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Microbiomes-Plant Interactions and K-Humate Application for Salinity Stress Mitigation and Yield Enhancement in Wheat and Faba Bean in Egypt's Northeastern Delta

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Abstract: Salinity, resulting from climate change and excessive mineral fertilization, burdens farmers and negatively impacts soil and water ecosystems in the Northeastern Nile Delta. Organic and biological approaches are crucial for addressing these issues. This study examined the effects of individual and combined inoculations with cyanobacteria, yeast, and Arbuscular Mycorrhizal Fungi (AMF), with or without K-Humate and reducing Nitrogen, phosphorus and potassium (NPK) mineral fertilizers application rates to crop quality of wheat and faba bean. In preliminary laboratory experiments, the interactive effects of these microbiomes on plant antioxidant and soil enzyme production were examined under salinity stress. Results showed that co-inoculation, especially with K-Humate, yielded superior outcomes compared to individual inoculations. These findings were validated by a field trial conducted in saline-alkaline soil in the Northeastern Nile Delta region. All biological treatments 25% of recommended doses, and enhancing salinity tolerance, increasing yield, and improving enhanced rhizosphere microbial activity, including soil enzyme activity, AMF colonization, spore density, and the total numbers of bacteria, cyanobacteria, and yeast. These effects were further amplified by K-Humate and were more pronounced with combined inoculations than with individual ones, leading to improved soil fertility and significant increases in both crop quantity and quality compared to control treatments. The triple treatment, combining cyanobacteria, yeast, and mycorrhizae in the presence of K-Humate while reducing the mineral NPK rate by 75%, achieved superior increases in the productivity of wheat grains and faba bean seeds, reaching 54.72% and 128.92%, respectively, compared to the 100% NPK mineral control. This treatment also significantly improved crop quality, with notable increases in nitrogen, potassium, phosphorus, and protein percentages in wheat grains and faba bean seeds. Microbiomes-interaction increased potassium uptake over sodium, enhancing the plant's potassium/sodium ratio and improving salt stress tolerance. This approach reduces reliance on costly mineral fertilizers, enabling bio-organic farming in marginal lands, optimizing resource utilization, and preserving natural resources.

Keywords: antioxidant enzymes; arbuscular mycorrhizal fungi; bio-organic farming; crop quality and yield; cyanobacteria; K-Humate; microbial activity; potassium uptake; salinity tolerance; yeast

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1. Introduction

Global climate change and intensive agricultural practices have intensified abiotic stresses, including drought, salinity, UV rays, and temperature extremes. These stresses particularly affect arid and semi-arid regions, increasing soil and water salinity and limiting

plant growth. As a result, agricultural productivity in these areas can decrease by 20% to 50% [1].These stressed conditions of soil salinization have grown in Egypt to represent about 46% of the total Nile Delta zone due to the complex environmental risks from climate change and human-caused developments in addition to water scarcity problems [2,3]. Nowadays in Egypt, there is a tendency to expand the cultivated area of many crops in the Northeastern Delta of Egypt, including wheat and faba bean, in newly-reclaimed soil, although most of these soils are affected by salinity, which is a real global problem that requires urgent solutions [4]. El Husseiniya Plain in Sharkia Governorate in the Nile Delta region of Egypt of about 141.6 square kilometers area and irrigated from El-Salam Canal (drainage water and Nile river water 1:1) suffers from soil degradation caused by salinization and alkalinity that limits plant growth and development leading to yield losses [5]. Despite the continued expansion of wheat (*Triticum aestivum* L.) cultivations in Egypt, the increase in consumption is much higher than the rate of production as the average consumption was 16.6 million tons, while the production was estimated at 9.3 million tons, which led to an increase in imports that constitutes a huge burden on the Egyptian agricultural trade balance [6]. Despite the continued expansion of wheat (*Triticum aestivum* L.) cultivations in Egypt, the increase in consumption is much higher than the rate of production, leading to an increase in imports that constitutes a huge burden on the Egyptian agricultural trade balance. The wheat gap is significant, with wheat and flour subsidies imposing a serious threat to the economy [7]. Additionally, salinity stress severely affects wheat cultivation, impacting food security [8]. Faba bean (*V. faba* L.), a rotational crop in the Mediterranean region and a major leguminous crop in Egypt, is an important source of crude protein and essential amino acids for human and animal nutrition [9]. *V. faba* plants are proved to be moderately sensitive legumes to salinity, exhibiting a reduction in plant growth up to 50% under 6.7 dS m^{-1} salinity [10]. Since the yields of faba bean and wheat are negatively affected by abiotic stresses such as salinity and drought, developing effective, low-cost, and adaptive methods for managing and coping with abiotic stresses is a major challenge [11]. In addition to the negative effect of salinity stress on plant growth which inhibit crop productivity in these soils, it may also negatively affect microbial diversity and decrease its fertility leading to soil erosion and deterioration. However, some microbes that may tolerate high salinity conditions and still maintain natural activity, which may positively affect plant growth such as cyanobacteria, yeast, and AMF, attach on/into the roots of plants and help in the absorption of water and nutrients from soil. Microorganisms could play an important role in adaptation strategies and increase tolerance to abiotic stresses in agricultural plants. Plant-growth-promoting rhizobacteria (PGPR) mitigate most effectively the impact of abiotic stresses on plants through the production of exopolysaccharides and biofilm formation. AMF and dual symbiotic systems (endophytic rhizospheric bacteria and symbiotic fungi) also tend to mitigate the abiotic stress in plants [12]. Humic acids (HA) also convert elements to more beneficial forms and make them more effective, like PGBR.

The beneficial effects of HA treatments on crops under salinity conditions are attributed to improvements in soil physical and chemical properties, such as promoting microbial growth, increasing nutrient availability, and enhancing enzyme activity, organic acid, and amino acid content. Although PGPRs and HA have demonstrated salinity damage reduction in some crops under controlled conditions, field results have often been insignificant and not reflective of productivity gains, underscoring the need for field validation of these biostimulants [13]. Due to their susceptibility to salt stress, crops may be affected adversely and farmers in areas experiencing increasingly severe droughts may need to implement new methods to minimize the harmful impact of salt stress on their crops. Crop types with higher drought resistance, more efficient irrigation methods, soil additives, and applications of biotechnologies that lower salinity are all examples of strategies for sustainable and safe economic production of strategic crops that ensure food security [14]. This work aimed to investigate the interactive effects of exogenously applied microbiomes, including cyanobacteria, yeast, and arbuscular mycorrhizal fungi (AMF), along with humic acids and a reduced dose (25%) of mineral fertilizers, to mitigate the harmful effects

of salinity stress on the productivity of faba bean and wheat crops. This study hypothesized that: (i) co-inoculation of cyanobacteria, yeast, and AMF with humic acids would significantly alleviate salinity stress and enhance antioxidant activity, growth, and productivity of bean and wheat under field conditions compared to single inoculations or conventional fertilization; (ii) this approach would improve microbial diversity in the rhizosphere and nutrient availability, leading to enhanced soil fertility, crop yields, and quality; and (iii) the use of reduced mineral fertilizers combined with microbiomes biostimulants and soil organic amendments would provide an eco-friendly solution for sustainable crop production under abiotic stress.

2. Materials and Methods

2.1. Preparation of Microbial Inoculation

Mixed cultures of cyanobacteria strains, yeast, and Arbuscular Mycorrhizal Fungi (AMF), individually or in combination, were tested as inoculants to ameliorate salinity stress and enhance the productivity of faba bean (*V. faba* L.), (cv-Nubaria 1) and wheat (*Triticum aestivum* L.) (cv-Sakha 93) in presence of K-Humate and stimulatory dose (25%) of the recommended mineral NPK fertilizer.

2.1.1. Cyanobacteria (Cyano)

Four heterocystous (*Nostoc muscorum* isolate HSSASE1, *Anabaena oryzae* isolate HSSASE6, *Anabaena* sp. HSSASE11 and *Wollea saccata* isolate HSSASE7) and two non-heterocystous (*Phormidium fragilis* isolate HSSASE9 and *Spirulina platensis* isolate HSSASE5) cyanobacteria strains were obtained from Microbiology Department, Soils, Water and Environment Res. Inst., Agric. Res., Center. The cyanobacteria strains were isolated from Egyptian soils and identified and deposited in GenBank under accession numbers (Table 1). All cyanobacterial strains were grown separately on BG11medium [15] except *Spirulina platensis*, which was grown on Zarrouk medium [16].

Table 1. GenBank accession number and some characterization parameters of different cyanobacteria and green microalgae species under study.

Ser.	Microalgal Strains	NCBI * Accession No.	Family	pH	Optical Density at 560 nm	Total Chlorophyll (mg•L^{-1})	Dry Weight (mg•L^{-1})
1	*Nostoc muscorum* isolate HSSASE1	KT277784.1	Nostocaceae	8.11	1.19	5.26	760.96
2	*Spirulina platensis* isolate HSSASE5	KT277788.1	Spirulinaceae	10.16	2.77	11.63	1772.80
3	*Anabaena oryzae* isolate HSSASE6	KT277789.1	Nostocaceae	7.14	0.87	4.03	557.76
4	*Wollea saccata* isolate HSSASE7	KT277790.1	Nostocaceae	6.82	2.40	9.82	1532.80
5	*Phormidium fragilis* isolate HSSASE9	KT277792.1	Phormidiaceae	8.67	2.09	3.00	1334.40
6	*Anabaena* sp. HSSASE11	KT277794.1	Nostocaceae	8.05	1.67	7.56	1065.60

* National Center for Biotechnology Information (NCBI).

The cultures were incubated in a growth chamber with continuous shaking (150 rpm) under continuous illumination with Philips Florescent 40-W cool-white fluorescent lamps, at a relatively low light intensity (400–500 lux) and incubated at 27 ± 2 °C for 30 days to be used as inoculums for laboratory experiments. Some characterization parameters of cyanobacteria strains in the stationary phase such as pH values, algal dry weight [17], and optical density by spectrophotometer at 560 nm [18] are shown in Table 1. Equal portions on a volumetric basis from the suspensions of the cyanobacterial cultures, in the stationary phase, were mixed together for use in laboratory and field experiments. Seeds were soaked 12 h before the laboratory germination experiment, while in the field

experiment, 24 L•ha^{-1}(mg•L^{-1}) of cyanobacteria mixture was added in 3 equal doses after one month, 45 days, and 60 days of sowing.

2.1.2. Yeast (Y)

Saccharomyces cerevisiae was kindly provided by the microbiology Department Soils, Water and Environment Research Institute, Agriculture Research Center, Giza, Egypt. *Saccharomyces cerevisiae* was grown on glucose peptone and yeast extract (GPY) mediums [19]. A total of 100 mL of yeast culture containing 1 × 10^8 cells.mL^{-1} (cell•mL^{-1}) was used for single treatments, while 50 mL was used just before inoculation for combined treatments.

2.1.3. Arbuscular Mycorrhizal Fungi (AMF)

Mixed spores of AMF (*Glomus mosseas*, *Glomus intraradices*, and *Glomus* sp.) were originally extracted from the rhizosphere of maize plants grown in Sahl El-Hossynia Research Farm Station, El-Sharkia Governorate. The inoculum contains 38 × 10^2 spores/Kg soil. AMF inoculated treatments received 100 g soil + 50 g infected maize roots + external hyphae. The inoculants were mixed with stickers (Arabic gum), uniformly coated on the seeds, and then air dried for 2 h before sowing.

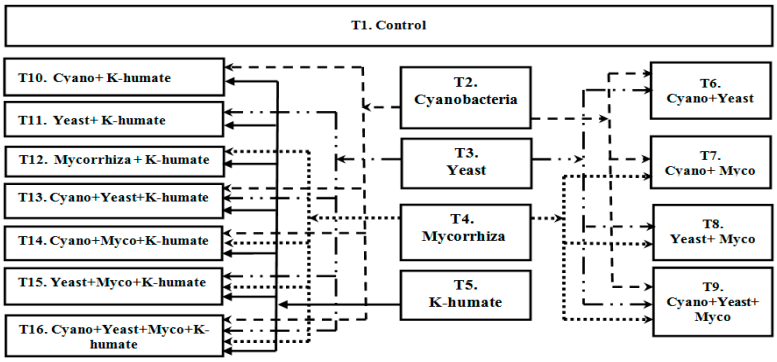
2.2. K-Humate Preparation (K-H)

Potassium humate (K-Humate) was extracted from rice straw compost and characterized according to the standard method described by Sanchez-Monedero et al. [20]. Rice straw compost was mixed with water at a rate of 1:5 (*w*/*v*) and then treated with 0.5 N of potassium hydroxide (KOH) solution to extract the humic substances.

2.3. In Vitro and Field Trials

2.3.1. In Vitro Experiment

A one-month lab experiment was conducted in pots to study the antagonistic or synergistic effects of microbial treatments on rhizosphere enzyme activity (dehydrogenase and nitrogenase), plant oxidative enzyme activity as peroxidase (POD) and catalase (CAT), and the lengths of shoots and roots of faba bean (*V. faba* L. cv. Nubaria 1) and wheat (*Triticum aestivum* L. cv. Sakha 93). The aim was to assess the most promising inoculation for field trial application under salinity stress and mineral NPK deficiency. The seeds were sterilized with 5% Clorox, rinsed thoroughly, and then presoaked in the biofertilizer treatments for 12 h before being germinated in plastic pots (25 cm × 25 cm) filled with saline soil (18.57 dS•m^{-1}) from the field experimental site. All treatments, except the control, were irrigated with saline water from the El-Salam Canal for one month under laboratory conditions. The experimental design is outlined in Scheme 1.



