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Nitrogen fixing bacteria, phosphate solubilizing bacteria, and their mixtures to reduce mineral fertilizers and improve yield, agronomic traits and nutrient uptake in barley under salinity stress

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Abstract: Under salinity conditions, growth and productivity of grain crops decrease, leading to inhibition and limited absorption of water and elements necessary for plant growth, osmotic imbalance, ionic stress, and oxidative stress. Microorganisms in bio-fertilizers have several mechanisms to provide benefits to crop plants and reduce the harmful effect of salinity. They can be effective in dissolving phosphate, fixing nitrogen, promoting plant growth, and can have a combination of all these qualities. During two successful agricultural seasons, two field experiments were conducted to evaluate the effect of bio-fertilizer application include phosphate solubilizing bacteria (PSB), nitrogen fixation bacteria and mixed of phosphate solubilizing bacteria and nitrogen fixation bacteria with three rates; 50, 75 and 100% NPK, of recommended dose of minimal fertilizer on agronomic traits, yield and nutrient uptake of barley (*Hordeum vulgare*) under saline condition at Village 13, Farafra Oasis, New Valley Governorate, Egypt, during the two successive seasons. The results showed that, the applied of Mycrobein + 75% NPK recorded the highest values of plant height, spike length, number of spikes/m2, grain yield (Ton ha^{-1}), straw yield (Ton ha^{-1}), biological yield (Ton ha^{-1}), protein content %, uptake nitrogen (N), phosphorus (P), potassium (K) in grain and straw (kg ha^{-1}), available nitrogen (mg/kg soil), available phosphorus (mg/kg soil), total microbial count of soil, antioxidant activity of soil (AOA), dehydrogenase, nitrogen fixers, and PSB counts. The application of bio-fertilizers led to an increase in grain yield and productivity, increase plant tolerance to salt stress, and an increase in various traits with applied the treatments that include bio-fertilizers and a 25% saving in the cost of mineral fertilizers used in barley production.

Keywords: biofertilizers; salinity; NPK fertilizers; microbial activity; soil antioxidant; barley (*Hordeum vulgare*).

1. Introduction

Barley (*Hordeum vulgare* L.) is one of the most important cereal crops. It is a du-al-purpose crop as it can be used as fodder and also one of the most important cover crops to enhance soil fertility. From a nutritional standpoint, its importance is because it is high in essential nutrients, protein, carbohydrates, fats and fiber [1]. Barley is a moderately salt-tolerant crop and has the ability to cultivate in a broad range of ecological stresses, including saline, drought, heating conditions [2,3]. In arid and semi-arid regions, salinity is one of the main abiotic stresses that negatively influence crop productivity and quality [4,5]. In Egypt the total salt affected area are about 0.9 M ha [6-8]. In Egypt, as a result of the prevailing environmental and climatic conditions, especially in reclamation areas, plants are showing to harsh climatic factors, such as high temperatures and drought. As a result of these conditions, dissolved salts often accumulate in the soil due to insufficient filtration of ions and because most of these areas are primarily affected by salinity [9,10]. Salinity stress lead to many effects, whether direct or indirect, on the physiological state of the plant by causing changes in metabolism, growth and development, and thus leads to decrease in productivity of crops [5,11-13].

Microbial activities and soil populations are negatively affected by high salinity, as it generally poses environmental stress on rhizospheric bacteria [12-14]. High salinity prevents and hampers various physiological processes, from nutrient absorption to inhibition of DNA replication and the biosynthesis of macromolecules [16,17]. In addition, high salinity leads to changes in the proteins concerned in the preliminary attachment steps of bacteria to plant roots, and it also works to inhibit the activity of bacterial nodules and fix nitrogen. It also prevents and reduces the movement of bacteria and their chemical attraction to plant roots [18-20].

In recent years the use of chemical fertilizers in agriculture has led to increase the rate of self-sufficiency in food production in the world, but this has been at the expense of the ecosystem and the safety of all living organisms [21-23]. Excessive use of chemical fertilizers over the past few decades has led to numerous negative impacts on soil quality and fertility and increased suitability of agricultural resources, such as increased soil salinity and negative impacts on the ecosystem and human health [24,25]. Environmental difficulties resulting from unbalanced applications of chemical fertilizers have had detrimental effects on biological cycles. Therefore, reducing the use of chemicals fertilizers in agricultural areas is of huge importance in order to protect plant health, reduce environmental pollution, and reduce production costs. To meet agricultural production requirements, beneficial microorganisms are the best alternatives to traditional farming methods. Microorganisms in the soil are very essential for the growth and development of plants. They play a major role in soil fertility, increasing the content and biodiversity of the soil, and facilitating nutrients for the plant [21-23]. Therefore, the importance of using bio-fertilizer with microorganisms such as phosphate-dissolving bacteria, fixing nitrogen, and plant growth-promoting rhizobacteria (PGPR) appears in order to increase productivity and preserve the environment. The use of these microorganisms represents environmentally friendly alternatives, and plant growth-promoting rhizobacteria (PGPR) are free-living bacteria in the roots of soil-borne plants that colonize them. They employment to improve plant growth and productivity either directly, by providing plants with nutrients they lack in the soil as an alternative to the use of chemical fertilizers, or indirectly by plant protection from plant pathogens through soil is widely used as an alternative to mineral fertilizers and chemical pesticides due to its environmentally friendly nature [26,27]. Moreover, they are incorporated into many bioactivities of the soil system and synthetic pesticides to make them active for nutrient movement and viable for crop production [28]. In addition, through its ability to fix N2, it works to enhance promote plant growth, soluble mineral phosphate and other nutrients, regulate soil nutrients, synthesize various plant growth hormones, protect plants from plant pathogens, improve soil structure, and mitigate the effect of abiotic stress e.g. salinity, drought, acidity, humidity, bioremediation of different classes of toxic heavy metals and degradation of synthetic chemical compounds [29-35]. In addition to the previously mentioned functions of PGPRs, it also play a major role in plant defense in various ways by producing antibiotics, volatiles, bio-surfactants, siderophores, and enzymes that denature the cell wall and bring systemic resistance [36,37]. Biologically, Azotobacter and Azospirillum are aerobic heterotrophs that have the ability to fix atmospheric nitrogen and make it available to plants. Azotobacter also secretes some hormones such as gibberellin, thiamine, indole acid, and some sugars that work to improve soil properties [38-42]. Some previous studies reported that the use of fertilizers containing Azotobacter bacteria led to a reduction in the recommendation for nitrogen fertilizers for barley by 20% [43-45].

Biofertilizers are distinguished from chemical fertilizers in that they are considered safer than chemical fertilizers for some reasons, including that they are less expensive, that they cause less environmental damage, that their activity is more concentrated, and that they are more efficient when used in smaller quantities [23,37,46]. Moreover, they also have the ability to reproduce while being simultaneously regulated by the plant and local microbes. Furthermore, microbial inoculants have faster decomposition processes and do not cause an increase in pathogens and pests to develop tolerance [23,37,47,48].

An additional advantage of bio-inoculants is that they do not show any harmful effect on plant and animal life in the soil. Therefore, they are considered environmentally friendly and highly efficient. They can also be used many times as biocides that do not have any damaging effect on plant productivity. In the direction of increase organic carbon in the soil, support sustainability in agricultural production, and meet the plant's need for mineral nutrients, which can only be provided when using chemical fertilizers directly or indirectly, you also need in addition to organic fertilizer and bio-fertilizers [37,48-50]. The expression bio-fertilizers refers to a broad range of products that contain live or inactive microorganisms, including bacteria, algae, fungi, and actinomycetes. These products are used and applied for many purposes. They work to fix atmospheric nitrogen, dissolve and mobilize soil nutrients, or one of these processes, in addition to it secretes substances that promote plant growth [51-54]. Currently, biofertilizers are available as alternatives to traditional chemical fertilizers or are used to reduce the amounts of chemical fertilizers used [55,56].

The production of biofertilizers is increasing year after year, and the size of the biofertilizer market is expected to increase and expand to include larger areas. The world's biofertilizer market value reached 3.14 billion US dollars in 2022, and its value is expected to reach 5.2 billion US dollars by 2032, at a compound annual growth rate (CAGR) is 11.3% between 2023 and 2032 [45,57]. Therefore, many researchers have attempted to reduce the doses used for chemical fertilizers by using bio-fertilizers in different treatments containing both in gradual and varied proportions [51,58]. It was found that the use of bacteria alone or in combination with mineral fertilizers enhanced the growth of roots and shoots of corn seedlings in sodium saline soil [59]. In another study, sunflower plant productivity was enhanced by reducing the use of chemical fertilizers and their harmful effects on soil health and the environment by combining PGPR with half the recommended dose of chemical fertilizers [60,61].

Nitrogen is one of the important nutrients for plants, as they need it in different stages of growth. It is also an important part of amino acids, proteins, enzymes, and protoplasm. In addition, N is part of a cofactor such as NAD and NADH in amino acid formation reactions in cells [23,62-65]. Phosphorus, it is an essential and main element in plant nutrition, as it plays an important role in the processes of growth, formation and division of cells, the formation and development of seeds, and in the synthesis of energy compounds such as CTP, ATP, and GTP, [64-67]. As for potassium, it plays a major role in the processes of plant growth and development, as it is of great importance in the process of photosynthesis. It also works to increase cell division and the plant's resistance to abiotic and biotic stresses [68-71].

Thus the present study was conducted with the objective of studying the impact of bio-fertilizer treatments and three diverse levels of mineral fertilizer (50, 75 and 100%) of the recommended dose on the growth, grain yield, productivity and chemical composition of barley (*Hordeum vulgare* L) under salinity conditions in newly reclaimed lands and attainment a treatment to reduce the amounts of mineral fertilization used and thus reduce the expenditure of production and improve the environment.

2. Materials and Methods

2.1. The experiment site:

During the two successive seasons in 2019 and 2020 years in new reclaimed sandy salt affected soil at Village 13, Farafra Oasis (Latitude: 27° 3' 24" N; Longitude: 27° 58' 11" E), New Valley Governorate, Egypt, two field experiments were conducted to study the response of barley plant (*Hordeum vulgare*, L. c.v. Giza-123) to three levels of recommended of mineral fertilizer NPK (50, 75 and 100%) and inoculation by Azotobacter chrococcum (in the form of a commercial biofertilizer compound for nitrogen fixation, Rhizobacterien), Bacillus subtilis as phosphate-solubilizing bacteria (PDB) (in the form of a commercial biofertilizer compound to fix nitrogen, Phosphorene), mixture of Pseudomonas, Azotobacter, Bacillus and Rhizobium (in the form of a commercial biofertilizer compound for nitrogen fixation, Mycrobein), and without inoculation as control. The three biofertilizers was used at the rate of Rhizobacterien 2.4 kg, Phosphorien 1.4 kg, Mycrobein 1 kg per 143 kg barley grains ha^{-1}. Inoculation of barley grains with Rhizobacterien, Phosphorien, and Mycrobein was carried out after coating the grains with an adhesive material (gum arabic 5%), then planting and irrigating them immediately after inoculation. The soil and irrigation water analysis for the experiment are described in Table 1.

Table 1. Physical and Chemical Analysis of Pre-cultivated Soil and Irrigated Water for the experiment site.

Sample Depth (cm)				Physical properties %							Soil texture	
0-30				**Sand**	**Silt**	**Clay**					**Sandy silt loam**	
				51.95	25.73	22.32						
Chemical analysis												
				Cation meq/l						**Anion meq/l**		
pH	E.C ds.m-1	Ca-Co3%	O.M%	T.N%	Ca+2	Mg+2	Na+	K+	Co3-2	HCo3	Cl-	So4-2
8.67	8.24	4.29	0.56	0.09	25.2	7.83	44.35	5.34	0.92	15.67	48.9	5.92
Chemical analysis of irrigation water												
			Cation meq./l						**Anion meq./l**			
pH	E.C ds.m-1	Ca+2	Mg+2	Na+	K+		CO3-2	HCO3-	Cl-	SO4-2		
7.62	2.34	5.37	3.12	5.43	0.25		1.63	12.37	4.23	6.18		

2.2. Experimental design and management:

Over two years, two field experiments with 12 treatments were conducted in three replications in a randomized complete block design (RCBD). The treatments included Without bio-fertilizer + 50% NPK (T1), Phosphorin + 50% NPK (T2), Rhizobacterien + 50% NPK (T3), Mycrobein + 50% NPK (T4), Without bio-fertilizer + 75% NPK (T5), Phosphorin + 75% NPK (T6), Rhizobacterien + 75% NPK (T7), Mycrobein+ 75% NPK (T8), Without bio-fertilizer + 100% NPK (T9), Phosphorin + 100% NPK (T10), Rhizobacterien + 100% NPK (T11), Mycrobein + 100% NPK (T12).

According to agricultural practices for growing barley in Egypt, the recommended dose of mineral fertilizers was as follows: nitrogen fertilizers were applied at a rate of 180 kg N ha^{-1} added in the form of ammonium nitrate fertilizer (33.5 % N) in three equivalent parts, the first part was fertilized 21 days after planting (DAS), the second was at 35 DAS, and the third part was added at 50 DAS. As for phosphorus, it was fertilized at a rate of

62 kg P_2O_5 ha^{-1}, in the form of calcium superphosphate fertilizer (15.5 % P_2O_5) at a rate of 400 kg per hectare. As for potassium fertilizer, it was added at a rate of 71 kg K_2O ha^{-1} in the form of potassium sulfate (48 % K_2O) in one dose with the first dose of nitrogen fertilizer. The seed rate used was 154.76 kg ha^{-1} of barley grain and sowing was done on 1st December for both seasons following all other cultural practices recommended for planting barley fields. Each plot had 4 ridges, where, the length of the ridge was 3.50 m and its width was 70 cm. thus, the area of the piece was 11.2 m^2.

2.3. Data Recoded

2.3.1. Agronomic traits

At harvest, ten plants were randomly selected from each plot to evaluate plant height (cm), spike Length (cm), spikes weight (g), number of grains $spike^{-1}$, number of spikes/m^2, 1000-grain weight, grain yield (Ton ha^{-1}), straw yield (Ton ha^{-1}), and biological yield (Ton ha^{-1}).

