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Effects of Phosphate Solubilizing Microorganisms and Phosphorus Chemical Fertilizer on Yield and Yield Components of Barely (*Hordeum vulgare* L.)

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Abstract: The effect of seed inoculation by phosphate solubilizing microorganisms and different levels of phosphorus chemical fertilizer on yield and yield components of barley (Karoon x Kavir cultivar) was studied in Experimental Farm of College of Agronomy and Animal Sciences, University of Tehran during 2006-2007 growing seasons. The experimental treatments were arranged in split plot factorial based on a complete randomized block design with four replications. Three phosphorus fertilizer levels of 0 (control), 30 and 60 kg/ha were allocated to the main plots and three levels of Phosphate solubilizing bacteria of 0 (control), *Pseudomonas petida* accessions number 9 and 41 along with two levels of Mycorrhiza: with and without Mycorrhiza (control) were assigned to the subplots in a factorial combination. Sole application of bacteria (accession 9) produced the maximum biological yield, while the application of the same bacteria along with Mycorrhiza achieved the maximum one thousand seed weight. Seed inoculation with sole bacteria positively affected the number of the seeds per kernel. Application of Mycorrhiza along with bacteria significantly increased leaf chlorophyll content. According to the results of this experiment, application of bacteria (accession 41) in absence of any chemical phosphorus fertilizer had an appropriate performance and could increase biomass production to an acceptable level, so it could be considered as a suitable substitute for chemical phosphorous fertilizer in organic agricultural systems.

Key words: Phosphate solubilizing bacteria % Mycorrhiza % Phosphorus chemical fertilizer % Barley % Yield % Yield components

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INTRODUCTION

Phosphorus is one the most essential elements for plant growth after nitrogen. However, the availability of this nutrient for plants is limited by different chemical reactions especially in arid and semi-arid soils. Phosphorus plays a significant role in several physiological and biochemical plant activities like photosynthesis, transformation of sugar to starch, and transporting of the genetic traits. Sharma [1] reported that one of the advantages of feeding the plants with phosphorus is to create deeper and more abundant roots. Phosphorus causes early ripening in pants, decreasing grain moisture, improving crop quality and is the most sensitive nutrient to soil pH [2]. Malakooti and Nafisi [3] declared that the best pH for phosphorous uptake by plants is 6.5. Arpana et al. [4] reported that a great proportion of phosphorus in chemical fertilizer becomes unavailable to the plants after its application in the soil. They referred this to formation of strong bonds between phosphorous with calcium and magnesium in alkaline pH and the same bonds with iron and aluminum in acidic soils. The mobility of this element is very slow in the soil and can not respond to its rapid uptake by plants. This causes the creation and development of phosphorus depleted zones near the contact area of roots and soil in rhizosphere. Therefore, the plants need an assisting system which could extend beyond the depletion zones and help to absorb the phosphorus from a wider area by developing an extended network around root system [5].

Biological fertilizers (phosphate solubilizing bacteria) are considered among the most effective plant assistants

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to supply phosphorus at a favorable level. These fertilizers are produced on the basis of selection of beneficial soil microorganisms which have the highest efficiency to enhance plant growth by providing nutrients in a readily absorbable form. Application of inoculants provided from these microorganisms enhances an abundant population of active and effective microorganisms to the root activity zone which increases plant ability to uptake more nutrients.

Phosphate solubilizing microorganisms refer to a group of soil microorganisms that as components of phosphorus cycle, can release it from insoluble sources by different mechanisms [5]. Phosphate solubilizing fungi and bacteria are known as effective organisms in this process [6]. Among the soil bacteria communities, *Pseudomonas strata* S., *Bacillus sircalmous* and intrubacters could be referred to as the most important strains [7]. In particular, Pseudomonas flourcents is considered as an important member of rhizosphere organism community. The positive effect of Pseudomonas inoculation on plant growth has been reported in many research trials. Phosphate solubilizing bacteria like *Z-ketuaxelalic* sp., *Malic* sp. and *Socsinic* sp. are able to affect the solubility of low dissolvable inorganic phosphorus compounds, by secreting different organic acids. The other bacteria of the same group are able to release phosphorus from phosphorus organic compounds, by producing phosphate enzymes. Regarding to conducted researches, the role of Phosphate solubilizing bacteria and their potential capacity to influence phosphorus cycle processes in plant-soil system, can not be ignored. Kim *et al.* [8] indicated that the population of Phosphate solubilizing bacteria depends on cultural activities and different soil properties (physical and chemical properties, organic matter, and soil phosphorus content). The Bacteria species *Pseudomonas* sp. has a considerable potential in phosphorus uptake efficiency. Due to the ecotype diversity of this species and its tolerance in some environmental stresses, this bacterium is of special importance as a biological fertilizer [1].

