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The Effects of Bio-Fertilizer by Arbuscular Mycorrhizal Fungi and Phosphate Solubilizing Bacteria on the Growth and Productivity of Barley under Deficit of Water Irrigation Conditions

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Abstract: Bio-fertilizers are the most important and effective method used to reduce the quantities of chemical fertilizers consumed and reduce dependence on them in agricultural production to avoid their harmful effects on the environment and public health as well as reduce the cost of agricultural production in light of increasing pollution and under adverse conditions for production and climate change. A bio-fertilizer depends primarily on the use of beneficial microorganisms such as arbuscular mycorrhizal fungi (AMF) to improve the uptake of nutrients, improve plant growth, productivity, and grain yield. Crop production faces many challenges, and drought is one of the majority of the significant factors limiting crop production worldwide, especially in semi-arid regions. The objective of this study was to evaluate the effects of AMF and phosphate solubilizing bacteria (PSB), plus three rates of the recommended dose of phosphorus (RDP) fertilizer on yield, yield components, and nutrients uptake, in addition to evaluating the beneficial effects of these combinations to develop Phosphorus (P) management under three levels of irrigation water, i.e., three irrigations (normal or well-watered), two irrigations (moderate drought), and one irrigation (severe drought) on barley (*Hordeum vulgare* L.). The results showed that the treatment with AMF bio-fertilizer yielded the highest values of plant height, spike length, spike weight, number of grains/spike, 1000-grain weight, grain yield, straw yield, biological yield, and harvest index. Moreover, the grain and straw uptake of nitrogen (N), P, and potassium (K) (kg ha^{-1}) in the two seasons under the three levels of irrigation, respectively, were superior followed by the inoculation by PSB. While the treatment without bio-fertilizer yielded the lowest values of these traits of barley, the treatment with bio-fertilizer yielded the increased percentage of the grain yield by 17.27%, 17.33% with applying AMF, and 10.31%, 10.40% with treatment by PSB. Treatment with AMF or PSB (Phosphorien), plus rates of phosphorus fertilizer under conditions of irrigation water shortage, whether irrigation was performed once or twice, led to an increase in grain yield and other characteristics compared to the same fertilization rates without inoculation. The results of this study showed that the use of bio-fertilizers led to an increase in plant tolerance to drought stress, and this was demonstrated by an increase in various traits with the use of treatments that include bio-fertilizers. Therefore, it is suggested to inoculate the seeds with AMF or PSB plus adding phosphate fertilizers at the recommended dose under drought conditions.

Keywords: barley (*Hordeum vulgare* L.); arbuscular mycorrhizal fungus; drought; grain yield; N, P and K uptake

1. Introduction

Barley (*Hordeum vulgare* L.) is one of the main and vital grain crops in the world. It grows in different environments and in many regions of the world ranging from the deserts of the Middle East to the high altitudes of the Himalayas [1–4]. Barley is considered as the main food source in many countries of North Africa [1,3,4]. It is also considered as a moderate stress-tolerant plant and is the main cereal crop in many arid regions of the world and is extremely important to the livelihoods of several farmers in these regions [1,4].

Grains are the most important products and the highest in economic value from the barley plant, which is used for food and fodder. Straw is often used as food for animals, and it may sometimes be used as an ingredient in compost. Grains are also used in manufacturing. Barley ranks fourth in grain production in the world after wheat, rice, and maize and is a major grain crop in many developing countries, but barley plants are often exposed to drought stress at different stages of growth [5–9].

Water is the main factor and the most important factor that determines the pattern and method of using agricultural land. The degree of productivity depends on the availability of water and its suitability for agriculture [10–12]. The biggest major global environmental problem in the 21st century is that of water scarcity [13,14]. Agriculture consumes about 80–90% of freshwater resources used by humans globally, and most of this water is used for crop production [15–18].

The major constraint of barley production worldwide is the lack of water or the insufficient availability of water. The production of barley in most areas of its cultivation is related to the degree of drought, and this is reflected in the amount of crop and the productivity of barley. This situation becomes an urgent field for research. Barley is also widely grown in semi-arid regions of the world as a rain-fed crop, but rain is often erratic in rainfall amounts and causes large variations and frequency of its events, which in turn affects barley yield productivity [16,19]. Like some regions in the world, Egypt suffers from water stress because of the limited renewable sources of fresh water, and precipitation falls mainly on a narrow strip of coastal areas [14,20]. Barley is grown in Egypt mostly in the northern coastal district and parts of the Nile Delta region; it is also cultivated in newly reclaimed lands due to its tolerance to drought and salinity that are widespread in these areas [21–23]. In order to produce a good and high yield of barley, the recommended number of irrigations must be applied at the stages of barley growth, whether vegetative or reproductive, in a timely and correct manner. Biological fertilizers play an essential role in reducing the effect of drought on barley productivity [24,25]. Therefore, yield is used as a benchmark for development under water shortage (drought) stress and as an indicator of the ability of bio-fertilizers to conserve water under drought stress conditions [16,26–28].

One of the most important advantages of bio-fertilizers is that they have the ability to improve the growth and productivity of the plant and its vital processes. Their use also leads to a decrease in the quantities of inorganic chemical fertilizers applied. Most bio-fertilizers consist of microorganisms that participate in the decomposition of organic matter and convert minerals into a soluble form that becomes soft and available to plants. The application of bio-fertilizers promotes fixation of nutrients in the root zone, recycling of nutrients, biodegradable organic matter, production of growth for plants, improvement of soil fertility, provision of biological control, and promotion of mycorrhiza symbiosis [4,16,28,29]. In natural N, P, and K cycles, microorganisms play an essential role, where the use of phosphate and potassium solubilizing microorganisms contributes in improving and increasing the absorption of plant nutrients [30]. Therefore, microorganisms are considered an important and effective tool in improving the absorption of nutrients and thus improving and increasing plant growth [16,28,31].

Among the most important microorganisms used in bio-fertilizers are arbuscular mycorrhizal fungi, AMF, which have the ability to form a symbiotic relationship with plants. This symbiotic relationship benefits both the fungus and the plant through the acquisition and absorption of nutrients, especially phosphorous, from the soil [4,16,32].

Other soil microorganisms are associated with AM fungi as free nitrogen fixers. In addition, the role of AMF and microorganisms is to convert organic compounds and unavailable minerals into forms available to plants. Moreover, they also increase root biomass, root length, and root system improvement resulting in higher plant growth and productivity [16,33,34]. With this case in mind, the use of arbuscular mycorrhiza (AMF) as a bio-fertilizer has been described as a possible and effective implement for food security and sustainable agriculture [35]. AMF is symbiotically associated with the roots of more than 80% of plant species. Barley like other cereal crops is associated with this ubiquitous fungus, which can lead to increased availability of nutrients and enhanced plant uptake of water and soil nutrients [35]. Increasing studies demonstrate the importance of inoculation with AMF and its ability to mitigate the harmful effects of abiotic stresses, including drought [36,37]. In this direction, Thaloonth et al. [38] mentioned that the use of phosphate solubilizing bacteria as a bio-fertilizer (Phosphorene) led to a significant improvement in the yield and yield components of barley crop compared to the control under water stress conditions. Numerous other studies have also shown that the use of arbuscular mycorrhizal fungi in semi-arid regions improved the uptake of phosphorus, nitrogen, potassium, zinc, copper, calcium, magnesium, iron, and manganese, which led to an increase in yield and its components of barley [4,39,40].

The main objective of the current study is to assess the influence of inoculation with AMF and PSB, in addition to three levels of phosphorus fertilizer under conditions of normal irrigation and water stress, and the extent of benefit of using arbuscular mycorrhizal fungi and phosphate solubilizing bacteria on plant growth, yield, and its components of barley (*Hordeum vulgare* L.) to enhance its production under normal irrigation and drought conditions in Egypt.

2. Materials and Methods

2.1. Experimental Design

After harvesting rice as a summer crop, a field experiment was carried out during the barley growing seasons of 2018/2019 and 2019/2020, at private Farm, Sakha, Kafr El-Sheikh Governorate, Egypt (latitude 31°06' N, longitude 30°56' E). To study the effect of combinations between phosphorus fertilizer rates and AMF as well as PSB on yield, yield components and nutrients uptake of barley (*Hordeum vulgare*, L. c.v. Giza-123) under three levels of irrigation water, i.e., three irrigations (normal), two irrigations (moderate drought), and one irrigation (drought) were recorded. Regarding the average monthly climate data for the site during the barley growing seasons of 2018/2019 and 2019/2020, they are tabulated in Table S1. The soil characteristics of the study site, which were clay soil (0–30 cm from the surface) according to US Soil Taxonomy (Soil Survey Staff 1999), ref. [41] were tabulated in Table S2.

In both seasons, on 1 December, barley grains were planted at a rate of 119 kg per hectare, and rice was the preceding crop in both seasons. The technical recommendations recommended for barley fields were followed in the implementation of all other agricultural practices.

Fertilization was carried out with an amount of phosphorus of 53.57 kg P₂O₅ ha⁻¹, and 357.14 kg of calcium superphosphate (15.5% P₂O₅) as a recommended dose in Egyptian agriculture.