2.3.2. Chemical composition

According to AOAC [72], the micro-Kjeldahl method was followed and used to evaluate total N in grains and straw. The protein content of grain and straw was estimated by multiplying the total nitrogen by 4.64 [73]. The reduced chlorostannous reduced molybdophosphoric blue color method as according to Chapman and Parker [74], was used to determine the phosphorus content (P%) colorimetrically. The flame photometric method according to Page et al [75], was used to determine the potassium content (K%) in digested plant materials. The uptake of N, P, and K (kg ha^{-1}) in grains and straw was determined and estimated by multiplying the yield of grains or straw by their N%, P%, and K% content, respectively.

2.3.4. Soil bio-activity

The method of Allen [76], was followed to estimate the total microbial counts. While the activity of dehydrogenase (DHA) enzyme in the rhizosphere soil was measured according to the method Casida et al [77], to estimate microbial activity, the antioxidant activity in the rhizosphere soil was also evaluated after the extraction procedure carried out by Rimmer and Abbott [78].

2.4. Statistical Analysis

The Casella method [79], was followed to analyze the data statistically using analysis of variance (ANOVA) for a randomized complete block design using Costat version 6.303 [80]. Treatment means were compared by performing Duncan's multiple range test at 0.05 probability level by Waller and Duncan [81].

3. Results

The treatment by bio-fertilizers with rates of the recommended dose of mineral fertilizers under salinity stress conditions gave the highest values of PH, Sp L, Sp W, G/Sp, 1000-GW, GY (Ton ha^{-1}), Straw Y (Ton ha^{-1}), biological Y (Ton ha^{-1}), N/Grain (Kg h^{-1}), K/Grain (Kg h^{-1}), P/Grain (Kg h^{-1}), N/Straw (Kg h^{-1}), K/Straw (Kg h^{-1}) and P/Straw (Kg h^{-1}) as well as studied traits at the second and first year, respectively.

The combinations of Mycrobein + 75% NPK (T8) followed by Rhizobacterien + 75% NPK (T7) next by fertilization with Mycrobein + 100% NPK (T12), gave the highest values for most of the studied traits, (Table 1, Table 2 and Figure 1,2,3 and 4).

The data showed that significant differences were obtained in plant height, spike length, and spikes weight of barley between the treatments in both studied seasons, Table 1. In this trend, the tallest plants were obtained with treatments; Mycrobein + 75% NPK (T8), Mycrobein + 100% NPK (T12) and Rhizobacterien + 75% NPK (T7), followed by Mycrobein + 50% NPK (T4), and the increase percentage was by 32.70, 30.69, 23.22, and 14.81% in the first year, while the increase in second was by 28.76, 24.76, 20.86 and 22.38% over recommended dose of mineral fertilizer. On the other hand, the increase over the same dose of mineral fertilizer application treatments were; 34.29, 30.69, 24.70, 42.29 and 33.82, 24.76, 25.62, 56.35%, in the first and second year, respectively.

The highest values of spike length were recorded at harvest with the applied parameters; Mycrobein + 75%NPK (T8), Mycrobein + 100% NPK (T12) and Rhizobacterien + 75% NPK (T7), with an increase of 32.32, 30.30, 23.23 and 28.85, 25.00, 21.15% compared with the recommended dose of mineral fertilizers i.e. treatment : without bio-fertilizer + 100% NPK (T9) in both seasons respectively. While, the increase over the same dose of mineral fertilizer application treatments without bio-fertilizer were; 33.67, 30.30, 24.49, and 34.00, 25.00, 26.00%, in the first and second year, respectively, (Table 1).

Table 1. The effect of treatment with bio-fertilizers and reducing mineral fertilizers by percentages of the recommended dose of mineral fertilizers (RDP) on plant height, spike length, and spikes weight of barley variety Giza 123 in the years 2018/2019 and 2019/2020.

Treatments	Plant height (cm)		Spike Length (cm)		Spikes weight (g)	
	2018/2019	2019/2020	2018/2019	2019/2020	18/2019	2019/2020
Without bio-fert.+50% NPK	68.1 ± 0.4	72.4 ± 1.3	8 ± 0.4	8.1 ± 0.2	1.6 ± 0.1	1.6 ± 0.1
Phosphorin+50% NPK	82.1 ± 1.6	87.4 ± 1.6	9.6 ± 0.2	9.8 ± 0.2	2.2 ± 0.1	2.3 ± 0.1
Rhizobacterien+50% NPK	92.4 ± 0.5	98.6 ± 1.8	10.8 ± 0.2	11.1 ± 0.5	2.4 ± 0.1	2.4 ± 0.1
Mycrobein+50% NPK	96.9 ± 0.4	113.2 ± 1.6	10.2 ± 0.3	12.7 ± 0.4	2.4 ± 0.1	2.5 ± 0.1
Without bio-fert.+75% NPK	83.4 ± 1.5	89 ± 0.8	9.8 ± 0.3	10 ± 0.5	2.3 ± 0.1	2.3 ± 0.1
Phosphorin +75% NPK	97.5 ± 1.7	105.3 ± 1.7	11.4 ± 0.5	11.8 ± 0.4	2.4 ± 0.1	2.6 ± 0.1
Rhizobacterien +75% NPK	104 ± 0.8	111.8 ± 2	12.2 ± 0.6	12.6 ± 0.6	3.8 ± 0.2	4 ± 0.2
Mycrobein+75% NPK	112 ± 1.8	119.1 ± 1.6	13.1 ± 0.6	13.4 ± 0.6	4 ± 0.2	4.1 ± 0.2
Without bio-fert.+100% NPK	84.4 ± 2.1	92.5 ± 1.4	9.9 ± 0.5	10.4 ± 0.5	3.1 ± 0.1	3.1 ± 0.1
Phosphorin +100% NPK	93.3 ± 2	101.3 ± 1.8	10.9 ± 0.5	11.4 ± 0.5	3.7 ± 0.2	3.9 ± 0.2
Rhizobacterien +100% NPK	98.2 ± 1.9	103.1 ± 1.8	11.5 ± 0.5	11.6 ± 0.5	4.1 ± 0.2	4.2 ± 0.2
Mycrobein+100% NPK	110.3 ± 1.7	115.4 ± 1.6	12.9 ± 0.6	13 ± 0.6	4.1 ± 0.2	4.2 ± 0.2

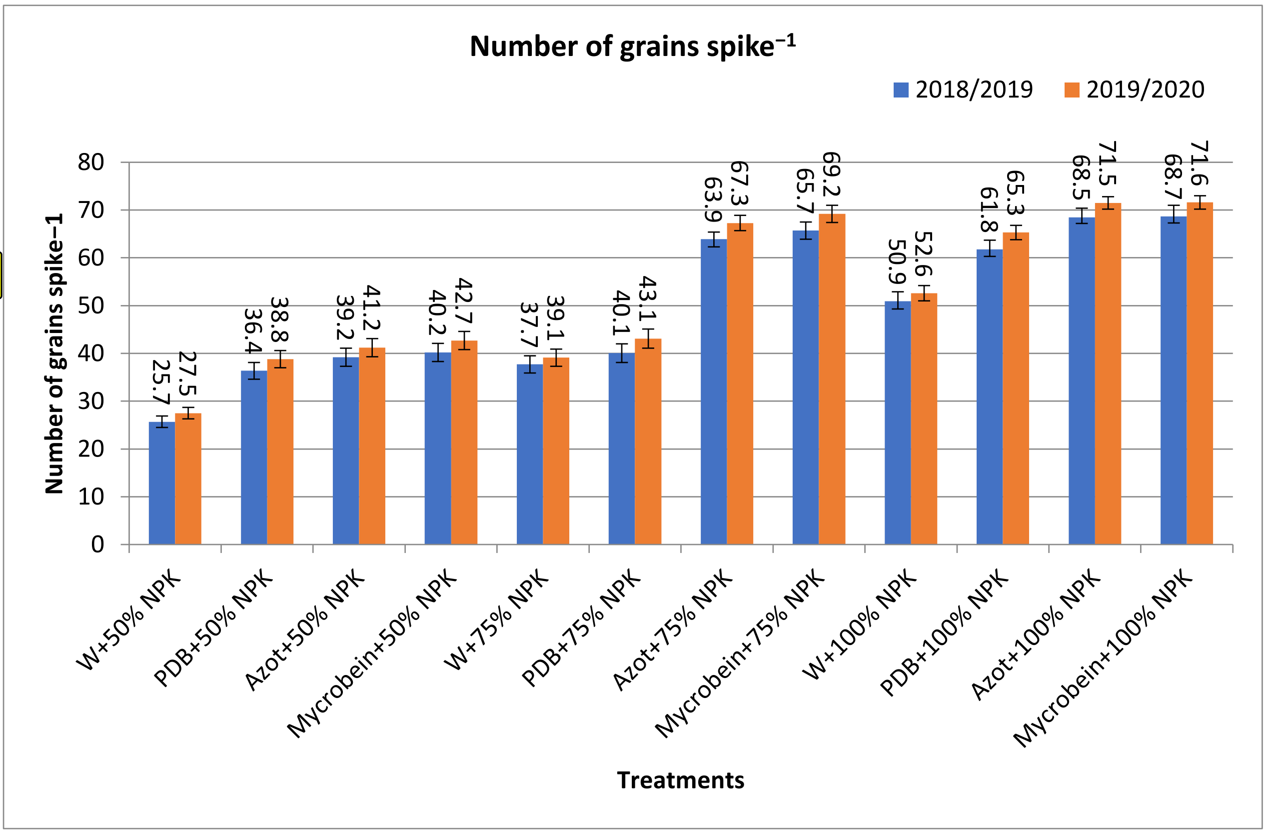
Different letters next to the values point to significant differences at $p \leq 0.05$ according to Duncan's multiple range test.

Rhizobacterien + 100% NPK (T11), Mycrobein + 100% NPK (T12), and Mycrobein + 75% NPK (T8) Rhizobacterien + 75% NPK (T7) achieved the heaviest weight of spikes. The increase in the spike weight compared with recommended dose of mineral fertilizer without bio-fertilizer (T9), was by the percentage of 32.26, 32.26, 29.03, 22.58, and 35.48, 35.48, 32.26, 29.03%, in the first and second year, while the percentage of increase compared with the same dose of mineral fertilizer without bio-fertilizer, was by 32.26, 32.26, 73.91, 65.22, and 35.48, 35.48, 78.26, 73.91 % in the first and second year, respectively (Table 1).

For number of grains spi Mycrobein + 100% NPK (T12), Rhizobacterien +100% NPK (T11), Mycrobein + 75% NPK (T8) and Rhizobacterien + 75% NPK (T7), gave the highest values 68.5, 68.7, 63.9, 65.7, and 71.5, 71.6, 67.3, 69.2, at the first year and second year, respectively, Figure 1. With increase percentage over to the recommended dose of mineral fertilizer without bio-fertilizer (T9); 34.97, 34.58, 76.98, and 72.12, 36.12, 35.93, 29.08, 25.54%, at the first year and second year, respectively. Meanwhile, the increase percentage over the same dose of mineral fertilizer without bio-fertilizer were; 34.97, 34.58, 74.27, 69.50, and 36.12, 35.93, 76.98, 72.12%, in the first year and second year, respectively, Figure 1.

With regard to number of spiks m^{-2}, the treatments Mycrobein +75% NPK (T8), Mycrobein + 100% NPK (T12), Rhizobacterien + 75% NPK (T7), Rhizobacterien + 100% NPK (T11), and Phosphorin + 75% NPK (T6), gave the highest values; 410.4, 395.4, 388.4, 384.5, 376.1, and 509.1, 473.3, 463.7, 444.8, 436, at the first year and second year, respectively, Figure 1. With increase percentage over to the recommended dose of mineral fertilizer without bio-fertilizer (T9); 23.39, 18.88, 16.78, 15.60, 13.08, and 50.00, 39.45, 36.62, 31.05, 28.46%, at the first year and second year, respectively. Meanwhile, the increase percentage compared the same dose of mineral fertilizer without bio-fertilizer were; 19.51, 18.88, 15.60, 13.10, 11.09, and 59.04, 39.45, 31.05, 44.86, 23.57%, in the first year and second year, respectively, Figure 1.

Concerning 1000-grain weight (g), Mycrobein + 100% NPK (T12), Rhizobacterien + 100% NPK (T11), Mycrobein + 75% NPK (T8), Rhizobacterien + 75% NPK (T7), and Phosphorin + 100% NPK (T10), gave the highest values 67.8, 67.3, 64.9, 63.1, 61, and 71.7, 71.6, 69.3, 67.4, 65.4, at the first year and second year, respectively, Figure 1. The percentage increase over the recommended dose (T9) were; 34.79, 33.80, 29.03, 25.45, 21.27, and 36.31, 36.12, 31.75, 28.14, 24.33%, in the first year and second year, respectively. Meanwhile, the increase over the same dose of mineral fertilizer without bio-fertilizer were; 34.79, 33.80, 74.93, 70.08, and 36.31, 36.12, 77.24, 72.38%, in the first year and second year, respectively, Figure 1.



