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Mitigation of Salinity Stress on Vetiver Grass (*Vetiveria zizanioides*) through Application of *Micrococcus yunnanensis* and Indole-3-Acetic Acid

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Abstract: Salinity represents an ever-challenging problem of agriculture in arid and semi-arid regions. This problem is considered a key limiting factor of agricultural production in the countries of Southwest Asia. In recent years, the use of alternative methods of chemical fertilizers has emerged as a promising approach to mitigate the negative effects of salinity on crop yield. In this research, the effect of *Micrococcus yunnanensis* and indole-3-acetic (IAA) acid on the growth and chemical composition of Vetiver grass (*Vetiveria zizanioides*) under salt stress has been investigated. Based on the results, application of IAA, *M. yunnanensis* and their interaction significantly increased the average plant growth, fresh and dry weight of aerial parts and root dry weight. Considering chemical properties of the plant, interaction between IAA and *M. yunnanensis* significantly increased shoot phosphorus, potassium and sodium absorption. Proline content, catalase, superoxide dismutase and peroxidase activity were significantly influenced by application of IAA, *M. yunnanensis* and their interaction. Follow-up experiments after vetiver harvest showed that IAA and *M. yunnanensis* treatments improved soil microbial biomass and respiration. In total, plant biomass improved by 34% and the activities of catalase, superoxide dismutase and peroxidase enzymes decreased by −20.61, −4.70 and −8.00%, respectively, which shows that the stress pressure on the plant has decreased. This study reinforces the previous literature on the positive effects of biological treatments to improve plant performance by providing new evidence of the positive effects of IAA and *M. yunnanensis* on mitigating the negative effects of salinity.

Keywords: salinity; Vetiver grass; *Micrococcus yunnanensis*; indole-3-acetic acid; soil microbial biomass

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1. Introduction

Salinity represents a major abiotic stress limiting plant growth and productivity [1]. The first effect of high salt concentration is ionic imbalance and severe osmotic stress in plants, and its subsequent effects often lead to oxidative damage [2]. Harmful environmental factors such as cold, salinity or drought prevent plant growth and cause a decrease in plant performance [3]. Salinity causes a change in the balance of nutrients, available water in the soil, a decrease in the quality of agricultural land and ecological structure of communities. The stress also causes a decrease in photosynthesis rate and plant growth [4]. During salinity stress, critical stages of plant growth such as photosynthesis, metabolism,

protein synthesis and lipid production are affected [5]. In Iran, salinity represents a crucial and limiting issue of sustainable agricultural production, so that many parts of arid and semi-arid regions of the country, especially the central plateau, suffer from different levels of salinity [6].

Water and soil salinity is a serious problem for agriculture in arid and semi-arid regions. Salinity stress is known as one of the most important factors in reducing agricultural production [7]. Currently, a significant amount of the world's water resources are affected by salinity, and subsequently, soil salinization is also considered a progressive phenomenon; meaning that the accumulation of salt increases the quality of agricultural soil [8]. Soil salinity is one of the most important environmental problems intensified in recent decades in Southwest Asia [9]. Considering that soil salinity is one of the most important causes of the decline in the quantity and quality of agricultural products, the continuation of this trend in the future can threaten food security in many countries. It should be noted that climate changes in recent years have led to the intensification of the soil salinization process; global warming and the resulting climate fluctuations have increased the rate of water evaporation in various lands and have brought saltiness to the soil in various regions of the world, including southwest Asia [10]. Due to the high rate of water evaporation in Iran, the country also struggles with soil salinity. The development of desalination plants in the country's seas and the farmers' neglect of the principles of water consumption reduction and soil protection have also fueled the intensification of soil salinity in Iran [11].

A promising approach to deal with salinity stress in plants and reduce its harmful effects is the introduction of microorganisms that improve plant growth. The portion of rhizosphere bacteria that directly and indirectly have beneficial effects on plants are called plant growth-promoting rhizosphere bacteria (PGPB) [12]. The PGPB can directly play a role in increasing crop growth and yield by means of different mechanisms. Increasing the dissolution of poorly soluble nutrients such as phosphorus, 1-aminocyclopropane-1-carboxylate deaminse (ACC deaminase) production, production of plant growth regulators such as auxin, nitrogen fixation and siderophore production (from the point of view of increasing iron absorption) are among the most important mechanisms used in this method [13–16]. Moreover, PGPB neutralize or modify the harmful effects of plant pathogens and increase plant growth by using different antagonism mechanisms. Competition for the absorption of nutrients and occupation of suitable positions for the activity of pathogens, production of antibiotics, production of siderophores and production of lytic enzymes and hydrogen cyanide are major mechanisms by which PGPB suppress the pathogens [17].

M. yunnanensis is a Gram-positive, aerobic, non-endospore-forming cocci. This non-motile bacterium is found in the roots of the plant *Polyspora axillaris* [18]. *M. yunnanensis* has been reported to have the ability to reduce oxidative stress produced by the plant in response to abiotic stress [19,20]. The use of this bacterium under different environmental conditions has increased the crops resistance to stress, which makes it a suitable candidate as an external agent to mitigate the adverse effects of stress [21–23]. For example, application of this bacterium increased salinity tolerance of rapeseed with a significant improvement in redox status and ion homeostasis [24]. Also, it has been reported that *M. yunnanensis* has a significant effect on remobilization nutrients from soil to developing seed of *Camelina sativa* under water deficit stress [25]. It has also been reported that *M. yunnanensis* in symbiosis with other bacterial strains improves tomato yield under water deficit stress [26]. Considering the increasing emphasis on sustainable agriculture [27] and considering the ability of *M. yunnanensis* to reduce the negative effects of abiotic stresses [28], it is expected that the use of this bacterium can enhance plant tolerance to salinity stress.

It is well documented that antioxidant enzymes play an effective role in mitigating the negative effects of biotic and abiotic stresses [29]. For example, it has been shown that catalase (CAT), NADH-dependent peroxidase (POD) and superoxide dismutase (SOD) play crucial role in mitigating the negative impact of biotic stress in wheat [30].

Vetiver grass (*Vetiveria zizanioides*), belonging to the *Poaceae* family, is a perennial plant with a height of more than two meters and a high density [31]. Vetiver grows naturally in all climates and in many soil types, even in the arid regions of India, where the plant has already demonstrated its capability to survive [32]. Due to its special vegetative structure, this plant is used to improve and rehabilitate degraded soils [33]. According to Vetiver's properties, it seems that this plant can adapt to many regions of Iran where it can play a great role in watershed management to reduce soil erosion. On the other hand, the plant can be used as fodder for livestock [34]. Vetiver has the ability to grow very fast under environmental stresses such as salinity, drought and presence of heavy metals [35]. Compared to most salt-resistant plants such as bermudagrass and barley, Vetiver is more resistant to salinity [36]. In many areas, especially in infertile soils, Vetiver can grow and survive without the use of nitrogen and phosphorus fertilizers. Additionally, the improvement of growth and the development of Vetiver was observed when its roots were inoculated by microorganisms [37].

The high level of salinity in Southwest Asia highlights the need for corrective methods to deal with salinity stress. Using such approaches, the resistance of plants to salinity stress is increased, and thus, the negative effects of salinity on plant performance are mitigated [38]. Bayomy et al. (2023), for instance, maintained that the agricultural economy of Saudi Arabia is being decreased by soil salinity by persistently decreasing the territory of crop cultivation. So, if this heavily influenced area is effectively utilized, it will be of significant economic benefit [39]. The use of biofertilizers using plant growth-stimulating bacteria is one of the effective ways to reduce the toxic effects caused by high salinity in plant growth [40]. Peng et al. (2023) reported that microorganisms in the rhizosphere, especially growth-promoting bacteria and fungi, can improve plant performance in stressful environments [41]. Growth-promoting bacteria can improve plant growth indirectly by reducing plant pathogens or directly by facilitating the absorption of nutrients from the environment or the production of hormones such as auxin, cytokinin and gibberellin [42].

