhance the nutritional density and functional quality of mustard microgreens, positioning them as sustainable, health-promoting foods. Future research should focus on validating these results under commercial-scale hydroponic systems and exploring the combined effects of *Trichoderma* with other biostimulants to maximize the functional potential of microgreens.

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