Scheme 1. In vitro experimental design illustrating the application of microbial and K-Humate treatments to faba bean and wheat plants under salinity stress and mineral NPK deficiency. The dotted arrows in the scheme illustrate the pathways of each treatment applied to the plants. Each arrow indicates the specific interactions between the treatments and the plants, highlighting their contributions to the overall experimental design.

Based on the results of this preliminary experiment, the most promising interactions of seeds with different biofertilizer formulas, as well as their combination with K-Humate under salinity stress, will be selected for the field trial.

2.3.2. Field Experiment

Experimental Location and Soil Analysis

The field experiment was conducted during the winter season of 2021/2022 in one of the experimental sites located at Khaled ben El-Waleed village, Sahl Al-Hussainiya 31°8′12.461″ N and 31°52′15.469″ E, Sharkia Governorate, Egypt (Figure 1). The soil was clayey textured, highly alkaline, saline, and poor in organic matter. Soil texture characterization and chemical analyses (Table 2) were determined according to Page et al. [21]. Irrigation for this location was sourced from the El-Salam Canal (Table 3), which is a mix of Nile fresh water and agricultural drainage water (1:1).

Figure 1. Field experiment site in the northeast Nile Delta, Sharkia Governorate, Egypt. (**A**) Location of the experimental sites at Khaled ben El-Waleed village; (**B**) Experimental plots before and after the growth of faba bean and wheat under salinity conditions.

Table 2. Soil Texture Characterization and Chemical Analyses of the Experimental Site in Khaled Ben El-Waleed Village, Sahl Al-Hussainiya, Sharkia Governorate, Egypt.

Coarse Sand (%)	Fin Sand (%)	Silt (%)	Clay (%)	Texture	O.M. (%)	$CaCO_3$ (%)
3.14	8.29	28.76	59.81	Clay	0.48	5.19
pH (1:2.5)	**EC ($dS \bullet m^{-1}$)**	**Cations (meq/L)**			**Anions (meq/L)**	
		Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	SO_4^{2-}
8.25	18.57	12.46	21.73	150	0.76	8.25	132	44.70
Macronutrients (ppm)					**Micronutrients (ppm)**			
N	P	K		Fe	Mn	Zn		Cu
37	5.67	189		1.37	3.25	0.73		0.048

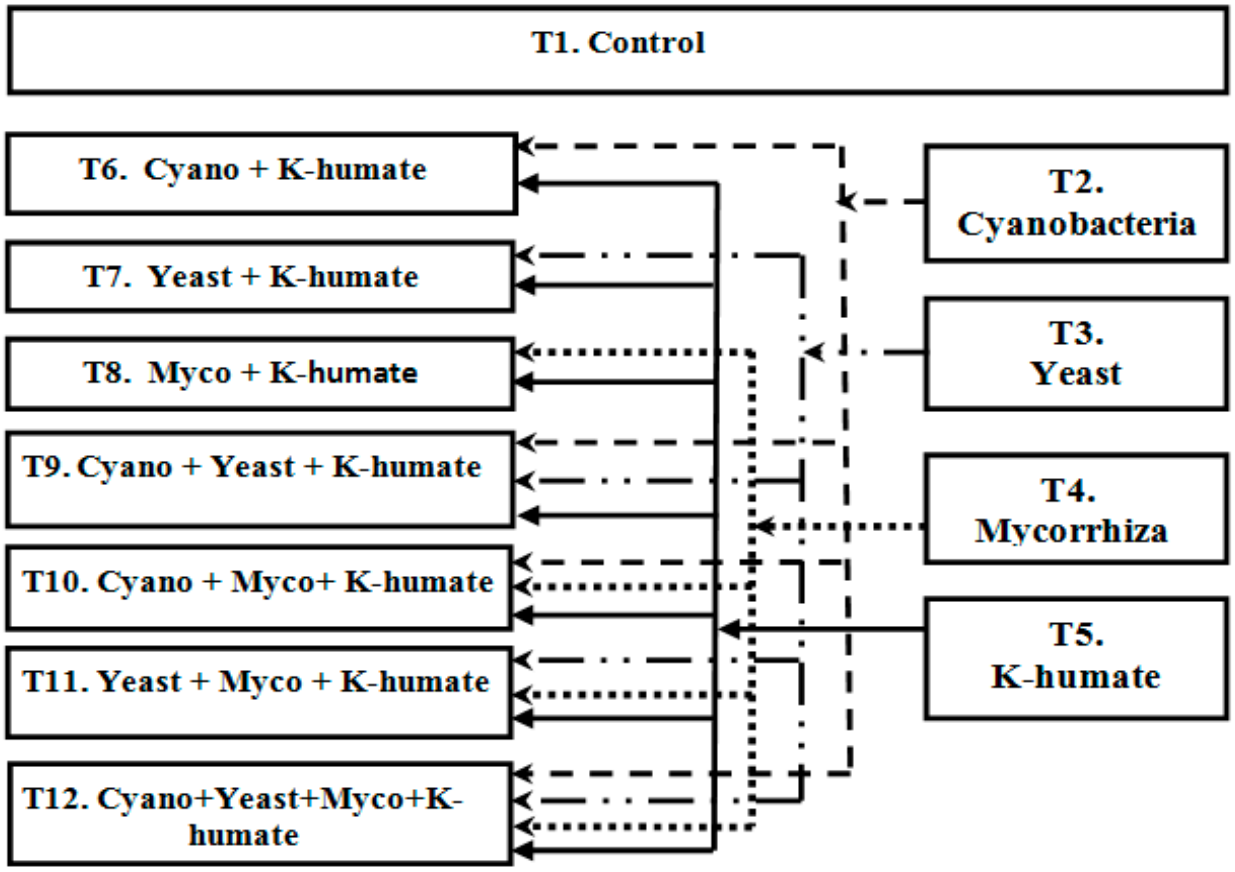
Table 3. Physical and Chemical Properties of Irrigation Water from El-Salam Canal.

pH	EC ($dS \bullet m^{-1}$)	Cations (meq/L)				Anions (meq/L)				R.S.C.	SAR
		Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	HCO_3^{-}	CO_3^{2-}	Cl^{-}	SO_4^{2-}		
9.00	4.94	2.12	2.29	39.00	2.40	12.45	6.60	16.27	10.49	14.64	26.25

Inorganic nitrogen (N), phosphorus (P), and potassium (K) fertilizers were sourced from the local market in Sharkia Governorate, Egypt, as ammonium sulfate (20.5% N), calcium superphosphate (15.5% P_2O_5), and potassium sulfate (48% K_2O), respectively. The recommended application rates of mineral fertilizers for the newly reclaimed saline soils were applied as follows: for wheat, 250 kg N, 500 kg P_2O_5, and 250 kg K_2O per hectare; for faba bean, 100 kg N, 250 kg P_2O_5, and 150 kg K_2O per hectare. These rates were determined based on crop-specific nutrient requirements to optimize growth and yield under saline conditions.

This experiment aimed to investigate the individual and combined effects of cyanobacteria, yeast, AMF, and K-Humate with 25% of the recommended mineral NPK fertilizers on the yield and yield components of faba bean and wheat under saline-alkaline soil conditions. Some soil biological activities were studied, including the total number of bacteria, cyanobacteria, and yeast, as well as soil enzymes (dehydrogenase and nitrogenase) after 75 days of cultivation. Additionally, AMF colonization was estimated after 45 and 75 days of cultivation, and spore numbers were assessed at harvest.

The field experimental is presented in Scheme 2 as follows:



Scheme 2. Experimental Design for Assessing the Effects of Biostimulants on Faba Bean and Wheat Yields Under Saline-Alkaline Conditions. The dotted arrows in the scheme represent the interactions between various treatments applied to the plants, indicating their pathways and contributions to the overall experimental setup.

2.4. Measurements

2.4.1. Soil Microbial Enzymes Analysis

Dehydrogenase activity was assayed in soil according to Glathe and Thalmann [22]. The activity of nitrogenase enzyme, as an indicator of free-living N_2-fixation potential in the rhizosphere of wheat soil (μmol C_2H_4 h^{-1} g^{-1} dry soil), was determined according to the method described by Dilworth [23].

2.4.2. Oxidative Enzymes Bioassay

POT activity was determined according to the method described by Allam and Hollis [24] and CAT activity assay was determined following the method of Góth [25].

2.4.3. Soil Biological Activity

Two soil enzyme activities(Dehydrogenase and Nitrogenase) were determined by the methods of Casida et al. [26] and Dilworth [23], respectively. Concerning the total count of bacteria, cyanobacteria, and yeast were counted after the 75thday from sowing. Total count of bacteria the dilution plate method on nutrient agar [27] and total cyanobacterial counts were conducted by plating ten-fold serial soil suspension-dilutions in triplicate onto agarized BG11 medium [28] and Watanabe and Barraquio [29] or nitrogen-fixing bacteria. AMF colonization (%) was estimated after 45 and 75 days of sowing by the method described by Phillips and Hayman [30] while spores numbers were determined at the harvest stage according to Gerdemann and Nicolson [31].

2.4.4. Crop Components and Some Chemical Analyses

At harvest, the collected wheat straw and grains samples were oven-dried at 70 °C to determine the total N, P, K, and Na contents in seeds as described by Van Schouwenburg [32]. Harvest index was calculated as a ratio of grain yield to total biological yield (straw yield + grain yield). Biological and grain yields were recorded at two central rows in each experimental unit. The subsequent sample was oven-dried at 70 °C for a maximum of 36 h to estimate dry matter yield. A total of 1000 grains were weighed on an electronic balance after drying for recording in the Seed Index [33].

2.4.5. Statistical Analysis

The obtained data were statistically analyzed using the COSTAT program and the Least Significant Difference (LSD) test at a 5% level of probability according to Gomez and Gomez [34].

3. Results

3.1. Effect of Microbiomes-Plant Interactions and K-Humate on Wheat and Faba Bean Seedlings under Salinity Stress In Vitro

3.1.1. Seedling Growth

Shoot Length

The shoot length of faba bean seedlings exhibited significant variation across different treatment groups (Figure 2). The Cyano + Yeast (T6) treatment resulted in the greatest shoot length, measuring 61.0 cm, which represents a 27.1% increase compared to the control (T1, 48.0 cm; $p \leq 0.05$). Other treatments that significantly influenced shoot length included K-Humate (T5), which yielded a shoot length of 43.0 cm, reflecting a 10.4% decrease relative to the control, and the Cyano + Yeast + AMF (T9) treatment, which resulted in a shoot length of 42.0 cm, corresponding to a 12.5% reduction. The Cyano + AMF + K-H (T14) treatment and Cyano + Y + AMF + K-H (T16) improved shoot length to 52.0 cm and 54.0 representing an 8.3 and 12.5%, respectively, increase compared to the control. Conversely, the individual AMF treatment (T4) severely inhibited shoot growth, with a shoot length of just 5.2 cm, marking an 89.2% reduction from the control ($p \leq 0.05$). A similar pattern was observed for wheat seedlings (Figure 3). The longest shoot length was recorded in the Cyano + Y + AMF treatment (T9, 34.01 cm), representing a 36.0% increase compared to the control (T1, 25.0 cm; $p \leq 0.05$).

Significantly increased shoot lengths were also noted in the Cyano (T2, 27.0 cm; 8.0% change), Cyano + AMF (T7, 29.0 cm; 16.0% change), and Cyano + K-H (T10, 29.0 cm; 16.0% change) treatments relative to the control. Consistent with the results for faba bean, the AMF treatment (T4) led to a complete suppression of shoot growth, with a shoot length of 0.0 cm, which was 100% lower than the control ($p \leq 0.05$).