Although the positive effects of biofertilizers and natural compounds in reducing the effects of salinity have been documented [9–11], due to the high number of bioactive compounds, there is a need to investigate their mutual effects during salinity stress. In addition, until now, the effects of biological compounds on the increase of Vetiver plant resistance during salinity stress have not been widely investigated. Although the effects of *M. yunnanensis* on mitigating the effects of stress have been investigated [24–26], to the best of our knowledge, the effects of this bacterium on improving the resistance of Vetiver to salinity stress have not been evaluated. In addition, the interaction of *M. yunnanensis* and IAA in reducing the adverse effects of salinity stress has not been investigated. Therefore, in this research, the effect of *M. yunnanensis* and IAA treatment on the growth, morphological and physiological characteristics of Vetiver is investigated. To better understand the interaction effects of *M. yunnanensis* and IAA, the chemical and biological characteristics of the soil are also investigated.

2. Materials and Methods

2.1. Preparation and Analysis of Soil

The soil for the research was collected from a depth of 0 to 30 cm of limestone soil located in Fars Bajgah area. The samples were mixed with sand (2:1 ratio) after air-drying and passing through a two-millimeter sieve. Organic matter was measured using an oxidation method with chromic acid, electrical conductivity in saturated extract with electrical conductivity meter (Milwaukee EC59 PRO), usable phosphorus by extracting with sodium bicarbonate and measuring with spectrophotometer. Concentration of cationic low consumption elements (manganese, copper, zinc and iron) was determined by reading with an atomic absorption device (Shimadzu-AA670) and potassium was determined by extracting with ammonium acetate and reading with a flame photometer (Sherwood Scientific, Model 410). Microbial respiration was measured using closed jars and carbon titration of microbial biomass was measured using incubation–fumigation method. In this

method, soil samples are fumigated with chloroform to kill microbial cells, inoculated with a non-fumigated soil suspension aliquot, and then incubated for 24 h. It is assumed that the newly inoculated cells will consume the dead biomass from fumigated samples and respire CO_2 [43]. Proline content was measured using the method proposed by Ref. [44]. Briefly, 0.1 g of fresh plant powder was ground with 10 mL of 3% sulfosalicylic acid and completely homogenized. The mixture was filtered and then 2 mL of the solution was mixed with 2 milliliters of Ninhydrin reagent and 2 milliliters of glacial acetic acid were added to each tube. Then, the samples were placed in a water bath at 100 °C for one hour; immediately after removing from the bath, they were placed in an ice bath for a few minutes. After cooling, 4 mL of toluene was added to each test tube and the samples were vigorously stirred with a finger shaker for 15–20 s until they were completely uniform. Then, the tubes were placed at room temperature until the upper and lower phases were completely separated. The upper phase was used to determine the concentration of proline with a spectrophotometer at wavelength of 520 nm using a toluene control solution.

The method developed by Ref. [18] was used to measure the activity of antioxidant enzymes. To measure the activity of superoxide dismutase, 3 mL of the reaction mixture, containing 50 µL of extracted enzyme extract, 50 mM potassium biphosphate (pH = 7.8), 13 mM L-methionine, 75 µM nitroblue tetrazolium (NBT), 0.1 mM EDTA and 4 µM riboflavin, was used. The riboflavin solution was made separately (and daily) in the dark and added to the reaction mixture in the last step. The cuvettes related to the samples were shaken and, in order to perform the reaction, these mixtures were placed in a light chamber with four 20-watt fluorescent lamps for 15 min. Then, the reaction was stopped by turning off the lamps and placing the samples in the dark. The absorbance value of each sample was read with a spectrophotometer model 7315 made by JENWAY UK at a wavelength of 560 nm.

Catalase enzyme activity was evaluated based on the decrease in light absorption due to the decomposition of hydrogen peroxide at a wavelength of 240 nm with a spectrophotometer model 7315 manufactured by JENWAY, England, during a period of 1 min with 10 s time periods. An amount of 3 mL of the reaction mixture containing 50 µL of extracted enzyme extract, 50 mM potassium phosphate buffer (pH = 7) and 10 mM hydrogen peroxide was prepared. Using the extinction coefficient (ε = 39.4 mM^{-1} cm^{-1}), enzyme activity was reported in terms of micromoles of hydrogen peroxide decomposed in one minute per gram of fresh weight of the sample.

Peroxidase enzyme activity was measured based on the increase in light absorption due to the oxidation of Guaiacol in the presence of hydrogen peroxide at a wavelength of 470 nm with a spectrophotometer model 7315 manufactured by JENWAY, England, during a period of 1 min with periods of 10 s. The reaction mixture containing 50 µL of enzyme extract, 2.9 mL of 10 mM potassium phosphate buffer (pH = 7) and 0.05 mL of 20 mM Guaiacol was prepared. The reaction was started by adding 20 microliters of 40 mM hydrogen peroxide. Using the extinction coefficient of 26.6 mM^{-1} cm^{-1}, the enzyme activity was calculated and reported in terms of micromoles of guaiacol oxidized in one minute per gram of fresh weight of the sample (Table 1).

Table 1. Physicochemical characteristics of the soil used in this present experiment.

Feature	The Amount
Sand	57.72%
Silt	12.56%
Clay	29.72
Texture class	Sandy clay loam
pH-saturated dough	7.6
Electrical conductivity of the saturated extract	2.15 dS/m
Cation exchange capacity	18 $cmol^{+}/kg$
Organic matter	1.3%

Table 1. *Cont.*

Feature	The Amount
Total N	0.07%
P extractable by $NaHCO_3$	15 mg/kg
K extractable by $C_2H_7NO_2$	420 mg/kg
Extractable Cu with DTPA	5.1 mg/kg
Extractable Mn with DTPA	0.6 mg/kg
Extractable Zn with DTPA	3 mg/kg
Extractable Cd with DTPA	1.3 mg/kg
Microbial respiration	5.2 mg CO_2-C $kg^{-1} \cdot h^{-1}$
Soil Microbial biomass carbon	15.15 mg of C/kg of soil

2.2. Preparation of Bacterial Inoculum

The PGPB bacterium (*M. yunensis*) with the ability to dissolve phosphate and produce siderophore without the ability to produce auxin was obtained from the laboratory of the soil science department in Shiraz, Iran, and was grown in a liquid culture medium. First, to culture and prepare bacterial suspension, we poured a certain amount of Nutrient Broth culture medium in a 1 L Erlenmeyer flask containing 500 mL and sterilized the culture medium in an autoclave for 15–20 min at a temperature of 121 degrees Celsius and a sterile pressure of 1.2 atmospheres. After the cooling of the culture medium (Nutrient Broth), a completely sterilized loop with an alcohol lamp (flame) was used to collect the colony of the desired bacteria (*M. yunensis*) from the Petri dish containing the bacteria, and inoculation in the culture medium (Nutrient Broth). It was kept at a temperature of 28 degrees Celsius on an incubator shaker at 120 rpm for 24 h. The broth was used after the complete growth of the bacteria and the population of 1×10^7 cfu/mL.

2.3. Plant Growth and Treatments

Two experiments were conducted as small blocks of soil in pots in the form of factorial $3 \times 4 \times 4$ in the form of a completely randomized design with three replications. The experiment included four levels of NaCl (0, 8, 16 and 24 dS/m) and four levels of modification treatment (control or not treated, IAA, *M. yunnanensis* (-inoculated) and interaction of IAA and the bacterium interaction). The control consisted of not using IAA and not using bacteria at all four salinity levels. Three technical replicates and three biological replicates were considered for each treatment. Four weeks after planting, salinity treatments were applied four times during four weeks. One week after the application of NaCl, two liters of 1mM indole-3-acetic acid solution was prepared and sprayed twice with an interval of one month. After 25 weeks from the beginning of cultivation, the plants were harvested and then the samples were taken from the roots and shoots near the soil surface, washed and then dried in an oven at a temperature of 65 °C for 48 h until reaching a constant weight.