Arbuscular mycorrhizal fungus spores containing a mixture of *Glomus* sp. and *Gigaspora* sp. from the root zone using the method described by Gerdemann and Nicholson [42] and then identified according to the Schenck and Perez [43] were distributed. Arbuscular mycorrhizal spores and their carriers were distributed in the soil to a depth of 5 cm immediately before planting. They are a dry powder and insoluble in water. The application rate of the AMF inoculum was 7 g/m⁻² (3550 spores m⁻²). Bio-fertilizer, known commercially as Phosphorien, was also used at a rate of 1.4 kg per 143 kg grains/ha, which is phosphate-dissolving bacteria (*Bacillus megaterium* var. *phosphaticum*). After coating the barley grains

with an adhesive material (gum arabic 5%), they were inoculated with Phosphorien, then planted and watered immediately after inoculation.

The dimensions of the experimental unit were (5×3 m), with an area of 15 m^2 and consisting of 15 rows, each row spaced 0.20 m. Nitrogen fertilizer was added in three equal doses and the total rate was $142.86 \text{ kg N ha}^{-1}$ added in the form of ammonium nitrate (33.5% N). After 21 days after sowing (DAS), the first dose from nitrogen fertilizer was added, the second was added at 35 DAS, and the third was added at 50 DAS. As for potassium applied fertilizer, it was added in one dose with the first dose of nitrogen fertilizer at a rate of $57.14 \text{ kg K}_2\text{O ha}^{-1}$ in the form of potassium sulfate (48% K_2O).

2.2. Studied Traits and Recorded Data

Immediately before harvest at 120 days after sowing, ten plants were randomly selected from each experimental plot to evaluate plant height (cm), spike length (cm), spike weight (g), grain number/spike, and 1000-grain weight (g). All plants in each experimental plot were then harvested and subsequently separated into straw and grain to determine straw yield and grain yield Mg ha^{-1} (ton ha^{-1}). Grain and straw samples were then taken from all experimental units and then dried at a temperature of $65 \text{ }^\circ\text{C}$ until the weight was constant and then pounded. The micro-Kjeldahl method was used to estimate total N in straw and grains according to Association of Official Analytical Chemists (AOAC) [44]. While the chlorostannous reduced molybdophosphoric blue color method was used to estimate the phosphorus content (P%) colorimetrically, as described by Chapman and Parker [45], flame photometry, according to Page et al. [46], was used to determine the potassium content (K%) in the digested plant material. By multiplying the grain or straw yield by its N%, P%, and K% content, the N, P, and K uptake (kg ha^{-1}) were calculated, respectively.

2.3. Statistical Analysis

Analysis of variance (ANOVA) for a randomized complete block design was used to analyze the data statistically as mentioned by Casella [47], using Costat version 6.303 [48]. Treatment means were compared using Duncan's multiple range test at a probability level of 0.05 according to Duncan [49].

3. Results

The bio-fertilizers by inoculation of AMF and PSB showed highest significant effects on agronomic traits, yield components, yield, grain N, P, and K uptake and straw N, P, and K uptake of barley and decreased negative impacts of drought on the grain yield and other studied traits as shown in Tables 1–3 and Figures 1–5.

3.1. The Effects of Bio-Fertilizers, Drought, and Phosphorus Fertilizers on Yield, Yield Components

The results in Tables 1 and 2 illustrated the mean effects of drought and normal irrigation conditions, bio-fertilizers by inoculation of AMF and PSB, phosphorus fertilizers of yield, yield components and harvest index of barley and interaction between these factors, whereas the mean effects of irrigation, bio-fertilizers, and phosphorus fertilizers were highly significant for all studied traits except the main effects for bio-fertilizers of biological yield in the first season (non-significant) and the second season (significant). Moreover, the interaction between irrigation conditions and bio-fertilizers by inoculation of AMF and PSB were non-significant for any studied traits.

Meanwhile, the interactions between irrigation conditions and phosphorus fertilizers were not significant for studied traits, see Tables 1 and 2.

Regarding the interaction between bio-fertilizers and phosphorus fertilizers which were significant and highly significant for the studied traits except spike length, number of grains plant^{-1} (second season), and straw yield (ton ha^{-1}), see Tables 1 and 2.

Table 1. Results of analysis of variance for bio-fertilization by inoculation of arbuscular mycorrhizal fungi (AMF) and phosphate-solubilizing bacteria (PSB) on yield, yield components and nutrients uptake in grain and straw of barley under drought and normal irrigation conditions and different percentages of the recommended dose of phosphorus fertilizers (RDP).

Source	PH (cm)		Sp L (cm)		Sp W (g)		G/Sp		1000-GW (g)		GY (ton ha ⁻¹)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Bio-fertilizer												
AMF	109.37 a	113.95 a	9.68 a	10.98 a	3.55 a	3.70 a	59.49 a	61.97 a	54.71 a	56.98 a	4.55 a	4.74 a
Phos.	105.24 b	109.58 b	9.01 b	10.38 b	3.16 b	3.29 b	55.91 b	58.25 b	53.91 b	56.13 a	4.28 b	4.46 b
WBF	99.87 c	103.96 c	8.19 c	9.53 c	3.00 c	3.13 c	51.40 c	53.51 c	51.86 c	53.98 b	3.88 c	4.04 c
Phosphorus (RDP) fertilizers												
100% SP	108.14 a	112.61 a	9.42 a	10.83 a	3.48 a	3.62 a	59.06 a	61.51 a	55.21 a	57.49 a	4.59 a	4.78 a
66% SP	106.14 b	110.51 b	9.11 b	10.38 b	3.31 b	3.45 b	56.91 b	59.29 b	53.84 b	56.06 b	4.33 b	4.52 b
0% SP	100.20 c	104.36 c	8.34 c	9.67 c	2.92 c	3.04 c	50.83 c	52.93 c	51.43 c	53.55 c	3.78 c	3.94 c
Irrigation												
Normal	111.01 a	115.59 a	9.88 a	11.35 a	3.59 a	3.74 a	60.58 a	63.12 a	58.31 a	60.71 a	4.94 a	5.15 a
2 Irrigations	106.89 b	111.30 b	8.93 b	10.26 b	3.21 b	3.35 b	55.79 b	58.09 b	53.67 b	55.88 b	4.08 b	4.25 b
1 Irrigation	96.58 c	100.60 c	8.07 c	9.27 c	2.91 c	3.03 c	50.43 c	52.51 c	48.51 c	50.51 c	3.69 c	3.84 c
Bio-F.	**	**	*	*	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
RDP	**	**	**	**	**	**	**	**	**	**	**	**
Irrigation	**	**	**	**	**	**	**	**	**	**	**	**
Bio-F. * RDP	*	**	Ns	Ns	*	*	*	Ns	**	**	**	**
Irrigation * Bio-F.	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	**	**
Irrigation * RDP	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
Irrigation * Bio-F. * RDP	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns

PH: plant height, Sp L: spike length, Sp W: spikes weight, G/Sp: number of grains spikes⁻¹, 1000-GW: 1000-grain weight, GY: grain yield (ton ha⁻¹), St Y: straw yield (ton ha⁻¹), BY: biological yield (ton ha⁻¹). Ns: non-significant at 95%. *, **: Point to significant and highly significant at 95% and 99% level of probability. Different letters indicate significant differences between values at $p \leq 0.05$ according to Duncan's multiple range test.

Table 2. Effect of bio-fertilizers and levels of the recommended dose of phosphorus fertilizers (RDP) on straw yield (ton ha⁻¹), biological yield (ton ha⁻¹), and harvest index of barley under drought and normal irrigation conditions.

Source	Straw Yield (ton ha ⁻¹)		Biological Yield (ton ha ⁻¹)		Harvest Index	
	1st	2nd	1st	2nd	1st	2nd
Bio-fertilizer						
AMF	6.04 a	6.29 a	11.02 a	11.48 a	52.50 a	54.87 a
Phos.	5.66 b	5.89 b	10.58 b	11.01 b	51.72 a	53.66 a
WBF	5.48 c	5.70 b	10.53 c	10.95 b	50.29 b	52.34 b
Phosphorus (RDP) fertilizers						
100% SP	6.12 a	6.37 a	11.81 a	12.30 a	54.88 a	55.92 a
66% SP	5.80 b	6.04 b	10.48 b	10.91 b	50.82 b	52.14 b
0% SP	5.25 c	5.47 c	9.83 c	10.23 c	48.81 c	50.19 c
Irrigation						
Normal	6.68 a	6.95 a	12.49 a	13.00 a	60.08 a	62.56 a
2 Irrigations	5.51 b	5.74 b	10.31 b	10.73 b	49.59 b	51.64 b
1 Irrigation	4.98 c	5.19 c	9.32 c	9.70 c	44.83 c	46.67 c
Bio-F.	**	**	Ns	*	**	**
RDP	**	**	**	**	**	**
Irrigation	**	**	**	**	**	**
Bio-F. * RDP	Ns	Ns	*	*	**	**
Irrigation * Bio-F.	**	**	Ns	Ns	Ns	Ns
Irrigation * RDP	Ns	Ns	Ns	Ns	Ns	Ns
Irrigation * Bio-F. * RDP	Ns	Ns	Ns	Ns	Ns	Ns

Ns: non-significant at 95%. *, **: Point to significant and highly significant at 95% and 99% level of probability. Different letters indicate significant differences between values at $p \leq 0.05$ according to Duncan's multiple range test.

Concerning the interaction between irrigation conditions, bio-fertilizers including inoculation of AMF and PSB and phosphorus fertilizers were not significant for any studied traits, see Tables 1 and 2.

The bio-fertilizers by inoculation of AMF showed the highest significant effects on agronomic traits, yield components, grain yield, of barley, and reducing drought damage to grain yield and other studied traits, followed by inoculation with PSB, see Tables 1 and 2.

