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Amelioration of Salinity Stress in Wheat Via Biofertilizer and Metal Complex Integration: Insights into Growth and Biochemical Modulation

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Abstract
This study investigates how metal complexes and biofertilizers affect the growth of wheat (Triticum aestivum L.) under various salinity stress levels. The study used a randomized factorial design and looked at the biochemical (total protein, amino acids, and catalase activity) and physiological (plant height, leaf and root number) responses at 50, 100, and 150 days after sowing (DAS). Plant development was gradually hampered by salinity, which was created by sodium-based salts. This was demonstrated by decreases in plant height, foliar expansion, and root architecture. However, these negative impacts were considerably lessened by biofertilizer treatments that included strains of Azotobacter chroococcum. Under minimal salt stress, biofertilizer treatments outperformed even control conditions in terms of morphological performance and nutrient uptake. Despite their advantages, metal complexes only showed modest gains. An indicator of osmotic regulation, amino acid concentration increased dramatically as salinity increased and was further increased by both treatments, especially biofertilizers. Under salinity, protein content decreased but increased after treatment, particularly at 0.4 M salt stress when biofertilizers brought levels back to almost normal levels. A crucial biomarker of oxidative stress, catalase activity, rose in response to salt exposure and was further enhanced by both treatments, indicating enhanced antioxidant defense systems. The significance of the observed changes was validated by statistical analysis. These results highlight the effectiveness of metal complexes and biofertilizers, particularly the former, in increasing wheat resistance to salt stress. This study backs the incorporation of chemical and microbiological supplements into agronomic procedures, providing a sustainable means of boosting crop yields in salty conditions and tackling upcoming climate change-related agricultural issues.

Key Words: Biofertilizers, Metal Complex, *Triticum aestivum* L., Salt Stress

Introduction

Agriculture remains the cornerstone of national economies worldwide, contributing significantly to food security, employment, and gross domestic product (GDP). Over 70% of Pakistan's economy depends either directly or indirectly on agriculture, which accounts for about 26% of the country's GDP [1]. The main crops rice, corn, wheat, cotton, and sugarcane are essential to maintaining the country's food security and bolstering agro-based businesses. Pakistan is among the most productive countries in the world thanks to its sophisticated irrigation system [2]. In order to increase crop yields, especially for staple crops like wheat and cotton, modern

technologies are being incorporated more and more. For example, the availability of water is crucial for the production of maize, which is commonly grown for its nutritious silage. When growth promoters are applied and irrigation is optimized, its yield significantly increases [3]. In a similar vein, sugarcane, an essential cash crop, is grown on 26 million hectares worldwide, with Pakistan producing the fourth most of it. In addition to sugar, its by-products, such as bagasse and molasses, are used in industrial manufacturing and energy production [4,5]. since of their high nutrient content, fruits and vegetables are essential for human health since they lower the risk of chronic conditions including cancer and heart disease [6]. Legumes and cereals have significant nutritional value as well. For almost 70% of the world's population, wheat in particular is a staple food that provides vital proteins and calories. Modern biotechnology interventions have significantly increased its production potential [7]. Wheat is frequently referred to as the "king of cereals," due to its nutritious profile and widespread cultivation. It is a staple cuisine in Pakistan and many other nations, with 60–80% protein and 2–5% carbohydrate [8]. Pakistan produces 23,888 kg of wheat per hectare on average, which accounts for 30% of the country's cereal consumption [9]. In order to meet the growing demand for food, government programs constantly seek to increase its output. Numerous biotic and abiotic stressors severely reduce the production of agricultural crops, especially wheat. Abiotic stressors that impact physiological and developmental processes include heat, cold, salinity, and drought. Heat stress disturbs the ideal temperature conditions required for plant growth, while drought stress significantly reduces wheat production at the heading stage [10,11]. These issues will worsen as a result of additional global temperature rises predicted by emerging climate models [12,9]. Herbivorous pests and microbial diseases are examples of biotic stresses that worsen production losses [13]. Particularly, salinity has become a significant barrier that disrupts plant growth and metabolism, hence reducing productivity [14]. Due mostly to sodium chloride and sulfate salts, salinity stress is particularly common in the Indo-Pak region, impacting approximately 6.3 million hectares in Pakistan alone [15]. Excessive concentrations of salt prevent plants from germinating, growing, and producing [16,17], even if trace salt levels may be advantageous. The main ways that salinity affects plant function are by causing osmotic stress and promoting the buildup of harmful ions [18]. This problem is made worse by saline irrigation water, which causes physiological problems that impede plant growth [19]. Although wheat can withstand a moderate amount of salt (7.0 dS/m), a small increase to 9.0 dS/m can result in a 25% yield drop [20]. In addition to providing vital nutrients, biofertilizers—which include living microbial inoculants—are essential for increasing plants' resistance to environmental challenges [21]. Biofertilizers that promote plant development and mobilize phosphate enhance microbial activity and nutrient availability in the rhizosphere [22]. Biofertilizers that fix nitrogen and mobilize potassium also help transform inorganic substances into forms that plants can use. In addition to enhancing nutrient uptake, these microbial agents also affect soil health and enzyme activity, which supports healthy plant growth [23, 24]. Nitrogen, in particular, is vital for chlorophyll synthesis, amino acid production, and cell development in wheat. Biofertilizers offer an eco-friendly alternative to chemical fertilizers, which although effective, pose environmental hazards [25,26]. Soil complexes represent an innovative approach in sustainable agriculture. These include encapsulated nutrients and growth enhancers delivered through novel methods such as polymer coatings and particulate systems, facilitating targeted delivery at the molecular level (Chinnamuthus et al., 2009) [27]. Salt stress leads to osmotic imbalance, ion toxicity, and oxidative stress, damaging cellular structures and photosynthetic capacity [28]. Plants combat this via antioxidant mechanisms, though excessive stress can overwhelm these defenses. Some plant species develop intrinsic adaptations for such stresses [29]. Recent studies emphasize the use of metal complexes—low-molecular-weight, heterocyclic compounds—for enhancing wheat