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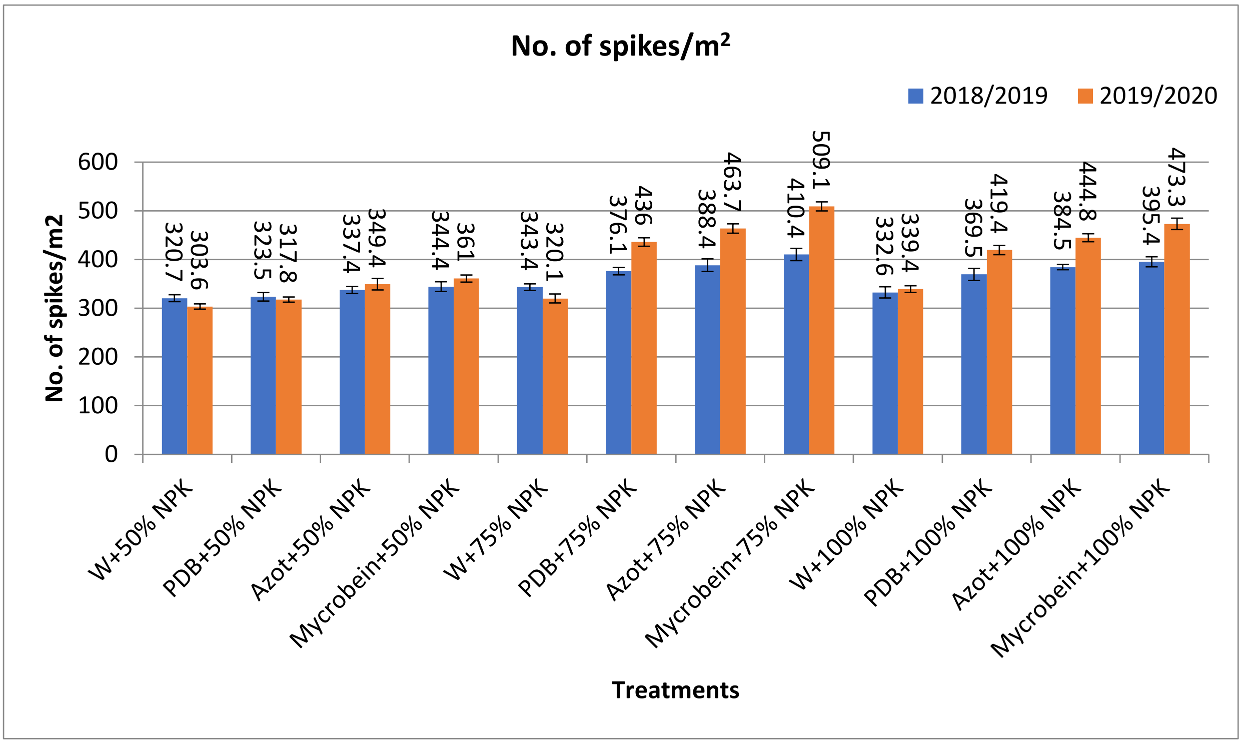

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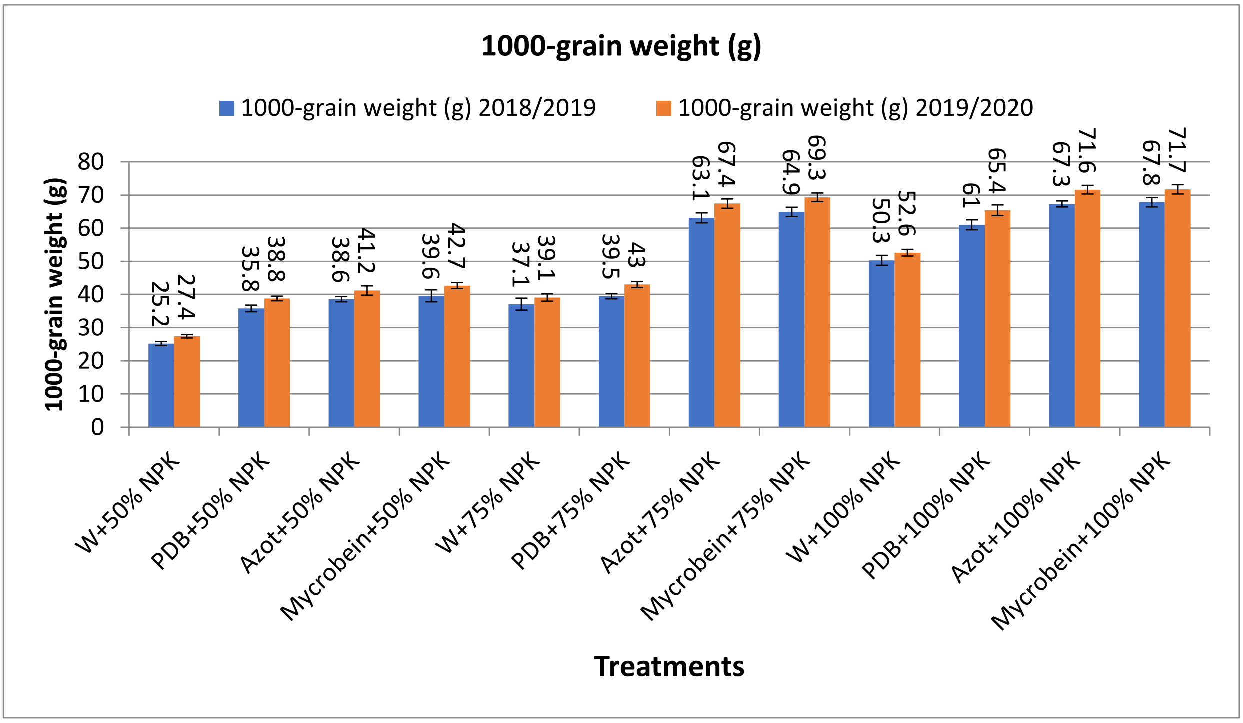
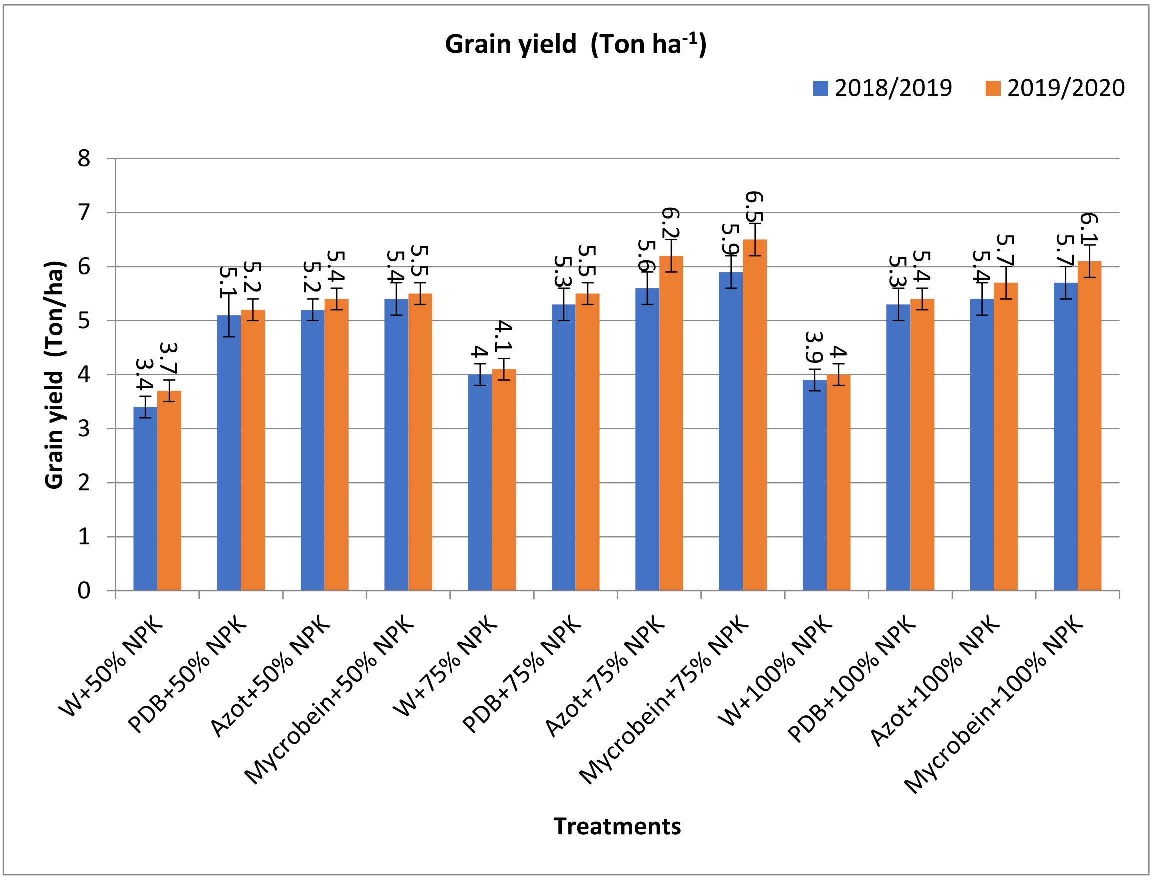
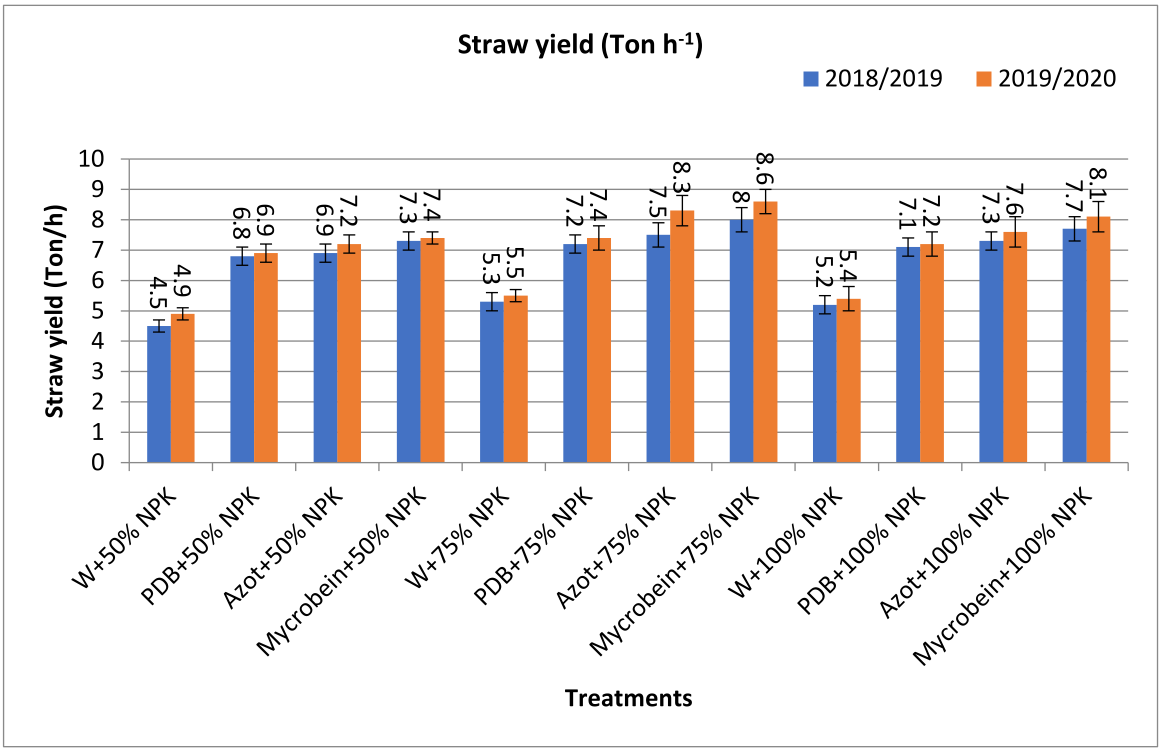


Figure 1. Effect of bio-fertilizers and reduction of the recommended dose of mineral fertilizers (RDP) on number of grains spike^{-1}, no. of spikes/m^2 and 1000-grain weight (g) of barley under saline conditions during 2018/2019 and 2019/2020 seasons. Different letters next to the values point to significant differences at $p \leq 0.05$ according to Duncan's multiple range test.

Regarding grain yield (Ton ha^{-1}), Mycrobein + 75% NPK (T8), Rhizobacterien + 75% NPK (T7), Mycrobein + 100% NPK (T12), gave the highest values 5.9, 5.6, 5.7, and 6.5, 6.2, 6.1 Ton ha-1, at the first year and second year, respectively, followed by Mycrobein + 50% NPK (T4), Rhizobacterien + 100% NPK (T11), Phosphorin + 100% NPK (T10), Phosphorin + 75% NPK (T6), Rhizobacterien + 50% NPK (T3), and Phosphorin + 50% NPK (T2), which gave the values 5.4, 5.4, 5.3, 5.3, 5.2, 5.1, and 5.5, 5.7, 5.4, 5.5, 5.4, 5.2 Ton ha-1, at the first year and second year, respectively, Figure 2. The percentage increase compared with the recommended dose (T9) were; 51.28, 46.15, 43.59, 38.46, 38.46, 35.90, 35.90, 33.33, 30.77, and 62.50, 52.50, 55.00, 37.50, 42.50, 37.50, 35.00, 35.00, 30.00%, in the first year and second year, respectively. Meanwhile, the increase compared with the same dose of mineral fertilizer without bio-fertilizer were; 47.50, 40.00, 46.15, 58.82, 38.46, 35.90, 32.50, 52.94, 50.00, and 58.54, 51.22, 52.50, 48.65, 42.50, 35.00, 34.15, 45.95, 40.54%, in the first year and second year, respectively, Figure 2.





