Effect of Different Levels of Nitrogen Fertilizer, Fungal Biofertilizer, and Algal Biofertilizer on Urease Enzyme Activity and Nutrient Availability for Barley (*Hordeum vulgare* L.)

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Abstract

A field experiment was conducted at Al-Muthanna University research station in Al-Najmi district, north of Al-Muthanna Governorate, during the winter growing season (2025–2026). The study aimed to investigate the effects of Trichoderma harzianum, Chara sp. algae, and nitrogen mineral fertilizer applied at four levels (0, 25, 50, and 75% of the full nitrogen recommendation, equivalent to 0, 50, 100, and 150 kg N ha^{-1} as urea 46%) on urease enzyme activity, soil NPK availability, plant growth, and grain yield of barley (Hordeum vulgare L.). The experiment was arranged in a randomized complete block design (RCBD) with three replicates. Results showed that dual inoculation with T. harzianum + Chara sp. (B3) significantly outperformed all other treatments across all studied parameters. Urease activity at 45 and 90 days after planting reached 124.00 and 116.67 µg $N\text{-}NH_4^+$ g^{-1} soil $2h^{-1}$, respectively. Soil available nitrogen, phosphorus, and potassium were 20.57 mg N kg^{-1} soil, 17.15 mg P kg^{-1} soil, and 185.57 mg K kg^{-1} soil, respectively. Plant height reached 101.31 cm and grain yield reached 7.70 Mg ha^{-1}. Application of nitrogen fertilizer at the 75% level (N3) recorded the highest urease activity (137.00 and 128.75 µg $N\text{-}NH_4^+$ g^{-1} soil $2h^{-1}$ at 45 and 90 days, respectively), soil NPK (19.72 mg N kg^{-1} soil, 16.27 mg P kg^{-1} soil, 174.49 mg K kg^{-1} soil), plant height (100.33 cm), and grain yield (7.27 Mg ha^{-1}).

Keywords: *Trichoderma harzianum*; Chara sp.; urease enzyme activity; soil nutrient availability; barley (*Hordeum vulgare* L.)

1. Introduction

The overuse of chemical fertilizers disrupts the environmental equilibrium, resulting in soil and water pollution [1], along with harmful effects on beneficial soil microorganisms. As a result, biological fertilization has emerged as an eco-friendly alternative with significant benefits and substantial influence on crop productivity [2, 3]. Biological fertilizers enhance soil properties, fix atmospheric nitrogen, increase nutrient availability (N, P, K, and micronutrients), and secrete growth-promoting hormones such as gibberellins and auxins, contributing to

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improved plant growth, increased crop yield, and better quality [4, 5].

Trichoderma is a prevalent soil fungus that produces extracellular enzymes and promotes root growth, thereby enhancing plant resilience to various stresses and adverse environmental conditions [6]. *T. harzianum* stimulates deeper root development to access water and nutrients, secretes growth-promoting hormones, and solubilizes micronutrients such as iron, zinc, copper, and manganese, making them available to plants even in alkaline soils [7].

Algae based soil amendment helps in nitrogen fixation in soil, which increases the fertility by making nutrients available and thus yield improvement in crop [8]. Chara sp. is one of the good algae for use as organic fertilizer because of its high nitrogen and potassium content, good water holding ability and the presence of natural plant growth regulating compounds [9].

Enzymes in the soil are indicators of soil health and fertility. Urease is one of the important enzymes which catalyze the hydrolysis of urea to ammonium so as to make the nitrogen available for plant uptake [10, 11]. Barley (Hordeum vulgare L.) is an important cereal crop used for green fodder, grain and for various industrial purposes. However, unstable cultivation areas and low productivity have hindered its expansion in Iraq [12, 13]. The price per ton of barley is lower than that of wheat (*Triticum aestivum* L.), even though agricultural costs for both crops are nearly equal; this economic disparity discourages Iraqi farmers from expanding barley cultivation [14]. Low yield per unit area remains the major obstacle to boosting production and achieving self-sufficiency.

2. Materials and Methods

2.1. Experimental Site and Soil Properties

A field experiment was conducted at the Al-Muthanna University research station in Al-Najmi district, north of Al-Muthanna Governorate (Iraq), during the winter growing season of 2025–2026. The station is situated on the Euphrates River, north of Samawah city. The soil at the experimental site was a silty clay loam. Selected physico-chemical properties of the soil before planting are presented in Table 1.

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Table 1. Physico-chemical properties of the experimental soil (0–30 cm depth) before planting.