The research on Mycorrhiza fungus and its role in soil and plant has been an interesting scientific subject since 1800. The presence of this fungus in rhizosphere provides with an advantageous and interactive symbiosis relationship between a higher plant root and a nonpathogenic fungus. Through receiving energetic carbon resources from plant, fungus facilitates the uptake of many inorganic nutrients such as phosphorus, zinc, molybdenum, copper and iron for it. The symbiotic relationship between Mycorrhiza and plants is one of the most abundant symbiotic activities in plant kingdom which exists in most of the ecosystems. Unfortunately, the neglectful interference of human activities such as over application of fungicides and frequent chemical phosphorous fertilizers application (mainly in intensive agricultural systems), have seriously threatened this advantageous symbiosis. Efforts to produce inoculants from Mycorrhiza fungi and to use it in proper environmental conditions, is a significant environmental friendly way to help plant growth and development through the enhancement of this natural phenomenon. The significance of this practice, especially under low fertility conditions, has been very obvious. Photosynthesis improvement in plants through Mycorrhiza symbiosis is mainly due to the increase in transporting of inorganic elements from soil to plants.

One of the most important means to achieve the goals of sustainable agriculture is to extent the application of biological fertilizers. To reach this goal, it is necessary to moderate the use of chemical fertilizers and pesticides through the time and in the mean time increase the soil organic matter content.

This experiment was conducted to compare the efficiency of different types of biological fertilizers (phosphate solubilising bacteria and Mycorrhiza) in association with different amounts of chemical phosphorous fertilizer on yield and yield components of barley (Karoon x Kavir cultivar).

MATERIALS AND METHODS

The experiment was conducted in Research field of College of Agriculture, University of Tehran. The height of the experimental site from the sea level was 1312 m. The mean annual precipitation was 265.9mm and the long term minimum and maximum monthly precipitation ranged from 46.9 to 108.2 mm, respectively. The mean annual temperature was 13.5°C while the mean maximum and minimum temperatures were 40 and -18°C, respectively.

The soil texture of the Research Site was clay loam with a pH of 8.2. The soil chemical properties before the start of the experiment are presented in (Table 1). The experimental treatments were arranged as split plot factorial on the basis of a Randomized Complete Block Design with four replications. Three levels of phosphorous chemical fertilizer (triple supper phosphate) consisting of P_1=30, P_2=60 kg haG^{1} and control (no chemical fertilizer) were allocated to the main plots and three levels of Phosphate solubilizing bacteria of

Table 1: Soil chemical properties of experimental area

Sample depth	pH $ds\ m^{-1}$	EC %	SAR meq/lit	Na meq/lit	Ca %	N mg/kg	P mg/kg	K %	O.M Meq/lit	Mg mg/kg	Fe mg/kg	Zn mg/kg	Mn (cm)
0-30	8.2	1.6	75	1.13	2.25	0.62	10.9	183	1.17	3.2	6.5	1.2	8.5

0 (control), *Pseudomonas petida* accessions 9 and 41 along with two levels of Mycorrhiza: with and without Mycorrhiza (control) were assigned to the subplots in a factorial combinations.

Seedbed preparation was done in early autumn. Nitrogen fertilizer of 250 kg ha^{-1} was used in the form of urea. Nitrogen fertilizer was top dressed in three portions, one third at the time of planting, one third before flowering and the remain at the time of grain filling. The barley was planted at 400 plants m^{-2} density. The hybrid barley cultivar (Karoon x Kavir) was used in this experiment. This cultivar has a high growth potential with a good drought tolerance. This cultivar has been recommended for cold and temperate regions of Iran.

The inoculants bacteria, *Pseudomonas potida,* have a good ability to facilitate phosphorus uptake and Auxine hormone secretion which effectively enhances the plant growth at 5×10^8 cell g^{-1} population. Micorrhiza is a Vesicular–Arbuscular fungus which can uptake the free and dissolved phosphorus at high levels.