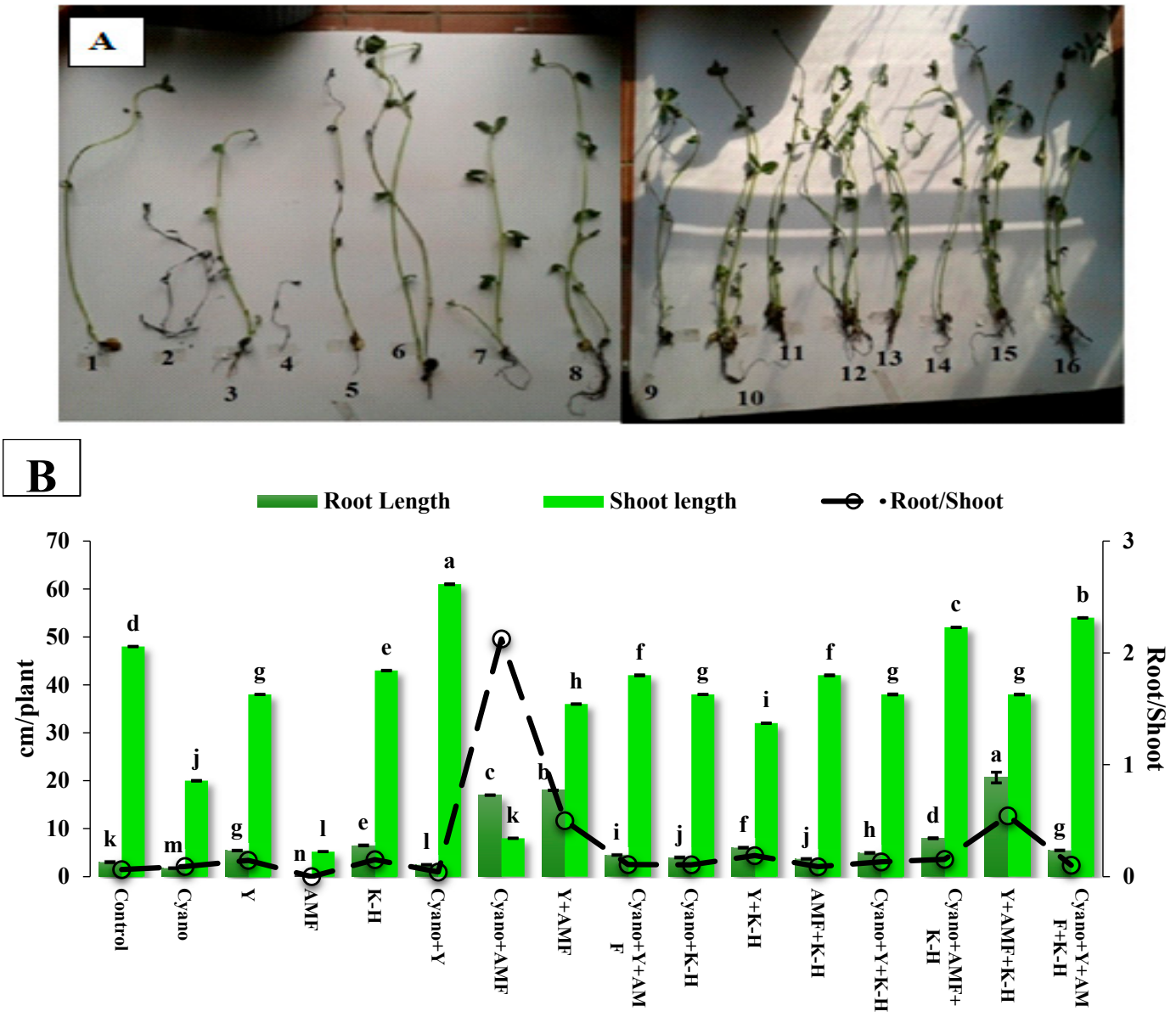


Figure 2. Impact of exogenous microbiomes and K-H on faba bean seedling growth after 30-day experiment under salinity stress. (**A**) An image of faba bean seedlings. The treatments are as follows: (1) Control, (2) Cyanobacteria (Cyano), (3) Yeast (Y), (4) Arbuscular Mycorrhizal Fungi (AMF), (5) K-Humate (K-H), (6) Cyano + Y, (7) Cyano + AMF, (8) Y + AMF, (9) Cyano + Y + AMF, (10) Cyano + K-H, (11) Y + K-H, (12) AMF + K-H, (13) Cyano + Y + K-H, (14) Cyano + AMF + K-H, (15) Y + AMF + K-H, (16) Cyano + Y + AMF + K-H. (**B**) faba bean shoot length, root length, and root-to-shoot ratio. Different letters (a, b, c, etc.) above the columns indicate significant difference between the treatments ($p \leq 0.05$). The letter "a" indicates the highest significant value, followed by letters in descending order of significance.

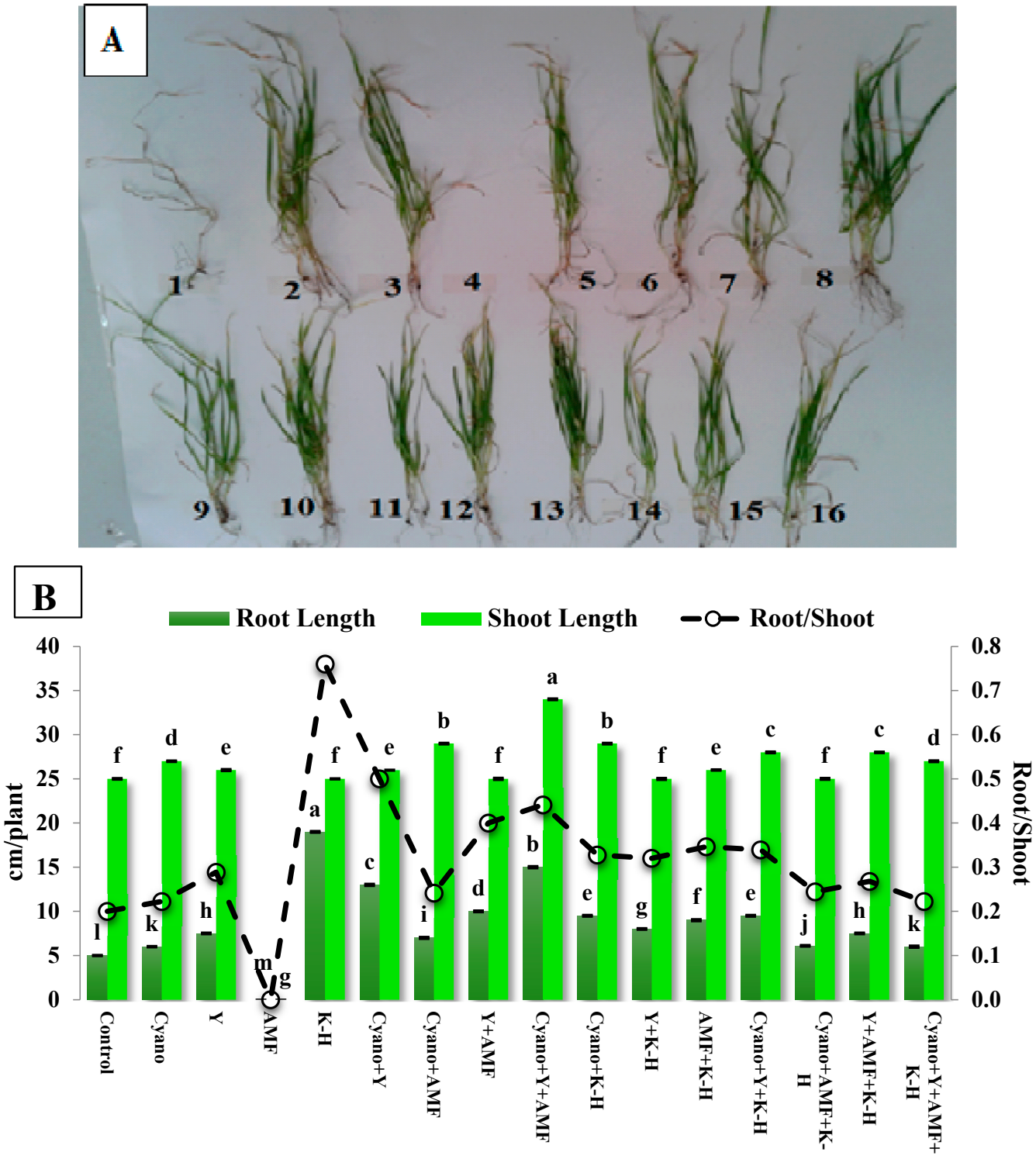


Figure 3. Effects of exogenous microbiomes and K-H on wheat seedling growth and morphology after 30-days experiment under salinity stress. (**A**) Visual assessment of wheat seedling morphology. The treatments are as follows: (1) Control, (2) Cyanobacteria (Cyano), (3) Yeast (Y), (4) Arbuscular Mycorrhizal Fungi (AMF), (5) K-Humate (K-H), (6) Cyano + Y, (7) Cyano + AMF, (8) Y + AMF, (9) Cyano + Y + AMF, (10) Cyano + K-H, (11) Y + K-H, (12) AMF + K-H, (13) Cyano + Y + K-H, (14) Cyano + AMF + K-H, (15) Y + AMF + K-H, (16) Cyano + Y + AMF + K-H. (**B**) Shoot length, root length, and root-to-shoot ratio in wheat seedlings. Different letters (a, b, c, etc.) above the columns and curve indicate statistically significant differences between the treatments ($p \leq 0.05$). The letter "a" represents the highest significant value, followed by letters in descending order of significance.

Root Length

The root length of faba bean seedlings also varied significantly among the different treatment groups (Figure 2). The longest root lengths were observed in the Y + AMF + K-H (T15, 20.67 cm) followed by Y + AMF (T8, 18.0 cm) treatments, which were 589% and 500%, respectively greater than the control (T1, 3.0 cm; $p \leq 0.05$). In contrast, the AMF treatment (T4) resulted in a complete inhibition of root growth, with a root length of 0.0 cm, which was 100% lower than the control ($p \leq 0.05$).

For wheat seedlings (Figure 3), the longest root length was recorded in the K-H treatment (T5, 19.0 cm), representing a 280% increase compared to the control (T1, 5.0 cm; $p \leq 0.05$). Increased root lengths were also observed in the Cyano + Y (T6, 13.0 cm; 160% change) and Cyano + Y + AMF (T9, 15.0 cm; 200% change) treatments relative to the control. Consistent with the results for faba bean, the AMF treatment (T4) led to a complete suppression of root growth, with a root length of 0.0 cm, which was 100% lower than the control ($p \leq 0.05$).

Root-to-Shoot Length Ratio

The root-to-shoot length ratio in faba beans (Figure 2) showed significant variation ($p \leq 0.05$) across the different treatments. The Cyano + AMF treatment (T7) exhibited the highest root-to-shoot ratio at 2.13, indicating a substantial enhancement of root growth relative to shoot growth under this treatment. Other treatments, such as Y + AMF (T8) and Y + AMF + K-H (T15), also resulted in relatively high root-to-shoot ratios of 0.50 and 0.54, respectively, suggesting a significantly greater allocation of resources towards root development compared to shoot growth in these treatments ($p \leq 0.05$).

In wheat, the root-to-shoot length ratio was generally lower compared to faba bean. The highest ratio in wheat (Figure 3) was observed under the K-H treatment (T5), with a ratio of 0.76, indicating a more balanced growth between roots and shoots. This treatment demonstrated significant improvements ($p \leq 0.05$) in both root and shoot lengths. Other treatments with relatively high root-to-shoot ratios in wheat included Cyano + Y (T6) and Cyano + Y + AMF (T9), with ratios of 0.50 and 0.44, respectively. These ratios reflect a more proportionate growth between roots and shoots in these treatments compared to others. The observed differences in root-to-shoot length ratios between faba bean and wheat highlight the varying responses of these two crop species to the applied treatments, with faba bean generally showing a greater tendency to allocate resources towards root growth under specific conditions.

3.1.2. Plant Antioxidant Enzymes

Catalase (CAT) Activity

The present study investigated the impact of various microbiome treatments, with and without K-Humate, on catalase (CAT) activity in faba bean and wheat seedlings (Figure 4). In faba bean, individual treatments with cyanobacteria (T2) and yeast (T3) increased CAT activity by 20.1% and 45.3%, respectively, compared to the control (T1). However, AMF treatment (T4) completely inhibited CAT activity. K-Humate alone (T5) enhanced CAT activity by 42.4%. Co-inoculation significantly boosted CAT activity. The Cyano + Y combination (T6) led to a 73.9% increase, followed by Y + AMF (T8) at 106.5%, and Cyano + AMF (T7) at 70.1%. The triple combination (Cyano + Y + AMF, T9) exhibited a remarkable 255.5% increase. Combining K-Humate with co-inoculations further amplified CAT activity. The Cyano + Y + K-H treatment (T13) showed the highest increase at 559.5%, followed by Cyano + AMF + K-H (T14) at 428.8%, Y + AMF + K-H (T15) at 403.0%, and Cyano + Y + AMF + K-H (T16) at 339.5%.

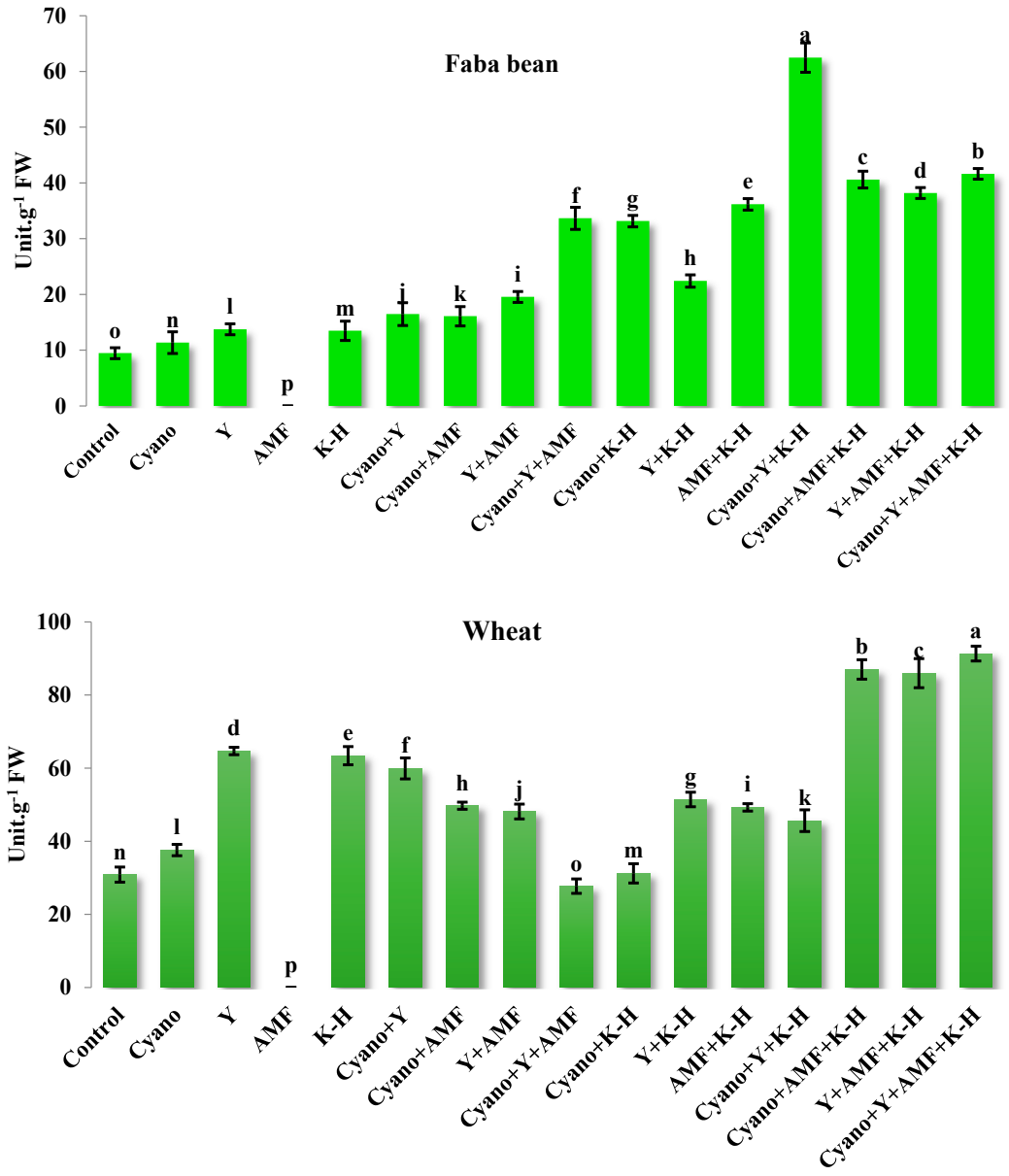


Figure 4. The effect of different microbiomes treatments and K-H on catalase (CAT) activity in faba bean (**upper**) and wheat seedlings (**bottom**). Different letters (a, b, c, etc.) above the columns indicate significant difference between the treatments ($p \leq 0.05$). The letter "a" indicates the highest significant value, followed by letters in descending order of significance.