Property	Value	Method
Soil texture	Silty clay loam	Hydrometer method
pH (1:2.5 soil:water)	7.6	Potentiometer
EC (dS m^{-1})	2.1	Conductivity meter
Organic matter (%)	0.82	Walkley–Black method
Available N (mg kg^{-1})	8.4	Kjeldahl distillation
Available P (mg kg^{-1})	6.2	Olsen method
Available K (mg kg^{-1})	98.5 ±	Flame photometer
$CaCO_3$ (%)	28.3	Calcimeter

2.2. Experimental Design and Treatments

The experiment was arranged in a split-plot design within a randomized complete block design (RCBD) with three replicates. Each experimental unit measured 3 × 3 m^2, with 1-m buffer strips between blocks and between experimental units. Barley cultivar Furat 5 was sown on 11 October 2025 at a seeding rate of 120 kg ha^{-1}, in rows spaced 20 cm apart (8 rows per unit). Crop was harvested at physiological maturity on 1 March 2026.

Factor 1 (main plot) — Biofertilizer treatments (B):

B0: Control (no biofertilizer); B1: T. harzianum alone (sprinkled at 2 g m^{-1} on planting lines before seeding); B2: Chara sp. alone (applied at 60 g peat $moss^{-1}$, soil-washed and dried before incorporation); B3: T. harzianum + Chara sp. (dual inoculation as described above).

Factor 2 (sub-plot) — Nitrogen mineral fertilizer levels (N): Urea (46% N) was applied at four rates representing 0, 25, 50, and 75% of the full nitrogen recommendation (200 kg N ha^{-1}), equivalent to N0 = 0, N1 = 50, N2 = 100, and N3 = 150 kg N ha^{-1}, respectively. Urea was applied in two equal splits: 10 days after planting and one month after planting. Phosphate fertilizer (superphosphate, 21% P_2O_5 at 100 kg ha^{-1}) and potassium fertilizer (potassium sulfate, 42% K_2O at 75 kg ha^{-1}) were applied as basal dressings before planting. Weeding was performed manually as required. The chemical composition of *Chara sp.* extract is presented in Table 2.

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Table 2. Chemical composition of Chara sp. algae extract.

Component	Concentration
Total N	1.567%
Total P	0.304%
Total K	0.397%
Total Ca	3.846%
Auxin	127.9 ppm
Gibberellins	149.13 ppm

2.3. Studied Parameters

2.3.1. Urease Enzyme Activity

Urease enzyme activity was determined by the method of Tabatabai and Bremner [16]. Briefly, 5 g of field-moist soil was combined with 0.2 mL of toluene, 9 mL of THAM buffer solution (pH 9.0), and 1 mL of 0.2 M urea solution in a 50 mL volumetric flask. After 2 h incubation at 37°C, 35 mL of 2.5 M KCl solution containing 100 ppm silver sulfate was added and the final volume was brought to 50 mL. A micro-Kjeldahl distillation apparatus with MgO and boric acid was used to quantify the ammonium nitrogen released. Results are expressed as µg $N\text{-}NH_4^+$ g^{-1} soil $2h^{-1}$.

2.3.2. Soil Available Nitrogen

Available soil nitrogen was extracted with 2 N KCl solution and determined using the micro-Kjeldahl distillation apparatus, following the method of Bremner [17]. Results are expressed as mg N kg^{-1} soil.

2.3.3. Soil Available Phosphorus

Soil available phosphorus was extracted with 0.5 N $NaHCO_3$ (pH 8.5) following the Olsen method [18] and determined colorimetrically using ammonium molybdate and ascorbic acid. Results are expressed as mg P kg^{-1} soil.

2.3.4. Soil Available Potassium

Soil available potassium was measured using a flame photometer following the method described by Page et al. [18]. Results are expressed as mg K kg^{-1} soil.

2.3.5. Plant Height (cm)

Plant height was determined at the flowering stage by measuring 10 randomly selected plants per experimental unit from the soil surface to the tip of the terminal spike.

2.3.6. Grain Yield (Mg ha^{-1})

Grain yield was determined by harvesting 1 m^2 from each experimental unit at maturity, threshing, cleaning, and weighing the grains. Results were converted to Mg ha^{-1}.

2.4. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using GenStat statistical software (17th edition). Differences between treatment means were compared using the least significant difference (LSD) test at the 5% probability level ($p < 0.05$). Data are presented as means ± standard error of three replicates.