growth under stress conditions [30]. These agents, applied during germination and seedling stages, mitigate salinity-induced damages and support healthy development. Salicylic acid-based metal complexes are particularly effective in increasing stress tolerance. Their application, even in minimal concentrations, proves beneficial in maintaining plant vitality and productivity under adverse environments [31].

Materials and Methods

A randomized factorial design experiment was carried out in November 2024 to investigate the effect of biofertilizers and metal complexes on wheat under salt stress. The study included three replicates per treatment. Each pot was filled with 10 kg of soil, and 25 wheat seeds were sown 5 cm deep. Salt stress was imposed 15 days post-sowing. For biofertilization, five strains of *Azotobacter chroococcum* were isolated from the wheat rhizosphere. Seeds were coated with Arabic gum to facilitate bacterial adhesion and uniformly inoculated with the bacterial suspension [31]. Samples were collected at three intervals: 50, 100, and 150 days after sowing (DAS). The first two samplings focused on vegetative traits, where plants were separated into roots and shoots for morphological observations. At 150 DAS, final harvest data were recorded to assess yield parameters including number of spikes per plant and 1000-grain weight.

Physiological Measurements

To evaluate the physiological impact of salt stress, five key growth parameters were assessed: plant height, number of leaves, leaf length, root number, and root length.

Biochemical Analysis

Biochemical assessments included quantification of total soluble proteins, total amino acids, and activities of specific antioxidant enzymes such as PAL (phenylalanine ammonia lyase), POD (peroxidase), and CAT (catalase), which are known to respond to abiotic stresses like salinity.

Protein Estimation and Enzyme Activity

Protein content was measured using the Bradford assay. A 0.2 g sample of plant tissue was homogenized in buffer and centrifuged at 11,500 rpm for 20 minutes at 4°C. The supernatant was treated with Bradford reagent, and absorbance was recorded at 595 nm. Enzyme activity was determined by grinding 0.2 g of fresh tissue in liquid nitrogen, suspending it in 1 mL of Tris-HCl buffer (pH 7.5), and centrifuging it at 13,000 rpm. The supernatant was used for enzyme activity assays [32-33].

Protein Extraction

Protein extraction involved crushing 2.0 g of callus tissue in phosphate buffer (0.1 M, pH 7.2) with 0.1 g polyvinyl polypyrrolidone (PVP). The homogenate was centrifuged at 14,000 rpm for 10 minutes at 4°C, and the supernatant was collected for further protein estimation.

Soluble Protein Quantification

Soluble protein content was assessed via the Biuret method [34]. The reaction mixtures, containing Biuret reagent and protein extract, were incubated at room temperature for 20 minutes. Absorbance was measured at 545 nm using a HITACHI spectrophotometer. Bovine serum albumin served as the standard.