Similar trends were observed in wheat. Individual treatments with cyanobacteria (T2) and yeast (T3) increased CAT activity by 21.7% and 109.4%, respectively. AMF treatment (T4) again inhibited CAT activity, while K-Humate alone (T5) enhanced it by 105.2%. In wheat, the Cyano + Y combination (T6) increased CAT activity by 94.0%. While Cyano + AMF (T7) and Y + AMF (T8) showed increases of 60.9% and 55.7%, respectively, the Cyano + Y + AMF combination (T9) only increased it by 11.4%. Incorporating K-Humate into wheat co-inoculations significantly enhanced CAT activity. The Cyano + Y + AMF + K-H treatment (T16) exhibited the highest increase at 195.6%, followed by Cyano + AMF + K-H (T14) at 181.6%, and Y + AMF + K-H (T15) at 178.3%. These findings demonstrate the potential of microbial inoculations, especially in combination with K-Humate, to enhance CAT activity and, consequently, antioxidant defense mechanisms in both faba bean and wheat seedlings.

Peroxidase (POD) Activity

The present study evaluated the impact of various microbiome treatments, both with and without K-Humate, on peroxidase (POD) activity in 30-day-old faba bean and wheat seedlings (Figure 5).

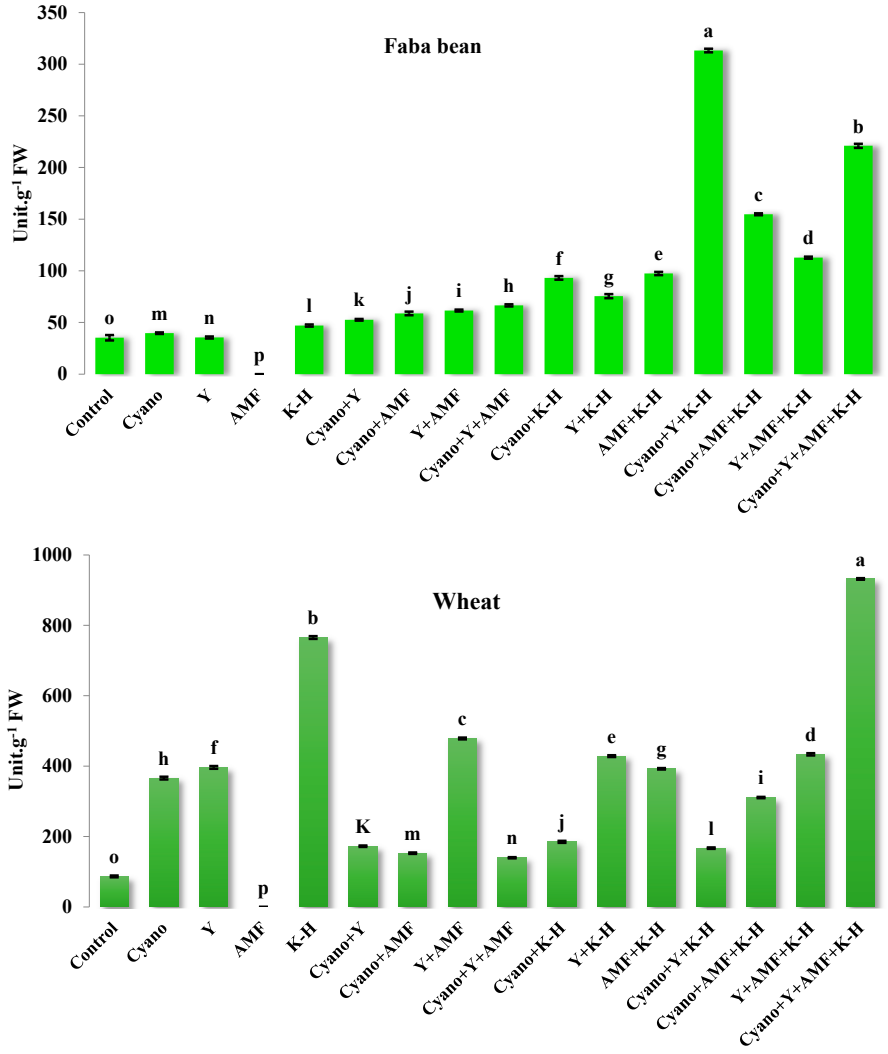


Figure 5. The effect of different microbiomes treatments and K-H on peroxidase (POD) activity in faba bean (**upper**) and wheat seedlings (**bottom**). Different letters (a, b, c, etc.) above the columns indicate significant difference between the treatments ($p \leq 0.05$). The letter "a" indicates the highest significant value, followed by letters in descending order of significance.

In faba bean, individual treatments with cyanobacteria (T2) and yeast (T3) resulted in marginal increases in POD activity by 12.7% and 0.9%, respectively, compared to the control (T1). However, the AMF treatment (T4) completely inhibited POD activity. K-Humate alone (T5) increased POD activity by 33.5%. Co-inoculation treatments showed significant enhancements in POD activity. The Cyano + Y combination (T6) led to a 49.4% increase, followed by Cyano + AMF (T7) at 66.8%, and Y + AMF (T8) at 74.7%. The Cyano+ Y + AMF treatment (T9) exhibited an 88.9% increase. When K-Humate was added to these co-inoculations, POD activity was further amplified. The Cyano + Y + K-H treatment (T13) showed an 789.1% increase, followed by Cyano + AMF + K-H (T14) at 339.2%, Y + AMF + K-H (T15) at 219.9%, and Cyano + Y + AMF + K-H (T16) at 527.4%.

In wheat, the individual treatments with cyanobacteria (T2) and yeast (T3) substantially increased POD activity by 320.9% and 356.0%, respectively. However, the AMF treatment (T4) again inhibited POD activity. K-Humate alone (T5) resulted in a 780.2% increase. Among the co-inoculation treatments, Y + AMF (T8) showed the highest increase at 450.6%, followed by Y + K-H (T11) at 392.2%. The Cyano + Y + AMF + K-H treatment (T16) showed the most significant enhancement with a 972.0% increase in POD activity. Other notable increases included Cyano + AMF + K-H (T14) at 257.6% and Y + AMF + K-H (T15) at 398.8%. These results highlight the synergistic effect of combining K-Humate significantly enhancing peroxidase activity in both faba bean and wheat seedlings, particularly under co-inoculation conditions.

3.1.3. Soil Enzymes

The present study evaluated the impact of various microbiome treatments, with and without K-Humate, on soil enzyme dehydrogenase (DHA-ase) and nitrogenase (N-ase) activity in faba bean and wheat seedlings (Figures 6 and 7).

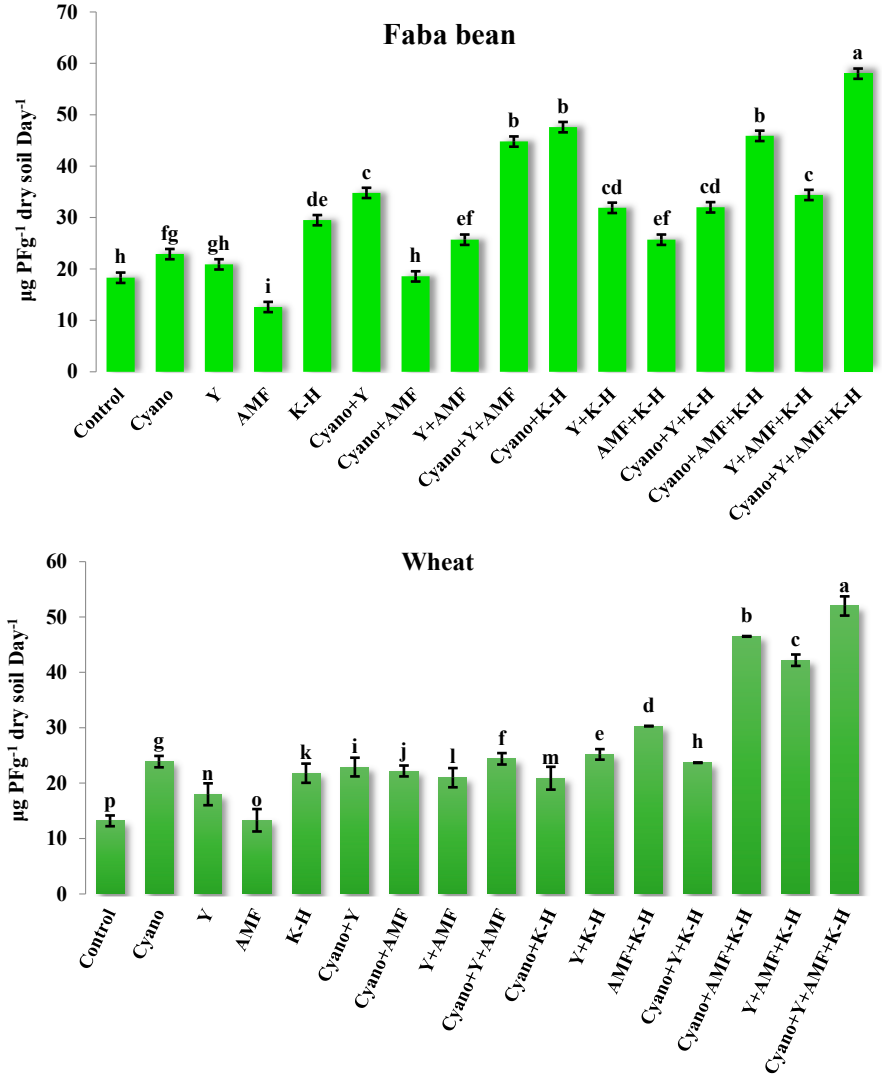


Figure 6. The effect of different microbiomes treatments and K-H on dehydrogenase (DHA-ase) activity in faba bean (**upper**) and wheat seedlings (**bottom**). Different letters (a, b, c, etc.) above the columns indicate significant difference between the treatments ($p \leq 0.05$). The letter "a" indicates the highest significant value, followed by letters in descending order of significance.

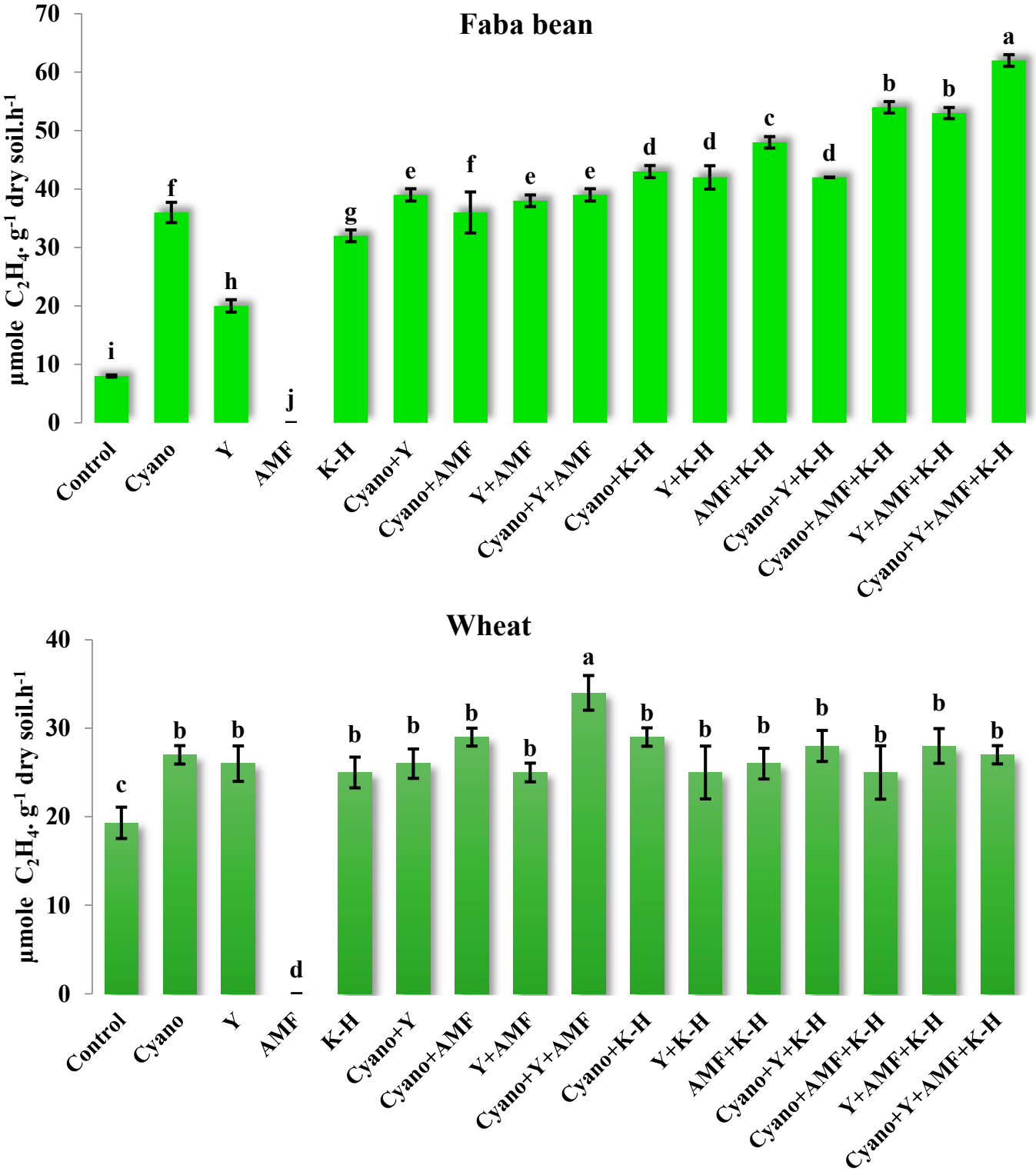


Figure 7. The effect of different microbiome treatments and K-H on nitrogenase (N-ase) activity in faba bean (**upper**) and wheat seedlings (**bottom**). Different letters (a, b, c, etc.) above the columns indicate significant difference between the treatments ($p \leq 0.05$). The letter "a" indicates the highest significant value, followed by letters in descending order of significance.