From results in Tables 1 and 2, the treatment with bio-fertilizer by inoculation of AMF yielded 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), harvest index, at the first and second year, respectively, followed by the inoculation by PSB, while the treatment without bio-fertilizer recorded the lowest values of the studied traits of barley. Compared with the treatment without bio-fertilizers (WBF), the percentage increases with applying AMF were 9.51%, 9.61%, and 5.38%, 5.41% with treated by Phosphorien as PSB for PH. For Sp length, the percentage increases were 18.19%, 15.22% with applying AMF and 10.01%, 8.92% with treatment by Phosphorien. Concerning, the percentage increases of G/Sp were 15.74%, 15.81% with applying AMF and 8.77%, 8.86% with treatment by Phosphorien.

The percentage increases of 1000-GW were 5.50%, 5.56% with applying AMF and 3.95%, 3.98% with treatment by Phosphorien. The grain yield (ton ha⁻¹) was increased by percentage 17.27%, 17.33% with applying AMF and 10.31%, 10.40% with treatment by Phosphorien. The treatment with bio-fertilizer yielded the percentage increase of 10.22%, 10.35% with treated by AMF and 3.28%, 3.33% with applying Phosphorien. Concerning, the percentage increases of biological yield (ton ha⁻¹) were 4.65%, 4.84% with applying AMF and 0.47%, 0.55%, with treatment by Phosphorien. Regarding the percentage increases of H Index, these were 4.39%, 4.83% with applying AMF and 2.84%, 2.52% with treatment by Phosphorien.

The use of different rates of the RDP fertilizers showed high differences in the studied traits of barley, see Tables 1 and 2. The results showed that the 100% RDP yielded 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), and harvest index, at the second and first year, respectively, followed by the 66% RDP, while the 0% RPD recorded the lowest values of these studied traits of barley.

The data showed that the normal irrigation condition yielded the highest main values of plant height (PH), spike length (Sp L), spike weight (Sp W), grain number/spike (G/Sp), 1000-grain weight (1000-GW), grain yield (GY) (ton ha⁻¹), straw yield (ton ha⁻¹), biological yield (ton ha⁻¹), and harvest index, at the second and first year, respectively. In addition, under condition of two irrigations, the studied traits observed moderate values at the second and first year, respectively. On the contrary, the one irrigation condition yielded the lowest values, see Tables 1 and 2, at the second and first year, respectively. The optimum irrigation regime (three times) for irrigating barley plants increased the studied traits by 14.94, 22.43, 23.37, 20.13, 20.20, 33.88, 34.14, 34.01, and 34.02%, respectively, in the first season, and by 14.90, 22.44, 23.43, 20.21, 20.19, 34.11, 33.91, 34.02, and 34.05%, respectively, in the second year compared with the control (one time irrigation).

3.2. The Effects of Bio-Fertilizers, Drought, and Phosphorus Fertilizers on Nutrients Uptake in Grain and Straw of Barley

The results of Table 3 illustrated the mean effects of drought and normal irrigation conditions, bio-fertilizers by inoculation of AMF and PSB, phosphorus fertilizers of nutrients uptake in grain and straw of barley, and interaction between these factors, whereas the mean effects of irrigation, bio-fertilizers, and phosphorus fertilizers were highly significant for nutrients uptake in grain and straw traits. Moreover, the interaction between irrigation conditions and bio-fertilizers by inoculation of AMF and PSB were non-significant for nutrients uptake in grain and straw traits.

Meanwhile, the interactions between irrigation conditions and phosphorus fertilizers were not significant for studied traits except for grain nitrogen uptake which was significant in the two seasons.

Table 3. Effect of bio-fertilizers and levels of the recommended dose of phosphorus fertilizers (RDP) on N/Grain (Kg h⁻¹), K/Grain (Kg h⁻¹), and P/Grain (Kg h⁻¹) of barley under drought and normal irrigation conditions.

Source	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)		P/Straw (Kg h ⁻¹)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Bio-fertilizer												
AMF	111.29 a	115.92 a	39.01 a	40.63 a	13.72 a	14.29 a	69.91 a	72.33 a	32.73 a	34.08 a	14.12 a	14.71 a
Phos.	103.24 b	107.49 b	31.73 b	33.04 b	12.14 b	12.65 b	56.00 b	58.79 b	29.98 b	31.21 b	12.81 b	13.34 b
WBF	88.50 c	92.13 c	25.49 c	26.53 c	9.44 c	9.83 c	53.79 b	55.99 b	23.96 c	24.94 c	9.99 c	10.39 c
Phosphorus (RDP) fertilizers												
100% SP	115.81 a	120.61 a	36.35 a	37.86 a	13.70 a	14.27 a	69.95 a	72.84 a	33.29 a	34.66 a	14.12 a	14.70 a
66% SP	107.95 b	112.40 b	33.89 b	35.29 b	12.78 b	13.31 b	66.24 b	68.97 b	31.60 b	32.90 b	13.41 b	13.97 a
0% SP	79.26 c	82.53 c	25.99 c	27.05 c	8.82 c	9.19 c	43.51 c	45.29 c	21.79 c	22.68 c	9.39 c	9.78 b
Irrigation												
Normal	117.85 a	122.71 a	37.42 a	38.97 a	13.73 a	14.30 a	69.88 a	72.76 a	33.70 a	35.09 a	14.36 a	14.95 a
2 Irrigations	97.27 b	101.29 b	30.89 b	32.16 b	11.33 b	11.80 b	57.68 b	60.06 b	27.82 b	28.97 b	11.85 b	12.34 b
1 Irrigation	87.92 c	91.54 c	27.92 c	29.07 c	10.24 c	10.67 c	52.13 c	54.29 c	25.15 c	26.18 c	10.71 c	11.15 c
Bio-F.	**	**	**	**	**	**	**	**	**	**	**	**
RDP	**	**	**	**	**	**	**	**	**	**	**	**
Irrigation	**	**	**	**	**	**	**	**	**	**	**	**
Bio-F. * RDP	**	**	**	**	**	**	**	**	**	**	**	**
Irrigation * Bio-F.	**	**	**	**	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns
Irrigation * RDP	**	**	**	**	**	**	**	**	**	**	**	**
Irrigation * Bio-F. * RDP	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns

Ns: non-significant at 95%. *, **: Point to significant and highly significant at 95% and 99% level of probability. Different letters indicate significant differences between values at $p \leq 0.05$ according to Duncan's multiple range test.

Regarding the interaction between bio-fertilizers and phosphorus fertilizers, they were highly significant for nutrients uptake in grain and straw.

Concerning the interaction between irrigation conditions, bio-fertilizers including inoculation of AMF and PSB and phosphorus fertilizers were not significant for nutrients uptake in grain and straw traits.

The bio-fertilizers by inoculation of AMF showed the highest significant effects on grain uptake of N, P, and K, straw uptake of N, P, and K, of barley, and reducing drought damage to grain yield and other studied traits, followed by inoculation with PSB, see Table 3.

From the results in Table 3, the treatment with bio-fertilizer by inoculation of AMF yielded the highest values of 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) at the second and first year, respectively, followed by the inoculation by PSB, while the treatment without bio-fertilizer recorded the lowest values of the studied traits of barley. The percentage increases of grain N uptake (kg ha⁻¹) of barley were 25.75%, 25.82% with applying AMF and 16.66, 16.67% with treated by Phosphorien for grain N uptake (kg ha⁻¹). Concerning the percentage increase of grain, K uptake of barley was 53.04%, 53.15% with applying AMF and 24.48%, 24.54% with treatment by Phosphorien. Regarding the percentage increase of grain, P uptake (kg ha⁻¹) of barley was 45.34%, 45.37% with applying AMF and 28.60%, 28.69% with treatment by Phosphorien. Concerning the percentage increase of straw N, uptake (kg ha⁻¹) of barley was 29.97%, 29.18% with applying AMF and 4.11%, 5.00% with treatment by Phosphorien. Regarding the percentage increase of straw, K uptake of barley was 36.60%, 36.65% with applying AMF and 25.13%, 25.14% with treatment by Phosphorien. For straw P uptake (kg ha⁻¹) of barley, the percentage increases were 41.34%, 41.58% with applying AMF and 28.23%, 28.39% with treatment by Phosphorien.

Use of different rates of the RDP fertilizers showed high differences of the studied traits of barley, see Table 3. The results showed that the 100% RDP yielded the highest values of 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) at the second and first year, respectively, followed by the 66% RDP, while the 0% RPD recorded the lowest values of these studied traits of barley.

The data showed that the normal irrigation condition yielded the highest main values of 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) at the second and first year, respectively. In addition, under the two irrigations condition, the studied traits observed moderate values at the second and first year, respectively. On the contrary, the one irrigation condition yielded the lowest values, see Table 3, at the second and first year, respectively. The optimum irrigation regime (three times) for irrigating barley plants increased the studied traits by 34.04, 34.03, 34.08, 34.05, 34.00 and 34.08%, respectively in the first season, and by 34.05, 34.06, 34.02, 34.02, 34.03 and 34.08%, respectively, in the second year compared with the control (one time irrigation).