To inoculate the seed by biological fertilizers, the seed were first covered by Arabic Gum solution and then either bacteria, Mycorrhiza or both were applied according to experimental treatments. All the seeds were sown soon after inoculation in experimental plots of 3 x 5 m in dimensions. The cultivation rows were 25 cm apart in each plot. Weeds were removed by hand and plots were irrigated as required through the two growing seasons.

Quantitative traits of barley forage were measured in April 2007. Grain yield and yield components were measured after plants reached their physiological maturity in late June 2007. Crop traits including plant height and leaf chlorophyll were measured during crop vegetative period and in various phonological stages. In every harvest an area of 2 square meter from 5 cm above the ground was harvested. To measure the grain yields components, 10 plants were randomly selected from each plot, and all the yield components were measured on them. After weighing the total biological crop weight (grain and stubble) in each plot, the grain was separated from stubble by experimental combine. To determine 1000-grain weight, five groups of 100-grain samples were randomly selected from each experimental plot and weighed after seed reached its physiological maturity. Data analysis and mean comparisons was done using SAS statistical software.

RESULTS AND DISCUSSION

The results of analysis of variance and the comparison of the means of main effects of treatments on different crop traits are presented in Tables 1 and 2, respectively.

Plant Height: The analysis of variance (Table 2) showed no significant effect of phosphorus fertilizers, bacterial strains and Mycorrhiza treatment and their interaction effects on plant height. It seems that phosphorus does not play an important role in enhancement of plant height. The application of chemical phosphorous fertilizer and phosphate solubilizing microorganism did not have any significant effect to increase the plant height.

Leaf Chlorophyll Content: Leaf chlorophyll was significantly affected by bacterial strains (Table 2). The most amount of leaf chlorophyll of 49.50 percent was found in S_2 treatment (bacteria accession 41) and its least amount was found in S_1 treatment (accession 9) (Table 3). The interaction effect of phosphorus and bacteria on leaf chlorophyll was significant (Table 2). The most amount of leaf chlorophyll of 49.55 was obtained by sole bacteria application (Table 4). The interaction effect of bacteria and Mycorrhiza on leaf chlorophyll was also significant. The most amount of leaf chlorophyll of 50.12 was obtained by co-application of S_{41} and Mycorrhiza and the least amount of leaf chlorophyll of 46.09 was obtained by co-application of S_9 and Mycorrhiza, respectively (Table 6).

Under absences of chemical phosphorus fertilizer, phosphate solubilizing microorganisms were able to significantly increase the leaf chlorophyll content. The maximum amount of leaf chlorophyll was obtained by co-application of bacterial strain 41 and Mycorrhiza. The results of this experiment showed that using Mycorrhiza increases leaf chlorophyll content and can positively affect photosynthesis [9]. Obtaining the maximum amount of chlorophyll through co-application of bacterial strain 41 and Mycorrhiza can suggest the existence of a synergic effect between bacteria and fungus. These results are supported by other study in which Glumus sp. fungus and phosphate solubilizing bacteria were used as inoculants. The maximum N and P uptake were observed in bacteria containing treatments. This suggests that there

Table 2: Analysis of variance of measured parameters

Variables	Degree of Freedom	Plant Height	One Thousand grain weight	Ear Weight	The number of grains in ear	Biological yield	Forage Yield	Grain yield	Leaf Chlorophyll Content	Harvest Index
Blocks	3	0.28	20.14	0.01	3.26	358300	7360563	1554615	5.31	28.11
Phosphorus Fertilizer	2	0.12ns	87.03**	0.01**	29.41ns	123466ns	1042629**	61484	3.27ns	7.16**
Error a	6	0.11	9.00	0.02	24.90	695005	2197636	458428	5.41	13.77
Accessions of bacteria	2	0.05ns	36.82**	0.00ns	26.25**	186553ns	142479ns	458428ns	35.8**	10.61**
Phosphorus × Accessions of bacteria	4	0.56ns	86.32**	0.04ns	40.49**	163318ns	124074ns	211484ns	14.8**	10.47**
Mycorrhiza	1	0.50ns	62.38**	0.01ns	16.16ns	261061ns	3609667**	529592ns	2.65ns	0.49ns
Phosphorus × Mycorrhiza	2	0.69ns	79.17**	0.00ns	3.08ns	291177**	5629454**	373810ns	7.23ns	7.31ns
Bacteria ×Mycorrhiza	2	0.54ns	35.74**	0.00ns	6.31	370610**	554364ns	45946ns	32.8**	57.19ns
Phosphorus x Bacteria × Mycorrhiza	4	0.41ns	138.9**	0.02ns	52.33**	110388	2763431**	592508ns	16.3**	58.13**
Error b	45	0.29	6.47	0.06	12.61	138948	1564758	429589	2.73	31.89
Coefficient of variation		0.67	6.80	19.1	8.78	10.68	8.09	16.66	4.52	15.87