Dehydrogenase (DHA-ase) Activity

In faba bean, individual treatments with cyanobacteria (T2) and yeast (T3) resulted in increases in DHA-ase activity by 25.1% and 14.2%, respectively, compared to the control (T1). However, the AMF treatment (T4) led to a decrease of 31.1% in DHA-ase activity. K-Humate alone (T5) increased DHA-ase activity by 61.2%. Co-inoculation treatments further enhanced DHA-ase activity, with the Cyano + Y combination (T6) leading to a 90.2% increase, followed by Cyano + AMF (T7) at 30.6%, and Y + AMF (T8) at 40.4%. The Cyano + Y + AMF treatment (T9) showed a 144.8% increase. When K-Humate was added to these co-inoculations, DHA-ase activity was further amplified, with the Cyano + K-H treatment (T10) showing a 160.7% increase, followed by Cyano + AMF + K-H (T14) at 150.8%, Y + AMF + K-H (T15) at 88.0%, and Cyano + Y + AMF + K-H (T16) at 216.9%.

For wheat, the individual treatments with cyanobacteria (T2) and yeast (T3) increased DHA-ase activity by 81.1% and 36.4%, respectively, compared to the control (T1). The AMF treatment (T4) showed a slight increase of 0.8%. K-Humate alone (T5) resulted in a 65.2% increase. Among the co-inoculation treatments, the Cyano + AMF + K-H (T14) combination showed the highest increase at 252.3%, followed by Y + AMF + K-H (T15) at 219.7%, and Cyano + Y + AMF + K-H (T16) at 293.9%. The Cyano + Y + AMF treatment (T9) resulted in an 84.8% increase in DHA-ase activity.

Nitrogenase Activity (N-ase)

Nitrogenase activity in faba bean was significantly influenced by the various microbiome treatments. Individual treatments with cyanobacteria (T2) and yeast (T3) led to substantial increases in N-ase activity by 350.0% and 150.0%, respectively, compared to the control (T1). The AMF treatment (T4) completely inhibited N-ase activity. K-Humate alone (T5) resulted in a 300.0% increase. Among the co-inoculation treatments, AMF + K-H (T12) showed the highest increase at 500.0%, followed by Cyano + AMF + K-H (T14) at 575.0%, Y + AMF + K-H (T15) at 562.5%, and Cyano + Y + AMF + K-H (T16) at 675.0%.

However, in wheat, the individual treatments with cyanobacteria (T2) and yeast (T3) resulted in moderate increases in N-ase activity by 8.0% and 4.0%, respectively. The AMF treatment (T4) again completely inhibited N-ase activity. K-Humate alone (T5) had no effect on N-ase activity. Co-inoculation treatments, such as Cyano + Y + AMF (T9), led to a 36.0% increase, while Cyano + AMF + K-H (T14) showed no significant change compared to the control. The highest increase was observed with the Cyano + Y + AMF + K-H treatment (T16), which showed an 8.0% increase.

3.2. Effect of Exogenously Applied Microbiomes and K-Humate on Alleviating Salinity Stress in Wheat and Faba Bean in the Field Trial

The in vitro results demonstrated that dual and triple co-inoculation treatments with cyanobacteria, yeast, and AMF, particularly when combined with K-Humate, significantly ($p \leq 0.05$) alleviated salinity stress and enhanced the growth of faba bean and wheat seedlings. These treatments also led to significant improvements in soil enzyme activities, indicating enhanced soil health compared to individual inoculations and control treatments. Based on these results, various combinations of these treatments were developed to create effective biofertilizers, organic fertilizers, or bio-organic fertilizers. These formulations are intended for use on wheat and bean crops in the saline-alkaline soils of the northeastern Delta of Egypt, which are irrigated with a 1:1 mixture of Nile River water and agricultural drainage water from the Al-Salam Canal.

The results in Figure 8 and Table 4 illustrated the effects of various treatments on soil biological activity in the rhizosphere of wheat and faba bean under salinity stress. These results are categorized into AMF colonization rates and spore, cyanobacterial counts, bacterial counts, yeast counts, and soil enzyme activity.

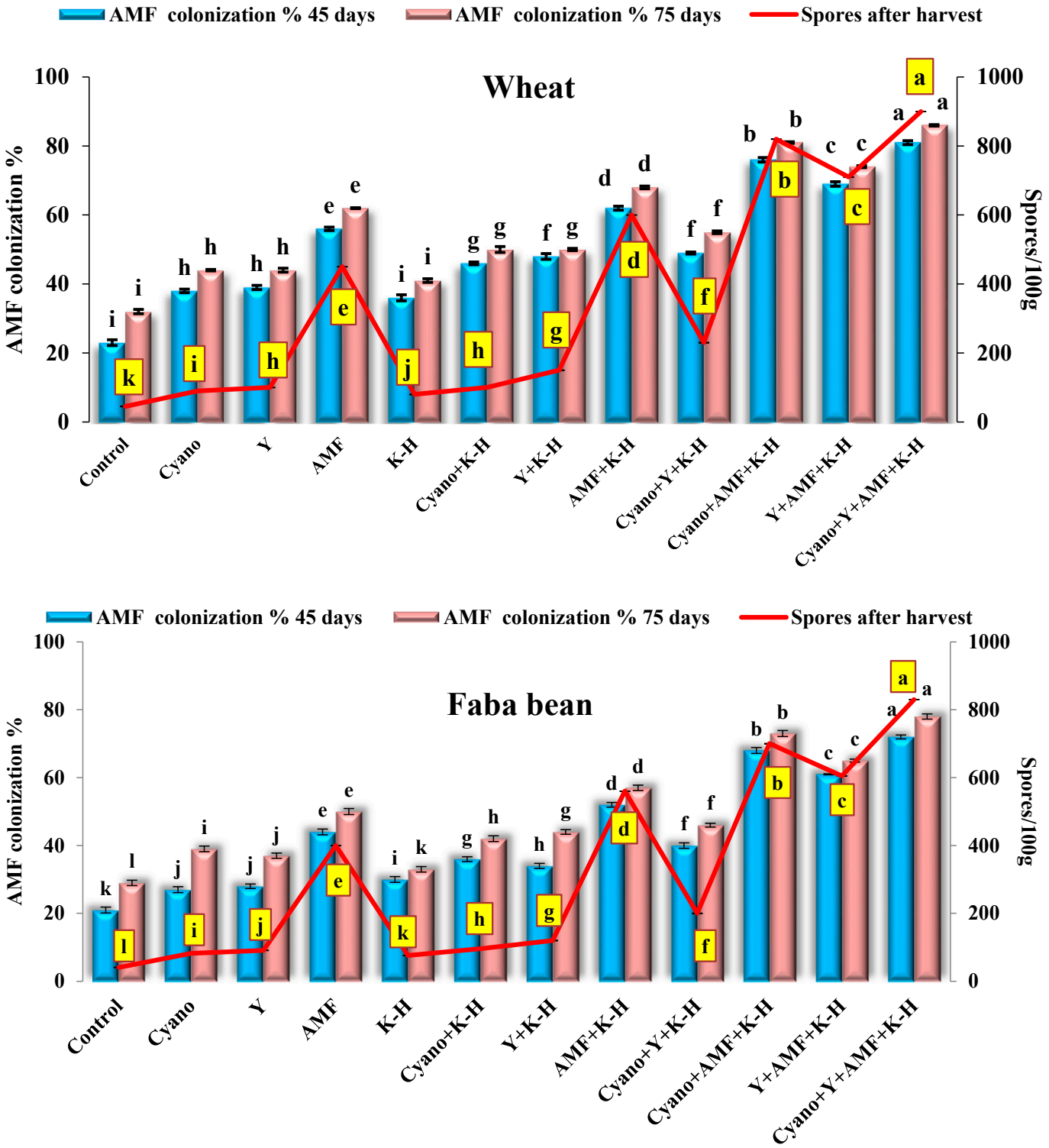


Figure 8. Influence of bio-inoculation and K-H on AMF infection rate and spore densities in the rhizosphere of wheat (**upper**) and faba bean (**bottom**) after 45 days, 75 days, and post-harvest. Different letters (a, b, c, etc.) above the columns and curve indicate statistically significant differences between the treatments ($p \leq 0.05$). The letter "a" represents the highest significant value, followed by letters in descending order of significance.

Table 4. Soil biological activity affected by cyanobacteria, yeast, AMF inoculation, and K-Humate after 75 days of wheat and faba bean growth. Different letters on the same column indicate significant difference between the treatments ($p \leq 0.05$).

Treatments		Wheat					Faba Bean				
		CyanoCount c.f.u. × 10^{-3}•g^{-1} Dry Soil	**Bacterial Count c.f.u. × 10^{-6}•g^{-1} Dry Soil**	**Yeast Count c.f.u. × 10^{-3}•g^{-1} Dry Soil**	**N-ase μmole C_2H_4•g^{-1} Dry Soil•h^{-1}**	**DHA-ase μg TPF•g^{-1} Dry Soil•Day^{-1}**	**CyanoCount c.f.u. × 10^{-3}•g^{-1} Dry Soil**	**Bacterial Count c.f.u. × 10^{-6}•g^{-1} Dry Soil**	**Yeast Count c.f.u. × 10^{-3}•g^{-1} Dry Soil**	**N-ase μmole C_2H_4•g^{-1} Dry Soil•h^{-1}**	**DHA-ase μg TPF•g^{-1} Dry Soil•Day^{-1}**
Control	T1	10.00 j	21.50 k	1.00 k	0.85 f	5.29 g	15.02 jk	108.00 j	2.00 l	3.46 gh	4.67 h
Cyano	T2	36.00 f	61.50 g	3.00 j	1.60 f	14.08 d	30.00 g	250.25 d	4.00 k	1.13 i	9.33 f
Y	T3	17.00 h	60.00 h	7.00 h	2.70 de	3.71 ghi	24.00 h	245.25 e	5.00 j	2.84 h	7.46 g
AMF	T4	12.00 i	26.00 j	6.00 i	0.70 f	2.87 i	14.00 k	147.33 i	7.00 i	4.8 fg	4.01 h
K-H	T5	12.00 i	35.00 i	3.00 j	2.34 de	3.36 hi	32.00 f	108.50 j	10.00 h	8.41 e	8.95 f
Cyano + K-H	T6	38.00 e	95.50 d	9.00 g	5.89 c	11.45 e	36.00 e	207.00 g	11.00 g	12.76 c	10.85 e
Y + K-H	T7	23.00 g	79.00 e	12.00 f	5.52 c	7.19 f	16.00 j	250.00 d	17.00 e	17.46 b	5.01 h
AMF + K-H	T8	18.00 h	66.00 f	13.00 e	3.12 d	4.84 gh	20.00 i	221.25 f	16.00 f	5.13 f	5.38 h
Cyano + Y + K-H	T9	60.00 c	95.50 d	18.00 c	5.48 c	21.82 b	50.00 b	194.00 h	32.00 c	11.14 d	18.19 b
Cyano + AMF + K-H	T10	64.00 b	126.00 c	14.00 d	8.42 b	19.69 c	42.00 c	307.00 b	23.00 d	25.30 a	12.92 d
Y + AMF + K-H	T11	48.00 d	194.00 b	22.00 b	16.98 a	18.22 c	38.00 d	287.00 c	35.00 b	25.11 a	14.42 c
Cyano + Y + AMF + K-H	T12	78.00 a	200.00 a	25.00 a	17.27 a	30.88 a	61.00 a	378.50 a	38.00 a	26.51 a	24.15 a
LSD		1.06	0.74	0.92	1.11	1.64	1.09	0.85	0.86	1.39	1.39

3.2.1. Soil Microbial Activity

AMF Colonization Rates and Spore Density

The results in Figure 8 highlighted the significant ($p \leq 0.05$) effects of bio-inoculation and K-Humate (K-H) on AMF colonization rates and spore densities in the rhizosphere of wheat and faba bean after 45 days, 75 days, and post-harvest.