3.3. The Interactions Effects between the Bio-Fertilizers, and Phosphorus Fertilizers Rates on Yield, Yield Components of Barley

Under normal irrigation and drought conditions, and as a general average of the effect of the bio-fertilizers under this study, bio-fertilization with AMF showed the highest values of the studied traits, followed by bio-fertilization with Phosphorene. Bio-fertilization with AMF also yielded the highest values of the studied traits under each irrigation period or irrigation treatment, as it led to decrease the harmful effect of water deficiency of irrigation water (drought), followed by treatment or bio-fertilization with Phosphorene under the conditions of the three irrigation treatments, see Figure 1. Under the treatment with the bio-fertilizers under this study, bio-fertilization with AMF +100% RDP showed the highest values of the studied traits, followed by bio-fertilization with AMF +66% RDP followed by bio-fertilization with Phosphorene +66% RDP, see Figure 1. The treatment by AMF under 100% RDP yielded the highest values of PH, Sp W, 1000-GW, GY (ton ha⁻¹), and biological Y (ton ha⁻¹), at the second and first year, respectively, followed by the treatment by AMF under 66% RDP, followed by the treatment of Phosphorene under 100% RDP.

Meanwhile, for G/Sp, the treatment with AMF +100% RDP showed the highest values of this trait, followed by bio-fertilization with AMF +66% RDP followed by bio-fertilization with Phosphorene + 66% RDP, at the first year, see Figure 1.

Concerning harvest index, the treatment with Phosphori +66% RDP followed by AMF +66% RDP and Phosphori + 0% RDP showed the highest values of the harvest index at the second and first year, respectively, see Figure 1.

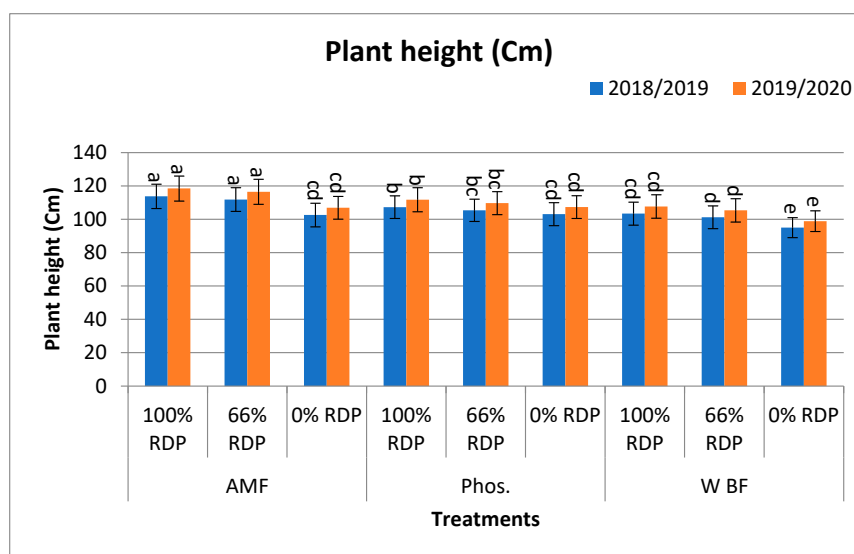


Figure 1. Cont.

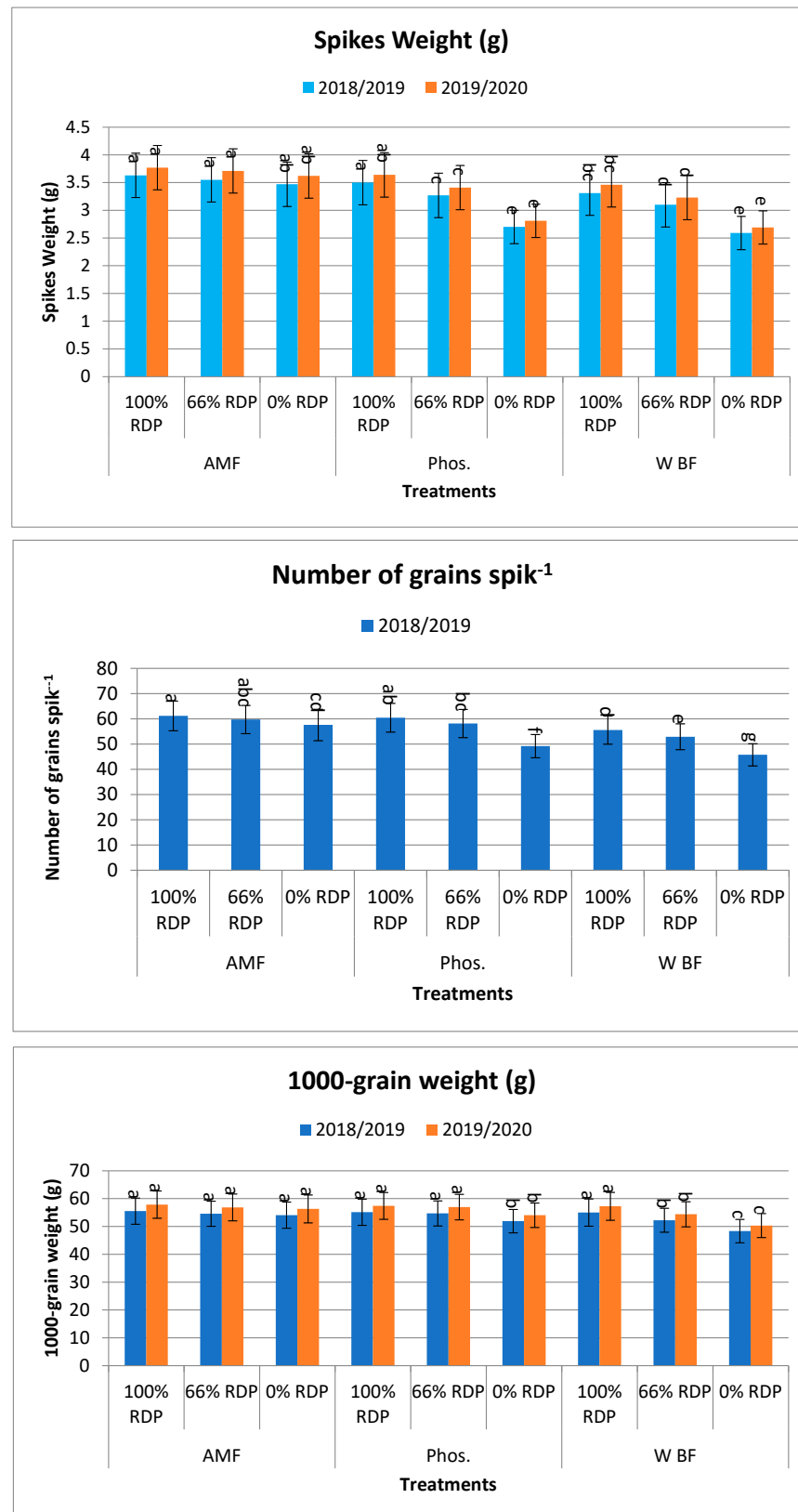


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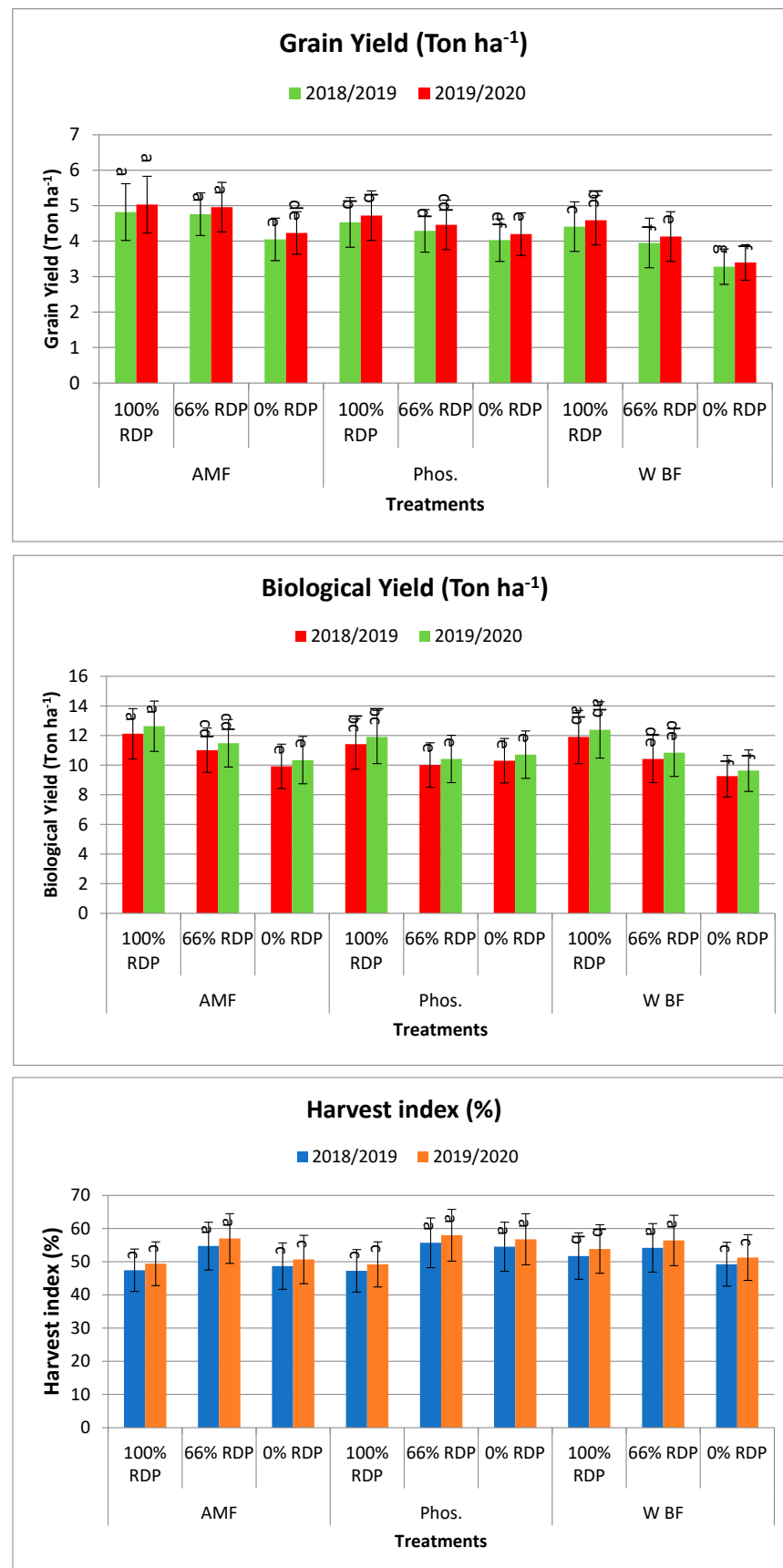


Figure 1. The interactions effects between the bio-fertilizers, phosphorus fertilizers rates on yield, and yield components of barley. Different letters indicate significant differences between values at $p \leq 0.05$ according to Duncan’s multiple range test. Bar represents the standard deviation.