2.4. Statistics

One-way analysis of variance (ANOVA) test was used to check the significance of the difference in the mean of different treatments. All statistical analyses were performed with SPSS 27.0.1 IF026 software. The graphs were drawn with Microsoft Office Excel 2010 software.

3. Results

3.1. The Effect of Salinity Levels, IAA Bacterial Inoculatiand on on Growth Characteristics and Yield of Vetiver

The effect of modifying treatments (IAA and the bacterium) and the interaction of sodium chloride (salinity) and modifying treatments on Vetiver height was significant ($p \leq 0.05$), while the effect of sodium chloride was not significant. The application of IAA and the combined application of IAA and bacterial inoculation increased the average plant height by 15.95% and 45.95%, respectively, compared to the control treatment (Figure 1a).

However, no statistically significant difference was observed between the levels of bacteria and the control, as well as the levels of growth regulators and bacteria.

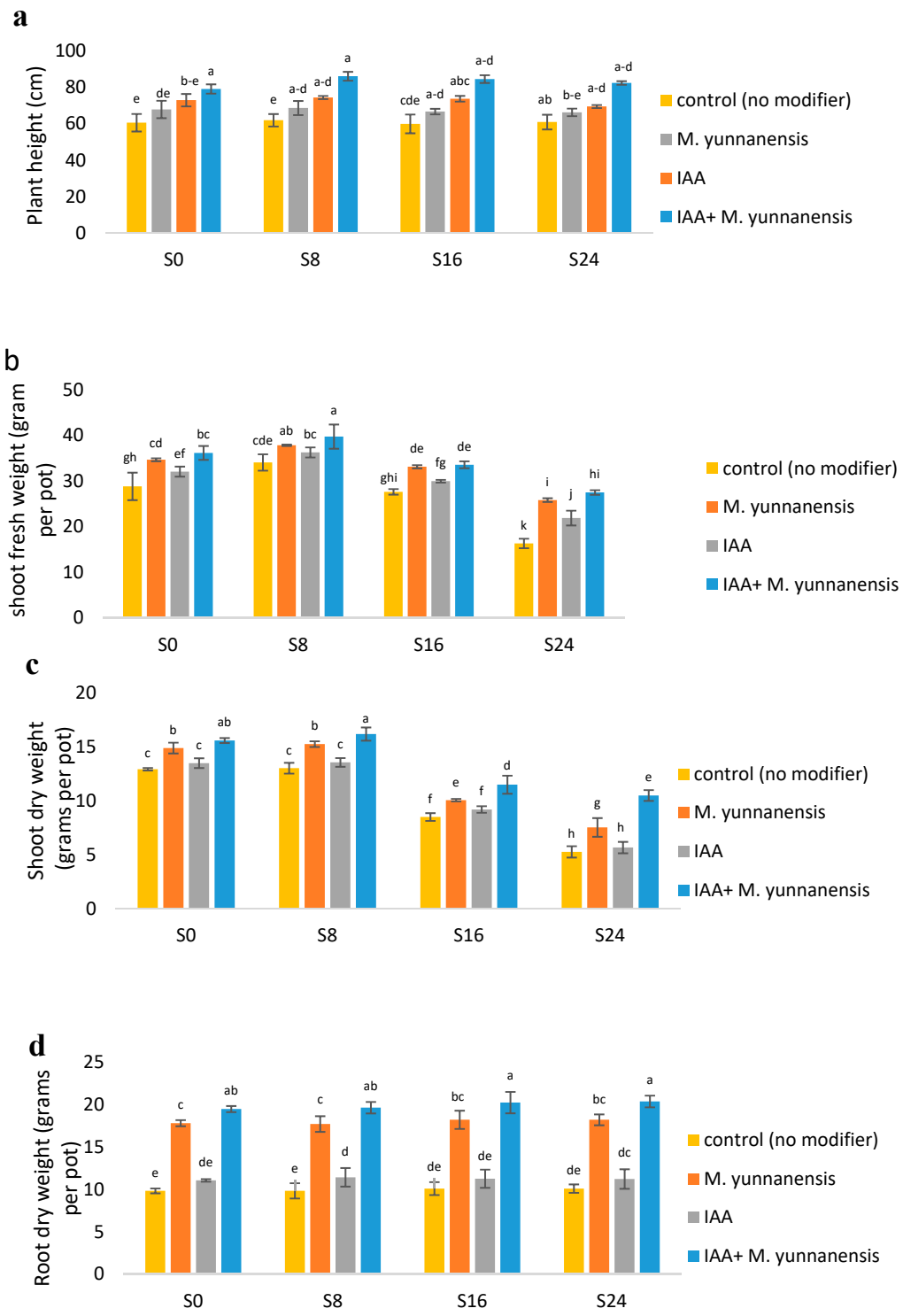


Figure 1. Effect of salinity levels, IAA and the bacterium on growth characteristics and yield of Vetiver. (**a**) Plant growth as affected by salinity levels, IAA and bacterial inoculation (data represented are average from all replications); (**b**) impact of salinity levels, IAA and *M. yunnanensis* on shoot fresh weight measured as grams per pot; (**c**) impact of salinity levels, IAA and bacterial inoculation on shoot dry weight measured as grams per pot; (**d**) impact of salinity levels, IAA and *M. yunnanensis* on root dry weight measured as grams per pot. S0, S8, S16 and S24 mean 0, 8, 16 and 24 dS/m of NaCl. The letters shown on the top of bars represent statistically significant differences.

The effect of sodium chloride levels and modifying treatments and their interaction on fresh weight of aerial parts of Vetiver was significant. The application of salinity of 8 dS/m significantly increased the average fresh weight of aerial parts by 12.26% compared to the control treatment. Compared to the control, the average fresh weight of aerial parts in salinity of 16 and 24 dS/m significantly decreased by 5.64 and 30.48%, respectively. The use of modifying treatments (IAA and the bacterium) also increased the weight of aerial parts. The highest fresh weight of shoot was related to salinity of 8 dS/m and the combined application of IAA and bacteria, although no significant difference was observed between this level of salinity and the application of bacteria (Figure 1b). Also, the lowest value of aerial fresh weight was related to salinity of 24 dS/m and without the use of modifying treatment.

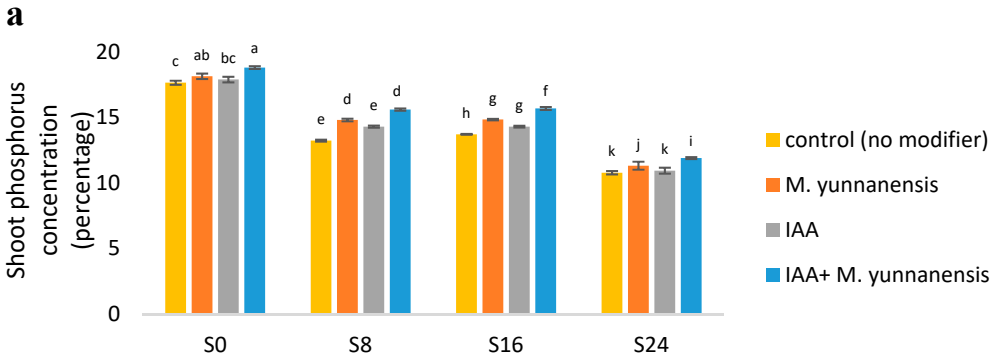
The individual effect of sodium chloride levels and modifying treatments, and their interaction effect was significant ($p < 0.05$). The use of modifying treatments (IAA and inoculation) also increased the dry weight of aerial parts (Figure 1c). Thus, the application of IAA and *M. yunnanensis* and the interaction of IAA and bacteria significantly increased the average dry weight of aerial parts by 5.50%, 20.20% and 35.40%, respectively, compared to the control treatment.