For wheat, AMF colonization in the control treatment (T1) was 23.00% after 45 days and 32.00% after 75 days, with a spore count of 45.00 per 100 g of soil after harvest. The individual AMF treatment (T4) showed a significant improvement, reaching 56.00% after 45 days and 62.00% after 75 days, alongside a substantial post-harvest spore density of 450.00 spores per 100 g of soil. Other individual treatments, such as cyanobacteria (T2), yeast (T3), and K-H (T5), also increased colonization compared to the control, but AMF alone (T4) proved more effective than most individual treatments.

The combined treatments produced even more notable results, with K-H further enhancing colonization and spore density. For instance, the Cyano + Y + AMF + K-H treatment (T12) reached the highest colonization rates, 81.00% at 45 days and 86.00% at 75 days, with a spore density of 900.00 spores per 100 g soil post-harvest.

Similar patterns were observed for faba bean, where the control group (T1) recorded an AMF colonization rate of 21.00% at 45 days, increasing to 29.00% at 75 days. Individual treatments such as Cyano (T2) and yeast (T3) achieved colonization rates of 27.00% and 28.00%, respectively, at 45 days, which rose to 39.00% and 37.00% after 75 days. The individual AMF treatment (T4) produced the highest colonization rates of 44.00% and 50.00% at 45 and 75 days, outperforming all other single treatments, including K-H (T5), which also significantly improved colonization compared to the control.

Regarding spore densities, treatments with Cyano (T2) and Y (T3) resulted in spore counts of 82.00 and 91.00 spores/100 g soil, respectively, both significantly higher than the control (T1), which recorded 41.00 spores/100 g soil. The AMF treatment (T4) notably increased spore density, achieving 400.00 spores/100 g soil, a tenfold increase over the control. K-H (T5) also showed an increase in spore density, reaching 76.00 spores/100 g soil, though it did not match the effect observed in combined treatments.

The highest spore density was achieved with the Cyano + Y + AMF + K-H treatment (T12), reaching 830.00 spores/100 g soil, which represented a remarkable increase compared to the control ($p \leq 0.05$). Overall, the combination treatments, especially those involving Cyano, AMF, and K-H, demonstrated substantial improvements in AMF colonization and spore density, underscoring their potential for enhancing plant growth and soil health. The significant increases ($p \leq 0.05$) in both parameters emphasize the effectiveness of these treatments in promoting beneficial mycorrhizal associations.

Cyanobacterial Counts

The total cyanobacterial count in the rhizosphere of wheat and faba bean (Table 4) was significantly ($p \leq 0.05$) enhanced by the solitary, dual, and triple co-inoculation of microbiomes with or without K-Humate under salinity stress, compared to the control (T1). This was true for all treatments except for the single treatment of K-Humate in faba bean (T4). For individual inoculation, the cyanobacterial density was significantly ($p \leq 0.05$) enhanced by the individual inoculation with cyanobacteria (36 and 30 c.f.u. $\times 10^{-3} \bullet g^{-1}$ dry soil for wheat and faba bean, respectively) compared to the other individual treatments and the 100% NPK control (T1).

The dual co-treatments of K-Humate with each of the cyanobacteria, yeast, and AMF significantly ($p \leq 0.05$) supported the cyanobacterial total count comparedto the individual microbiomes inoculation and 100% NPK control (T12). However, the superior significant ($p \leq 0.05$) total count of cyanobacteria in the rhizosphere of wheat and faba bean (78.00 and 61.00 c.f.u. $\times 10^{-3} \bullet g^{-1}$ dry soil, respectively) was due to the co-treatment of K-Humate with the consortium microbiomes (T12).

Bacterial Counts

Compared to the control (T1), all treatments led to significant ($p \leq 0.05$) increases in total bacterial counts in the rhizosphere of both wheat and faba bean. Notably, the treatment with K-Humate and the microbiomes consortium (T12) resulted in the highest bacterial counts, reaching 200.00 and 378.50 c.f.u. $\times 10^{-3} \bullet g^{-1}$ dry soil for wheat and faba bean, respectively (Table 4). This suggested that the combined effect of K-Humate and multiple microbiomes had the most substantial influence on bacterial proliferation under salinity stress.

Among individual treatments, cyanobacteria (T2), yeast (T3), and AMF (T5) also significantly ($p \leq 0.05$) increased bacterial populations in both crops, though their effect was relatively less pronounced compared to the consortium treatments. For instance, bacterial counts in wheat rhizosphere were 61.50, 60.00, and 35.00 c.f.u. $\times 10^{-3} \bullet g^{-1}$ dry soil for T2, T3, and T5, respectively, while faba bean rhizosphere had bacterial counts of 250.25, 245.25, and 108.50 c.f.u. $\times 10^{-3} \bullet g^{-1}$ dry soil for the same treatments. These results emphasize the potential synergistic effects of microbiomes combinations, particularly when combined with K-Humate.

Yeast Counts

The total yeast count in the rhizosphere of wheat and faba bean(Table 4) was significantly ($p \leq 0.05$) increased by all microbiomes inoculations, either individually or in combination with K-Humate. The dual inoculations of K-Humate with each microbiomes (cyanobacteria, yeast, AMF) led to notable improvements in yeast populations, but the most significant ($p \leq 0.05$) increases were recorded with the triple co-treatment of K-Humate and the microbiomes consortium (T12), achieving yeast counts of 25.00 and 38.00 c.f.u. $\times 10^{-3} \bullet g^{-1}$ dry soil for wheat and faba bean, respectively.

When comparing individual treatments, the application of yeast alone (T3) resulted in yeast counts of 7.00 and 5.00 c.f.u. $\times 10^{-3} \bullet g^{-1}$dry soil in the rhizospheres of wheat and faba bean, respectively. These values, while significantly ($p \leq 0.05$) lower than the consortium treatments, were still higher than the control (T1). In the dual microbiomes treatments with K-Humate, yeast counts reached up to 18.00 and 22.00 c.f.u. $\times 10^{-3} \bullet g^{-1}$dry soil for T9 (Cyanobacteria + Yeast + K-Humate) and T11 (Yeast + AMF + K-Humate) in the rhizosphere of wheat. In the faba bean rhizosphere, these treatments resulted in yeast counts of 32.00 and 35.00 c.f.u. $\times 10^{-3} \bullet g^{-1}$dry soil, respectively. These findings underscore the significant role of yeast in enhancing microbial diversity under salinity stress.

Soil Enzyme Activity

There was a significant variation in the response of soil enzymes (nitrogenase and dehydrogenase) to all treatments in both wheat and faba bean crops (Table 4). Among the individual treatments, AMF (T4) failed to significantly increase nitrogenase activity in the wheat rhizosphere, while cyanobacteria (T2) and yeast (T3) treatments led to significant ($p \leq 0.05$) increases in N-ase activity. Conversely, in the faba bean rhizosphere, only the AMF treatment (T4) resulted in a significant ($p \leq 0.05$) increase in N-ase, while the cyanobacteria and yeast treatments did not surpass the control (T1). The dual inoculations involving K-Humate further enhanced N-ase activity, with the most significant ($p \leq 0.05$) increases observed in T11 (Yeast + AMF + K-Humate) and T12 (Cyanobacteria + Yeast + AMF + K-Humate) of 16.98 and 17.27 µmole $C_2H_4 \bullet g^{-1}$ dry soil$\bullet h^{-1}$ in wheat. The same treatments, along with T10 (Cyanobacteria + AMF + K-Humate), also exhibited superior N-ase activity in faba beans (Table 4).

Dehydrogenase activity (DHA-ase), a key indicator of microbial activity and soil health, was significantly enhanced in many treatments compared to the control (T1) as shown in Table 4. Among individual microbiomes, cyanobacteria (T2) led to increases of 166.16% in wheat and 99.71% in faba bean. However, the yeast treatment (T3) resulted in a 29.87% decrease in wheat but a 59.74% increase in faba bean. The AMF treatment (T4) caused a decrease in both wheat (45.75%) and faba bean (14.13%).

In treatments where K-Humate was combined with individual microbiomes, such as cyanobacteria (T6), yeast (T7), or AMF (T8), DHA-ase activity improved further. In combination with cyanobacteria (T6), DHA-ase increased by 116.41% in wheat and 132.27% in faba bean. In the yeast + K-Humate treatment (T7), wheat saw a 35.91% increase, while faba bean showed a modest 7.28% rise. The AMF + K-Humate treatment (T8) caused a slight 8.51% decrease in wheat but a 15.21% increase in faba bean. The most significant improvements were observed in multi-microbiomes treatments. For instance, the combination of cyanobacteria, yeast, and K-Humate (T9) led to DHA-ase increases of 312.51% in wheat and 289.30% in faba bean. The triple consortium with K-Humate (T12) showed the highest DHA-ase enhancements, with increases of 483.37% in wheat and 417.98% in faba bean, demonstrating the powerful synergy between multiple microbiomes and K-Humate in boosting soil enzyme activity under salinity stress.

3.3. Effects of Microbiomes and K-Humate on Wheat and Faba Bean Yield and Yield Components under Saline Soil Conditions

The individual, dual, and triple interactions between cyanobacteria, yeast, AMF inoculation, and K-Humate significantly enhanced the yield components of both faba bean and wheat under saline conditions, as shown in Tables 5 and 6.

Table 5. Impact of microbiomes and K-Humate on crop components and faba bean productivity. Different letters on the same column indicate significant difference between the treatments ($p \leq 0.05$).

Treatments		Number of Pods•Plant^{-1}	Number of Seeds•Plant^{-1}	Weight of Seeds•Plant^{-1} (g)	Weight of 100 Seeds (g)	Seeds Yield (ton•ha^{-1})	Straw Yield (ton•ha^{-1})	Biological Yield (ton•ha^{-1})	Harvest Index (%)
T1	Control	6.83 g	16.10 j	5.83 g	31.56 l	1.21 j	1.45 k	2.66	0.45
T2	Cyano	14.00 d	33.75 g	19.00 e	50.41 h	1.33 g	1.63 h	2.96	0.45
T3	Y	11.00 ef	23.43 i	11.67 f	46.61 j	1.22 i	1.62 i	2.84	0.43
T4	AMF	10.00 f	14.00 k	6.76 g	42.92 k	0.98 k	1.41 l	2.39	0.41
T5	K-H	13.50 de	28.00 h	17.76 e	48.78 i	1.29 h	1.45 j	2.75	0.47
T6	Cyano + K-H	19.73 c	55.24 c	33.50 c	61.90 e	1.42 e	1.66 f	3.08	0.46
T7	Y + K-H	18.20 c	42.22 f	23.57 d	54.04 g	1.42 e	1.71 e	3.13	0.45
T8	AMF + K-H	20.30 c	52.78 e	33.42 c	59.28 f	1.38 f	1.66 g	3.04	0.45
T9	Cyano + Y + K-H	24.20 b	54.21 d	39.10 b	70.75 c	1.93 d	2.11 d	4.03	0.48
T10	Cyano + AMF + K-H	25.74 b	57.14 b	43.87 a	75.45 b	2.36 b	2.53 b	4.89	0.48
T11	Y + AMF + K-H	24.97 b	52.94 e	37.74 b	69.88 d	2.21 c	2.55 a	4.77	0.46
T12	Cyano + Y + AMF + K-H	28.86 a	58.09 a	46.20 a	78.24 a	2.77 a	2.51 a	5.28	0.52
	LSD 0.05	2.96	0.65	3.82	0.59	0.57	0.72		

Table 6. Impact of microbiomes and K-Humate on crop components and wheat productivity. Different letters on the same column indicate significant difference between the treatments ($p \leq 0.05$).

Treatments		Number of Spike•Plant^{-1}	Number of Grains•Spike^{-1}	Weight of 1000-Grain (g)	Grain Yield (ton•ha^{-1})	Straw Yield (ton•ha^{-1})	Biological Yield (ton•ha^{-1})	Harvest Index (%)
T1	Control	18.00 f	35.57 f	55.80 j	3.07 g	4.44 i	7.51	0.41
T2	Cyano	26.00 a	60.17 a	62.30 f	3.53 def	5.28 g	8.81	0.40
T3	Y	19.00 ef	33.96 g	61.00 g	3.34 efg	4.92 h	8.26	0.40
T4	AMF	23.00 b	33.90 g	59.50 h	3.14 fg	5.02 h	8.16	0.39
T5	K-H	19.00 ef	44.89 d	60.98 g	3.43 efg	5.64 ef	9.07	0.38
T6	Cyano + K-H	22.00 bc	40.76 e	64.90 e	3.90 bcd	5.86 de	9.77	0.40
T7	Y + K-H	26.00 a	29.80 h	62.60 f	3.70 cde	5.59 f	9.31	0.40
T8	AMF + K-H	25.00 a	29.21 h	58.26 i	3.56 def	5.30 g	8.86	0.40
T9	Cyano + Y + K-H	14.00 g	34.81 fg	66.50 c	4.06 bc	6.07 cd	10.13	0.40
T10	Cyano + AMF + K-H	21.00 cd	44.20 d	68.10 b	4.10 bc	6.34 ab	10.44	0.39
T11	Y + AMF + K-H	20.00 de	48.23 c	65.80 d	4.10 bc	6.17 bc	10.27	0.40
T12	Cyano + Y + AMF + K-H	18.00 f	52.24 b	70.20 a	4.75 a	6.43 a	11.18	0.42
	LSD 0.05	1.47	1.17	0.46	0.18	0.11		

3.3.1. Faba Bean

Among the individual treatments, i.e., T2, T3, T4, and T5, cyanobacteria alone had the most pronounced effect on faba bean yield components. This included the number of pods per plant, seeds per plant, seed weight per plant, 100-seed weight, biological yield per hectare, and harvest index all of which were significantly improved compared to the control (T1) with 100% NPK (Table 5).