3.4. The Interactions Effects between the Bio-Fertilizers, and Phosphorus Fertilizers Rates on Nutrients Uptake in Grain and Straw of Barley

Applications of the bio-fertilizers with AMF + 100% RDP showed the highest values of the nutrients uptake in the grain and straw of barley, followed by bio-fertilization with AMF + 66% RDP followed by bio-fertilization with Phosphorene + 66% RDP, see Figure 2. The treatment by AMF with 100% RDP yielded the highest values of N/grain (Kg h^{-1}), K/grain (Kg h^{-1}), P/grain (Kg h^{-1}), K/straw (Kg h^{-1}), and P/straw (Kg h^{-1}) at the second and first year, respectively, followed by the treatment by AMF under 66% RDP, followed by the treatment of Phosphorine under 100% RDP.

Regarding N/straw uptake (Kg h^{-1}), the applied bio-fertilizer with Phosphori + 100% RDP followed by Phosphori + 66% RDP recorded the highest values of the N/straw uptake (Kg h^{-1}), at the second and first year, respectively, see Figure 2.

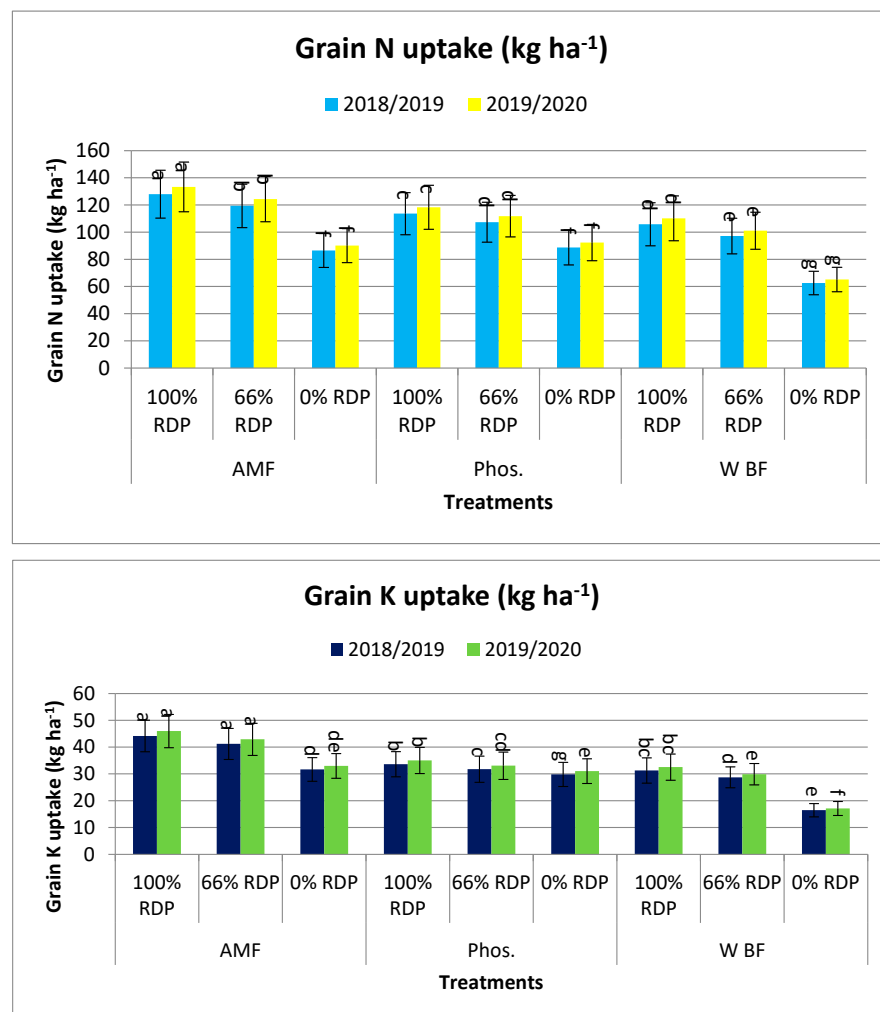


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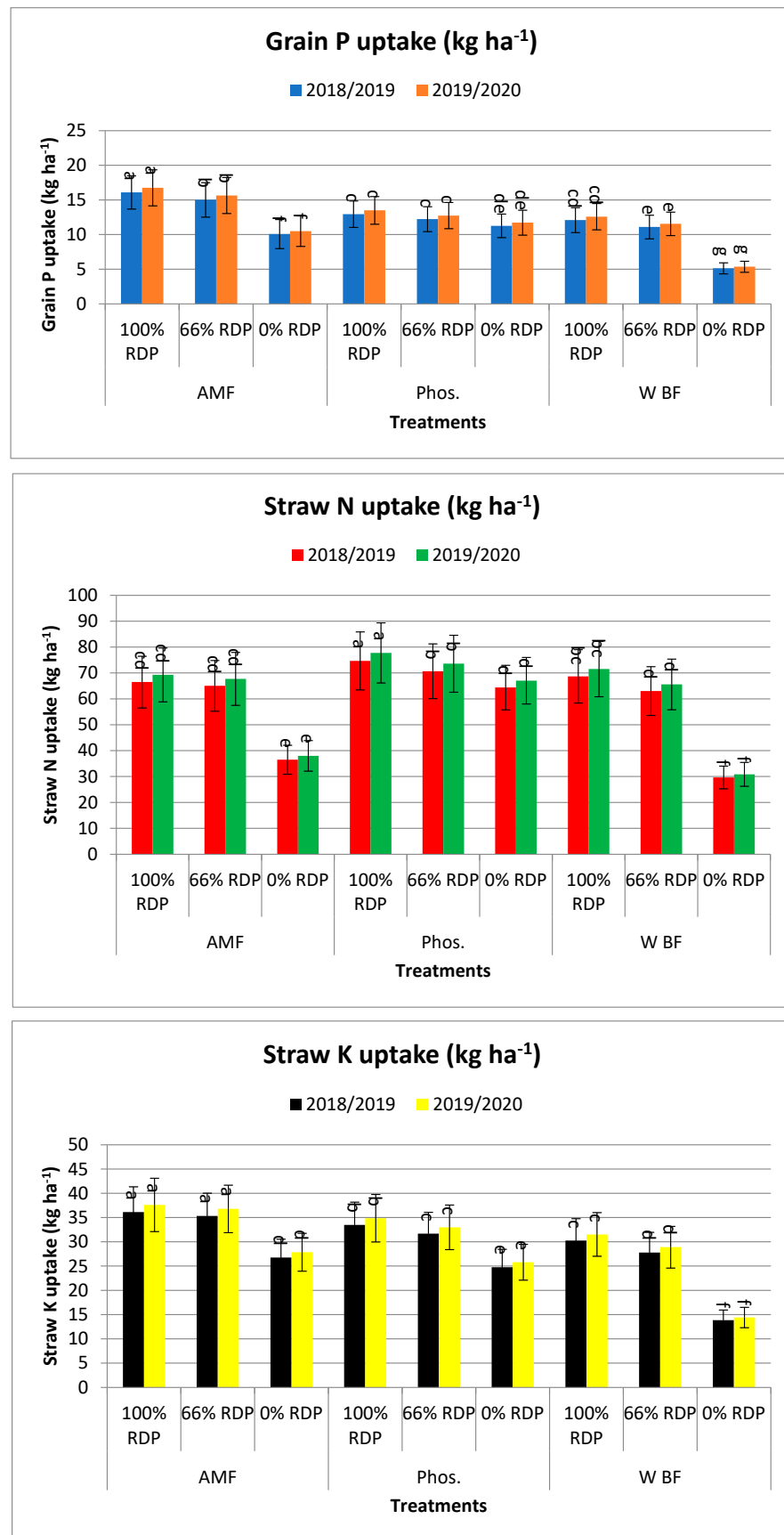


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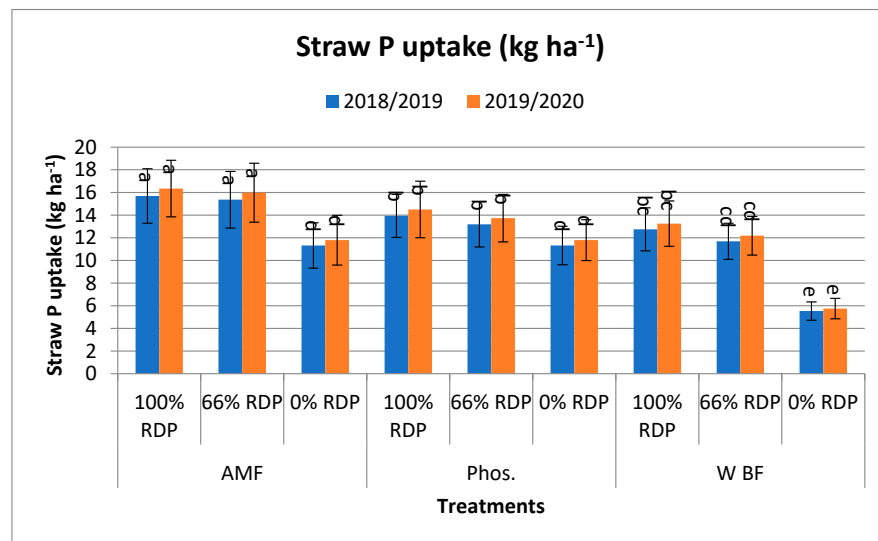


Figure 2. The interactions effects between the bio-fertilizers, and phosphorus fertilizers rates on nutrients uptake in grain and straw of barley. Different letters indicate significant differences between values at $p \leq 0.05$ according to Duncan’s multiple range test. Bar represents the standard deviation.