Total Amino Acids
Amino acid content was determined by the Hamilton and Van Slyke method [35]. The assay included protein extract, 2% ninhydrin, pyridine, and distilled water. Absorbance values were compared to a standard curve to quantify amino acids.

Catalase Assays
To analyze CAT activities, 2 g of callus tissue was homogenized in phosphate buffer with PVP. The homogenate was centrifuged at 14,000 rpm for 10 minutes at 4°C. The supernatant was used for enzymatic studies.
Catalase activity was determined at 240 nm following the Beers and Sizer method [36], where the decomposition rate of hydrogen peroxide was used to calculate enzyme activity.

Test Blank Preparation
Blank assays were performed using 3.0 mL phosphate buffer and 2.9 mL of 0.036% hydrogen peroxide. Absorbance was stabilized at 25°C before actual readings were recorded at 240 nm [37].

Statistical Analysis
Statistical evaluations were conducted using ANOVA. Duncan's New Multiple Range Test (DNMRT) was applied where necessary to determine significant differences among treatments.

Results

Effects of Biofertilizer and Metal Complexes on Average Plant Height of Wheat Under Salt Stress
The effects of metal complex treatments, biofertilizer, and salt stress on wheat plant height at 50 and 100 days after sowing (DAS) are shown in Table 1. As the standard for comparison, the control group, which was not exposed to either salt stress or treatment, had the tallest plants at both intervals (28.78 cm at 50 DAS and 35.69 cm at 100 DAS). Plant height significantly decreased under increasing salt stress in the absence of treatment; values gradually decreased from 27.10 cm at 0.2 M salinity to 22.81 cm at 1.0 M at 50 DAS. At 100 DAS, a similar pattern continued, with plant height under 1.0 M stress dropping to 28.12 cm. This demonstrates how salt buildup negatively affects wheat shape and growth kinetics. However, the application of biofertilizers led to a discernible rise in plant height under salt stress. At a 0.2 M salt concentration, for instance, plants fed with biofertilizer fared better than the control group, growing 30.47 cm at 50 DAS and 38.34 cm at 100 DAS. This suggests that biofertilizers might not only mitigate salt stress but also encourage growth beyond unstressed conditions, most likely due to improved nutrient uptake and stress tolerance. Metal complexes also shown a positive effect, albeit not as strongly as biofertilizers. Under 0.2 M salt stress, plant heights were 27.51 cm at 50 DAS and 37.58 cm at 100 DAS. These results lend credence to the notion that metal complexes provide some physiological protection against salt-induced development retardation. Strong treatment effects across all salinity levels and applications are suggested by the statistical validation of all pairwise mean differences using Least Significant Difference (LSD) values.Overall, the data clearly demonstrate the advantages of both metal complexes and biofertilizers in enhancing plant height in saline environments, with biofertilizers performing somewhat better.

Table 1: Effects of Biofertilizer and Metal complexes on Average Plant Height of Wheat Plant under Salt Stress

Salt Treatments	Plant Height (cm)					
	After 50 days			After 100 days		
	Salt stress	Biofertilizer application	Metal Complex	Salt stress	Biofertilizer application	Metal Complex
Control	28.78±0.57a	---	---	35.69±0.64a	---	---
0.2	27.100±0.34b	30.47±0.91a	27.51±0.50a	33.76±0.58b	38.34±0.25a	37.58±0.25a
0.4	26.36±0.100c	28.50±0.3b	26.24±0.72c	32.89±0.36c	37.24±0.66b	37.35±0.41b
0.6	25.53±0.7d	27.44±0.91c	26.86±0.73b	30.14±0.49d	36.07±0.64c	35.30±0.60c
0.8	24.08±0.74e	26.09±0.66d	25.35±0.37d	28.67±0.3e	33.100±0.27e	32.71±0.55d
0.10	22.81±0.49f	23.98±0.35e	22.49±0.49e	28.12±0.43f	34.43±0.24d	28.00±0.75e
LSD	1.2347	1.8925	1.3550	1.5058	1.3975	1.0393
Results	All pairwise mean differences are significant because there values are larger than the LSD test score					

Effects of Biofertilizer and Metal Complexes on Average Leaf Number of Wheat Under Salt Stress