The dual interaction of K-H with each of the microbiomes cyanobacteria, yeast, and AMF (T6, T7, and T8) resulted in even greater increases in yield productivity, biological yield, and yield index compared to the single inoculations (T2, T3, T4, and T5). The most significant increase was observed when K-H was combined with cyanobacteria (T6), surpassing the other dual treatments. Moreover, co-treatments involving two microbiomes with K-Humate (T9, T10, and T11) led to significant improvements in key yield indicators. The weight of 100 seeds increased by 124.18%, 139.07%, and 121.42%, respectively, compared to the full recommended NPK mineral fertilizers (Control). Seed yield per hectare rose by 59.50%, 95.04%, and 82.64%, while straw yield per hectare increased by 45.52%, 74.48%, and 75.86% compared to the control (T1). The highest yield and productivity were achieved with the combined treatment of the microbiomes consortium and K-H (T12). This treatment resulted in a remarkable increase in the number of pods per plant (322.55%), seeds per plant (260.81%), seed yield per plant (692.45%), 100-seed weight (147.91%), seed yield per hectare (128.93%), and straw yield per hectare (73.10%) compared to the full-dose mineral NPK control (T1).

3.3.2. Wheat

In wheat, Table 6 illustratesthe impact of microbiome inoculation and K-H application on yield components compared to plants fertilized with the recommended full dose of NPK fertilizers (T1). The highest significant number of spikes per plant (26) was recorded in plants treated with cyanobacteria alone (T2), followed by co-treatments of K-H with yeast (T7) and AMF (T8). Additionally, cyanobacteria alone (T2) produced the highest number of grains per spike (60.17). The weight of 1000 grains increased by 4.41% to 25.81% across all treatments compared to the full recommended NPK mineral fertilizers (Control). The most significant increase in 1000-grain weight (70.20 g) was achieved in plants treated with the microbiomes consortium and K-H (T12).

Inoculating wheat with microbiomes, either with or without K-Humate, also increased straw and grain yields by 10.81% to 44.86% and 2.34% to 54.68%, respectively, compared to the control (T1). The highest grain yield (4.75 tons/ha) and straw yield (6.43 tons/ha) were recorded in the consortium treatment with K-Humate (T12). This boost in yield also led to an increase in biological yield per hectare by 8.62% to 48.88% compared to the control.

3.4. Effect of Different Treatments on Nutrient Composition, Protein Content, and K^+/Na^+ Ratio in Wheat and Faba Bean

The percentages of nitrogen (N), phosphorus (P), potassium (K), protein, and sodium (Na), as well as the K/Na ratio in wheat grains and faba bean seeds, are key indicators of crop quality. As shown in Figures 9 and 10, the combination of exogenously applied microbiomes (cyanobacteria, yeast, AMF) with K-Humate in the plant rhizosphere significantly enhanced N, P, and K levels while reducing Na content. This treatment not only improved the nutrient profile but also alleviated the effects of salinity stress, resulting in the highest NPK percentages and the lowest Na levels in both faba bean seeds and wheat grains.

Both crops exhibited increased nitrogen content with treatments, although faba bean showed higher overall N levels. In faba bean, nitrogen increased by 48.21% in the consortium treatment (T12) compared to the control, rising from 3.07% to 4.55%. For wheat, the increase was more moderate, with N rising by 26.27% from 1.18% (T1) to 1.49% (T12). Treatments combining microbiomes with K-Humate consistently outperformed individual inoculations.

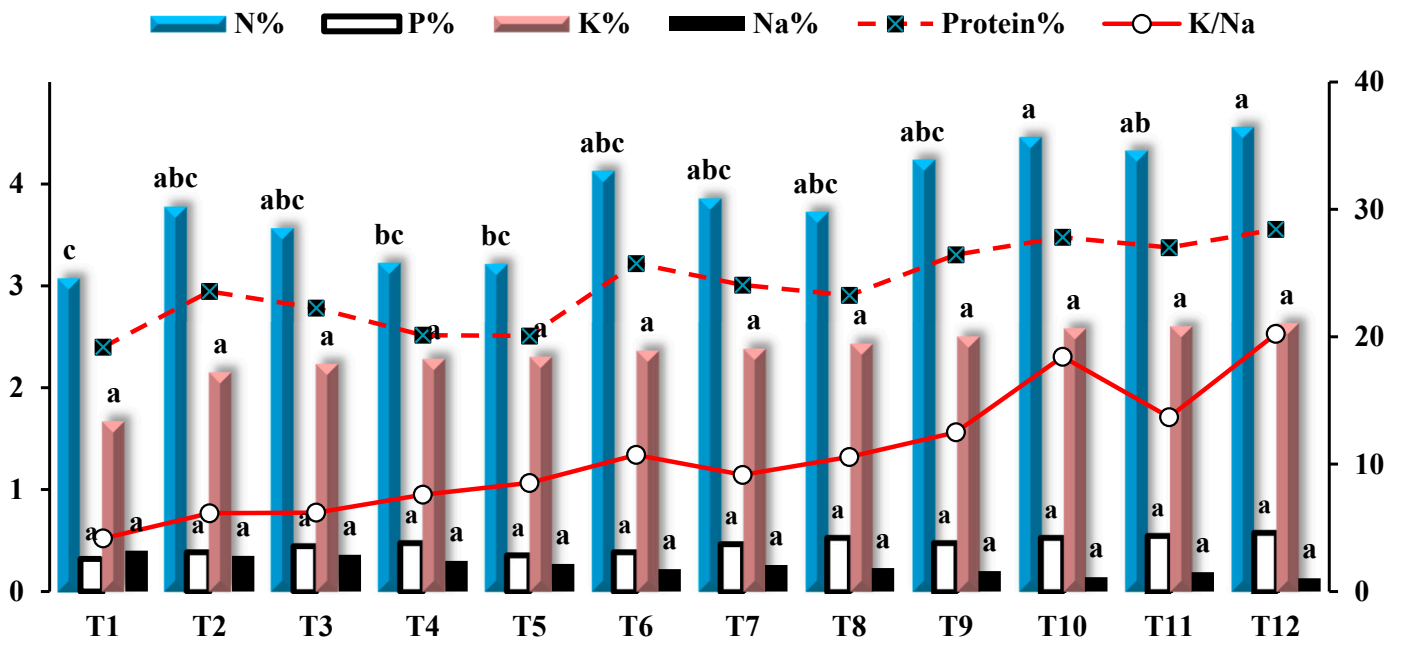


Figure 9. Effect of Treatments on K/Na Ratio and Percentages of Protein, Nitrogen (N), Phosphorus (P), Potassium (K), and Sodium (Na) in Faba Bean Seeds. Different letters (a, b, c, etc.) above the columns and curves indicate statistically significant differences between the treatments ($p \leq 0.05$). The letter "a" represents the highest significant value, followed by letters in descending order of significance.

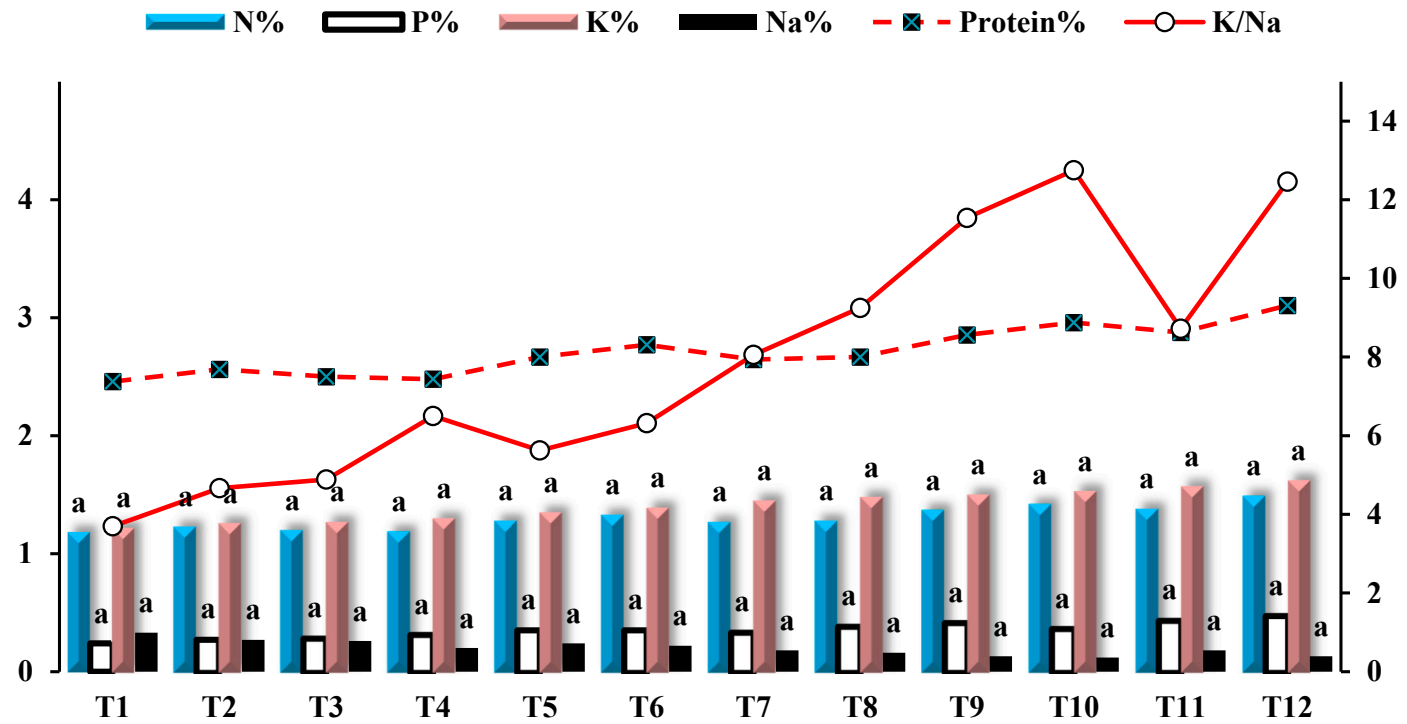


Figure 10. Effect of Treatments on K/Na Ratio and Percentages of Protein, Nitrogen (N), Phosphorus (P), Potassium (K), and Sodium (Na) in Wheat Grains. Different letters (a, b, c, etc.) above the columns and curve indicate statistically significant differences between the treatments ($p \leq 0.05$). The letter "a" represents the highest significant value, followed by letters in descending order of significance.

Phosphorus content was enhanced in both crops, but the increases were more pronounced in faba beans. Faba bean's P content increased from 0.32% (T1) to 0.58% (T12), an 81.25% increase, while wheat's P content increased from 0.24% to 0.48%, a 100% increase. AMF treatments, particularly when combined with K-Humate, had a significant impact on both crops.

In faba bean, potassium increased by 57.49% (from 1.67% to 2.63%), while in wheat, it rose by 32.79% (from 1.22% to 1.62%) under the consortium treatment (T12). The combination of cyanobacteria and K-Humate (T6) provided notable improvements in both crops, though faba bean consistently showed higher K values across treatments.

Sodium levels were significantly reduced in both crops, with faba bean showing a 67.5% decrease (from 0.40% to 0.13%) and wheat showing a 60.61% decrease (from 0.33% to 0.13%) under the consortium treatment (T12). Across treatments, faba beans tended to have slightly higher Na levels compared to wheat.

Protein content increased significantly in both crops. In faba bean, the protein content rose by 48.16% (from 19.19% to 28.44%), while in wheat, it increased by 26.15% (from 7.38% to 9.31%) under the consortium treatment (T12). Single inoculations showed modest increases, but combining microbiomes with K-Humate maximized protein content.

The K/Na ratio improved substantially in both crops, with the most significant increases seen in the consortium treatment. In faba bean, the K/Na ratio rose from 4.18 (T1) to 20.23 (T12), a 384.21% improvement, while in wheat, it increased from 3.70 to 12.46, a 236.76% increase. The dual and triple combinations of microbiomes with K-Humate consistently produced the highest ratios, enhancing the balance of essential nutrients in both crops.

These findings revealed that both wheat and faba bean responded positively to treatments, with the most significant improvements in nutrient composition, protein content, and K/Na ratio achieved under the consortium of microbiomes and K-Humate (T12). Faba bean generally exhibited higher nutrient concentrations and protein content compared to wheat, although both crops benefited substantially from the dual and triple combinations of microbiomes with K-Humate. These results highlight the potential of integrated microbiome treatments to improve crop quality and resilience under saline conditions.

4. Discussion

In arid and semi-arid regions like Egypt, salinity is a critical stressor that severely reduces crop yields by disrupting plant metabolism and inducing excess reactive oxygen species (ROS), which damage cells [35]. Salinity imposes both ionic stress, where high Na^+ levels disrupt protein functions, and osmotic stress, which hinders water and nutrient uptake [36]. These stresses trigger secondary signals like Ca^{2+}, ROS, and ABA to activate stress-responsive genes [37,38]. Uncontrolled ROS accumulation can lead to oxidative damage and cell death. To counteract salinity, plants employ defense mechanisms such as osmotic adjustment, ion homeostasis, and antioxidant defenses. Catalase (CAT) and peroxidases (POD) are key enzymes that decompose H_2O_2, protecting plants from oxidative stress [39]. POD is more efficient at lower H_2O_2 levels, while CAT excels at quenching higher concentrations [40]. CAT's high catalytic efficiency and resistance to environmental fluctuations make it crucial for protecting cells from ROS. It primarily functions in peroxisomes, where H_2O_2 is generated through processes like photorespiration [41,42].

Microbiomes exhibit a higher NaCl tolerance than plants, employing mechanisms like compatible solute accumulation and diverse Na^+ transporters to maintain osmotic balance and reduce cytoplasmic Na^+ levels. In addition to tolerating high salinity, many bacterial and fungal strains enhance plant salinity tolerance by mitigating growth inhibition induced by salt stress [43].