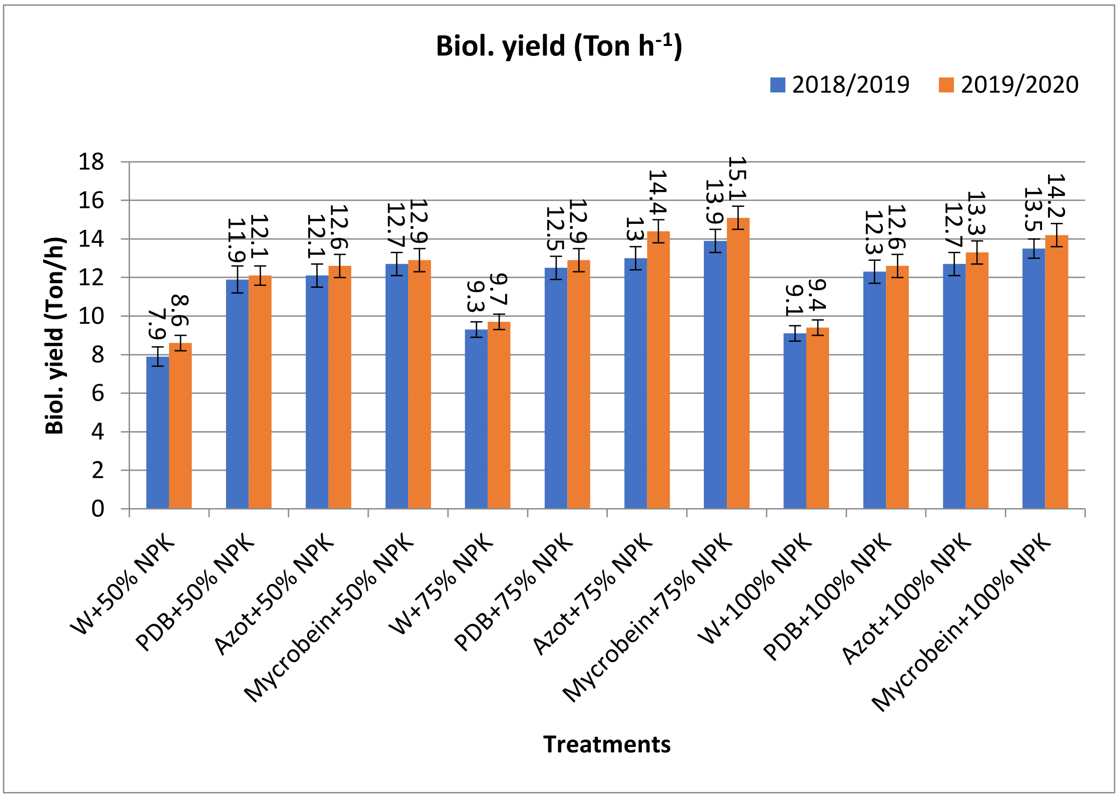


Figure 2. Effect of bio-fertilizers and reduction of the recommended dose of mineral fertilizers (RDP) on grain yield (Ton ha^{-1}), straw yield (Ton ha^{-1}) and biological yield (Ton ha^{-1}) of barley under saline conditions during 2018/2019 and 2019/2020 seasons. Different letters next to the values point to significant differences at $p \leq 0.05$ according to Duncan's multiple range test.

For straw yield (Ton ha^{-1}), Mycrobein + 75% NPK (T8), Mycrobein + 100% NPK (T12), Rhizobacterien + 75% NPK (T7), Mycrobein + 50% NPK (T4), Rhizobacterien + 100% NPK (T11), Phosphorin + 75% NPK (T6), Phosphorin + 100% NPK (T10), Rhizobacterien + 50% NPK (T3), Phosphorin + 50% NPK (T2), and Without bio-fertilizer + 75% NPK (T5), gave the highest values 8, 7.7, 7.5, 7.3, 7.3, 7.2, 7.1, 6.9, 6.8, 5.3, and 8.6, 8.1, 8.3, 7.4, 7.6, 7.4, 7.2, 7.2, 6.9, 5.5 Ton ha-1, at the first year and second year, respectively, Figure 2. The percentage increase compared with the recommended dose (T9) were; 53.85, 48.08, 44.23, 40.38, 40.38, 38.46, 36.54, 32.69, 30.77, and 59.26, 50.00, 53.70, 37.04, 40.74, 37.04, 33.33, 33.33, 27.78%, in the first year and second year, respectively. Meanwhile, the increase over the same dose of mineral fertilizer without bio-fertilizer were; 50.94, 48.08, 41.51, 62.22, 40.38, 35.85, 36.54, 53.33, 51.11, and 56.36, 50.00, 50.91, 51.02, 40.74, 34.55, 33.33, 46.94, 40.82%, in the first year and second year, respectively, Figure 2.

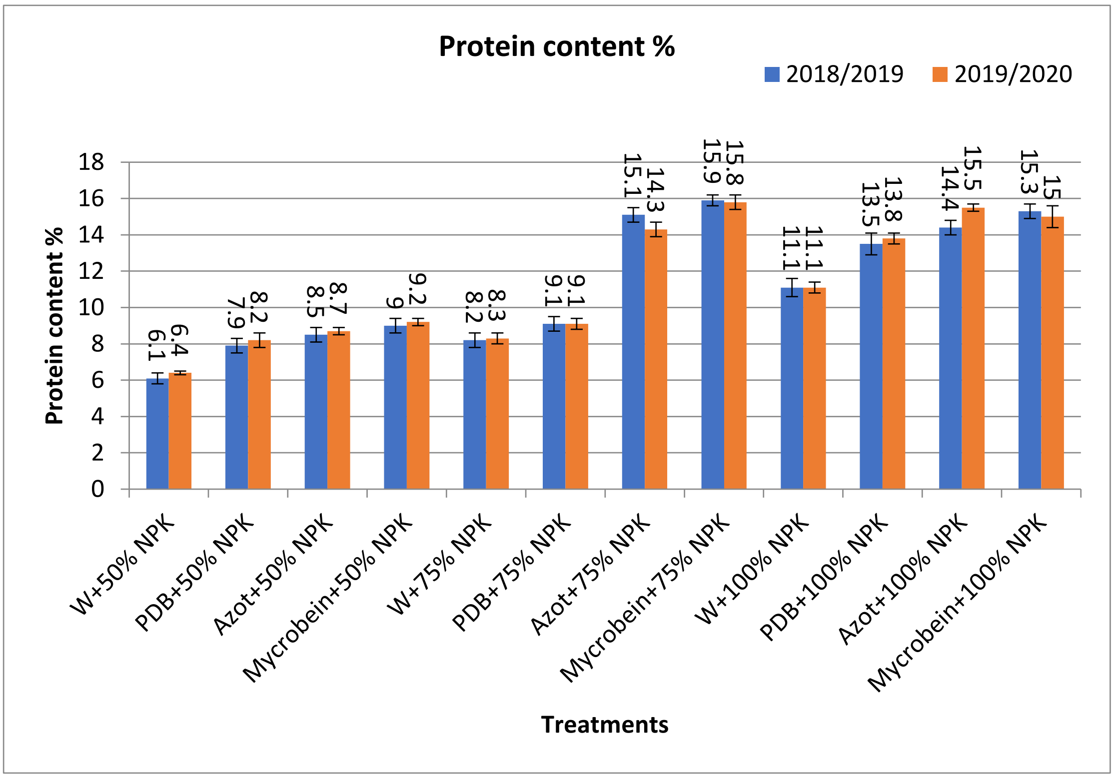
Concerning biological yield (Ton ha^{-1}), Mycrobein + 75% NPK (T8), Mycrobein + 100% NPK (T12), Rhizobacterien + 75% NPK (T7), Mycrobein + 50% NPK (T4), Rhizobacterien + 100% NPK (T11), Phosphorin + 75% NPK (T6), Phosphorin + 100% NPK (T10), Rhizobacterien + 50% NPK (T3), Phosphorin + 50% NPK (T2), and W+75%NPK (T5), gave the highest values 13.9, 13.5, 13, 12.7, 12.7, 12.5, 12.3, 12.1, 11.9, 9.3, and 15.1, 14.2, 14.4, 12.9, 13.3, 12.9, 12.6, 12.6, 12.1, 9.7 Ton ha-1, at the first year and second year, respectively, Figure 2. The percentage increase compared with the recommended dose (T9) were; 52.75, 48.35, 42.86, 39.56, 39.56, 37.36, 35.16, 32.97, 30.77, 2.20, and 60.64, 51.06, 53.19, 37.23, 41.49, 37.23, 34.04, 34.04, 28.72, 3.19%, in the first year and second year, respectively. In the meantime, the increase over the same dose of mineral fertilizer without bio-fertilizer were; 49.46, 48.35, 39.78, 60.76, 39.56, 34.41, 35.16, 53.16, 50.63, 55.67, 51.06, 48.45, 50.00, 41.49, 32.99, 34.04, 46.51, 40.70%, in the first year and second year, respectively, Figure 2.

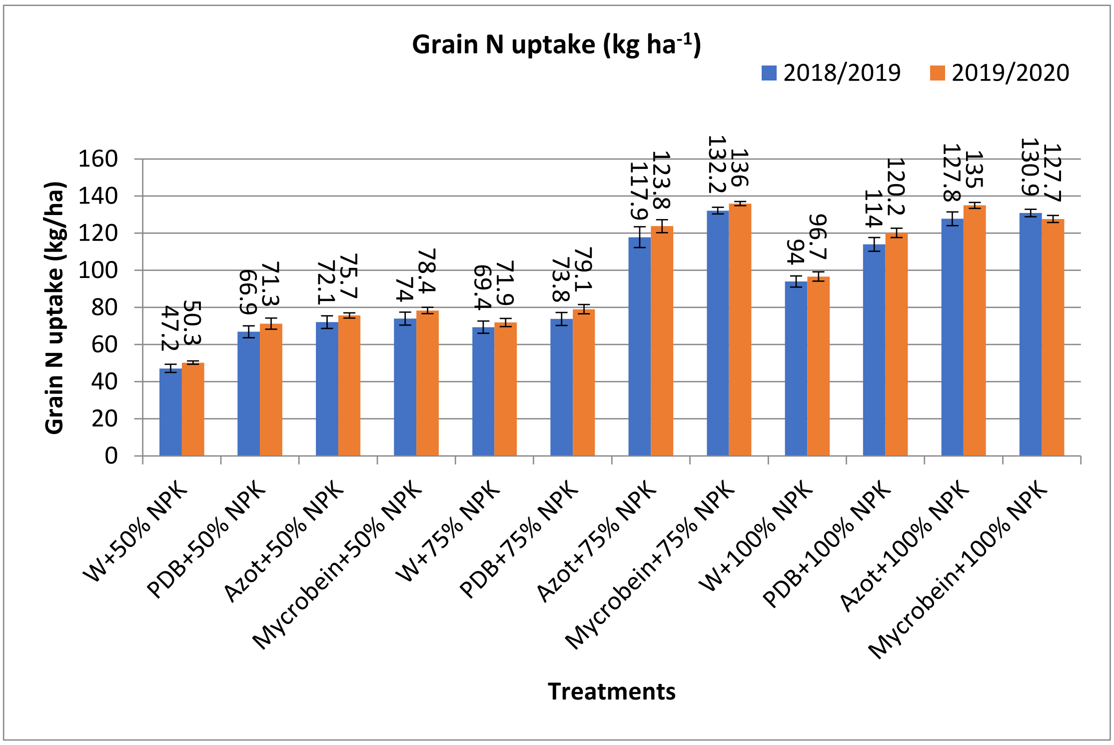
In relation to protein content %, Mycrobein + 100% NPK (T12), Rhizobacterien + 100% NPK (T11), Mycrobein + 75% NPK (T8), Rhizobacterien + 75% NPK (T7), Phosphorin + 100% NPK (T10), gave the highest values 15.5, 15.3, 14.3, 13.9, 13.5, and 15.7, 15.5, 14.7, 14.2, 13.8%, at the first year and second year, respectively, Figure 3. The percentage increase compared with the recommended dose (T9) were; 39.64, 37.84, 28.83, 25.23, 21.62, and 41.44, 39.64, 32.43, 27.93, 24.32%, in the first year and second year, respectively. In the interim, the increase compared with the same dose of mineral fertilizer without bio-fertilizer were; 39.64, 37.84, 74.39, 69.51, 21.62, and 41.44, 39.64, 77.11, 71.08, 24.32%, in the first year and second year, respectively, Figure 3.

Concerning grain N uptake (kg ha^{-1}), Mycrobein + 100% NPK (T12), Rhizobacterien + 100% NPK (T11), Mycrobein + 75% NPK (T8), Rhizobacterien + 75% NPK (T7), Phosphorin + 100% NPK (T10), gave the highest values 131.4, 129.1, 121.2, 117.8, 114, and 136.4, 135, 127.4, 123.8, 120.2 kg ha^{-1}, at the first year and second year, respectively, Figure 3. The percentage increase compared with the recommended dose (T9) were; 40.99, 38.52, 30.04, 26.39, 22.32, and 41.05, 39.61, 31.75, 28.02, 24.30 %, in the first year and second year, respectively. While, the increase over the same dose of mineral fertilizer without bio-fertilizer were; 40.99, 38.52, 74.64, 69.74, 22.32, and 41.05, 39.61, 77.19, 72.18, 24.30%, in the first year and second year, respectively, Figure 3.

For grain K uptake (kg ha^{-1}), Mycrobein + 75% NPK (T8), Mycrobein + 100% NPK (T12), Phosphorin + 75% NPK (T6), Rhizobacterien + 75% NPK (T7), Phosphorin + 100% NPK (T10), Rhizobacterien + 100% NPK (T11), Mycrobein + 50% NPK (T4), and Rhizobacterien + 50% NPK (T3), gave the highest values 41.6, 40, 38.5, 37.7, 37.7, 36.9, 33, 28.3, and 54.2, 53.4, 44, 42.9, 42.1, 41.4, 34.6, 31.6 kg ha^{-1}, at the first year and second year, respectively, Figure 3. The percentage increase compared with the recommended dose (T9) were; 51.27, 45.45, 40.00, 37.09, 37.09, 34.18, 20.00, 2.91, and 89.51, 86.71, 53.85, 50.00, 47.20, 44.76, 20.98, 10.49%, in the first year and second year, respectively. Meanwhile, the increase compare with the same dose of mineral fertilizer without bio-fertilizer were; 60.62, 45.45, 48.65, 45.56, 37.09, 34.18, 55.66, 33.49, and 94.96, 86.71, 58.27, 54.32, 47.20, 44.76, 53.10, 39.82%, in the first year and second year, respectively, Figure 3.