The effect of modifying treatments (IAA and bacteria) was significant on the dry weight of Vetiver roots. The use of modifying treatments (IAA and *M. yunnanensis*) increased the dry weight of the roots so that the application of IAA, *M. yunnanensis* and their interaction significantly increased the average root dry weight by 12.88, 80.61 and 100%, respectively ($p < 0.05$) (Figure 1d and Table A1).

3.2. Effect of Salinity Levels and IAA and M. yunnanensis on the Chemical Composition of Vetiver

The effect of sodium chloride levels (salinity), modifying treatments (IAA and *M. yunnanensis*) and their interaction on shoot phosphorus concentration was significant. The use of sodium chloride caused a significant decrease in the concentration of phosphorus in aerial organs. The application of IAA and bacterial inoculation caused a significant increase in the concentration of phosphorus in the aerial parts of Vetiver (Figure 2a). Also, the lowest phosphorus concentration of aerial parts was related to 24 dS salinity and without the use of modifiers.

The effect of sodium chloride levels, modifying treatments (IAA and bacteria) and their interaction in the potassium concentration of the shoot was significant. The use of sodium chloride significantly reduced the average concentration of potassium in aerial parts compared to the control. The application of IAA and the bacterium caused a significant increase in potassium concentration in aerial parts of Vetiver (Figure 2b). The highest concentration of potassium in aerial parts was related to the non-use of sodium chloride and the combined use of IAA and bacteria. In total, shoot phosphorous, shoot potassium and shoot sodium were increased by 10.36%, 44.49% and 68.61%, respectively.



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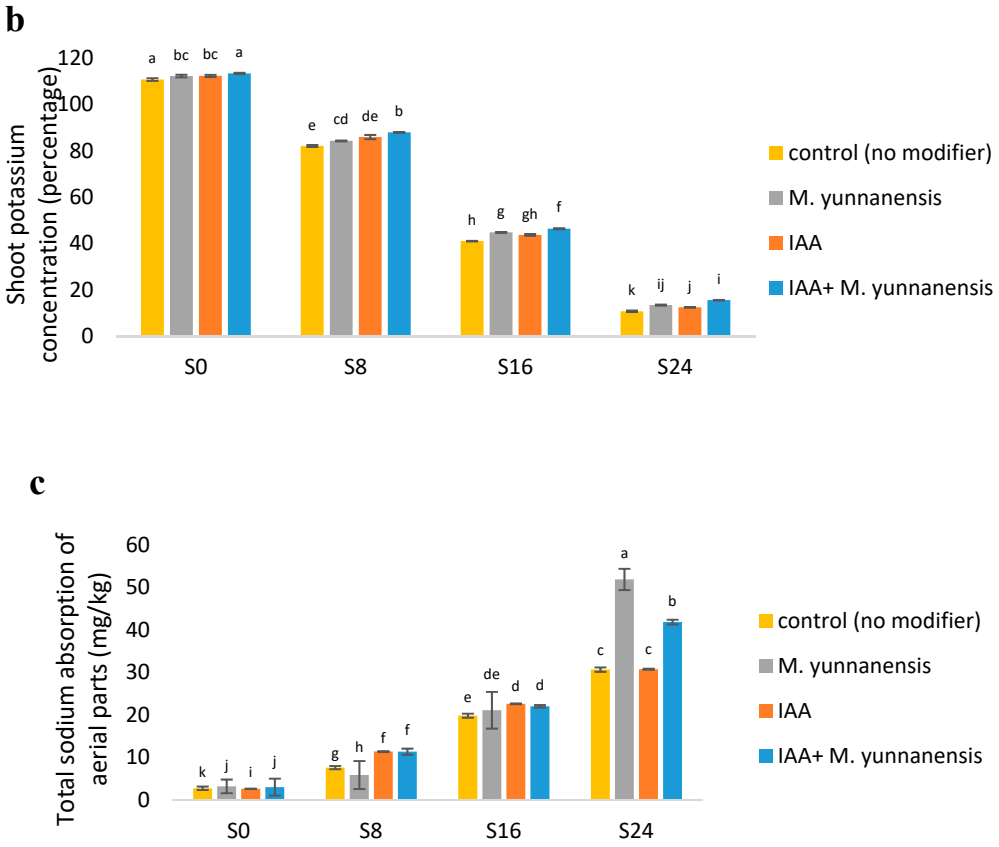

Figure 2. Effect of salinity levels, IAA and bacterial inoculation on the chemical composition of Vetiver. (**a**) Shoot phosphorus content under salinity, IAA and *M. yunnanensis* treatments (the values are represented as percentage); (**b**) impact of salinity, IAA and *M. yunnanensis* on potassium content of aerial part (the values are represented as percentage); (**c**) sodium concentration affected by salinity, IAA and *M. yunnanensis* measured as mg per kilogram. S0, S8, S16 and S24 mean 0, 8, 16 and 24 dS/m of NaCl. The letters shown on the top of bars represent statistically significant differences.

Only the effect of sodium chloride levels on the sodium concentration of aerial organs was significant ($p < 0.05$). Figure 2c shows that the effect of sodium chloride levels, modifying treatments (IAA and bacteria) and their interaction on the total sodium absorption of aerial organs was significant.

3.3. Effect of Salinity Levels, IAA and M. yunnanensis on Physiological Properties of Vetiver

The effect of salinity levels, IAA and bacterial inoculation and their interaction in plant proline concentration was significant. The application of sodium chloride levels increased the proline concentration of the plant. The use of IAA, *M. yunnanensis* treatments and the combined use of these two treatments also decreased the proline concentration of the plant so that the average proline concentration of the plant, with the application of IAA, *M. yunnanensis* and the combined application of IAA and bacteria, decreased by 6.80%, 11.41% and 14.44%, respectively, compared to the control treatment (Figure 3a). While no significant difference was observed between the level of bacteria application and the combined application of IAA and bacteria.