Table 2 shows how the number of wheat leaves responded to varying salt concentrations at 50 and 100 DAS, with or without the use of metal complex and biofertilizer. As the physiological optimum, the control group, which was not subjected to salt stress, had the highest average number of leaves (5.54 after 50 days and 11.26 after 100 days). Just salt stress caused a significant drop in the quantity of leaves. Untreated plants under 0.2 M stress had 4.70 and 9.95 leaves at 50 and 100 DAS, respectively. At 1.0 M stress, these leaves rapidly decreased to 0.31 and 5.38. This pattern demonstrates how salt stress negatively impacts wheat's vegetative proliferation. It's interesting to see that applying biofertilizer significantly increased leaf yield at all salt concentrations. For instance, treated plants at 0.2 M outperformed even the control at a later stage, exhibiting 7.06 leaves at 50 DAS and 12.01 at 100 DAS. Likewise, metal complexes enhanced leaf development, albeit not as well as biofertilizers. Plants treated with metal showed 5.08 and 11.49 leaves at 0.2 M, respectively. When compared to salt stress alone, both treatments maintained their considerable improvement in leaf count at higher stress levels (0.6–1.0 M). The statistical significance of these data is supported by the LSD values. Therefore, the inhibitory effects of salinity on leaf production are significantly counteracted by both metal complexes and biofertilizers, with biofertilizers showing greater effectiveness in fostering vegetative resilience.

Table 2: Effects of bio-fertilizer and metal complexes on average leaf number of wheat plant under salt stress

Salt stress Treatment	Leaf number					
	After 50 Days			After 100 Days		
	Salt Stress	Biofertilizer Application	Metal Complex	Salt Stress	Biofertilizer Application	Metal Complex
Control	5.54±0.48a	---	----	11.26±0.63a	---	---
0.2	4.70±0.6b	7.06±0.74a	5.08±0.92a	9.95±0.52c	12.01±0.89a	11.49±0.89a
0.4	2.77±0.57c	6.17±0.92b	4.43±1.08b	10.00±0.74b	10.48±0.74b	10.69±0.74b
0.6	1.91±0.74d	4.94±0.48c	4.33±0.74c	8.66±0.63d	10.33±0.74c	8.74±0.48c
0.8	1.22±0.63e	4.31±0.74d	2.72±0.57d	6.80±0.52e	8.84±0.92d	7.48±0.92d
0.10	0.31±0.48f	2.22±0.57e	1.23±0.56e	5.38±0.63f	6.14±0.48e	6.38±0.63e
LSD	**1.1267**	**1.4367**	**1.0568**	**1.4896**	**1.5382**	**1.2121**
Result	All pairwise mean differences are significant because there values are larger than the LSD test score					

Effect of Biofertilizer and Metal Complexes on Average Root Number of Wheat Under Salt Stress

Table 3 examines how the quantity of roots in wheat reacts to rising salinity and whether root inhibition can be lessened by metal complexes or biofertilizers. The largest root counts were seen in control plants, which had 4.10 after 50 DAS and 8.03 after 100 DAS. A significant decrease was noted as salinity increased. For example, root numbers dropped to 0.85 and 3.12 at 50 and 100 DAS, respectively, at 1.0 M salinity. However, plants showed a significant improvement in root development at all stress levels when biofertilizer was applied. Biofertilized plants outperformed even the non-stressed control at 100 DAS, exhibiting 4.96 and 8.37 roots at 0.2 M stress. This implies that biofertilizers improve rhizogenesis, possibly via improving nutrient solubilization and microbial symbiosis. Although not as much as biofertilizers, metal complex treatments also produced improvements. Plants treated with metal complex generated 3.93 and 6.49 roots at 0.2 M, respectively. Both treatments confirmed their protective impact by helping to preserve root numbers above those observed in salt-only treatments at higher salinity levels (0.8–1.0 M). The veracity of these discrepancies is confirmed by statistical analysis (LSD values). With metal complexes acting as a secondary but still useful amendment, biofertilizers generally greatly enhanced root architecture under salinity. This emphasizes how crucial it is to regulate the rhizosphere when there is abiotic stress.