3. Results and Discussion

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3.1. Urease Enzyme Activity at 45 Days After Planting (μg N-NH_4^+ g^{-1} soil $2h^{-1}$)

Data presented in Table 3 indicate that inoculation with *T. harzianum* and/or *Chara sp.* exerted a significant effect on urease activity at 45 days after planting (DAP). Dual inoculation (B3) recorded the highest mean enzyme activity of 124.00 μg N-NH_4^+ g^{-1} soil $2h^{-1}$, significantly surpassing single algae inoculation (B2: 110.92), single fungal inoculation (B1: 101.58), and the un-inoculated control (B0: 82.58 μg N-NH_4^+ g^{-1} soil $2h^{-1}$). This enhancement can be attributed to the role of organic and biological inputs in increasing the supply of energy, carbon, and nutrients to soil microorganisms, thereby stimulating their metabolic activity and enzyme secretion [19].

With respect to mineral nitrogen fertilizer, the N3 level (150 kg N ha^{-1}) produced the highest significant urease activity (137.00 μg N-NH_4^+ g^{-1} soil $2h^{-1}$), followed by N2 (118.00), N1 (93.92), and the control N0 (71.17 μg N-NH_4^+ g^{-1} soil $2h^{-1}$). Nitrogen fertilization stimulates plant physiological processes and photosynthetic capacity, resulting in a larger root system and increased rhizosphere microbial populations that secrete greater quantities of urease [20].

The interaction between biofertilizer treatments and nitrogen levels significantly influenced urease activity ($p < 0.05$). The B3N3 combination achieved the highest mean of 160.67 μg N-NH_4^+ g^{-1} soil $2h^{-1}$, while the un-treated control (B0N0) recorded the lowest mean of 52.00 μg N-NH_4^+ g^{-1} soil $2h^{-1}$. This synergistic effect is consistent with findings showing that combined mineral and biological fertilization increases biomass production and stimulates urease-producing microbial communities [21].

Table 3. Effect of biofertilizer treatments (B) and nitrogen levels (N) on urease activity at 45 DAP (μg N-NH_4^+ g^{-1} soil $2h^{-1}$).

Treatment	N0 (0 kg N ha^{-1})	N1 (50 kg N ha^{-1})	N2 (100 kg N ha^{-1})	N3 (150 kg N ha^{-1})	B Mean
B0 (Control)	52.00	77.00	91.00	110.33	82.58
B1 (T. harzianum)	68.00	95.33	113.00	130.00	101.58
B2 (Chara sp.)	78.67	98.00	124.00	143.00	110.92
B3 (T. harzianum + Chara sp.)	86.00	105.33	144.00	160.67	124.00
N Mean	71.17	93.92	118.00	137.00	—
$LSD_{0.05}$	B = 1.56	N = 1.56	B×N = 3.13		

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3.2. Urease Enzyme Activity at 90 Days After Planting (μg N-NH_4^+ g^{-1} soil $2h^{-1}$)

Table 4 shows that dual inoculation (B3) continued to record the highest urease activity at 90 DAP (116.67 μg N-NH_4^+ g^{-1} soil $2h^{-1}$), representing a 54.53% increase over the control (B0: 75.50 μg N-NH_4^+ g^{-1} soil $2h^{-1}$). Single-organism treatments recorded intermediate values: B2 (*Chara sp.*): 102.50; B1 (*T. harzianum*): 95.25 μg N-NH_4^+ g^{-1} soil $2h^{-1}$. The physiological basis for this enhancement lies in the role of *T. harzianum* in secreting organic acids that solubilize nutrients and stimulate rhizosphere microbial communities, while *Chara sp.* provides additional organic nitrogen that serves as substrate for urease-producing organisms [22].

Nitrogen fertilizer application at different levels significantly increased urease activity at 90 DAP compared to the unfertilized control. The N3 level recorded the highest activity (128.75 μg N-NH_4^+ g^{-1} soil $2h^{-1}$), followed by N2 (111.00) — representing a 72.09% increase over the control (64.50 μg N-NH_4^+ g^{-1} soil $2h^{-1}$). The urease enzyme specifically catalyzes the hydrolysis of urea substrate; therefore, urea additions stimulate the proliferation of urease-secreting microbial communities, thereby enhancing overall biological activity and enzyme expression [23, 24].

The B3N3 interaction produced the highest combined mean of 152.00 μg N-NH_4^+ g^{-1} soil $2h^{-1}$ at 90 DAP, compared to the lowest of 44.00 μg N-NH_4^+ g^{-1} soil $2h^{-1}$ in the control (B0N0). This synergistic enhancement reflects the contribution of *Chara sp.* nutrients and urea fertilizer alongside plant root secretions (amino acids, sugars, organic acids) that collectively boost microbial activity in the rhizosphere.