Table 3: Mean comparisons of the main effects

Treatments	Harvest index	Grain yield (kg/ha)	Forage dry matter yield (kg/ha)	Biological yield (kg/ha)	Grains per ear	Ear weight (g)	1000-grain weight (g)	Plant height (cm)	Leaf chlorophyll content (mg/g of fresh tissue)
P60	36.17^{a}	3966^{a}	15717^{ab}	10965^{a}	41.66^{a}	1.38^{a}	39.27^{a}	80.80^{a}	48.00^{A}
P30	35.11^{a}	3876^{a}	14724^{b}	11041^{a}	40.22^{a}	1.23^{b}	36.9^{b}	80.90^{a}	47.90
P0	35.65^{a}	3960^{a}	15971^{a}	11108^{a}	39.48^{a}	1.23^{b}	35.49^{b}	80.81^{a}	48.60
S0	35.55^{a}	3828^{a}	15534	10768^{a}	40.77^{ab}	1.28^{a}	38.96^{a}	80.83^{a}	48.74^{a}
S9	36.42a	4125^{a}	15492	11325^{a}	38.93^{b}	1.27^{a}	37.80^{a}	80.90^{a}	46.79^{b}
S41	34.93^{a}	3850^{a}	15385	11021^{a}	41.65^{a}	1.30^{a}	35.84^{b}	80.91^{a}	49.47^{a}
M0	35.80^{a}	4020^{a}	16178^{a}	11228^{a}	40.92^{a}	1.27^{a}	38.17^{a}	80.97^{a}	48.44
M1	35.46^{a}	3848^{a}	14763^{b}	10847^{a}	39.98^{a}	1.27^{a}	36.31^{b}	80.80^{a}	47.94

Table 4: Mean comparisons of the interaction effects of phosphorus and bacteria on the number of grains per ear, 1000-grain weight and leaf chlorophyll content

Phosphorus	Accessions of bacteria	Leaf chlorophyll content (kg/ha)	1000-grain weight (mg/g)	Number of grains per ear (g)
60	S0	47.49^{abc}	41.35^{a}	42.90^{ab}
60	S9	48.33^{ab}	36.90^{bcd}	39.47^{abcd}
60	S41	48.17^{ab}	39.58^{ab}	42.61^{ab}
30	S0	49.18^{ab}	38.61^{abc}	41.67^{abc}
30	S9	45.27^{c}	36.19^{cd}	39.87^{abcd}
30	S41	49.43^{a}	3.11^{cd}	39.13^{bcd}
0	S0	49.55^{a}	34.33^{de}	37.75^{cd}
0	S9	46.78^{bc}	40.33^{a}	37.46^{d}
0	S41	49.53^{a}	31.84e	43.24^{a}

Table 5: Mean comparisons of the interaction effects of phosphorus and Mycorrhiza on 1000-grain weight, biological yield and forage dry matter of barley

Phosphorus	Mycorrhiza	Forage dry matters yield (kg/ha)	1000-grain weight (g)	Biological yield (kg/ha)
60	M0	16910^{a}	38.79^{a}	10820^{ab}
60	M1	14530^{b}	39.76^{a}	11110^{ab}
30	M0	14950^{b}	39.95a	11210^{ab}
30	M1	14500^{b}	33.99b	10880^{ab}
0	M0	16680^{a}	35.79b	11660^{a}
0	M1	15260^{b}	35.20^{b}	10560^{b}

No significant difference among means with the same letter in each column

Table 6: Mean comparisons of the interaction effects of bacteria and Mycorrhiza on leaf chlorophyll content, 1000-grain weight and Biological yield