Table 3: Effect of biofertilizer, metal complex s on average root number of wheat

Salt Stress Treatment	Root Number					
	After 50 Days			After 100 Days		
	Salt Stress	Biofertilizer Application	Metal Complex	Salt Stress	Biofertilizer Application	Metal Complex
Control	4.10±0.25a	---	---	8.03±0.43a	---	---
0.2	3.66±0.57b	4.96±0.92a	3.93±0.92a	6.36±0.74b	8.37±0.62a	6.49±0.92a
0.4	2.62±0.85c	3.63±0.74b	3.14±0.74b	5.67±0.92c	8.16±0.92b	5.26±0.63b
0.6	0.85±0.26f	2.75±0.57c	2.72±0.74c	5.00±0.92d	6.41±0.52d	4.59±92c
0.8	1.13±0.32d	2.17±0.74e	1.49±0.7d	4.12±0.63e	7.16±0.92c	3.34±0.74e
0.10	0.85±0.63e	2.22±0.26d	0.56±0.2e	3.12±0.57f	4.34±0.92e	4.09±0.63d
L.S.D	**1.3234**	**1.2635**	**1.2385**	**1.8242**	**1.2113**	**1.04409**
Result	All pairwise mean differences are significant because there values are larger than the LSD test score					

Effect of Biofertilizer and Metal Complexes on Total Amino Acid Content of Wheat (mg/g Tissue)

The variations in total amino acid content at 50 and 100 DAS under treatment settings and salinity stress are examined in Table 4. The body's known defense mechanism in reaction to osmotic stress is the buildup of amino acids. The concentrations of amino acids under control were 10.88 mg/g at 50 DAS and 14.37 mg/g at 100 DAS. It's interesting to note that amino acid concentration increased with increasing salt stress; 1.0 M stress produced 22.6 and 23.18 mg/g, respectively. The plant's struggle to preserve osmotic equilibrium and safeguard cellular structures is reflected in this elevation. The use of biofertilizer increased the production of amino acids much further. At 50 and 100 DAS, the amino acid concentration at 0.6 M salt was 23.01 and 23.96 mg/g, respectively. Likewise, plants treated with metal complex showed increased levels: 22.74 mg/g and 24.31 mg/g at similar intervals. Both treatments confirmed their osmoprotective functions by increasing the amino acid content above untreated stress conditions at the greatest stress level (1.0 M). According to LSD values, every change was statistically significant. These findings highlight the vital functions that metal complexes and biofertilizers play in improving protein metabolism and nitrogen uptake in salinized environments. By enhancing the plant's adaptation mechanisms, their use improves performance and survival in high-stress situations.

Table 4: Effect of biofertilizer and metal complex on total amino acid content of wheat (mg/g of tissue)

SALT STRESS TREATMENT	Investigation After 50 Days			Investigation After 100 Days		
	In Salt Stress	Biofertilizer Application	Metal Complex	In Salt Stress	Biofertilizer Application	Metal Complex
Control	10.88±0.60e	---	---	14.37±0.81e	---	---
0.2	12.27±0.72d	16.08±0.21c	15.50±0.03c	16.29±0.60d	20.15±0.02c	20.29±0.01c
0.4	14.13±0.52c	21.91±0.71b	21.29±0.04b	19.98±0.57c	23.49±0.03b	23.08±0.02b
0.6	20.06±0.60b	23.01±0.04a	22.74±0.02a	20.14±0.51b	23.96±0.3a	24.31±0.01a
0.8	21.50±0.01a	23.20±0.71^{ba}	21.60±0.04^{ba}	22.73±0.036a	24.76±0.03^{ab}	24.44±0.02^{ab}
0.10	22.6±0.01^{a}	24.35±0.04^{a}	23.12±0.02^{a}	23.18±0.036^{a}	25.28±0.3^{a}	25.19±0.01^{a}
ANOVA	Significant	Significant	Significant	Significant	Significant	Significant
LSD	**1.3335**	**1.4240**	**1.6064**	**1.2113**	**1.2314**	**1.08422**
Result	All pairwise mean differences are significant because there values are larger than the LSD test score					

Effects of Biofertilizer and Metal Complexes on Total Protein Content of Wheat (mg/g Tissue)