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Table 4. Effect of biofertilizer treatments (B) and nitrogen levels (N) on urease activity at 90 DAP (µg N-NH_4^+ g^{-1} soil $2h^{-1}$).

Treatment	N0	N1	N2	N3	B Mean
B0 (Control)	44.00 d	69.00 cd	85.00 bc	104.00 b	75.50 D
B1 (T. harzianum)	62.00	86.00	108.00	125.00	95.25
B2 (Chara sp.)	71.00	88.00	117.00	134.00	102.50
B3 (T. harzianum + Chara sp.)	81.00	99.67	134.00	152.00	116.67
N Mean	64.50	85.67	111.00	128.75	—
$LSD_{0.05}$	B = 0.982	N = 0.982	B×N = 1.964		

3.3. Soil Available Nitrogen (mg N kg^{-1} soil)

The results in Table 5 show that dual inoculation (B3) achieved the highest post-harvest soil available nitrogen (20.57 mg N kg^{-1} soil), representing a 48.95% increase over the control (B0: 13.81 mg N kg^{-1} soil). Single *Chara sp.* (B2) and single *T. harzianum* (B1) recorded 17.76 and 15.90 mg N kg^{-1} soil, respectively. The increase in soil nitrogen attributable to *T. harzianum* is mediated by its capacity to form chelated compounds and break down complex organic molecules, converting them to readily plant-available forms, while *Chara sp.* contributes directly to the organic nitrogen pool through its decomposition [25].

Mineral nitrogen fertilizer application significantly increased soil available nitrogen at harvest (Table 5). The N3 level recorded the highest post-harvest value (19.72 mg N kg^{-1} soil), a 34.75% increase over the control (N0: 13.86 mg N kg^{-1} soil). The N2 level recorded 18.51 mg N kg^{-1} soil (27.58% increase). The lower-than-applied soil nitrogen values at harvest reflect losses through plant uptake, leaching, volatilization, and immobilization, consistent with the findings of Al-Yasariu [26].

The B3N3 interaction produced the highest post-harvest soil nitrogen content (24.29 mg N kg^{-1} soil), while the control (B0N0) recorded the lowest value (11.55 mg N kg^{-1} soil). This synergism can be explained by the capacity of biofertilizers to reduce nitrogen losses through immobilization while simultaneously stimulating phosphorus solubilization, creating a favorable rhizosphere environment that enhances overall nitrogen retention [27].

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Table 5. Effect of biofertilizer treatments (B) and nitrogen levels (N) on soil available nitrogen at harvest (mg N kg^{-1} soil).

Treatment	N0	N1	N2	N3	B Mean
B0 (Control)	11.55	13.06	14.82	15.82	13.81
B1 (T. harzianum)	13.04	14.64	17.28	18.66	15.90
B2 (Chara sp.)	14.54	16.65	19.76	20.12	17.76
B3 (T. harzianum + Chara sp.)	16.32	19.51	22.19	24.29	20.57
N Mean	13.86	15.96	18.51	19.72	—
$LSD_{0.05}$	B = 0.020	N = 0.020	B×N = 0.040		

3.4. Soil Available Phosphorus (mg P kg^{-1} soil)

Table 6 shows that dual inoculation (B3) produced the highest post-harvest soil available phosphorus (17.15 mg P kg^{-1} soil), a 49.82% increase over the control (B0: 13.44 mg P kg^{-1} soil). Single *T. harzianum* (B1: 15.58 mg P kg^{-1} soil) outperformed single *Chara sp.* (B2: 14.37 mg P kg^{-1} soil). The phosphorus-solubilizing ability of *T. harzianum* is well established; it increases tricalcium phosphate availability by secreting organic acids that lower rhizosphere pH, thereby boosting phosphorus release from insoluble compounds [28].

Application of mineral nitrogen fertilizer significantly increased soil available phosphorus compared to the control (Table 6). The N3 level achieved the highest mean (16.27 mg P kg^{-1} soil, 16.46% increase) followed by N2 (15.74 mg P kg^{-1} soil, 13.64% increase), whereas the control (N0) recorded the lowest value (13.97 mg P kg^{-1} soil). The reduction in soil phosphorus at harvest compared to pre-planting levels reflects enhanced plant uptake stimulated by mineral fertilizer application, compounded by adsorption to $CaCO_3$ minerals in the alkaline calcareous soil. These results are consistent with those of Dergahm et al. [29].

The B3N3 interaction yielded the highest post-harvest soil phosphorus (18.48 mg P kg^{-1} soil), while the control (B0N0) recorded the lowest value (12.00 mg P kg^{-1} soil). This combined effect underscores the importance of integrating biological phosphorus solubilization with adequate mineral nutrition to optimize phosphorus cycling in calcareous soils [30].