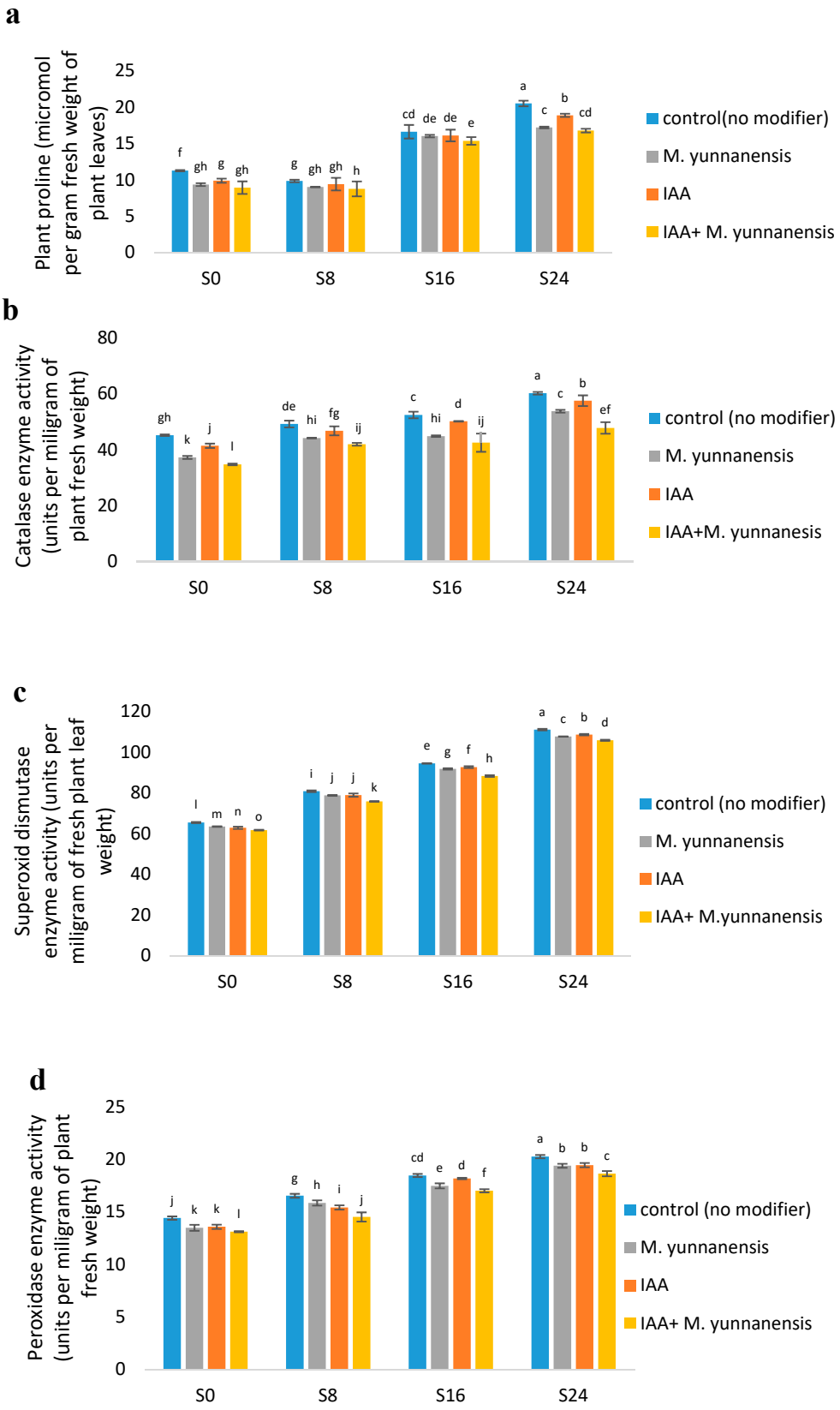

Figure 3. Effect of salinity levels, IAA and *M. yunnanensis* on physiological properties of Vetiver. (**a**) Proline content represents an ascending trend in line with increase in salinity; (**b**) catalase activity is enhanced with increase in salinity level; (**c**) increase in SOD activity as a result of salinity increase; (**d**) peroxidase activity is stimulated by increase in salinity level. S0, S8, S16 and S24 mean 0, 8, 16 and 24 dS/m of NaCl. The letters shown on the top of bars represent statistically significant differences.

The effect of sodium chloride levels modifying treatments (IAA and *M. yunnanensis*) on catalase enzyme activity was significant. Also, their interaction in catalase activity was significant. The application of sodium chloride levels increased the activity of plant catalase enzyme. The use of IAA, *M. yunnanensis* treatments and their interaction also caused a decrease in plant catalase enzyme activity. The highest level of catalase enzyme activity of the plant was related to the salinity level of 24 dS/m and without the use of modifying treatments. The lowest level of plant catalase activity was related to the surface without salinity stress and the combined application of IAA and *M. yunnanensis* (Figure 3b).

The effect of modifier treatments (IAA and *M. yunnanensis*) and the interaction effect of sodium chloride and modifier treatments on superoxide dismutase enzyme activity of Vetiver were significant. The application of sodium chloride levels increased the activity of superoxide dismutase enzyme of Vetiver plant. The application of IAA, *M. yunnanensis* treatments and their interaction also decreased the activity of plant superoxide dismutase enzyme. The highest level of superoxide dismutase activity was observed in 24 dS/m NaCl without the use of modifying treatments. The lowest level of superoxide dismutase enzyme activity of the plant was related to the surface without salinity stress and the combined application of IAA and *M. yunnanensis* (Figure 3c).

The effect of the levels of IAA and bacteria treatments and the interaction effect of sodium chloride and modifying treatments on the peroxidase enzyme activity of Vetiver plant was significant at the level of 1%. The application of sodium chloride levels increased the activity of Vetiver peroxidase enzyme, while the use of IAA and *M. yunnanensis* treatments and the combined use of these two treatments also caused a decrease in the activity of the peroxidase enzyme of the plant (Figure 3d).

3.4. Chemical and Biological Properties of Soil after Vetiver Harvest

Figure 4a shows that the effect of sodium chloride levels on phosphorus concentration in the soil after Vetiver harvesting is significant, while the effect of application of modifying treatments and the interaction effect of salinity levels and modifying treatments were not significant.

The effect of NaCl, modifying treatments (IAA and *M. yunnanensis*) and their interaction in potassium concentration in the soil after Vetiver harvesting was significant ($p < 0.01$) (Figure 4b).