3.5. The Interactions Effects between the Bio-Fertilizers, and Irrigation Intervals on Yield, Yield Components of Barley

The applications of the bio-fertilizers with AMF under normal irrigation conditions (N), followed by the treatment with Phosphorene under normal irrigation conditions (N), followed by WBF under normal irrigation conditions (N), followed by the treatment with AMF under two irrigation conditions (D 2), the treatment with Phosphorene under two irrigation conditions (D 2), and the treatment with AMF under one irrigation condition (D 1) showed the highest values of GY (ton ha⁻¹) and straw Y (ton ha⁻¹) at the second and first year, respectively, see Figure 3.

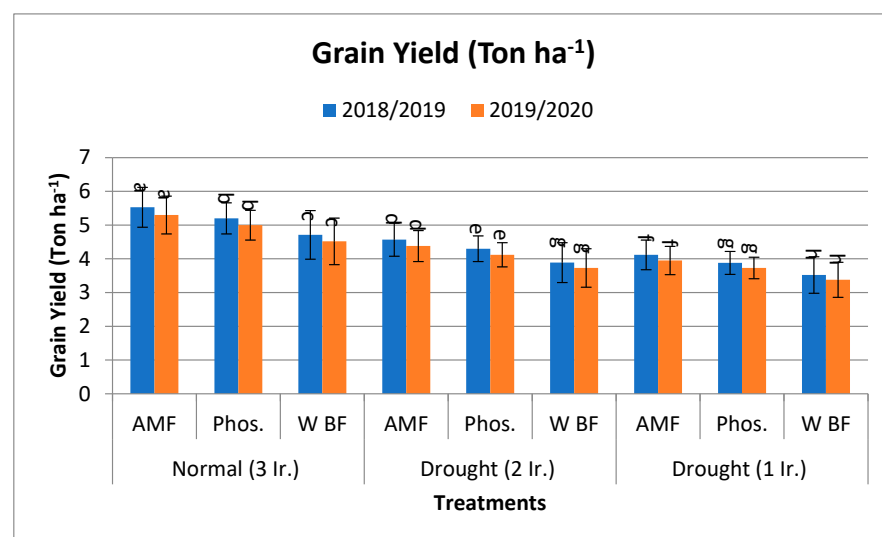


Figure 3. Cont.

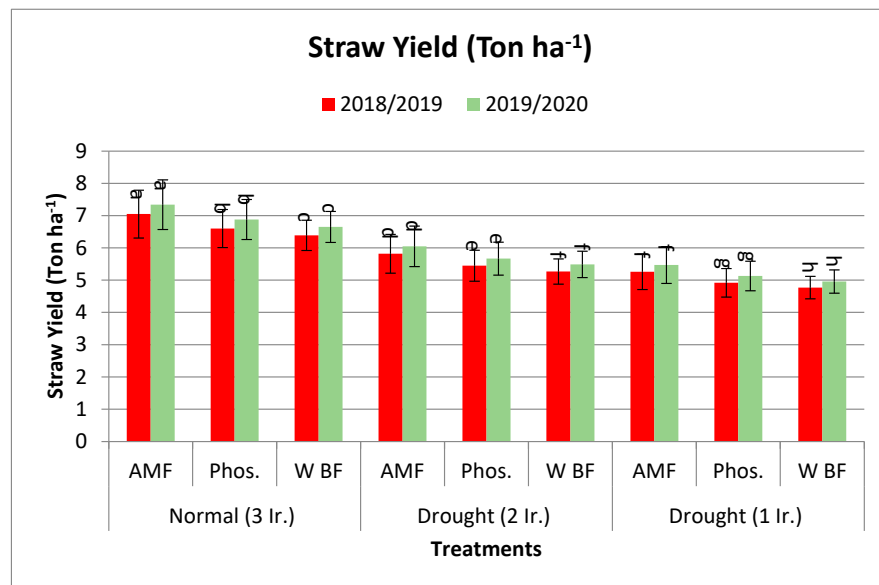


Figure 3. The interactions effects between the bio-fertilizers, irrigation intervals on yield, and yield components of barley. Different letters indicate significant differences between values at $p \leq 0.05$ according to Duncan’s multiple range test. Bar represents the standard deviation.

3.6. The Interactions Effects between the Bio-Fertilizers, Irrigation and Drought Conditions on Nutrients Uptake in Grain and Straw of Barley

The treatment by AMF under normal irrigation conditions (N), followed by the treatment with Phosphorene under normal irrigation conditions (N), followed by the treatment with AMF under two irrigation conditions (D 2), followed by WBF under normal irrigation conditions (N), followed by the treatment with Phosphorene under two irrigation conditions (D 2), followed by the treatment with AMF under two irrigation conditions (D 1), and the treatment with Phosphorene under one irrigation condition (D 1) yielded the highest values of N/grain (Kg h⁻¹), at the first and second year, respectively, see Figure 4.

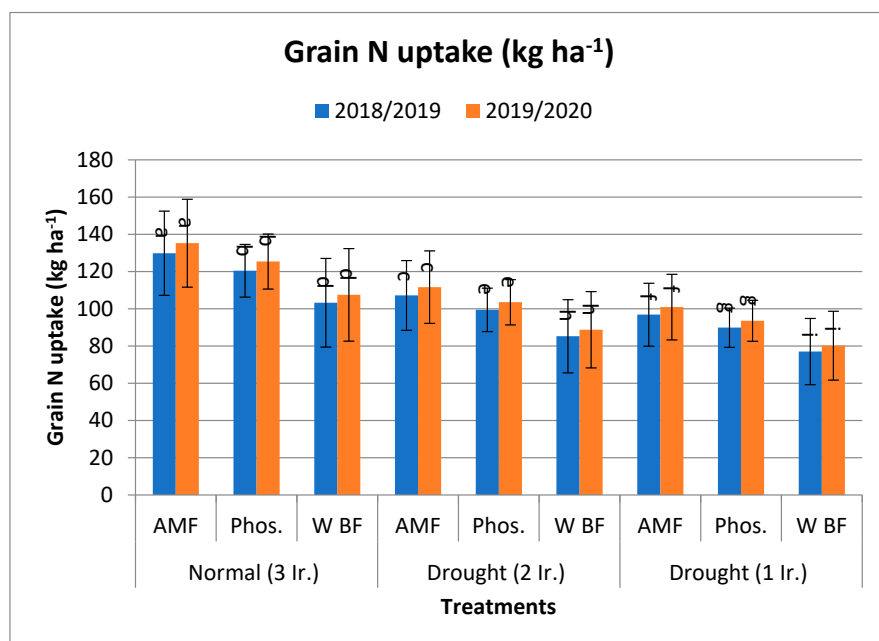


Figure 4. Cont.

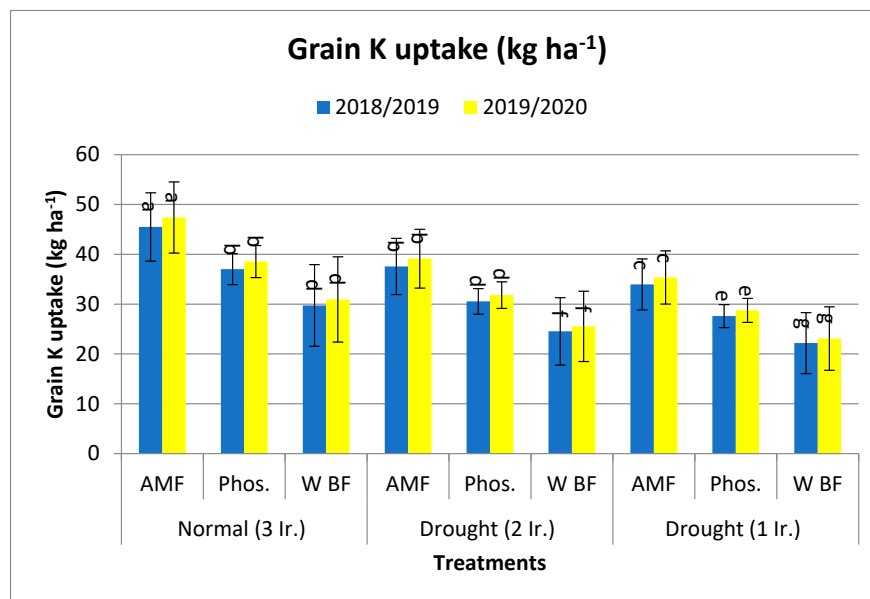


Figure 4. The interactions effects between the bio-fertilizers, and irrigation intervals on nutrients uptake in grain and straw of barley. Different letters indicate significant differences between values at $p \leq 0.05$ according to Duncan's multiple range test. Bar represents the standard deviation.