Concerning grain P uptake (kg ha^{-1}), Mycrobein + 75% NPK (T8), Mycrobein + 100% NPK (T12), Phosphorin + 75% NPK (T6), Phosphorin + 100% NPK (T10), Rhizobacterien + 75% NPK (T7), Rhizobacterien + 100% NPK (T11), Mycrobein + 50% NPK (T4), and Rhizobacterien + 50% NPK (T3), gave the highest values 16.1, 15.9, 15.5, 15, 14.6, 14.2, 12.7, 10.9, and 16.5, 16.2, 16, 15.2, 15.1, 14.7, 13.1, 12.2 kg ha^{-1}, at the first year and second year, respectively, Figure 3. The percentage increase compared with the recommended dose (T9) were; 51.89, 50.00, 46.23, 41.51, 37.74, 33.96, 19.81, 2.83, and 50.00, 47.27, 45.45, 38.18, 37.27, 33.64, 19.09, 10.91%, in the first year and second year, respectively. While, the increase over the same dose of mineral fertilizer without bio-fertilizer were; 61.00, 50.00, 55.00, 41.51, 46.00, 33.96, 54.88, 32.93, and 58.65, 47.27, 53.85, 38.18, 45.19, 33.64, 50.57, 40.23%, in the first year and second year, respectively, Figure 3.





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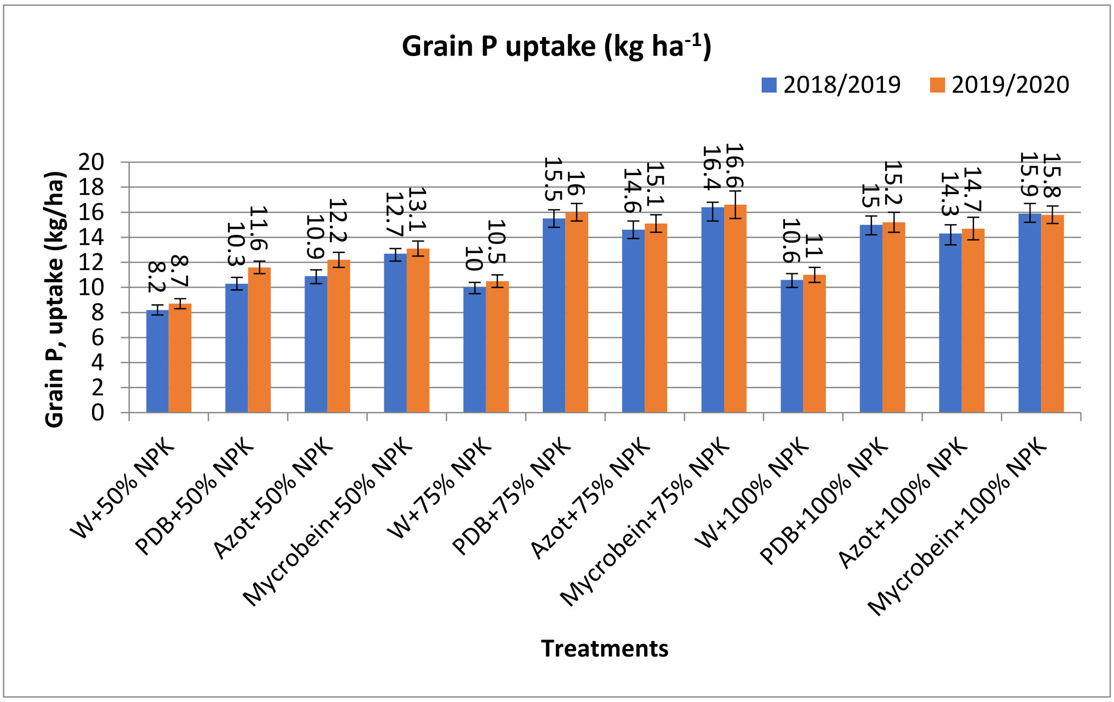

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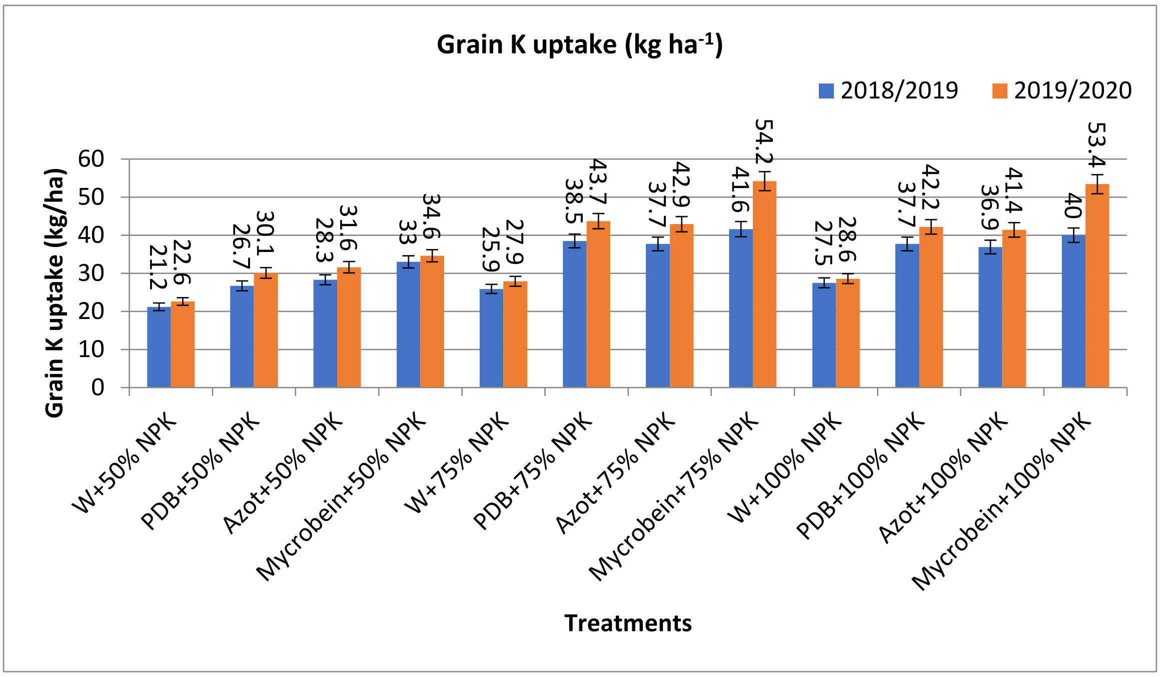


Figure 3. Effect of bio-fertilizers and reduction of the recommended dose of mineral fertilizers (RDP) on protein content %, Grain N, P, and K uptake (kg ha^{-1}) of barley under saline conditions during 2018/2019 and 2019/2020 seasons. Different letters next to the values point to significant differences at $p \leq 0.05$ according to Duncan's multiple range test.

Regarding straw N uptake (kg ha^{-1}), Mycrobein + 100% NPK (T12), Rhizobacterien + 100% NPK (T11), Mycrobein + 75% NPK (T8), Rhizobacterien + 75% NPK (T7), Phosphorin + 100% NPK (T10), gave the highest values 83.9, 82.5, 77.5, 75.4, 72.9, and 88, 87.1, 82.3, 80, 77.7 kg ha^{-1}, at the first and second year, respectively, Table 2. The percentage increase compared with the recommended dose (T9) were; 39.14, 36.82, 28.52, 25.04, 20.90, and 40.13, 38.69, 31.05, 27.39, 23.73%, in the first year and second year, respectively. In the same time, the percentage increase compared with the same dose of mineral fertilizer without bio-fertilizer were; 39.14, 36.82, 72.61, 67.93, 20.90, and 40.13, 38.69, 75.11, 70.21, 23.73 %, in the first year and second year, respectively, Table 2.

Regarding straw K uptake (kg ha^{-1}), Mycrobein + 50% NPK (T4), Mycrobein + 100% NPK (T12), Mycrobein + 75% NPK (T8), Phosphorin + 100% NPK (T10), Rhizobacterien + 100% NPK (T11), and Rhizobacterien + 75% NPK (T7), gave the highest values 31.9, 31.3, 28.7, 28.3, 27.5, 26.8, and 33.2, 32.6, 29.9, 29.5, 28.6, 27.9 kg ha^{-1}, at the first year and second year, respectively, Table 2. The percentage increase compared with the recommended dose (T9) were; 19.92, 39.14, 28.52, 20.90, 36.82, 25.04, and 19.86, 40.13, 31.05, 23.73, 38.69, 27.39%, in the first year and second year, respectively. In the interim, the increase over the same dose of mineral fertilizer without bio-fertilizer were; 58.71, 17.67, 15.26, 6.39, 3.38, 7.63, and 58.85, 17.69, 15.00, 6.50, 3.25, 7.31%, in the first year and second year, respectively, Table 2.

For straw P uptake (kg ha^{-1}), Mycrobein + 75% NPK (T8), Mycrobein + 100% NPK (T12), Phosphorin + 75% NPK (T6), Phosphorin + 100% NPK (T10), Rhizobacterien + 75% NPK (T7), Rhizobacterien + 100% NPK (T11), Mycrobein + 50% NPK (T4), Phosphorin + 50% NPK (T2), and Rhizobacterien + 50% NPK (T3), gave the highest values 17.1, 16.8, 16.7, 16, 15.6, 15.3, 13.4, 12.1, 11.2, and 17.4, 16.8, 16.8, 15.8, 15.8, 15.1, 13.7, 12.6, 12.1 kg ha^{-1}, at the first year and second year, respectively, Table 2. The percentage increase compared with the recommended dose (T9) were; 52.68, 50.00, 49.11, 42.86, 39.29, 36.61, 19.64, 8.04, 0.00, and 50.00, 44.83, 44.83, 36.21, 36.21, 30.17, 18.10, 8.62, 4.31%, in the first year and second year, respectively. In the meantime, the increase compared with the same dose of mineral fertilizer without bio-fertilizer were; 64.42, 50.00, 60.58, 42.86, 50.00, 36.61, 55.81, 40.70, 30.23, and 58.18, 44.83, 52.73, 36.21, 43.64, 30.17, 53.93, 41.57, 35.96%, in the first year and second year, respectively, Table 2.

Table 2. The effect of treatment with bio-fertilizers and reducing mineral fertilizers by percentages of the recommended dose of mineral fertilizers (RDP) on nitrogen, phosphorus, and potassium uptake of straw of Giza 123 barley cultivar in the 2018/2019 and 2019/2020 seasons.

Treatments	N/Straw		K/Straw		P/Straw	
	2018/2019	2019/2020	2018/2019	2019/2020	2018/2019	2019/2020
W+50% NPK	30.9 ± 1.5	33.3 ± 1	20.1 ± 1	20.9 ± 1	8.6 ± 0.4	8.9 ± 0.4
Phosphorin +50% NPK	43.3 ± 2.1	46.6 ± 0.8	25.6 ± 1.2	26.6 ± 1.2	12.2 ± 0.6	12.6 ± 0.6
Rhizobacterien +50% NPK	46.6 ± 2.2	49.4 ± 0.6	25.6 ± 1.2	26.8 ± 1.2	11.2 ± 0.5	12.1 ± 0.6
Mycrobein +50% NPK	47.1 ± 2.2	50.6 ± 0.8	31.9 ± 1.5	33.2 ± 1.5	13.4 ± 0.6	13.7 ± 0.6
W+75% NPK	44.9 ± 2.1	47 ± 1.2	24.9 ± 1.2	26 ± 1.2	10.5 ± 0.5	11 ± 0.5
Phosphorin +75% NPK	47.7 ± 2.3	51.6 ± 1.6	25.8 ± 1.2	26.9 ± 1.3	16.7 ± 0.8	16.8 ± 0.8
Rhizobacterien +75% NPK	75.3 ± 3.5	80 ± 2.9	26.8 ± 1.3	27.9 ± 1.3	15.6 ± 0.7	15.8 ± 0.7
Mycrobein +75% NPK	85.7 ± 2.9	86.9 ± 2.8	32.1 ± 1.6	32.8 ± 1.5	17.1 ± 0.8	17.4 ± 0.8
W+100% NPK	60.3 ± 2.9	62.8 ± 2.9	26.6 ± 1.3	27.7 ± 1.3	11.2 ± 0.5	11.6 ± 0.5
Phosphorin +100% NPK	72.9 ± 3.5	77.7 ± 3.6	28.3 ± 1.4	29.5 ± 1.4	16 ± 0.8	15.8 ± 0.7
Rhizobacterien +100% NPK	82.5 ± 3.9	85.5 ± 3.2	27.5 ± 1.3	28.6 ± 1.3	15.3 ± 0.7	15.1 ± 0.7
Mycrobein +100% NPK	83.9 ± 4	85.6 ± 2.9	31.3 ± 1.5	31.3 ± 0.9	16.8 ± 0.8	16.8 ± 0.8

Different letters next to the values point to significant differences at p ≤ 0.05 according to Duncan's multiple range test.

Regarding available nitrogen (mg/kg soil), application of bio-fertilizer combined with rates of mineral fertilizer significantly improved the availability of nitrogen in soil, the treatments; Mycrobein + 75% NPK (T8), Mycrobein + 100% NPK (T12), Phosphorin + 75% NPK (T6), Phosphorin + 100% NPK (T10), Rhizobacterien + 75% NPK (T7), Rhizobacterien + 100% NPK (T11), Mycrobein + 50% NPK (T4), Phosphorin + 50% NPK (T2), and Rhizobacterien + 50% NPK (T3), gave the highest values 27.3, 26.7, 24.1, 24.1, 21.3, 20.4, 18.4, 17.3, 16.3, and 28.8, 26.9, 25.6, 24.3, 21.6, 20.9, 19.4, 17.5, 16.7 mg/kg soil, at the first year and second year, respectively, Figure 4. The percentage increase compared with the recommended dose (T9) were; 71.70, 67.92, 51.57, 51.57, 33.96, 28.30, 15.72, 8.81, 2.52, and 72.46, 61.08, 53.29, 45.51, 29.34, 25.15, 16.17, 4.79, 0.00%, in the first year and second year, respectively. Meanwhile, the increase compared with the same dose of mineral fertilizer without bio-fertilizer were; 76.13, 67.92, 55.48, 51.57, 37.42, 28.30, 84.00, 73.00, 63.00, and 78.88, 61.08, 59.01, 45.51, 34.16, 25.15, 81.31, 63.55, 56.07 %, in the first year and second year, respectively, Figure 4.