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Table 6. Effect of biofertilizer treatments (B) and nitrogen levels (N) on soil available phosphorus at harvest (mg P kg^{-1} soil).

Treatment	N0	N1	N2	N3	B Mean
B0 (Control)	12.00	13.53	13.94	14.32	13.44
B1 (T. harzianum)	14.34	14.54	16.21	17.24	15.58
B2 (Chara sp.)	13.85	14.12	14.46	15.07	14.37
B3 (T. harzianum + Chara sp.)	15.71	16.05	18.36	18.48	17.15
N Mean	13.97	14.56	15.74	16.27	—
$LSD_{0.05}$	B = 0.014	N = 0.014	B×N = 0.029		

3.5. Soil Available Potassium (mg K kg^{-1} soil)

Data in Table 7 show that the dual inoculation treatment (B3) achieved the highest post-harvest soil available potassium (185.57 mg K kg^{-1} soil), a 51.45% increase over the control (B0: 122.53 mg K kg^{-1} soil). Single algae treatment (B2: 167.52 mg K kg^{-1} soil) outperformed single fungal treatment (B1: 144.91 mg K kg^{-1} soil). This superiority is attributable to the high potassium concentration inherent in *Chara sp.* (Table 2), and to the oxidation–reduction activities of *T. harzianum* that lower soil pH, facilitating the displacement of potassium from exchange sites through competitive interactions with calcium and magnesium ions [31].

Levels of mineral nitrogen fertilizer had a significant effect on the soil available potassium at all levels when compared to the control. The mean for N3 level was highest (174.49 mg K kg-1 soil) while the mean for N0 level (control) was lowest (136.26 mg K kg-1 soil). The application of fertilizer will increase plant biomass, which will increase the microbial activity and the production of exudates from the roots, indirectly mobilizing the exchangeable potassium from soil reserves. The results are in line with the results obtained by Shams Allah [32].

The B3N3 interaction produced the highest mean potassium availability (196.30 mg K kg^{-1} soil), while the control (B0N0) recorded the lowest (104.23 mg K kg^{-1} soil). The synergistic effect of biological agents and mineral fertilizers in stimulating rhizosphere microbial communities enhances the

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release of all major nutrients, including potassium [33, 34].

Table 7. Effect of biofertilizer treatments (B) and nitrogen levels (N) on soil available potassium at harvest (mg K kg^{-1} soil).

Treatment	N0	N1	N2	N3	B Mean
B0 (Control)	104.23	117.84	126.47	141.60	122.53
B1 (T. harzianum)	121.33	138.87	147.67	171.80	144.91
B2 (Chara sp.)	146.83	149.67	185.33	188.27	167.52
B3 (T. harzianum + Chara sp.)	172.67	183.20	190.13	196.30	185.57
N Mean	136.26	147.39	162.40	174.49	—
$LSD_{0.05}$	B = 0.021	N = 0.021	B×N = 0.043		

3.6. Plant Height (cm)

Table 8 shows that dual inoculation (B3) produced the tallest plants (101.31 cm), a 16.05% increase over the control (B0: 87.30 cm). Single *T. harzianum* (B1: 98.10 cm) ranked second, followed by single *Chara sp.* (B2: 93.70 cm). The enhancement in plant height attributable to biofertilizers is physiologically linked to the secretion of auxin-like hormones by *T. harzianum* and *Chara sp.* (gibberellins and auxins; Table 2), which promote meristematic cell elongation, root development, and enhanced nutrient absorption [35, 36].

Mineral nitrogen fertilizer significantly increased plant height at all application levels. The N3 level produced the tallest plants (100.33 cm, 15.81% increase over N0: 86.63 cm), followed by N2 (99.15 cm, 14.44% increase) and N1 (94.30 cm). The beneficial effects of nitrogen fertilization on plant height operate through enhanced soil nutrient supply, support of plant metabolic pathways (particularly protein and chlorophyll synthesis), and promotion of vigorous vegetative growth.

The effect of interaction was significant and the tallest plant height was obtained in B3N2 (107.50 cm) and the shortest in B0N0 (un-treated control) (81.20 cm). The combined use of biological and mineral fertilizers shows that this combination results in optimum growing conditions because of the complementary mechanism of hormone production, nutrient mobilization and increased root activity [36].

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Table 8. Effect of biofertilizer treatments (B) and nitrogen levels (N) on plant height (cm).