a

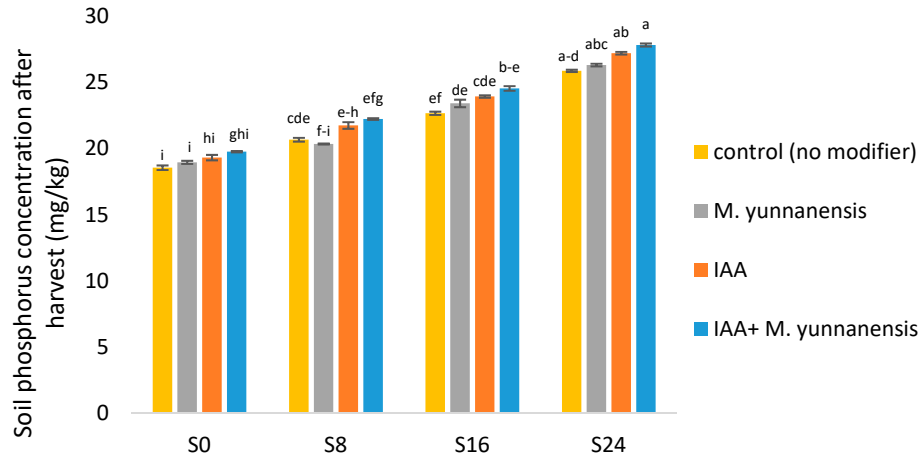

Figure 4. *Cont.*

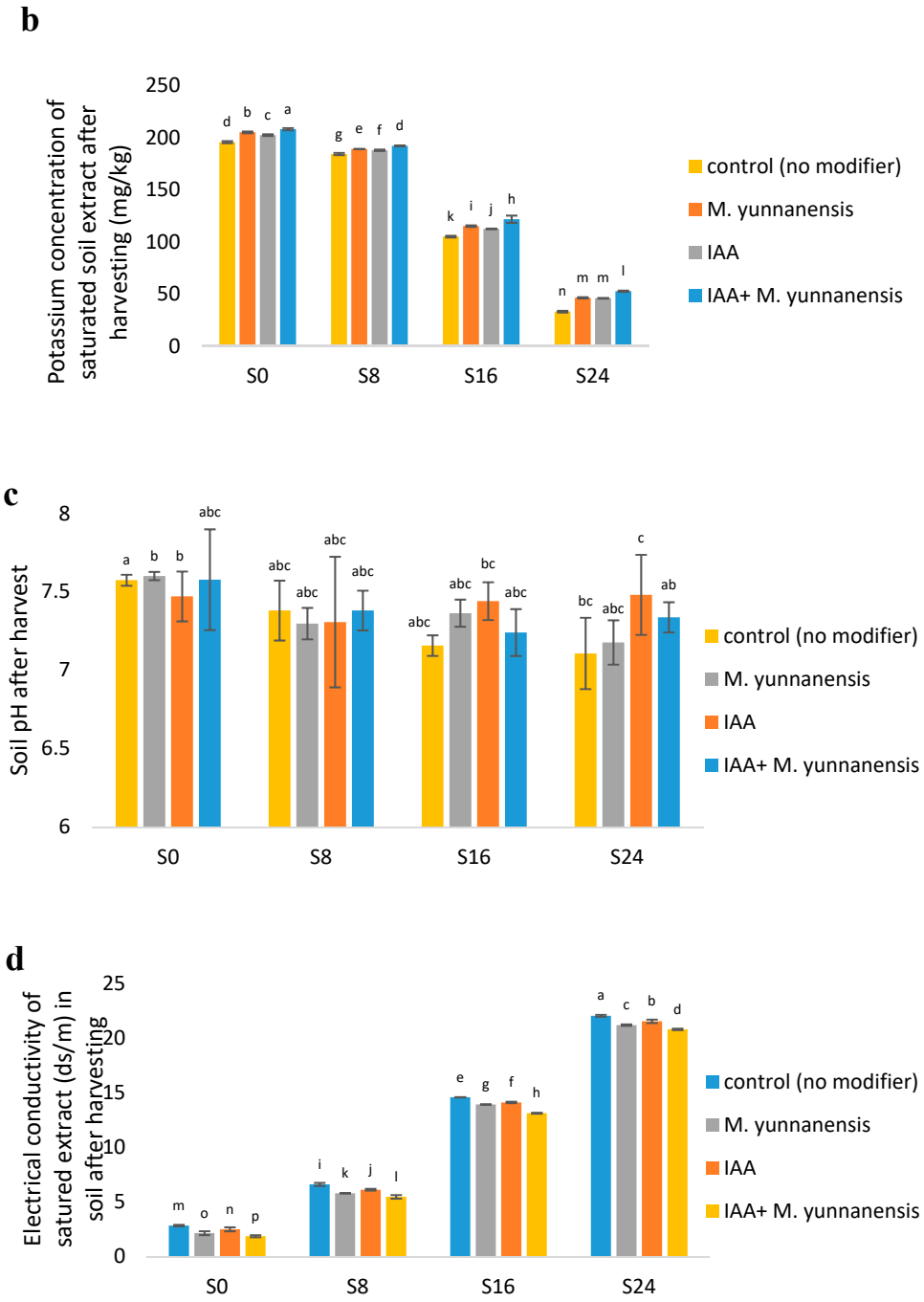

Figure 4. Chemical properties of soil after Vetiver harvest. (**a**) Individual and combined effects of treatments results in increased soil phosphorus content as salinity level increases. (**b**) Significant decrease in potassium concentration under salinity, IAA and *M. yunnanensis* treatments. (**c**) fluctuation in soil pH under the treatments; increase in salinity is accompanied with pH decrease. (**d**) Increase in soil electrical conductivity after Vetiver harvest under NaCl treatment. S0, S8, S16 and S24 mean 0, 8, 16 and 24 dS/m of NaCl. The letters shown on the top of bars represent statistically significant differences.

The use of IAA and *M. yunnanensis* treatments and their interaction also caused a significant decrease in sodium concentration in the soil after Vetiver harvest so that the average concentration of sodium in the soil after harvesting, with the application of IAA, *M. yunnanensis* and their interaction decreased by 0.88, 0.76 and 2.40%, respectively, compared to the control. Regarding soil acidity, a decrease in pH is seen as the salinity level is increased.

The effect of the levels of modifying treatments (IAA and bacteria) and the interaction effect of sodium chloride on the electrical conductivity of the saturated soil extract after Vetiver harvest were significant. The application of NaCl increased the electrical conductivity of the saturated soil extract after Vetiver harvest. The use of IAA and *M. yunnanensis* treatments and their interaction also caused a significant change in electrical conductivity of saturated soil extract after harvesting (Figure 4d).

The effect of modifying treatments (IAA and bacteria) and the interaction effect of sodium chloride in the electrical conductivity of the saturated soil extract after Vetiver harvesting were significant. The application of NaCl increased the electrical conductivity of the saturated soil extract after Vetiver harvest. The use of IAA and *M. yunnanensis* treatments and their interaction caused a significant decrease in electrical conductivity of saturated soil extract after harvesting.

The use of NaCl, modifying treatments (IAA and *M. yunnanensis*) and their interaction in soil microbial respiration after harvest were significant. The use of IAA, *M. yunnanensis* treatments and their interaction also caused a significant increase in soil microbial respiration after harvesting so that the average amount of soil microbial respiration after harvesting increased by 9.39, 22 and 30.54 percent with the application of IAA, bacteria and the combined application of IAA and bacteria, respectively, compared to the control treatment (Figure 5a).

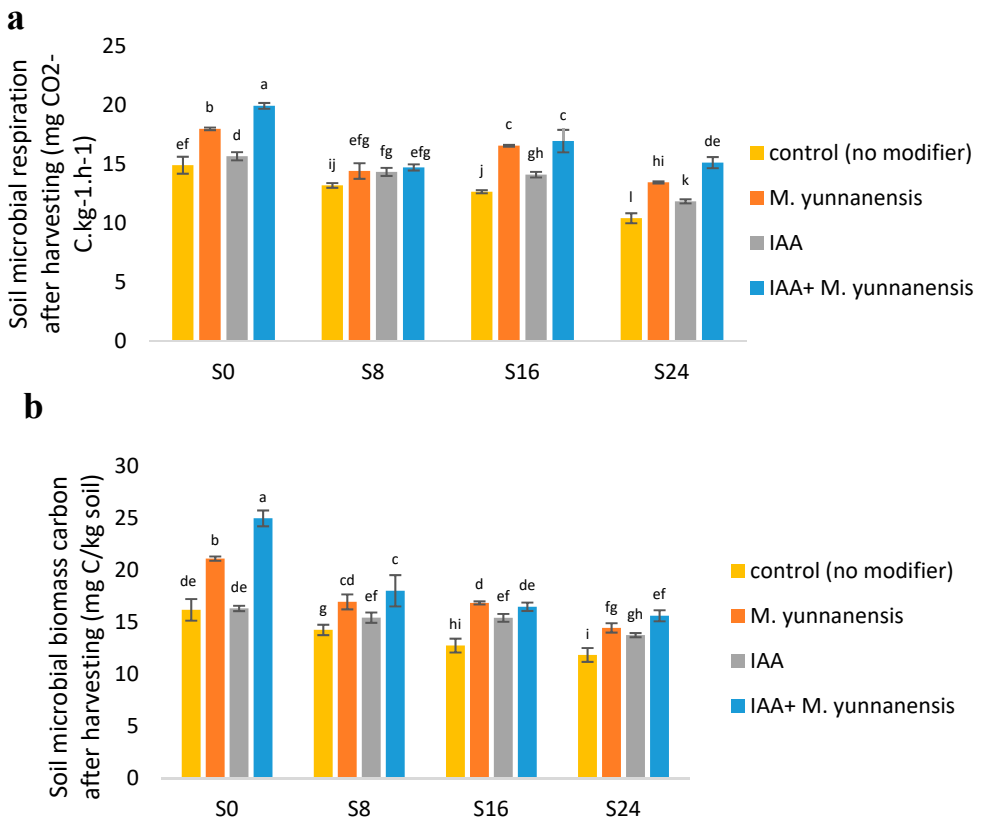

Figure 5. Effect of biological properties of soil after Vetiver harvest. (**a**) Increase of soil microbial respiration under interaction of IAA and *M. yunnanensis*. (**b**) Soil microbial biomass is improved by IAA and interaction of IAA and *M. yunnanensis*. Positive impact of the treatments is reduced as salinity increases. S0, S8, S16 and S24 mean 0, 8, 16 and 24 dS/m of NaCl. The letters shown on the top of bars represent statistically significant differences.