For available phosphorus (mg/kg soil), application of treatments of bio-fertilizer combined with rates of mineral fertilizer significantly improved the availability of phosphorus in soil, the treatments Mycrobein + 75% NPK (T8), Mycrobein + 100% NPK (T12), Rhizobacterien + 75% NPK (T7), Phosphorin + 75% NPK (T6), Rhizobacterien + 100% NPK (T11), Phosphorin + 100% NPK (T10), Mycrobein + 50% NPK (T4), and Phosphorin + 50% NPK (T2), gave the highest values 10.3, 10.1, 9.7, 9.6, 9.1, 8.9, 7.9, 7, and 11, 10.7, 9.3, 10.2, 9.4, 9.4, 8.4, 7.4 mg/kg soil, at the first year and second year, respectively, Figure 4. The percentage increase compared with the recommended dose (T9) were; 53.73, 50.75, 44.78, 43.28, 35.82, 32.84, 17.91, 4.48, and 52.78, 48.61, 29.17, 41.67, 30.56, 30.56, 16.67, 2.78%, in the first year and second year, respectively. In the same direction, the increase over the same dose of mineral fertilizer without bio-fertilizer were; 58.46, 50.75, 49.23, 47.69, 35.82, 32.84, 51.92, 34.62, and 59.42, 48.61, 34.78, 47.83, 30.56, 30.56, 52.73, 34.55%, in the first year and second year, respectively, Figure 4.

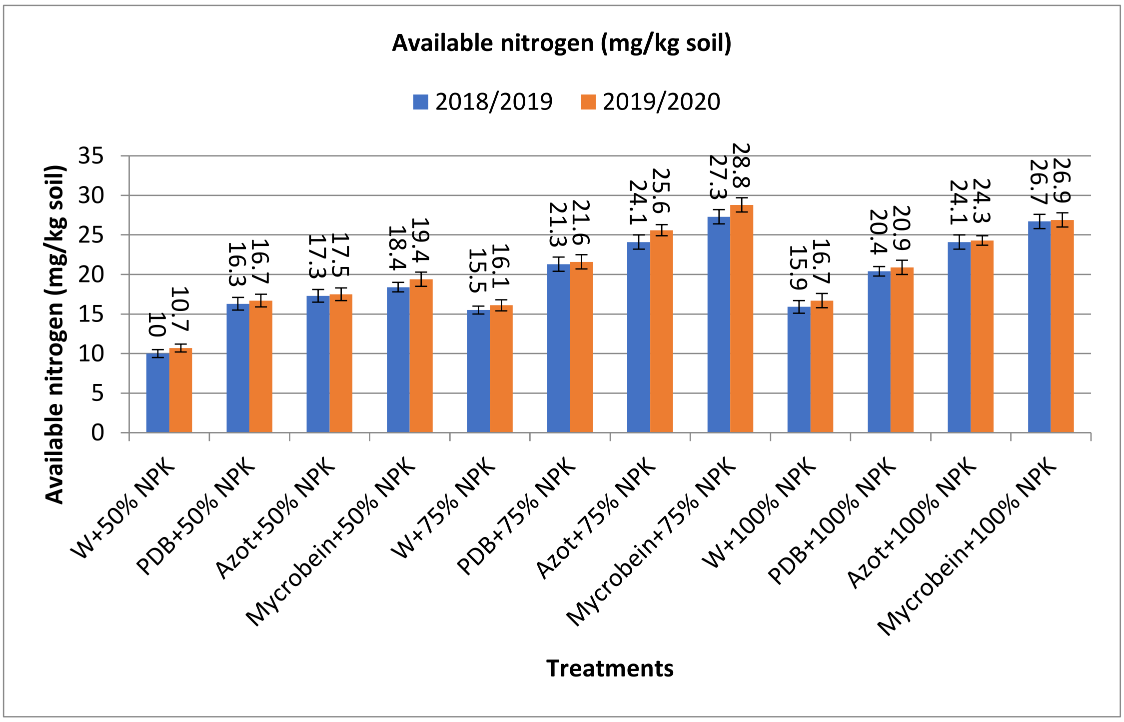
Concerning total microbial count (×10^5 cfu /g dry soil) was greatly affected by the application of biofertilizer, the treatments; Mycrobein + 75% NPK (T8), Rhizobacterien + 75% NPK (T7), Mycrobein +100% NPK (T12), Rhizobacterien + 100% NPK (T11), Phosphorin + 75% NPK (T6), Phosphorin + 100% NPK (T10), Mycrobein + 50% NPK (T4), Rhizobacterien + 50% NPK (T3), and Phosphorin + 50% NPK (T2), gave the highest values 201.8, 194.7, 175.9, 158.6, 124.9, 117.6, 90.6, 78.1, 61.4, and 207.3, 197.1, 180.7, 160.6, 126.6, 118.4, 93.9, 80.7, 63.3, 10^5 cfu /g dry soil, at the first year and second year, respectively, Figure 4. The percentage increase compared with the recommended dose (T9) were; 361.78, 345.54, 302.52, 262.93, 185.81, 169.11, 107.32, 78.72, 40.50, and 394.75, 370.41, 331.26, 283.29, 202.15, 182.58, 124.11, 92.60, 51.07%, in the first year and second year, respectively. Meanwhile, the increase compared with the same dose of mineral fertilizer without bio-fertilizer were; 350.45, 334.60, 302.52, 262.93, 178.79, 169.11, 135.32, 102.86, 59.48, and 341.06, 319.36, 331.26, 283.29, 169.36, 182.58, 135.93, 102.76, 59.05%, in the first year and second year, respectively, Figure 4.

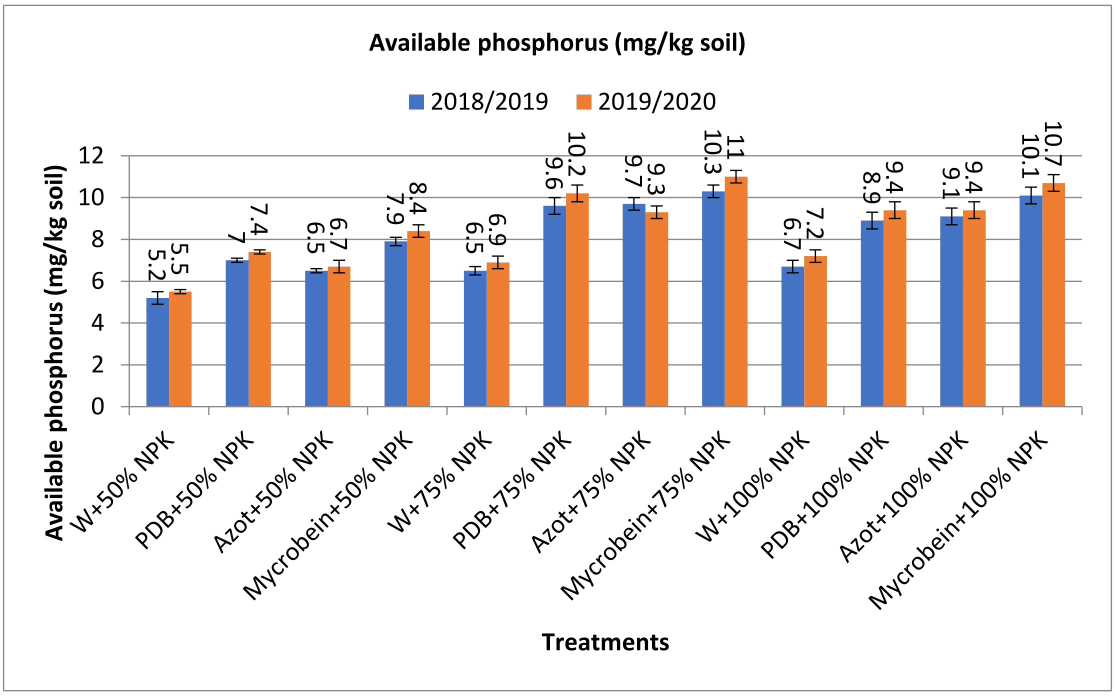
Regarding antioxidant activity of soil ug ascorbic acid /g dry soil (AOA), Mycrobein + 75% NPK (T8), Rhizobacterien + 75% NPK (T7), Mycrobein + 100% NPK (T12), Rhizobacterien + 100% NPK (T11), Phosphorin + 75% NPK (T6), Phosphorin + 100% NPK (T10), Mycrobein + 50% NPK (T4), Rhizobacterien + 50% NPK (T3), and Phosphorin + 50% NPK (T2), have the maximum impact on increasing the antioxidant activity of soil in both seasons with values 4.4, 4.2, 4, 3.6, 2.7, 2.5, 2.3, 2.2, 1.9, and 4.6, 4.4, 4.3, 4.1, 2.8, 2.6, 2.4, 2.3, 2.2 ug ascorbic acid /g dry soil, in the first year and second year, respectively, Figure 4. The percentage increase compared with the recommended dose (T9) were; 144.44, 133.33, 122.22, 100.00, 50.00, 38.89, 27.78, 22.22, 5.56, and 142.11, 131.58, 126.32, 115.79, 47.37, 36.84, 26.32, 21.05, 15.79%, in the first year and second year, respectively. Meanwhile, the increase compared with the same dose of mineral fertilizer without bio-fertilizer were; 193.33, 180.00, 122.22, 100.00, 80.00, 38.89, 91.67, 83.33, 58.33, and 187.50, 175.00, 126.32, 115.79, 75.00, 36.84, 84.62, 76.92, 69.23%, in the first year and second year, respectively, Figure 2.

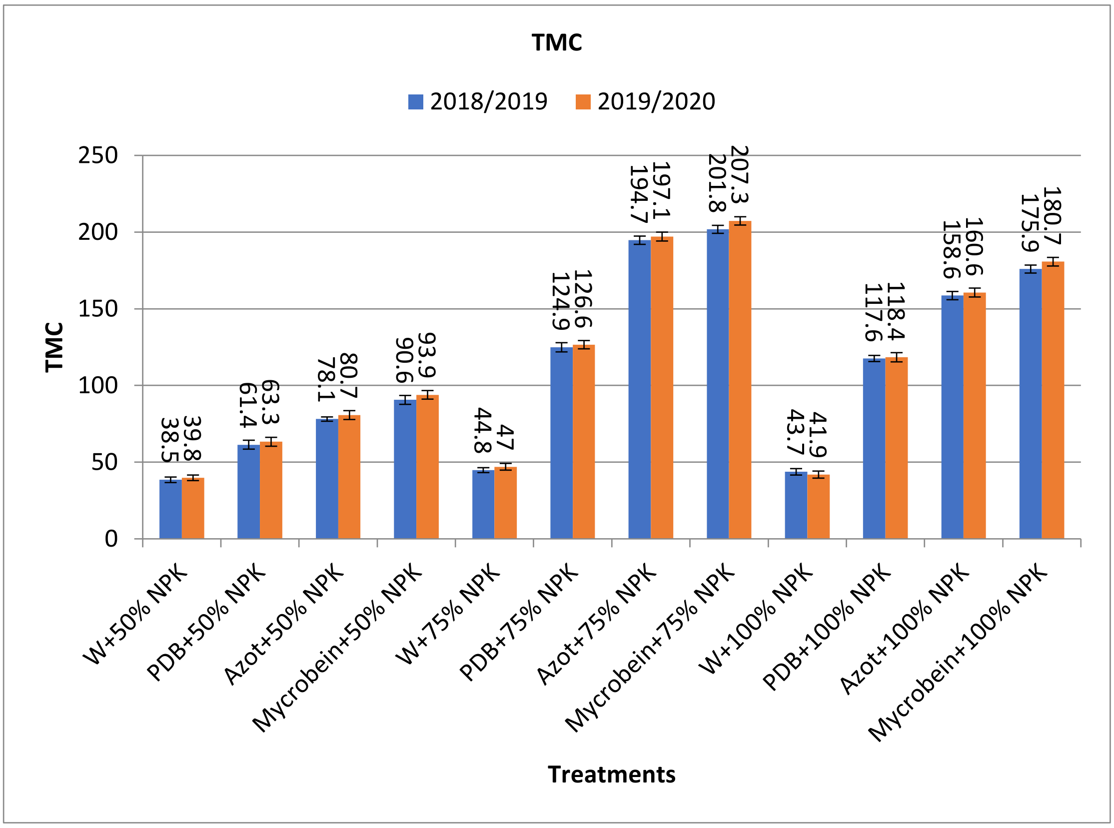
Concerning dehydrogenase (enzyme µg TPF/g dry soil/24h), results indicated that the treatments; Rhizobacterien + 75% NPK (T7), Mycrobein + 75% NPK (T8), Rhizobacterien + 100% NPK (T11), Mycrobein + 100% NPK (T12), Rhizobacterien + 50% NPK (T3), Phosphorin + 75% NPK (T6), Mycrobein + 50% NPK (T4), Phosphorin + 100% NPK (T10), and Phosphorin + 50% NPK (T2), obtained the highest values 29.5, 27.3, 26.7, 23.8, 22.5, 20.7, 20.4, 19.6, 16.3, and 30.3, 28, 27.4, 24.4, 23.1, 21.2, 20.9, 20.1, 16.7 µg TPF/g dry soil/24h, at the first year and second year, respectively, Figure 4. The percentage increase compared with the recommended dose (T9) were; 383.61, 347.54, 337.70, 290.16, 268.85, 239.34, 234.43, 221.31, 167.21, and 380.95, 344.44, 334.92, 287.30, 266.67, 236.51, 231.75, 219.05, 165.08%, in the first year and second year, respectively. While, the increase compared with the same dose of mineral fertilizer without bio-fertilizer were; 383.61, 347.54, 337.70, 290.16, 341.18, 239.34, 300.00, 221.31, 219.61, and 380.95, 344.44, 334.92, 287.30, 344.23, 236.51, 301.92, 219.05, 221.15%, in the first year and second year, respectively, Figure 4.