For K/grain uptake (Kg h^{-1}), the treatments of AMF under normal irrigation conditions (N), followed by the treatment with AMF under two irrigation conditions (D 2), followed by Phosphorene under normal irrigation conditions (N), followed by the treatment with AMF under one irrigation condition (D 1), followed by Phosphorene under two irrigation conditions (D 2), followed by WBF under normal irrigation conditions (N), and the treatment with Phosphorene under one irrigation condition (D 1) yielded the highest values of N/grain (Kg h^{-1}), at the first and second year, respectively, see Figure 4.

3.7. The Interactions Effects between Irrigation Intervals and the Ratios of the Recommended Dose of Phosphorus Fertilizers (RDP) on Yield, Yield Components and Nutrients Uptake in Grain and Straw of Barley

The interaction between irrigation intervals and the ratios of the recommended dose of phosphorus fertilizers (RDP) were not significant for yield and yield components of barley under this study.

The interaction between irrigation intervals and the ratios of the recommended dose of phosphorus fertilizers (RDP) on nutrients uptake in grain and straw of barley were significant for studied traits at the two studied seasons.

The treatment by 100% RDP under normal irrigation conditions (N), followed by the treatment with 66% RDP under normal irrigation conditions (N), followed by the treatment with 100% RDP under two irrigation conditions (D 2), followed by 66% RDP under normal irrigation conditions (N), and the treatment with 100% RDP under one irrigation condition (D 1) yielded the highest values of N/grain (Kg h^{-1}), K/grain (Kg h^{-1}), and P/grain (Kg h^{-1}), at the first and second year, respectively, see Figure 5.

Concerning nutrients uptake in straw of barley, i.e., N/straw (Kg h^{-1}), K/straw (Kg h^{-1}), and P/straw (Kg h^{-1}), the treatments content of 100% RDP under normal irrigation conditions (N), followed by the treatment with 66% RDP under normal irrigation conditions (N), followed by the treatment with 100% RDP under two irrigation conditions (D 2), followed by 66% RDP under normal irrigation conditions (N), and the treatment with 100% RDP under one irrigation condition (D 1) yielded the highest values of N/straw (Kg h^{-1}), K/straw (Kg h^{-1}), and P/straw (Kg h^{-1}), at the first and second year, respectively, see Figure 5.

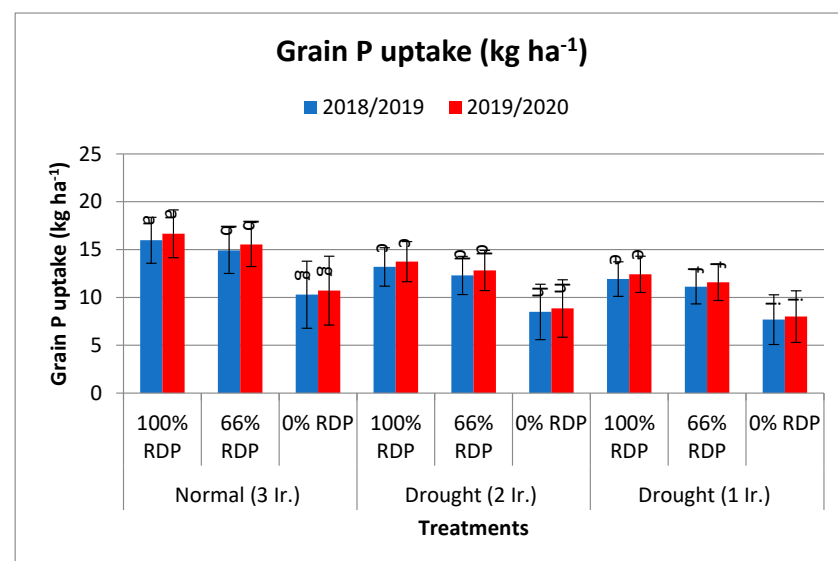
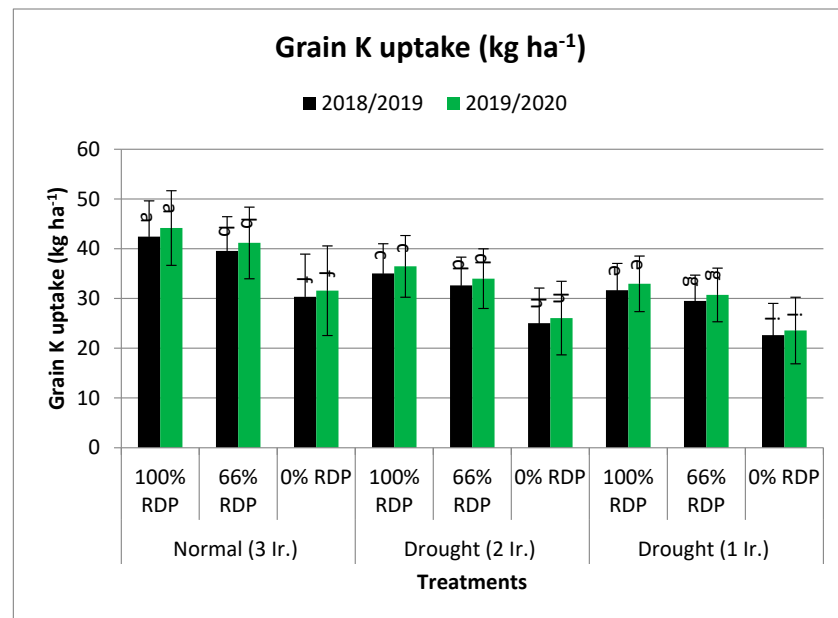
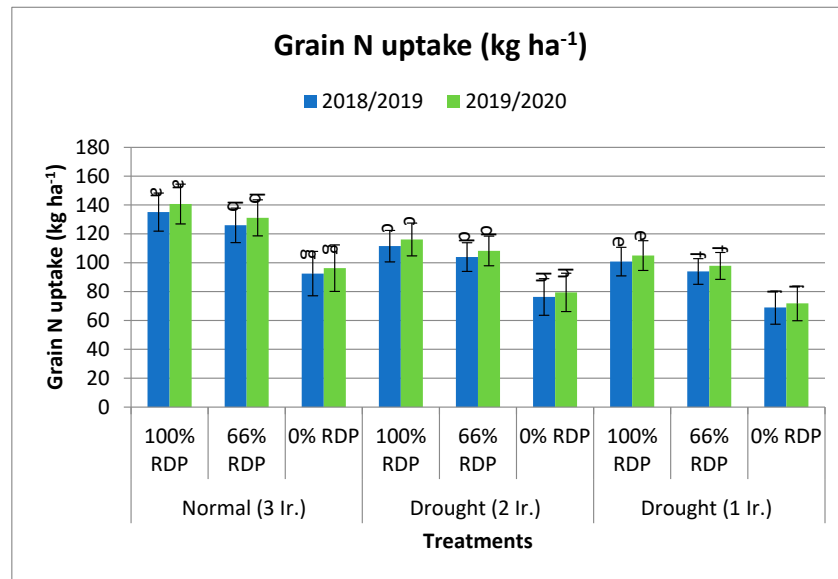


Figure 5. Cont.