The effects of metal complex treatments, biofertilizer, and salt stress on the overall protein concentration at 50 and 100 DAS are shown in Table 5. Protein content was 2.17 mg/g and 1.32 mg/g under control circumstances, which corresponded to baseline levels. Protein concentration was first lowered by salt stress alone; 0.2 M treatment produced 1.23 mg/g at 50 DAS and 1.08 mg/g at 100 DAS. The use of biofertilizer, however, stopped this drop. Among all treatments and timepoints, the highest values, 3.29 mg/g and 3.00 mg/g, were found in plants treated with biofertilizer at 0.4 M, indicating improved nitrogen absorption and metabolic recovery. Although not as much as biofertilizers, metal complexes also increased the amount of protein. Metal-treated plants showed 3.00 mg/g at 50 DAS and 2.59 mg/g at 100 DAS at 0.4 M salinity. Both treatments demonstrated their efficacy in preventing protein breakdown under salinity by maintaining higher protein levels at the greatest stress (0.5 M). LSD values are used to statistically validate the results. These results highlight the fact that protein biosynthesis is susceptible to oxidative stress caused by salt, but that it may be considerably restored by the use of metal complexes and biofertilizers, improving physiological function and growth sustainability.

Table 5: Effects of biofertilizer and metal complex on total protein content of wheat (mg/g of tissue)

Salt Stress Treatment	Investigation After 50 Days			Investigation After 100 Days		
	In Salt Stress	Biofertilizer Application	Metal Complex	In Salt Stress	Biofertilizer Application	Metal Complex
Control	2.17±0.66b	---	---	1.32±0.31d	---	---
0.1	2.15±0.57c	2.08±0.70c	1.68±0.84e	1.76±0.56c	2.31±0.057b	1.82±0.56d
0.2	1.23±0.5f	2.23±0.61b	2.69±0.86a	1.08±0.50f	2.69±0.86a	1.92±0.52c
0.3	1.78±0.51e	2.26±0.75a	1.74±0.63d	1.11±0.54e	2.00±0.50c	1.77±0.50e
0.4	1.89±0.71d	3.29 ± 0.61^{a}	3 ± 0.86^{b}	1.94±0.49b	3 ± 0.86^{b}	2.59 ± 0.52^{b}
0.5	2.20 ± 0.71^{a}	3.17 ± 0.75^{bc}	2.72 ± 0.63^{cd}	2.28 ± 0.49^{a}	2.55 ± 0.50^{a}	2.52 ± 0.50^{a}
LSD	**1.2311**	**1.2237**	**1.21002**	**1.1340**	**1.4250**	**1.3409**
Result	All pairwise mean differences are significant because there values are larger than the LSD test score					

Effects of Biofertilizer and Metal Complexes on Specific Activity of Catalase (CAT) in Wheat (mg/g Tissue)

The specific activity of the antioxidant enzyme catalase (CAT), a crucial indicator of the oxidative stress response, is shown in Table 6. CAT activity in control plants was 5.67 mg/g at 100 DAS and 4.46 mg/g at 50 DAS. Increased CAT activity following salinity exposure suggests higher amounts of reactive oxygen species (ROS), which plants try to detoxify. Plants under salt stress showed a slight increase in CAT activity at 0.1 M stress. Nevertheless, plants fed with biofertilizer exhibited additional increases, reaching 5.97 mg/g at 50 DAS and 6.45 mg/g at 100 DAS. The administration of metal complex produced comparable results, demonstrating the function of both therapies in boosting antioxidant defenses. Following 100 DAS, the maximum CAT activity was noted at 0.5 M stress with applications of metal complex (7.48 mg/g) and biofertilizer (7.63 mg/g). The significant contribution of both treatments to the upregulation of enzymatic antioxidant machinery is confirmed by these higher values. Specifically, biofertilizers were marginally more successful, most likely because they promote microbial-induced tolerance and systemic acquired resistance. According to LSD thresholds, every variation was statistically significant. According to the research, catalase activity is a valid biochemical indicator of stress response, and increasing it through treatments helps plants survive in harsh saline environments.

Table 6: Effects of biofertilizer and metal complex on specific activity of cat on wheat (mg/g of tissue)

Salt Stress Treatment	Investigation After 50 Days			Investigation After 100 Days		
	In Salt Stress	Biofertilizer Application	Metal Complex	In Salt Stress	Biofertilizer Application	Metal Complex
Control	4.46±0.01f	---	---	5.67±0.75e	---	---
0.1	6.01±0.09d	5.97±0.67d	6.19±0.98b	6.32±0.64b	6.45±0.50d	5.77±0.43e
0.2	6.13±0.57b	6.25±0.23c	6.15±0.57d	6.17±0.51c	6.89±0.61b	6.77±0.54a
d0.3	7.39±0.79a	6.76±0.63a	6.16±0.53c	5.55±0.54f	6.65±0.56c	6.08±0.50b
0.4	6.07±0.95c	7.61±0.23^{ab}	7.33±0.57^{ab}	7.27±0.81a	7.70±0.61^{ab}	7.37±0.54^{ab}
0.5	7.50±0.95^{a}	7.63±0.63^{a}	7.31±0.53^{a}	7.52±0.81^{a}	7.65±0.56^{a}	7.48±0.50^{a}
LSD	**1.1038**	**1.1303**	**1.3014**	**1.2104**	**1.1339**	**1.0313**
Result	All pairwise mean differences are significant because there values are larger than the LSD test score					