Regarding nitrogen fixers (MPN/gm dry soil) 10^3, application of treatments of; Mycrobein + 75% NPK (T8), Rhizobacterien + 75% NPK (T7), Mycrobein +100% NPK (T12), Phosphorin + 100% NPK (T10), Rhizobacterien +100% NPK (T11), Phosphorin + 75% NPK (T6), Mycrobein + 50% NPK (T4), Rhizobacterien + 50% NPK (T3), and Phosphorin + 50% NPK (T2), showed the highest values 177.7, 176.7, 143, 129.7, 126.6, 123.6, 73.5, 70.5, 61.3, and 177.7, 177.7, 145, 130.7, 128.7, 125.6, 75.6, 71.5, 63.3 (MPN/gm dry soil) 10^3, at the first year and second year, respectively, Figure 4. The percentage increase compared with the recommended dose (T9) were; 412.10, 409.22, 312.10, 273.78, 264.84, 256.20, 111.82, 103.17, 76.66, and 382.88, 382.88, 294.02, 255.16, 249.73, 241.30, 105.43, 94.29, 72.01%, in the first year and second year, respectively. In the same time, the increase over the same dose of mineral fertilizer without bio-fertilizer were; 427.30, 424.33, 312.10, 273.78, 264.84, 266.77, 148.31, 138.18, 107.09, and 382.88, 382.88, 294.02, 255.16, 249.73, 241.30, 138.49, 125.55, 99.68%, in the first year and second year, respectively, Figure 4.

The phosphate solubilizing bacteria (PSB) count (CFU/gm of dry soil) was significantly affected by the use of biofertilizers as application of treatments; Phosphorin + 100% NPK (T10), Phosphorin + 75% NPK (T6), Mycrobein + 75% NPK (T8), Rhizobacterien + 75% NPK (T7), Mycrobein + 100% NPK (T12), Rhizobacterien + 100% NPK (T11), Phosphorin + 50% NPK (T2), Mycrobein + 50% NPK (T4), and Rhizobacterien + 50% NPK (T3), to obtain the highest values 49, 45.9, 44.9, 38.8, 36.8, 28.6, 22.5, 18.4, 15.3, and 50, 48, 47, 39.8, 36.8, 29.6, 23.5, 20.4, 17.4 (CFU/gm dry soil), at the first year and second year, respectively, Figure 4. The percentage increase compared with the recommended dose (T9) were; 497.56, 459.76, 447.56, 373.17, 348.78, 248.78, 174.39, 124.39, 86.59, and 443.48, 421.74, 410.87, 332.61, 300.00, 221.74, 155.43, 121.74, 89.13%, in the first year and second year, respectively. Meanwhile, the increase compared with the same dose of mineral fertilizer without bio-fertilizer were; 497.56, 546.48, 532.39, 446.48, 348.78, 248.78, 268.85, 201.64, 150.82, and 443.48, 485.37, 473.17, 385.37, 300.00, 221.74, 230.99, 187.32, 145.07 %, in the first year and second year, respectively, Figure 4.




Available phosphorus (mg/kg soil)
2018/2019
2019/2020
Available phosphorus (mg/kg soil)
0
2
4
6
8
10
12
5.2
5.5
7
7.4
6.5
6.7
7.9
8.4
6.5
6.9
9.6
10.2
9.7
9.3
10.3
11
6.7
7.2
8.9
9.4
9.1
9.4
10.1
10.7
W+50% NPK
PDB+50% NPK
Azot+50% NPK
Mycrobein+50% NPK
W+75% NPK
PDB+75% NPK
Azot+75% NPK
Mycrobein+75% NPK
W+100% NPK
PDB+100% NPK
Azot+100% NPK
Mycrobein+100% NPK
Treatments


TMC
2018/2019
2019/2020
TMC
0
50
100
150
200
250
38.5
39.8
61.4
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47
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197.1
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117.6
118.4
158.6
160.6
175.9
180.7
W+50% NPK
PDB+50% NPK
Azot+50% NPK
Mycrobein+50% NPK
W+75% NPK
PDB+75% NPK
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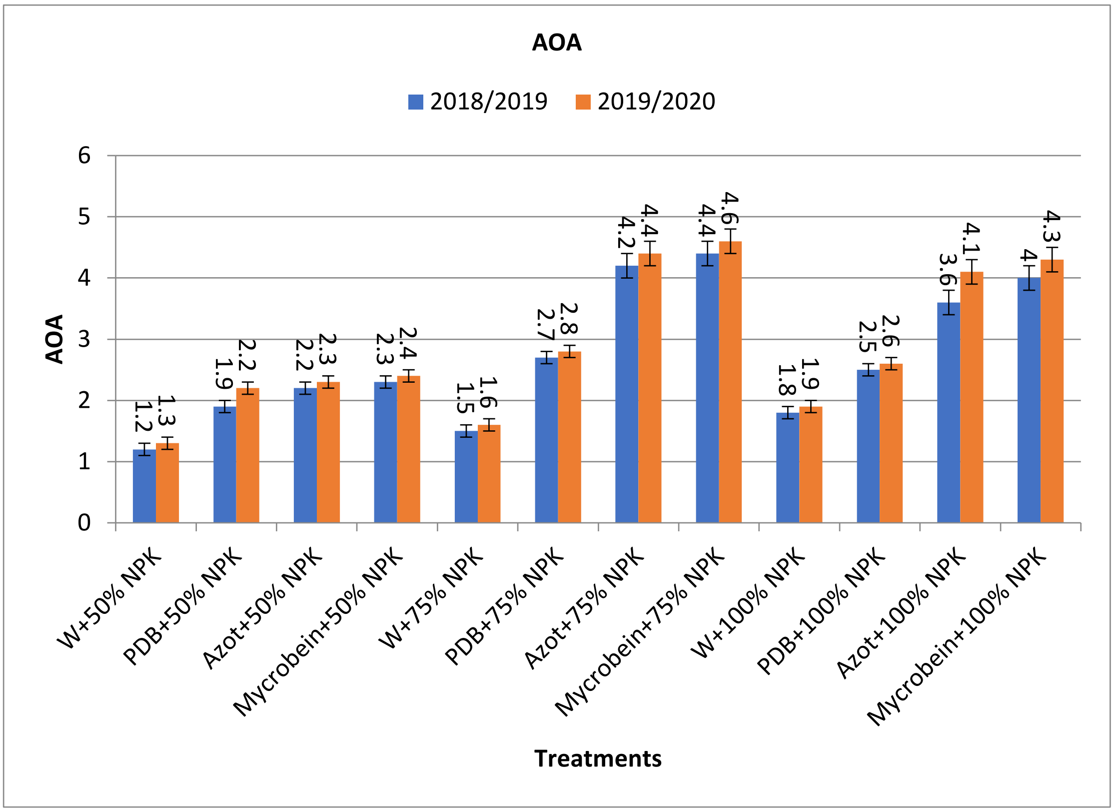
Treatments



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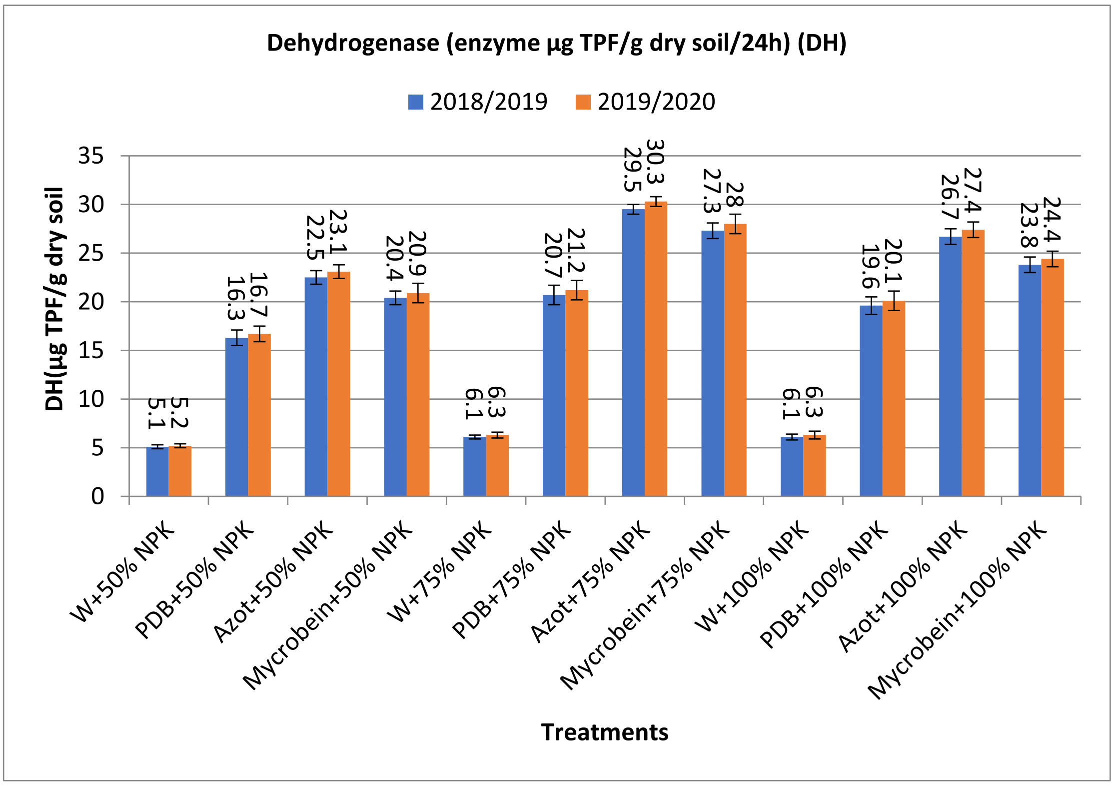

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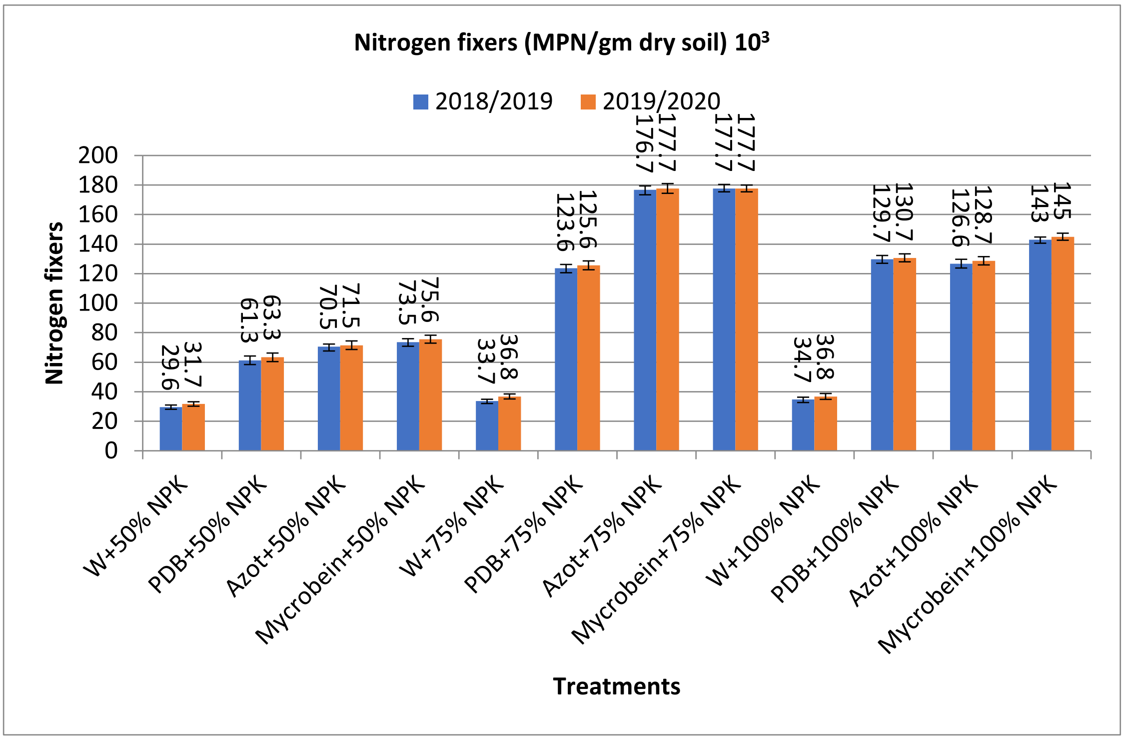

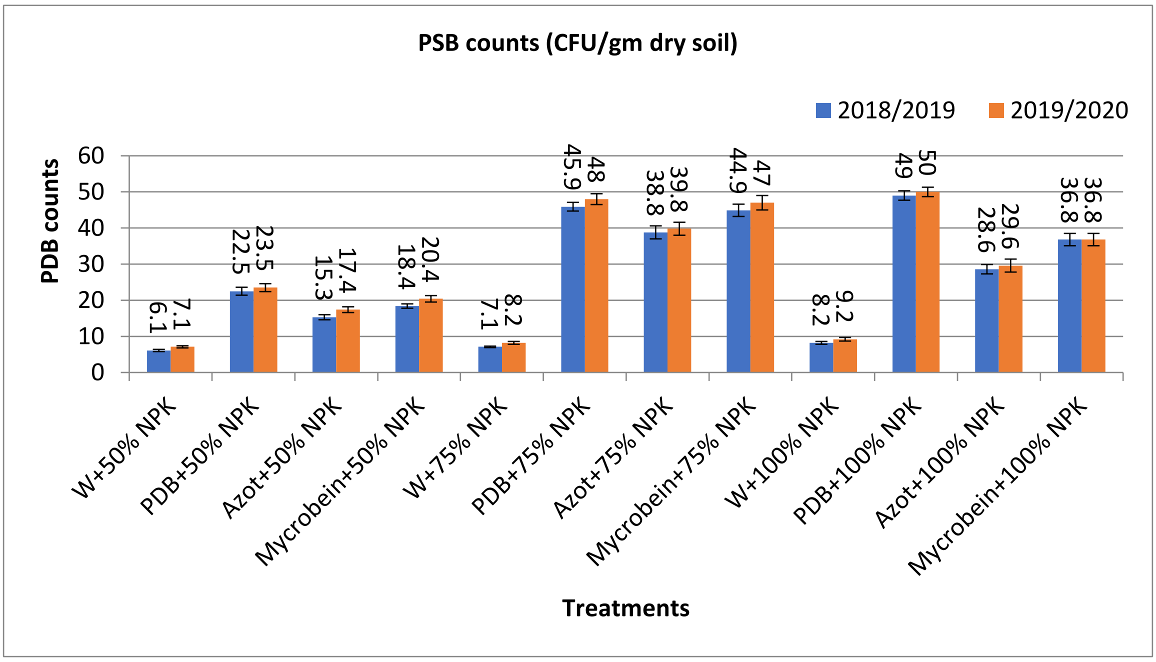

Figure 4. Effect of bio-fertilizers and reduction of the recommended dose of mineral fertilizers (RDP) on available nitrogen (mg/kg soil), available phosphorus (mg/kg soil), total microbial count $\times 10^5$ cfu /g dry soil, antioxidant activity of soil ug ascorbic acid /g dry soil (AOA), dehydrogenase (enzyme µg TPF/g dry soil/24h), nitrogen fixers (MPN/gm dry soil) 10^3 and phosphate solubilizing bacteria (PDB) counts (CFU/gm dry soil) of barley under saline conditions during 2018/2019 and 2019/2020 seasons. Different letters next to the values point to significant differences at $p \leq 0.05$ according to Duncan's multiple range test.