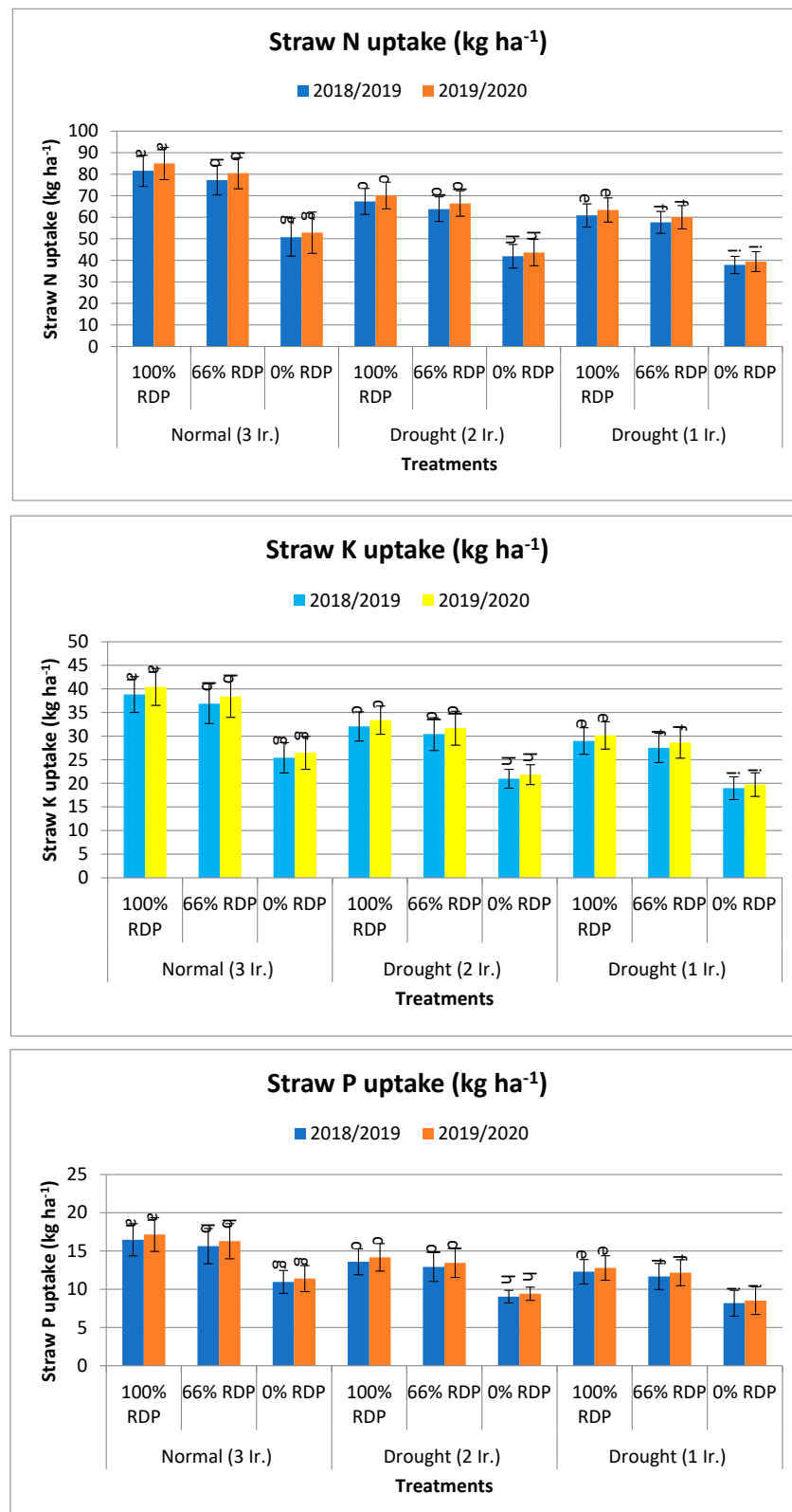


Figure 5. The interactions effects between irrigation intervals and the ratios of the recommended dose of phosphorus fertilizers (RDP) on nutrients uptake in grain and straw of barley. Different letters indicate significant differences between values at $p \leq 0.05$ according to Duncan’s multiple range test. Bar represents the standard deviation.

4. Discussion

Irrigation is the most important factor that can significantly affect crop growth and productivity. Therefore, determining the optimal irrigation regime for the grown crop is crucial regarding improving and obtaining the highest crop productivity. Irrigation regimes, by their nature, include periods between irrigations or their numbers. Therefore, the correct decision regarding irrigation must be carried out based on a comprehensive study and understanding of the environmental factors that affect plant growth, development, various characteristics, productivity, and quality. The current study provides important and useful information about the use of bio-fertilization with AMF and PSB under the application of different water regimes and the impact of this on the growth, various characteristics, and productivity of the barley crop in Egypt, see Tables 1 and 2 and Figures 1 and 3. Water deficiency leads to negative effects, reducing the process of photosynthesis, accelerating leaf senescence, and reducing cell division, leaf area, and transpiration, and all of this leads to a significant decrease in both grain and straw yield [50,51].

Water supply is considered the most expensive input for irrigated crops, and it is also considered the most important factor limiting the growth and productivity of cereal crops in general, and in rainfed areas and barley production and cultivation areas in particular. The regions of the barley-growing areas of Egypt are also characterized by cruel agricultural and environmental conditions. The main obstacle to grain production in these areas is the inadequate soil moisture content in the root area to meet the water needs of the crops. The presence and occurrence of periods of severe water stress are very ordinary in barley-growing areas and often coincide with the majority of the sensitive growth stages. Consequently, water must be provided through supplemental irrigation, applied in sufficient quantities at the right time, and some other processes, such as those that can enhance crop productivity potential and avoid the negative effect of water stress. Also among these methods is the use of biological fertilization [52–55].

Drought conditions lead to negative effects and a significant decrease in the number of grains/spike, the weight of the spike and 1000-grain weight, as it accelerates the contribution and absorption of carbon reserves, that is, the leaves, the grains, and the roots, which lead to an accelerated filling process and, consequently, a decrease in the amount stored in the grains [56–59].

Irrigation water shortage (drought) affects the process of transfer of photosynthetic substances from leaves to grains and grain filling, which in turn greatly affects grain yield, straw yield, and nitrogen uptake [60–62]. Since there is a lack of humidity, it forces the plant to complete grain formation and nitrogen uptake quickly and in relatively less time [63–65]. Under drought conditions, current assimilation is often available to expand grain packing, and it is severely reduced [66–68].

On the other hand, there is a positive and significant impact of bio-fertilizers and therefore attention should be paid to adopting innovative practices that develop crop productivity and soil fertility [69–71]. Among these practices is the use of arbuscular mycorrhizal fungi and bacteria. Different types of bio-fertilization for improving productivity and yield are used. Where microorganisms stimulate the growth of root hairs, and thus the longitudinal growth of mycorrhizal fungi and their penetration into the deeper layers of the soil, this increases the availability of plant nutrients [72].

These results are consistent with those obtained by Najafi et al. [73] who reported useful effects of bio-fertilizers on barley root which increase growth, water absorption, and nutrition. Additionally, several studies observed the positive effects of bio-fertilizers and the importance of adopting innovative practices that improve crop productivity and soil fertility [69–71].

There are positive effects of the coexistence of microorganisms on the growth of barley roots, water absorption, and nutrition [61,66]. The results of many previous studies also indicated a positive effect of the use of AMF on crop productivity and explained that AMF stimulates the growth of hair roots, and thus the longitudinal growth of mycelium fungi

and their penetration into the deeper layers of the soil, which increases the absorption and availability of plant nutrients [72].

The results of this study showed that the N, P, K, uptake in grains and straw was much higher in inoculated plants than in non-pollinated plants under the three irrigation treatments: three irrigations, two irrigations, and one irrigation, see Table 3 and Figures 2, 4 and 5. Where the mycorrhizal inoculation treatment was +100% RDP, then the mycorrhizal inoculation treatment was +66% RDP, followed by the phosphorene inoculation treatment at +100% RDP, then the phosphorene inoculation treatment at +66% RDP yielded the highest values in the nitrogen content of grains under each irrigation treatment for the two seasons respectively, see Table 3 and Figures 2, 4 and 5. This demonstrates that the role of AMF in plant nitrogen nutrition is significant in various symbiotic systems, but AMF can transfer large amounts of nitrogen to their hosts [74].

These findings agreed with earlier studies that showed that mycorrhizal fungi improved water uptake by plants under drought conditions [75–78]. In addition, various reports have indicated that higher water content in mycorrhizal plants is associated with either the ability of soil-growing hyphae to increase the absorption area of host plant roots and low-potential water absorption from the rhizosphere [77–80] or to increase the plant's ability to control water loss through stomata regulations [81] to conserve water under severe drought conditions.

Phosphorus is considered the least mobile element in the soil and thus causes obstruction and prevention of plant growth when soil water and P decrease in dry soil. Treatment with AMF leads to fungal reproduction and increased plant growth and thus increases biomass under abiotic stress [82]. AMF symbiosis can reduce the effects of stress using different defense mechanisms [83,84].

Among these mechanisms, AMF improves phosphatase secretion, which leads to increased P absorption efficiency [85,86]. Increasing the exposed surface of the roots as a result of the union of fungal hyphae and increasing the depth of the roots allows the absorption of a sufficient amount of water and mineral supplements from dry soil [74,82,87].

Some researchers explained the positive effect of AMF to provide sufficient amounts of nitrogen for plant growth and development in an ideal manner, and indicated that the inorganic nitrogen taken up by fungi can be incorporated into amino acids that are transferred to the plant [72,88–90]. In general, the results of the study conducted by Zhang et al. [91] indicated that under normal irrigation conditions, the symbiosis and coexistence of AMF with plant roots leads to an increase in the root surface area, and this in turn leads to an increase in the uptake of plant nutrients.

The resulting improvement in the growth and productivity of barley plants treated with AMF may be due to the relations of plant roots with AMF, which may lead to a change in the expression of genes concerned in biotic resistance and abiotic tolerance responses [78,92]. During periods of drought, phytohormones, such as abscisic acid (ABA), are essential for regulating stress tolerance by stimulating stomatal closure, thus reducing transpiration water loss [78,93]. Moreover, ABA is also essential to mitigate stress harm by activating several stress-responsive genes that encode enzymes for the biosynthesis of osmotically active metabolites and late embryogenesis abundant proteins [78,93,94].

5. Conclusions

The consequences of the current study concluded that the application of arbuscular mycorrhizal or phosphate-dissolving bacteria under normal irrigation conditions, lack of irrigation water (drought), and different levels of phosphate fertilization were very useful and effective in overcoming the harmful effects of drought and led to a significant and highly significant improvement in the growth and productivity of barley plants, their various characteristics, and the level of absorption of the plants for nutrients, NPK, and the productivity of grain and straw crops. The use of bio-fertilization led to saving a third of the amount of phosphate fertilizer, and this in turn leads to reducing the cost of production and increasing the net return. Accordingly, the importance of developing sustainable

bio-fertilizer technology and increasing its application becomes clear to achieve maximum crop production in a healthy way, reduce pollution, preserve the environment, and sustain the soil.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy14091973/s1>, Table S1: The physical and chemical properties for the soil of study site; Table S2: Means monthly of climatic parameters during barely growth and development at the study site (2018/2019 and 2019/2020 seasons).

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