The use of NaCl, modifying treatments (IAA and *M. yunnanensis*) and their interaction were significant at the 1% probability level on the amount of soil microbial biomass after harvest. The use of sodium chloride caused a significant decrease in the amount of soil microbial biomass after harvesting, while the use of IAA and *M. yunnanensis* treatments

and their interaction caused a significant increase in soil microbial biomass after harvest (Figures 5b and 6).

Figure 6. Phenotypic comparison of Vetiver grass at the end of experimental period before removal from the soil. (**Right**) Plant treated with bacteria and IAA under saline conditions; (**Left**) plant without bacteria and IAA treatment under saline conditions.

4. Discussion

The results showed that the individual application and interaction of IAA and *M. yunnanensis* had a significant effect on plant height. Probably, IAA led to an increase in plant height by increasing cell proliferation and cell size. Growth-promoting bacteria through increasing the absorption of nutrients such as nitrogen, phosphorus and potassium [45,46] and the production of plant growth regulators such as gibberellin (effect on the longitudinal growth of cells, especially the longitudinal growth of cells, especially the internodes of the stem), auxin and cytokinin (effect on cell division) increase the vegetative traits of the plant [47]. Findings reported by other authors also show that IAA is effective in increasing the height of rice plants [48,49].

The effect of salinity levels and modifying treatments and their interaction on fresh weight of aerial parts of Vetiver were significant. In some studies, the tolerance of this plant to salinity equal to 8 dS has been reported [34], which is consistent with our results. In line with our results, it has been reported that with the increase of sodium chloride concentration, the fresh weight of Vetiver increased [50]. This can be justified by the fact that with increasing soil salinity due to lack of water, the length of most plants decreases, but the leaves become thick and stiff to adapt to the stress [51]. According to previous studies, the fresh weight of shoots of *Salicornia bigelovii* increased under salt stress. Ion toxicity or nutritional imbalance in plants under salt stress is one of the most important reasons for the reduction in length and weight of most plants [52]. In peppers treated with *Bacillus subtilis*, the fresh weight of the plant was significantly higher than that of untreated plants, which was due to the ability to produce siderophore and increase the absorbable iron for the plant [53]. In plants inoculated with bacteria, more water is absorbed, and as a result, the turgor pressure increases, which leads to improved nutrition and more growth of shoots and roots [54]. Corn fresh weight and yield were affected and increased by increasing the level of auxin hormone application. The reason for this increase could be due to the role of auxin in photosynthesis and, most importantly, the greenness of corn and having greener leaves [55]. Growth-promoting bacteria grow and act through various methods, including the production of vitamins, amino acids, the production of the acid amino acid enzyme, the dissolution of insoluble phosphates and the formation of the cydophore–iron complex. They increase the fertilizer of plants [14,56]. The effect

of modifying treatments (IAA and bacteria) was significant on the dry weight of Vetiver roots. Rhizosphere bacteria that stimulate growth by producing growth-stimulating IAAs and ACC-deaminase increase the root levels by increasing the weight of the root mass, longitudinal growth and the production of lethal threads, and as a result, the absorption of water and nutrients is increased [13,15,16].

Shoot phosphorus concentration significantly increased under IAA and *M. yunnanensis* treatments. Previous reports also show that under salinity conditions, phosphorus concentration in Vetiver and lettuce decreased [57]. With increasing salinity, phosphorus concentration increased in salt-sensitive rice variety but decreased in salt-tolerant rice variety [44]. The increase in phosphorus concentration in bacterial treatments is attributed to the production of inorganic acids (carbonic acid and sulfuric acid), organic acids (oxalic, citric and lactic) and the production of phosphatase enzymes, resulting in the dissolution of organic and inorganic phosphates [58].

Considering that phosphorus is an immobile element in the soil, its absorption increase can be attributed to the increase in the dry weight of this plant in saline conditions. Pseudomonas bacteria isolates, increased phosphorus absorption in tomatoes [59]. Increase in phosphatase enzyme activity and then an increase in phosphorus availability as a result of corn inoculation with a phosphate-dissolving bacterium has also been reported [60]. Also, the increase in phosphorus availability due to the use of growth-promoting microorganisms through the production of phytase enzyme has been reported [61].

The application of NaCl significantly reduced potassium concentration in aerial parts compared to the control, while the application of IAA and *M. yunnanensis* caused a significant increase in potassium concentration in aerial parts of Vetiver. The main reason for the decrease in potassium concentration can be attributed to conflict with sodium. The amount of potassium in plants with high salt concentrations is an advantage and can be used as a suitable criterion for selecting plants in terms of salinity tolerance [62]. Many microorganisms, including bacteria, fungi and yeasts, are able to decompose silicate and release elements such as potassium, phosphorus, iron, zinc and silicon. Therefore, it can be concluded that the bacterium caused the release of potassium through the decomposition of silicates and the dissolution of minerals, and consequently caused the greatest increase in the amount of potassium in aerial organs [63].

The most important effect of NaCl is to increase sodium concentration in plant tissue. Excess sodium can lead to changes in the nutritional status of other elements. For example, decreasing potassium absorption and decreasing plant growth and performance are the results of increasing sodium concentration [64]. With increasing salinity, the amount of sodium in wheat aerial parts increased [65]. The reduction of shoot sodium in the treatment of bacterial application can be attributed to the reduction of sodium transfer from roots to shoots. The increase in the population of bacteria producing exopolysaccharides in the root zone reduces the amount of sodium available for plant absorption and thus contributes to the resistance of plants grown in saline environments [66].

With increasing salinity, osmotic regulators increase the osmotic pressure of the cytoplasm and stabilize proteins and membranes in such conditions [67]. Proline is considered one of the osmotic-regulating compounds produced in response to salinity stress, and its accumulation in tissue is one of the most induced changes caused by water or salinity stress in plants [68]. High levels of proline in plants under stress allow cells to balance the osmotic tensions of their cytoplasm and prevent water shortage [69]. Moreover, proline stabilizes phospholipid membranes during salt stress [70]. The change in proline content by hormones is the reason for increasing the relative water content of the plant [71]. IAA can decrease proline content by enhancing the plant's stress tolerance mechanisms, reducing the need for proline accumulation. It may also regulate the enzymes involved in proline metabolism, such as proline dehydrogenase (which degrades proline) and pyrroline-5-carboxylate synthetase (which synthesizes proline). For example, it has been reported that IAA produced by Klebsiella oxytoca reduced the proline content in cotton under salt stress [72]. This finding matches with that observed in the present study.

Catalase is an important enzyme that, together with superoxide dismutase, converts superoxide anion radicals and H_2O_2 into water and oxygen molecules in the defense system [73]. The decrease in catalase activity in *M. yunnanensis* treatment indicates that the bacteria placed the plant in a less stressful conditions. In the presence of bacteria due to the balancing of the environmental and internal conditions (such as the increase in the absorption of food and also the increase of some plant hormones), stress can be mitigated by antioxidant enzymes [74]. Research on rapeseed showed that with increasing salinity, catalase enzyme activity increased in the plant and the presence of bacteria reduced the need for catalase enzyme activity [75]. A decrease in catalase enzyme activity in barley seedlings inoculated with *Azospirillium* bacteria has been reported [76]. Salinity stress caused a significant increase in catalase enzyme activity in corn plants but the use of PGPR bacteria reduced the oxidative damage caused by salt stress in inoculated corn compared to non-inoculated plants [57].