4. Discussion

Barley is considered a type of crop that tolerates salt, but barley productivity is affected by salinity stress and osmotic pressures. Salt stress leads to a decrease in the germination rate and all growth parameters in barley. Treatment and inoculation with PGP bacteria led to enhancing and increasing plant growth under salinity conditions through the production of growth-promoting nutrients and growth regulators [82].

The results in Table 1, 2, and Figure 1, 2, 3, 4 showed that the application of combination of bio-fertilizers with levels of mineral fertilizers led to a significant increase in productivity and yield contributed traits of barley plants growing under saline stress.

The results of the current study showed that the most of studied traits showed the highest values when applying the treatments Mycrobein + 75% NPK (T8), Rhizobacterien + 75% NPK (T7), Mycrobein + 100% NPK (T12), especially grain yield (Ton ha^{-1}) and some related traits, or it may be assigned T12 higher than T8 with a little and insignificant difference. This indicates the importance of applying bio-fertilizer in reducing NPK mineral fertilizers doses by 25% without affecting the yield and other characteristics or their decrease (Table 1, 2, and Figure 1, 2, 3, 4). The inoculation by bio-fertilizer significantly improved agronomic traits and yield components than the recommended dose of mineral fertilizer without bio-fertilizer (T9 : without bio-fertilizer + 100% NPK) treatment, and compared with the same dose of mineral fertilizer without bio-fertilizer. Under saline conditions, bio-fertilizers promoted plant growth by facilitating enhanced nutrients and produce growth-promoting substances and growth regulators [37,83,84].

The reason for the increase in yield and its components in treatments received with bio-fertilizers is mainly due to the beneficial effect of applying bio-fertilizers to the soil, as this improved the biological, chemical and physical properties of the soil. All of these effects resulted in an increase in the availability and release of more nutrients available to the roots plants [85-87].

In the past and during the past years, the focus was on the use of chemical fertilization in agricultural production, but with the emergence of problems of environmental pollution and concern for environmental safety, the search began for alternative methods, the most important of which, of course, is the use of microorganisms in the soil for bio-fertilization. Studies have continued around the world on bio-fertilization, where seeds or soil can be used to inoculate with PGPR, in which microorganisms can multiply their numbers and participate in nutrient cycling by increasing phosphate solubilization, nitrogen fixation, and secretion of plant hormones (gibberellins, indole acetic acid, cytokinin, and ethylene), which are necessary for growth and adaptation in a stressful environment, which is reflected in increasing the growth and productivity of plants and various crops for sustainable agriculture [61,88-91]. To increase productivity, plant growth and sustainable food production, inoculation is carried out with PGPR, which involves the use of a wide range of soil bacteria. As live, symbiotic rhizobacteria, they exert their function by colonizing the rhizosphere extracellularly and/or intracellular in search of a source of carbon [29,88,92].

Microorganisms in bio-fertilizers provide benefits to crop plants in several ways and mechanisms. It can be effective in fixing nitrogen and dissolving soluble nutrients that plants need, such as phosphate, zinc, and potassium, in addition to secreting plant growth-promoting substances, including various hormones, all of which lead to enhanced plant growth, or it can have a combination of all these qualities [37,88,93-95].

Bio-fertilizer are distinguished from their chemical counterparts in that they have several advantages [96,97]. They are important for maintaining the health of the soil, its fertility and its biological content, as they are considered sound sources of renewable nutrients necessary for plant growth, in addition to being environmentally friendly and harmless to it [90,98]. Moreover, microorganisms in Bio-fertilizer show resistance and antigenic activity against many plant pathogens and combat abiotic stresses [99,100]. Many microbial strains and genera have been used commercially as effective bio-fertilizers, based on their ability to stimulate the solubilization and uptake of nutrients in soil, fix nitrogen from the atmosphere, and act as bio-control agents [88,101,102].

The results showed that bio-fertilizers have the ability to enhance plant growth, due to their ability to solubilize phosphate and produce organic acids, exopolysaccharides, and IAA. Therefore, microorganisms with PGP activities can lessen biotic and abiotic stresses, thus being useful in agricultural fields [103-105]. PGP microbes improve stress tolerance in plants through various mechanisms; such as the production of antioxidants that can detoxify reactive oxygen species, which leads to improved growth standards, the production of growth regulators, and most importantly, increased nutrient content through nitrogen fixation and dissolution of elements. All of these mechanisms lead to improving plant health under salt stress [82,106,107].

Effect of Biofertilizers and Mineral Fertilizer Levels on NPK uptake and protein Contents:

The results in (Figure 3 and Table 2) showed that the effects of applying the three bio-fertilizers containing N2-fixing and P- solubilizing PGPR strains led to a significant increase in the grain and straw contents of nitrogen, phosphorus, potassium, as well as protein in the barley plants under study. The highest values of N, P, K and protein % contents were obtained from Mycrobein bio-fertilizer inoculation (mixed strains) combined with 75% of NPK level, which increased N contents of grain by 40.64 and 40.64%, P content by 54.72 and 50.91%, K content by 51.27 and 89.51% and protein content by 43.24 and 42.34% compared with the recommended dose (control) T9 (without bio-fertilizer + 100% NPK) in the two seasons, respectively, Table 6. In the same time, The highest values of N, P, and K, uptake were obtained from Mycrobein bio-fertilizer + 75 % NPK (T8), which increased N contents of straw by 42.12 and 38.38%, P content by 52.68 and 50.00%, and K content by 20.68 and 18.41% compared with the same dose of mineral fertilizer without bio-fertilizer in the two seasons, respectively.

A significant increase was recorded in the levels of nitrogen, phosphorus, and potassium when applying bio-fertilized to barley, corn and wheat plants compared with the recommended dose (control). A significant increase was also recorded and observed in the percentage of water- and salt-soluble protein fractions (albumin and globulin) when applying bio-fertilization [108-110].

Presence of minerals available in the Rhizosphere of barley plants under saline conditions

One of the negative effects of high salinity in natural conditions is that it greatly affects microbial communities in the soil and prevents the mineralization of organic matter in the soil. This leads to sharp changes in the process of turnover of organic substances [12,15,59], thus reducing the release of plant nutrients. Here comes the importance of applying bio-fertilizers are added to the soil under conditions of salinity stress to decrease the damaging effects of salinity and increase the numbers of beneficial microbes in the soil. The data in Figure 4, revealed that application of Mycrobein + 75% NPK (T8) contained bio-fertilizer combined with 75% mineral fertilizer significantly improved the availability of nitrogen and phosphorus in soil under current study (Figure 4). Application of All treatments containing bio-fertilizers gave values of available nitrogen and phosphorus much higher than the control treatment (T9), which is the recommended rate of mineral fertilizers without adding bio-fertilizers. Application of Mycrobein + 75% NPK (T8) gave the highest values of available nitrogen and phosphorus with increased percentage by 71.70, 72.46 for available nitrogen and by 53.73 and 52.78% for available phosphorus over the control at the studied season, respectively, Figure 4. On the hand, the application of bio-fertilizer gave values of available nitrogen and phosphorus much higher than the same dose of mineral fertilizer without bio-fertilizer, the increased percentage for T8 were; 76.13 and 78.88% for available nitrogen, and 58.46 and 59.42% for available phosphorus over the same dose of mineral fertilizer without bio-fertilizer at the studied season, respectively, Figure 4 .

Bio-fertilizers containing PGPR bacteria play an necessary role in providing and cycling nutrients in the soil through several processes, including that they participate in processes such as nitrogen fixation, nitrification, oxidation, ammonification, and other processes that lead to the decomposition of organic matter in the soil and the release of vital inorganic plant nutrients in the soil [111-113]. Kurokura et al. [114], shown that the secretion of organic acids by PGPR contributes significantly to increasing the solubility of phosphorus and converting it from insoluble to soluble forms [115-117].

The availability of essential nutrients in the soil is very important for plants, as they mainly depend on them for their development and growth, which serves as a main reservoir through which plants obtain the nutrients necessary for their growth and development. Though, the majority of nutrients in soil are in insoluble and un-absorbable forms, making the availability of these nutrients restricted for plants. Whereas mineral fertilization is considered an effective and quick method, it is not currently recommended due to its harmful effects on the ecosystem and soil [118,119]. On the other side, over time, bio-fertilizer has proven to be an effective means of providing the necessary nutrients to plants and maintaining their the sustainability of the agricultural system and the ecosystem in general. Bio-fertilizers consist of a variety of beneficial microorganisms that are able to decompose essential nutrients from insoluble compounds, making them available to plants. As a result of successive studies on this, there are a large number of microorganisms, including bacteria, fungi, and actinomycetes, that have properties that enhance the dissolution of metal ions through various mechanisms, such as changing the pH of the soil or direct heavy metal removal from metal cations [118-120].

One of the useful indicators for knowing the biological condition and quality of the soil is to rely on the antioxidant system to determine this [121]. Increased antioxidant activity in the soil protects the plant from oxidative stress resulting from high salinity [11]. Many researchers have also confirmed that there is a close correlation between the total antioxidant capacity of soil and the number of microbes in different soil types. In addition, enzymatic and non-enzymatic antioxidants play an vital function in development of plant and growth, especially in protection mechanisms. Protracted exposure to ecological stress led to oxidative stress, causing a pathological condition [122]. Several studies reported that peroxidases and phenolic compounds contributed to protecting the plant from oxidative stress [123,124]. High salinity affects the soil in many ways, including its effect on microbial communities and their growth in the soil. It also prevents the mineralization of organic matter in the soil, this results in severe changes in the rate of organic matter turnover, thus reducing the release of plant nutrients [84,125]. The results of the current study showed that the application of bio-fertilizer farms mixed with mineral fertilizer levels led to a significant enhancement in the availability of nitrogen and phosphorus in the soils examined (Figure 4).

Biological activities in the Rhizosphere of barley plants under salinity-affected soil conditions

The numbers of microorganisms, or what is known as microbial density, in the soil are affected by the presence of mineral fertilizers levels, mainly NPK, as well as the levels of applied bio-fertilizers, as shown in Figure 4. Microbial density increases with the addition of mineral fertilizers as well as bio-fertilizers, but it increases to a certain limit and is not a continuous increase. The high dose of mineral fertilizers used in agricultural systems may lead to restricting the growth of microbes and their activities in the soil [126,127]. In the same direction, with regard to the effect of bio-fertilizers, the results showed an increase in microbial density when adding bio-fertilizers in the presence of the same rate of mineral fertilizer, with a relative decrease in the greater rate of 100% NPK. The results indicated that the total number of microbes was higher when 75%

NPK/ha were applied than that obtained at 50% NPK/ha or 100% NPK/ha. Several studies have revealed that microbial community structure, microbial activity, and soil microbial biomass are decreased by high salinity [128-130]. However, the use of halo tolerant bacteria with PGP properties enhanced soil microbial biomass through osmolyte production [131-133].

The antioxidant system is a very useful indicator of soil quality and the biological condition of the soil [134-136]. The dehydrogenase enzyme increases with the addition of bio-fertilizers with the increase in the addition of mineral fertilizers, but it increases to a certain extent and not a continuous increase. In the same direction and with regard to the effect of bio-fertilizers, the results showed an increase in the dehydrogenase enzyme when adding bio-fertilizers in the presence of the same rate of mineral fertilizers, with a relative decrease in the rate greater than 100%. The results indicated that the dehydrogenase enzyme was higher when using 75% NPK/ha compared to that obtained at 50% NPK/ha or 100% NPK/ha, (Figure 4). Increased antioxidant activity in the soil protects the plant from oxidative stress resulting from high salinity [137,138]. Enzymatic and non-enzymatic antioxidants play an essential function in plant development and growth, especially in protection mechanisms [139-141]. Growth of plants under environmental stresses leads to oxidative stress, causing a pathological condition. Numerous studies have confirmed the existence of a strong relationship between the number of microbes and the total antioxidant capacity in soil and in different soil types [142,143].

5. Conclusions

Bio-fertilizers were applied to barley with three compounds. The first compound contains a various of phosphate solubilizing bacteria, the second compound contains a variety of bacteria that fixed nitrogen, and the third compound contains many species from both groups. This led to a significant improvement in all the characteristics under study, including productivity and grain yield. The results showed that the application of bio-fertilizers with the addition of 75% of the recommended dose of the mineral fertilizer was a higher production quantity than that obtained from applying 100% of the mineral fertilizer with the recommended dose of chemical fertilizers. This reduces the cost of mineral fertilizers used in barley production under saline stress and provides the nutritional requirements of the plant, which lead to an increase in the growth and productivity of the barley plant. The application of bio-fertilizers also increased uptake and content of nutrients in straw, grains and increased the number of microorganisms in the soil, which led to an increase in the enzyme dehydrogenase and antioxidants and increased availability of nutrients.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: title; Table S1: title; Video S1: title.

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