Treatment	N0	N1	N2	N3	B Mean
B0 (Control)	81.20	84.20	89.50	94.30	87.30
B1 (T. harzianum)	87.70	98.30	102.80	103.60	98.10
B2 (Chara sp.)	86.30	92.30	96.80	99.40	93.70
B3 (T. harzianum + Chara sp.)	91.33	102.40	107.50	104.00	101.31
N Mean	86.63	94.30	99.15	100.33	—
$LSD_{0.05}$	B = 0.593	N = 0.593	B×N = 1.186		

3.7. Grain Yield (Mg ha⁻¹)

The highest grain yield (7.70 Mg ha-1) was obtained from dual inoculation (B3) which was 37.26% higher than the control (B0: 5.61 Mg ha-1) (Table 9). Algae treatment (B2: 6.95 Mg ha-1) proved to be superior than fungal treatment (B1: 6.33 Mg ha-1). The higher grain production under dual inoculation could be attributed to the production of auxin-like hormones and proteins secreted by T. harzianum which stimulate root elongation and proliferation, increasing water and nutrient uptake capacity. In addition, chara sp. helps in nitrogen mineralization and soil water holding capacity, which together leads to enhanced vegetative growth and transport of assimilates to grains [36].

Application of mineral nitrogen fertilizer significantly increased grain yield at all levels. The N3 level (150 kg N ha^{-1}) produced the highest mean yield (7.27 Mg ha^{-1}, 23.43% increase over N0: 5.89 Mg ha^{-1}), followed by N2 (7.03 Mg ha^{-1}, 19.35% increase). Nitrogen fertilization enhances vegetative growth, stimulates nitrogen and phosphorus uptake, increases starch, sugar, and protein synthesis, and promotes grain filling, all contributing to higher grain weight per unit area [37].

The B3N3 interaction resulted in the highest grain yield (8.52 Mg ha^{-1}), and the lowest grain yield (5.10 Mg ha^{-1}) was found for the control (B0N0). The integrated action of T. harzianum and Chara sp. and mineral N fertilizer is synergistic in which the pathogen suppression, hormone production, and vegetative and root system development stimulate plant growth resulting in an increase in the harvestable yield [36].

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Table 9. Effect of biofertilizer treatments (B) and nitrogen levels (N) on barley grain yield (Mg ha^{-1}).

Treatment	N0	N1	N2	N3	B Mean
B0 (Control)	5.10	5.49	5.88	5.98	5.61
B1 (T. harzianum)	5.72	6.08	6.60	6.91	6.33
B2 (Chara sp.)	5.92	6.70	7.52	7.68	6.95
B3 (T. harzianum + Chara sp.)	6.83	7.33	8.14	8.52	7.70
N Mean	5.89	6.40	7.03	7.27	—
$LSD_{0.05}$	B = 0.039	N = 0.039	B×N = 0.078		

4. Conclusion

The results of this study demonstrate that dual inoculation with *T. harzianum* + *Chara sp.* (B3) consistently produced the highest values across all studied parameters: urease activity at 45 and 90 DAP (124.00 and 116.67 µg $N\text{-}NH_4^+$ g^{-1} soil $2h^{-1}$, respectively), soil available NPK (20.57 mg N, 17.15 mg P, and 185.57 mg K kg^{-1} soil, respectively), plant height (101.31 cm), and grain yield (7.70 Mg ha^{-1}). Among mineral nitrogen levels, N3 (75% of full recommendation = 150 kg N ha^{-1}) recorded the highest values for all studied parameters: urease activity (137.00 and 128.75 µg $N\text{-}NH_4^+$ g^{-1} soil $2h^{-1}$), soil NPK availability (19.72 mg N, 16.27 mg P, and 174.49 mg K kg^{-1} soil), plant height (100.33 cm), and grain yield (7.27 Mg ha^{-1}). The B3N3 interaction treatment consistently achieved the highest combined values, confirming the synergistic benefits of integrated biological and mineral fertilization for barley production under the prevailing agroecological conditions of southern Iraq.

References

[1] Hussain, I., Ali, I., Ullah, S., Iqbal, A., Al Tawaha, A. R., Thangadurai, D., Sangeetha, J., Rauf, A., Saranraj, P., Al Sultan, W., AL-Taey, D. K. A., Youssef, R. A., & Sirajuddin, S. N. (2021). Agricultural soil reclamation and restoration of soil organic matter and nutrients via application of organic, inorganic and bio fertilization. IOP Conference Series: Earth and Environmental Science, 788, 012165. https://doi.org/10.1088/1755-1315/788/1/012165

ISSN 2072-3857

[2] Suhag, M. (2016). Potential of biofertilizers to replace chemical fertilizers. International Advanced Research Journal in Science, Engineering and Technology, 3(5), 163–167.