Microbiomes promote ion and osmotic homeostasis, regulate ROS, and support photosynthesis, facilitating plant growth under salinity through three main mechanisms: re-establishing homeostasis, preventing cellular damage, and enabling growth under stress. Microbial inoculation also activates the plant's ROS detoxification pathways, enhancing

both the enzymatic antioxidant system including catalase (CAT) and peroxidase (POD). These plant-microbes interactions form a diverse ecosystem with mutualistic benefits, improving nutrient uptake, stress tolerance, and pathogen defense [43–45].

These findings support our hypothesis that exogenous inoculation with beneficial microbiomes can enhance the plant's antioxidant defense system specifically by increasing the activity of catalase (CAT) and peroxidase (POD) enzymes while simultaneously enriching the soil microbiomes. This synergistic mechanism not only improves soil fertility but also facilitates nutrient uptake, which ultimately leads to increased plant productivity under saline conditions [46,47].

In this study, the effects of microbiome treatments, including cyanobacteria, yeast, and AMF were assessed on the resilience of faba bean and wheat seedlings under salinity stress. The results demonstrated that the exogenous application of these microbiomes, either individually or in combination with K-Humate, significantly improved plant tolerance to salinity. This was evidenced by enhanced stress mitigation, increased antioxidant enzyme activity, and overall improved plant performance under saline conditions [48,49].

The in vitro experiment demonstrated that biological treatments, both with and without K-Humate, significantly ($p \leq 0.05$) enhanced the activity of key antioxidant enzymes, including catalase (CAT) and peroxidase (POD). These enhancements were reflected in substantial increases in root and shoot lengths of faba bean and wheat seedlings (Figures 2–5). These findings were also accompanied by significant ($p \leq 0.05$) improvements in soil enzyme activities, particularly dehydrogenase and nitrogenase (Figures 6 and 7), in response to microbiome treatments. While all treatments showed significant ($p \leq 0.05$) improvements compared to the control, the inclusion of K-Humate resulted in more pronounced enhancements, further supporting the synergistic effect of microbiomes and K-Humate on plant resilience under stress conditions. These results align with previous studies demonstrating the role of beneficial microbiomes in enhancing plant tolerance to abiotic stresses by boosting antioxidant enzyme activity and improving soil health [46,50,51].

However, the AMF treatment alone was insufficient to confer salinity tolerance under the high-salinity conditions tested in this study (Figures 2 and 3). This was evident as faba bean seedlings displayed very weak growth, while wheat seeds failed to germinate entirely, resulting in no observable growth in the AMF-treated plants. This outcome aligns with previous research showing that AMF can enhance salt tolerance in host plants by activating protective enzyme systems, increasing photosynthetic efficiency, and improving nutrient uptake [52]. Nonetheless, the efficacy of AMF in mitigating salinity stress can vary greatly. Some studies, for example, have highlighted *Funneliformis mosseae* as particularly effective in alleviating salt stress [53], while others have found *Glomus fasciculatus* more beneficial for species like *Acacia nilotica*, various herbs, and woody or perennial plants [54]. These variations likely stem from differences in salt tolerance among AMF species, as well as differences in the salt tolerance of their respective host plants [55]. However, the combination of AMF and KH enhanced the antioxidant defense system by increasing antioxidant enzymes and antioxidant capacity and, thus, could be used to enhance plant growth [56].

The root-to-shoot length ratio is a crucial indicator of plant adaptation, particularly under saline conditions where stress often leads to reduced shoot growth. A higher root-to-shoot ratio suggests increased resource allocation to root development, enhancing water and nutrient uptake under challenging conditions [57].

Microbiomes, including cyanobacteria, yeast, and arbuscular mycorrhizal fungi (AMF), play a significant role in influencing this ratio. These beneficial microbes often enhance root architecture, improve nutrient uptake, and increase access to water in saline soils [43].

For instance, cyanobacteria, known for their nitrogen-fixing ability, improved soil fertility and supported the development of more extensive root systems, thereby increasing the root-to-shoot ratio under salinity stress [58].

Similarly, certain yeast strains produce phytohormones like auxins, which can stimulate root growth and bolster plant resilience to saline conditions, leading to a higher

root-to-shoot ratio that reflects the plant's adaptive response to stress [59]. AMF, through their symbiotic relationships with plant roots, enhances nutrient uptake (particularly phosphorus) and improves water absorption, which is particularly beneficial in saline environments. This symbiosis often results in better root development and a more favorable root-to-shoot ratio [54].

Cyanobacteria are capable of solubilizing microbial nutrients and dissolving insoluble carbonate nodules through the secretion of oxalic acid. In this respect, Han and Lee [50] showed that some cyanobacteria produce polysaccharide products, binding Na^+ in the root zone and hence alleviating the salinity stress on plant and microbial growth and activities. Cyanobacterial exopolysaccharides improve soil aggregation by lowering the pH and electrical conductivity and increasing the hydraulic conductivity of saline and alkali soil [60]. Exopolysaccharide production by the cyanobacteria increased when exposed to higher concentrations of salt which seems to play a role in metal biosorption and the enrichment of saline soils with cyanobacteria improved the soil quality by decreasing pH, exchangeable sodium, Na/Ca, conserving organic C, organic N, and organic P as well as moisture and converts Na^+ to Ca^{2+}. These metabolites produced by the cyanobacteria affect the gene expression of the host plants and thereby bring about qualitative and quantitative changes in the phytochemical composition of the plants. Experiments carried out with live inoculum or with the extracts of cyanobacterial strains on several plant species, such as rice, wheat, maize, cotton, etc., have demonstrated the synthesis of signaling metabolites [61–63]. On the other hand, yeasts have been found in different soils and rhizosphere of various plants [64,65]. Although the numbers of yeasts are low in comparison with other microorganisms, many investigators claim that this group of organisms appears to play an important role in soil fertility and iscapable of producing certain growth-promoting substances such as hormones, amino acids, vitamins, proteins, organic acids, and soluble and volatile exudates [66,67]. Nonetheless, despite the known ability of yeasts to produce organic acids, there have been very few reports on their ability to solubilize inorganic phosphate [68].

The combined application of these microbiomes, particularly with K-Humate, produced promising results in promoting plant growth and improving root-to-shoot ratios under salinity stress. K-Humate is known to improve soil structure, enhance nutrient availability, and support microbial activity, contributing to plant resilience [13,69].

The same findings were reported by Kthiri et al. [70], after conducting pots experiment for wheat germination, they found that inoculation with mixtures of microorganisms had a significant effect on increasing the biomass of stems and roots of wheat seedlings compared to untreated seedlings due to the enhancement of the antioxidative system (phenols and POD enzymes) and reduce the harmful effect of NaCl on wheat seedlings and improve the resistance of seedlings to saline-alkali stress [71]. Recent studies also demonstrate that using stress-tolerant microbiomes is a sustainable and eco-friendly approach for mitigating abiotic stresses, including salinity. These microbiomes enhance crop productivity by improving antioxidant potential, nutrient uptake, and the production of plant hormones like ACC deaminase and siderophores, while also accumulating osmoprotectants that stimulate biomass production and increase crop yields [72].

The application of humic acid (HA) in saline soils further improves key variables affected by salinity, such as nitrate, nitrogen, and phosphorus uptake, while reducing soil electrical conductivity and proline leakage. This improves root and shoot growth and increases plant dry weight by allowing nutrients and water to be released as needed. HA has also been shown to mitigate salinity effects in crops like strawberry, maize, and pepper seedlings by acting as a growth regulator that improves hormone balance, plant growth, and stress tolerance [73–76].

The combined application of cyanobacteria, yeast, AMF, and K-Humate significantly influenced AMF spore numbers and root colonization in wheat and faba beans under salt stress (Figure 8). The consortium of microorganisms and K-Humate resulted in higher mycorrhizal root infection rates and spore densities in the rhizosphere of both crops. These findings align with those of De Carvalho Neta et al. [77], who demonstrated that PGPR microbiomes enhance salinity tolerance through mechanisms such as ACC deaminase production, nitrogen

fixation, and phosphate solubilization. These microbiomes also serve as mycorrhization helpers, promoting AMF hyphal growth, root colonization, and spore production.

Furthermore, yeasts may enhance AMF development by supplying vitamin B12 to the rhizosphere, as AMF hasbeen shown to be stimulated by this vitamin. Thus, vitamin B12 produced by soil yeasts might have resulted in better plant growth and yield in plants treated with both AMF and soil yeasts [78]. Boby et al. [67] found that inoculation with *S. cerevisiae* significantly increased root colonization and spore count in AMF plants but had negligible effects on non-AMF plants. This suggests that yeast specifically stimulates AMF development rather than the host plant, which upholds Larsen and Jacobsen's [79] observations. Vitamin B12 production by soil yeasts could be the main reason for AMF development observed in this study, warranting further investigation [78].

The results of yield and yield components of faba bean (Table 5) and wheat (Table 6) indicated that the integrated application of microbiomes (cyanobacteria, yeast, AMF) and K-Humate, with only 25% of the recommended NPK fertilizers, significantly improved faba bean and wheat yield components compared to individual treatments or 100% NPK. Hamed et al. [80] reported similar results, demonstrating that cyanobacteria and yeast inoculation combined with reduced N levels (50% or 75%) enhanced wheat growth (chlorophyll a, b), upgraded the soil microbial community (nitrogenase activity, CO_2 evolution), and improved NPK uptake and protein content in grains. This combination resulted in wheat yield components similar to those produced by 100% N fertilization. Ghazal et al. [81] also found that combining cyanobacteria with *R. radiobacter* and reduced nitrogen fertilization achieved comparable grain yields to 100% N, with higher NPKuptake in both wheat grains and straw.

In general, nitrogen-fixing cyanobacteria inoculation in cereals, such as wheat and rice, has proven to increase Navailability in soil, resulting in vigorous seedlings and optimal yields at harvest [82,83]. The combined application of biofertilizers also significantly increased plant height and tillering compared to single treatments. Similarly, spikes per m^2, grains per spike, and 1000-grain weight improved in wheat when biofertilizers were applied alone or in combination, with the highest grain yield recorded in plants receiving the combined treatments [84].

The findings of this study support the hypothesis that the combined application of microbiomes (cyanobacteria, yeast, AMF) and K-Humate (T12) significantly improved wheat and faba bean yield and quality under salinity stress. This combination markedly ($p \leq 0.05$) boosted antioxidant enzyme activities and increased soil microbial activity, primarily dehydrogenase and nitrogenase. Furthermore, the combination improved nutrient concentrations, including N, P, and K, in both crops under salinity stress. These findings align with Alharbi et al. [13], who showed that the combined application of plant growth-promoting microbes (PGPMs, *Bradyrhizobium japonicum*, *Trichoderma harzianum*) and K-Humate increased K^+ levels in leaves compared to Na^+, enhanced antioxidant defense systems (CAT, POD, SOD), and reduced oxidative stress markers (H_2O_2, MDA, EL%). Salt stress induces ionic imbalances in plants due to excessive Na^+ and Cl^- accumulation, reducingthe uptake of essential nutrients like K^+, Ca^{2+}, and Mn^{2+}. Excess Na^+ disrupts membrane stability and increases ROS production, causing oxidative damage to cellular macromolecules [85]. To counteract salinity stress, plants trigger adaptive responses, such as enhanced K^+ transport, ROS-scavenging enzyme activation, and the production of compatible solutes to maintain osmotic balance [85–88]. Enhanced K^+ uptake and a reduced Na^+/K^+ ratio in treated plants indicated that microalgae-cyanobacteria formulations helped reestablish ion homeostasis, stimulated plant tolerance responses through antioxidant enzyme activity, and improved root growth and nutrient uptake [89].

Therefore, combined cyanobacteria–yeast–AMF formulations could be a sustainable alternative to boost nutrient uptake, growth, and crop adaptability under both normal and saline conditions [83,90,91]. Nutrient accumulation in the grain increased in plants treated with the biofertilizer consortium (cyanobacteria + yeast + AMF) plus K-Humate with quarter doses of chemical fertilizers, compared to those of complete mineral fertilization [92]. A clear benefit of biofertilizer application was observed in the improvement of protein,

N, P, and K contents versus Na in wheat grains and faba bean seeds, particularly under stress conditions. Strikingly, there were no significant differences between biofertilizer and chemical fertilizer treatments for most parameters [93]. Moreover, the biofertilizer consortium's overall response was accompanied by greater changes in biological yield and harvest index [94,95]. In conclusion, the biofertilizer consortium + 25% NPK improved the yield and nutrient status of wheat and faba bean to a similar extent as 100% chemical fertilizers, particularly under stress conditions, demonstrating the value of integrating microbiomes and K-Humate as sustainable fertilization treatments.

5. Conclusions

Salinity stress severely limits crop productivity by disrupting plant metabolism, reducing nutrient uptake, and causing oxidative damage. In vitro and field trials demonstrated that applying microbiomes (cyanobacteria, yeast, AMF) with K-Humate significantly boosted antioxidant enzyme activities (CAT, POD), root and shoot growth, and overall tolerance to salinity stress in wheat and faba bean. The treatment also improved key yield components and nutrient uptake (N, P, K), and reduced sodium content, enhancing the K^+/Na^+ ratio. Additionally, soil biological health was enhanced through increased enzyme activities and microbial populations. This integrated microbiomes–K-Humate approach presents a sustainable, eco-friendly biofertilizer strategy for improving crop yield and soil health in saline soils, reducing the need for chemical fertilizers. It is strongly recommended as a robust solution for improving crop productivity and sustainability in saline-affected and challenging environments.

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