In many crops, including wheat [77] and cotton [78], an increase in the level of superoxide dismutase has been reported during salinity stress. Superoxide dismutase converts superoxide into H_2O_2 and atomic oxygen and catalase and peroxidase enzymes convert H_2O_2, which is toxic to cells, into water and oxygen [79,80]. Therefore, under salinity stress, superoxide dismutase alone cannot protect the plant against oxidative stress, and the increase of enzymes involved (catalase and peroxidases) in the detoxification of H_2O_2 is necessary [79]. In line with our results, it has been reported that with the increase of NaCl concentration, the activity level of superoxide dismutase enzyme increased in aloe vera plants [81].

Peroxidase activity is a key factor for protecting plants against environmental stresses [82]. Salt stress increases the peroxidase enzyme activity in leaves and reduces the adverse effects of salinity [83]. In general, plants treated with salinity in the presence of growth regulators show more resistance in stress conditions than plants exposed to salt alone because growth regulators minimize the damage caused by sodium chloride [84]. It seems that under bacterial treatment, the content of ROS decreased and, as a result, the activity of antioxidant enzymes decreased. In a study, inoculation of soybean plant with PGPR bacteria under salt stress conditions caused a significant decrease in the activity of antioxidant enzymes compared to control plants [85]. Regarding reduction of antioxidant enzymes activity as a result of the bacterium and IAA treatments, it should be remarked that the presence of IAA—either as exogenous agent or produced by the bacteria—may mitigate proline content or antioxidant enzymes activity. A study by Chandra et al. (2018) demonstrated that IAA-producing bacteria could reduce the accumulation of proline and antioxidant enzymes such as superoxide dismutase (SOD) in wheat under salinity stress. This reduction in proline and antioxidant enzyme activity is attributed to the bacteria's modulation of stress-responsive genes, which affected the plant's overall stress tolerance and growth regulation [86].

Moreover, Al-Turki et al. (2023) found that PGPRs that produce IAA can lead to a reduction in the activity of antioxidant enzymes such as catalase (CAT) and ascorbate peroxidase (APX) under salinity stress. This reduction is attributed to the PGPR-mediated modulation of hormonal pathways that prioritize growth over stress defense [87].

According to the present results, the treatment of IAA and bacteria caused a significant increase in potassium concentration. One of the reasons for the increase in potassium concentration could be considered to be less absorption of usable soil potassium by the plant during salinity stress conditions, which causes more potassium to remain in the soil. Different microorganisms, such as bacteria, fungi, algae and lichens, are able to decompose silicates and release elements such as potassium, phosphorus, iron, zinc and silicon [63].

Studies have demonstrated that symbiosis with PGPB can reduce nutrient uptake imbalances and enhance plant growth and yield by improving external hyphae, hormonal adjustments, the solubilization of minerals and mineral nutrition uptake [88]. Moreover, it has been shown that plants inoculated with PGPB under saline conditions show improved antioxidant enzyme activities. This is the case for alfalfa [89], wheat [90], common bean [91]

and lettuce [92]. In addition, PGPR inoculation enhances gas exchange and the contents of photosynthetic pigments by increasing nutrient uptake through the root, which maintains the osmotic balance in cells, thereby improving the metabolism [24]. Moreover, PGPB can improve plant growth by increasing chlorophyll synthesis [93]. In a recent study, it was shown that *M. yunnanensis* is able to produce plant growth-promoting factors (PGPFs) such as IAA [28]. IAA is regarded a multifunctional hormone for plant growth and development under stress and non-stress conditions. IAA accelerates the activities of antioxidant enzymes such as POX and CAT and increases the defense system [94].

Increasing the salinity of the irrigation water is expected to increase the electrical conductivity of the soil because salts have an ionic structure, and when added to the soil, they increase the number of solutes and thus the electrical conductivity of the soil. The addition of PGPR bacteria—through the production of siderophores and the increase in nutrient uptake by various mechanisms—reduces the destructive effects of salinity and increases plant growth [95].

The highest rate of soil microbial respiration after harvesting was observed without salinity stress and interaction of growth regulator and bacteria. The effect of salts in soil on microbial respiration is due to osmotic stress and ion toxicity, which impact the physiology and metabolic pathways of microbial cells [96]. The decrease in the growth of microorganisms in saline soils is caused by high salinity [97]. In a previous study, application of NaCl significantly reduced soil microbial respiration after harvesting spinach [98]. The presence of growth-promoting bacteria increased the amount of organic carbon in the soil and increased the microbial respiration compared to the control [99].

The highest rate of soil microbial respiration after harvesting was related to the surface without salinity stress and with the combined application of a growth regulator and bacteria. Microbial biomass carbon is a direct unit for expressing the number of microorganisms, especially bacteria, and represents the carbon fixed in microbial cells [100]. In accordance with these findings, it was previously reported that the addition of bacteria caused a significant increase in soil respiration and microbial biomass compared to the absence of bacterial fertilization [101].

Salinity is one of the most important non-biological stresses that disrupts various biochemical and physiological processes in plants and ultimately leads to a decrease in production and performance. Hence, it is a serious threat to agricultural productivity, especially in arid and semi-arid regions of Southwest Asia. The obtained results can have practical guidelines because it introduces a cost-effective and practical method to reduce the negative effects of salinity stress. Some countries in arid and semi-arid regions are facing economic problems and it is difficult for them to use expensive soil desalination methods; thus, the biological methods—in addition to reducing negative environmental effects—can also contribute to sustainable development from an economic point of view.

5. Conclusions

Salinity is the main environmental factor limiting plant growth and productivity. Based on the results obtained in this research, it was found that the use of IAA and *M. yunnanensis* has a significant effect on improving the performance of Vetiver under salt stress conditions. Follow-up experiments after Vetiver harvest showed that IAA and *M. yunnanensis* treatments improved soil microbial biomass and respiration. Considering the importance of developing non-chemical inputs in achieving sustainable development goals, the results obtained in this research are promising. Also, according to the research findings, bacteria and IAA treatments can be investigated to improve the performance of other crops. Undoubtedly, conducting more research in this field can pave the way for the development of environmentally friendly agents. Finally, it should be mentioned that this present study was conducted only at four salinity levels; therefore, caution should be taken in generalizing its results. Also, plants have different physiological mechanisms, so the results obtained about Vetiver may not apply to all other plants.

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Appendix A

Table A1. The effect of individual treatments and interaction on the morphological and physiological characteristics of Vetiver grass. Salt stress (S), Bacteria and IAA as modifiers (M), Interaction (S × M). Plant height (PH), Fresh weight (FW), Dry weight (DW), Root dry weight (RDW), Shoot phosphorous (ShP), Shoot potassium (ShK), Shoot sodium (ShNa), Proline (P), Catalase (CAT), Dismutase (DIS), Peroxidase (POD), Soil phosphorous (SP), Soil potassium (SK), Soil Ph (SpH), Electrical conductivity (EC), Microbial respiration (MR), Microbial Biomass (MB) (* stands for significance at $p < 0.05$; ** represents significance at $p < 0.01$; ns means non-significant effect).

	S	**M**	**S × M**
PH (cm)	ns	**	*
FW (g)	**	**	*
DW (g)	**	**	**
RDW (g)	ns	**	ns
ShP (%)	**	**	**
ShK (%)	**	**	**
ShNa (mg/kg)	**	ns	ns
Pr (µmol/g)	**	**	**
CAT (U/mg)	**	**	*
DIS (U/mg)	**	**	**
POD (U/mg)	**	**	**
SP (mg/kg)	**	ns	ns
SK (mg/kg)	**	**	**
SpH	**	ns	ns
EC (ds/m)	**	**	**
MR (mg CO_2-C/Kg·h)	**	**	**
MB (mgC/kg)	**	**	**

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