[3] AL-Taey, D. K. A., AL-Azawi, S. S. M., AL-Shareefi, M. J. H., & AL-Tawaha, A. R. (2018). Effect of saline water, NPK and organic fertilizers on soil properties and growth, antioxidant enzymes in leaves and yield of lettuce (Lactuca sativa var. Parris Island). Research on Crops, 19, 441–449. https://doi.org/10.31830/2348-7542.2018.0001.14

[4] Al-Taey, D. K. A., Kamaluddin, Z. N. A., & Al-Kazemy, H. H. S. (2022). Impact of Zytonic-M and organic fertilizer on growth and yield of hybrid tomato Awahr (N1) under saline conditions. International Journal of Vegetable Science, 28(4), 1–6. https://doi.org/10.1080/19315260.2022.2070569

[5] Saad, T. A. (2013). The use of Rhizobium bacteria to reduce the infestation of crops with some fungal diseases. Al-Muthanna Journal of Agricultural Sciences, 1(2), 109–118.

[6] Abdullah, N. A., & Al-Kartani, A. K. O. S. (2017). Effect of inoculation with Pseudomonas fluorescens, Azospirillum brasilense and Trichoderma harzianum on some growth traits and plant hormones of maize. Tikrit University Journal of Agricultural Sciences, 17(1), 212–224.

[7] Dawar, S., Wahab, S., Tariq, M., & Zaki, M. J. (2010). Application of Bacillus species in the control of root rot diseases of crop plants. Archives of Phytopathology and Plant Protection, 43(4), 412–418.

[8] Al-Dulaimi, W. A. A. (2013). Study of the environment of algae attached to aquatic plants in the Tigris River, Baghdad/Iraq [Master's thesis, Diyala University, College of Education for Pure Sciences].

[9] Sohail, F. M. (2013). Effect of the interaction between inoculation with Trichoderma harzianum and vegetable fertilization with Chara sp. and chemical fertilizers on the growth of onion plants (Allium cepa L.). Diyala Journal of Agricultural Sciences, 5(2), 223–231.

[10] Hui, D., Mayes, A. M., & Wang, G. (2013). Kinetic parameters of phosphatase: A quantitative synthesis. Soil Biology and Biochemistry, 65, 105–113.

[11] Yang, L., Li, T., Li, F., Lemcoff, J. H., & Cohen, S. (2008). Fertilization regulates soil enzymatic activity and fertility dynamics in a cucumber field. Scientia Horticulturae, 116, 21–26.

[12] Farooq, M., Khan, I., Ahmed, S., Ilyas, N., Saboor, A., Bakhtiar, M., & Khan, A. Y. (2018). Agronomical efficiency of two wheat (Triticum aestivum L.) varieties against

ISSN 2072-3857

different levels of nitrogen fertilizer in the subtropical region of Pakistan. International Journal of Environmental and Agriculture Research, 4(4), 28–36.

[13] Al-Juthery, H. W. A., Ali, E. H. A. M., Al-Ubori, R. N., Al-Shami, Q. N. M., & AL-Taey, D. K. A. (2020). Role of foliar application of nano NPK, micro fertilizers and yeast extract on growth and yield of barley. International Journal of Agricultural and Statistical Sciences, 16(Suppl. 1), 1295–1300.

[14] FAO. (2020). Summary of cereal supplies and demand. Food and Agriculture Organization of the United Nations. https://www.fao.org

[15] Al-Hafez, H. Z. A. (2001). Integration in the control of charcoal rot disease on sesame caused by Macrophomina phaseolina [Master's thesis, University of Baghdad, College of Agriculture].

[16] Tabatabai, M. A., & Bremner, J. M. (1972). Assay of urease activity in soils. Soil Biology and Biochemistry, 4, 479–487.

[17] Black, C. A. (1965). Methods of soil analysis. Part 2: Chemical and microbiological properties. American Society of Agronomy.

[18] Page, A. L., Miller, R. H., & Keeney, D. R. (1982). Methods of soil analysis. Part 2: Chemical and microbiological properties (2nd ed.). American Society of Agronomy / Soil Science Society of America.

[19] Jian, S., Li, J., Chen, J., Wang, G., Mayes, M. A., Dzantor, K. E., Hui, D., & Luo, Y. (2016). Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. Soil Biology and Biochemistry, 101, 32–43.

[20] Al-Juthery, H. W. A., & Al-Taweel, L. S. J. (2022). Effect of integrated nano-complete, mineral fertilizer, and seaweed extracts on the activity of urease in the reduction and oxidation layers of soil planted with rice. IOP Conference Series: Earth and Environmental Science.