Discussion

The growth results clearly showed that wheat shape and biochemical properties were considerably affected by salinity stress. Table 1 shows that plant height significantly decreases as salt concentration rises, supporting previous research showing that salinity reduces wheat cell elongation and metabolic activity [38]. Plant height decreased to 22.81 cm and 28.12 cm at 50 and 100 DAS, respectively, at 1.0 M salt. At lower salinities, however, the treatment of biofertilizer not only reduced this loss but substantially surpassed the control, reaching 30.47 cm and 38.34 cm at 0.2 M salinity. This is consistent with research by Stone et al. (1994), who observed improved morphological characteristics in stressed plants treated with biofertilizer [29], [39]. A similar pattern was seen in leaf number, a crucial morphological indication (Table 2). Leaf production was greatly decreased by salt stress; under 1.0 M, the lowest count (0.31 at 50 DAS) was recorded. However, plants treated with biofertilizer outperformed untreated stressed plants by a large margin, reaching 2.22 leaves at 1.0 M. Although somewhat slight, metal complexes also demonstrated improvement. In line with the previous claim that biofertilizers provide vital nutrients and preserve osmotic equilibrium, these findings corroborate the idea that both treatments enhance vegetative resilience [40]. Under salinity, root growth was also hindered (Table 3). At 50 DAS, the roots of the control plants were 4.10, but under 1.0 M stress, they fell to 0.85. At the same stress level, the application of biofertilizer brought the root numbers back to 2.22. According to Stone et al. (1994) and the current work [39], this suggests improved rhizospheric interactions, most likely as a result of microbial activity encouraging nutrient solubilization. Different stress responses were displayed by biochemical measures. Table 4 shows how total amino acids gradually accumulate under salt stress, increasing from 10.88 mg/g in control to 22.6 mg/g at 1.0 M. This adaptive mechanism is consistent with earlier findings that amino acids function as osmoprotectants, preserving metabolic activities and turgor in the face of osmotic stress [41–42]. Reaffirming their osmoprotective effectiveness, it is noteworthy that both metal complexes and biofertilizers further enhanced amino acid accumulation, with the biofertilizer reaching up to 25.28 mg/g at 100 DAS. In contrast, there was an inverse pattern in the protein content (Table 5). At 0.2 M salinity, salt stress decreased protein production by at least 1.08 mg/g. Protein metabolism is disrupted by ROS generation and poor carbon absorption,

which are associated to this reduction [43]. With the highest protein level recorded at 0.4 M salinity (3.29 mg/g), biofertilizers dramatically reverted this trend, demonstrating their function in promoting nitrogen uptake and preventing oxidative damage [44–45]. A key measure of oxidative stress, catalase activity (Table 6), increased in direct proportion to salinity, peaking at 0.5 M. CAT activity was further increased by treatments, especially biofertilizers, which at 100 DAS reached 7.63 mg/g. According to earlier research, this implies that biofertilizers trigger systemic resistance pathways [46]. Additionally, metal compounds increased CAT activity, confirming their function in oxidative stress management.

Conclusion

This study confirms that wheat growth is significantly hampered by salt stress, which suppresses morphological and biochemical features. Metal complexes and biofertilizers, however, greatly lessen these negative consequences. Plant height, the quantity of leaves and roots, the accumulation of amino acids, the amount of protein, and the antioxidant activity were all found to be significantly improved by biofertilizers. Although not as much, metal complexes also made a beneficial contribution. Thus, there is great potential for improving wheat resilience by incorporating biofertilizer technology into agroecosystems impacted by salt. They are a sustainable, environmentally acceptable method of increasing crop output in salinity because of their dual function in physiologic support and stress reduction. These results offer a strong foundation for additional agronomic interventions in saline environments in addition to validating earlier research.

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