[21] Al-Karaawi, N. J. K. (2020). Effect of adding different sources of fertilizers on biomass and enzymatic activity in the root zone of Vigna radiata L. [Master's thesis, University of Al-Qadisiyah, College of Agriculture].

[22] Aeschbacher, M. S., & Schwarzenbach, R. P. (2010). Novel electrochemical approach to assess the redox properties of humic acids. Environmental Science and Technology, 47, 87–93.

[23] Al-Ziadi, H. A. H., & Al-Jubouri, A. K. J. (2023). Effect of Trichoderma harzianum, Chara sp. algae and levels of mineral fertilizer on plant NPK concentration and wheat yield (Triticum aestivum L.). IOP Conference Series: Earth and Environmental Science.

ISSN 2072-3857

[24] Al-Taey, D. K. A., & Majid, Z. Z. (2018). Study effect of kinetin, bio-fertilizers and organic matter application in lettuce under salt stress. Journal of Global Pharma Technology, 10(1), 148–164.

[25] Morel, M. A., Brana, V., & Castro-Sowinski, S. (2012). Legume crops, importance and use of bacterial inoculation to increase production. In A. Goyal (Ed.), Plant crop (pp. 217–240). InTech.

[26] Al-Yasariu, M. N. H. (2012). Effect of batches and levels of nitrogen and potassium fertilizers on the availability and release of ammonium and potassium in the soil and the growth and yield of wheat Triticum aestivum [Master's thesis, University of Baghdad, College of Agriculture].

[27] Panpatte, D. G., Jhala, Y. K., Vyas, R. V., & Shelat, H. N. (2017). Microorganisms for green revolution. Volume 1: Microbes for sustainable crop production. Anand Agricultural University.

[28] Kapri, A., & Tewari, L. (2010). Phosphate solubilization potential and phosphatase activity of rhizospheric Trichoderma spp. Brazilian Journal of Microbiology. https://doi.org/10.1590/S1517-83822010000100015

[29] Dergahm, O. S., Al-Tememe, M. S. O., & Hussain, M. A. (2023). Comparative study of the effect of biological and chemical fertilization on some growth characteristics of wheat crop (Triticum aestivum L.). IOP Conference Series: Earth and Environmental Science.

[30] Al-Aarthy, A. N. B., Al-Jebory, A. K. J., & Al-Kaby, A. H. J. (2023). Effect of adding phosphate-dissolving bacteria on the activity of phosphatase enzyme and some soil characteristics and the yield of eggplant (Solanum melongena L.) under protected cultivation conditions. IOP Conference Series: Earth and Environmental Science.

[31] Al-Samarrai, F. H. S. (2002). Effect of Trichoderma harzianum isolates on the germination and growth of citrus seedlings [Master's thesis, University of Baghdad, College of Agriculture].

[32] Shams Allah, J. A. (2007). Comparison between potassium sulfate and potassium chloride and their relationship to balanced fertilization in growth and yield of tomato in protected cultivation [Doctoral dissertation, University of Baghdad, College of Agriculture].

[33] Al-Rishawi, N. H. O., Noni, G. B., & Hadi, B. J. (2023). Effect of applying biofertilizers and salicylic acid on NPK in soil and fruits of eggplant (Solanum melongena L.) under protected conditions. IOP Conference Series: Earth and Environmental Science.

[34] Al-Maliki, S., Al-Amery, A., Sallal, M., Radhi, A., & Al-Taey, D. K. A. (2021). Effects of arbuscular mycorrhiza and organic wastes on soil carbon mineralisation, actinomycetes and nutrient content in maize plants (Zea mays L.). Malaysian Journal of Soil Science, 25, 107–124.

[35] Nora, A., Hamza-Meddad, A., & Rachid, D. (2017). Impact of arbuscular mycorrhizal fungi and fertilization levels on biochemical changes in potato (Solanum tuberosum L.). Biodiversity Journal, 8(2), 689–696.

[36] Muhaisin, M. A. K. (2019). Effect of adding Trichoderma harzianum and NPK fertilizers on some indicators of growth and yield of wheat (Ibaa 99 variety) [Master's thesis, University of Al-Muthanna, College of Agriculture].

[37] Al-Hrkane, H. T., Mohammed, I. Q., & Al-Taweel, L. S. J. (2025). Effect of adding spent mushroom, biofertilizers and mineral fertilizers on the availability of some nutrients and the soil aggregates stability. Basrah Journal of Agricultural Sciences.

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