Accessions of bacteria	Biological yield (kg/ha)	Leaf chlorophyll (g)	Mycorrhiza (mg/kg of fresh tissue)	1000-grain weight content
S0	M0	11280^{ab}	39.72^{a}	49.88^{a}
S0	M1	10250^{b}	36.47^{b}	47.61^{b}
S9	M0	11630^{a}	37.33^{b}	47.50^{b}
S9	M1	11020^{ab}	38.28^{ab}	46.09^{b}
S41	M0	10780^{ab}	37.48^{b}	47.97^{b}
S41	M1	11270^{ab}	34.20^{c}	50.12^{a}

No significant difference among means with the same letter in each column

is a direct and positive synergic effect between fungus and bacteria on soil phosphorous availability [8].

The interaction effect of phosphorus, bacteria and Mycorrhiza on leaf chlorophyll was also significant (Table 2). Due to the antagonistic effect of high amounts of chemical phosphorus fertilizer on bacteria and Mycorrhiza, the maximum amount of leaf chlorophyll was obtained either in low sole chemical phosphorus application or low sole chemical phosphorus application along with S41 and Mycorrhiza, while the minimum amount of leaf chlorophyll was obtained by co-application of 30 kg ha^{-1} phosphorus fertilizer, bacterial strain 9 and Mycorrhiza. It seems that bacterial strains have higher potential to establish antagonistic relations with chemical phosphorus fertilizer.

Some agricultural practices like over application of chemical fertilizers, fungicides, herbicides and pesticides have negative effects on life and development of Mycorrhiza fungus [4]. On the basis of this study, it seems that phosphorous fertilizer did not have much positive effects on leaf chlorophyll content. It can be also concluded that the initial soil phosphorus content was already adequate without phosphorus fertilizer application. It means that the adequate amount of phosphorus in the soil provided with enough resources for phosphate solubilizing microorganisms to provide high levels of available phosphorous while application of high levels of chemical phosphorous fertilizer (60 kg ha^{-1}) led to an antagonistic interaction with bacteria and fungus. Thus, to make a significant increase in leaf chlorophyll content in barley (Karoon x Kavir cultivar) under the conditions of present experiment, it is recommended to use the co-application of bacterial strain 41 and Mycorrhiza without any chemical phosphorous chemical fertilizer.

Forage Yield: The effects of phosphate chemical fertilizer on forage dry matter was significant (Table 2). The maximum dry matter yield of 15971 kg ha^{-1} was obtained in control plots (no phosphorus fertilizer application) which was not significantly different from 60 phosphorus fertilizer level, and the minimum yield of 14724 kg ha^{-1} was obtained using 30 kg ha^{-1} phosphorus fertilizer (Table 3). The interaction effect of phosphorus fertilizer and Mycorrhiza on forage dry matter was also significant. The maximum dry matter yield of 16910 kg ha^{-1} was obtained by sole application of 60 kg ha^{-1} phosphorus fertilizer and the minimum yield of 14500 kg ha^{-1} was gained by using 30 kg ha^{-1} phosphorus fertilizers along with Mycorrhiza (Table 5). In fact the application of Mycorrhiza, decreased forage dry matter yields. The triple interaction effect of phosphorus fertilizer, bacteria and Mycorrhiza on forage dry matter was also significant (Table 6). The maximum dry matter yield of 17220 kg ha^{-1} was obtained by sole application of 60 kg ha^{-1} phosphorus fertilizers which was not significantly different from using bacterial strains and Mycorrhiza. Thus, it can be concluded that the high rates of chemical phosphorus fertilizers application, lead to antagonistic interaction with bacteria and Mycorrhiza. In absence of chemical phosphorus fertilizer, phosphate solubilizing microorganisms were able to increase the forage dry matter yield significantly. In fact the results of using biological fertilizer treatment (inoculation by bacterial strain 41 along with Mycorrhiza in absence of chemical phosphorus fertilizer) was not significantly different from the high rate of chemical phosphorous fertilizer application (best treatment). So it could be concluded that applying biological fertilizers can reasonably increase the forage dry matter yield and are more recommendable than chemical phosphorous fertilizers in terms of environmental effects under present experimental conditions.

Ear Weight: The effect of triple supper phosphate fertilizer on ear weight was significant (Table 2). The maximum ear weight was obtained using 60 kg ha^{-1} phosphorus fertilizer and the minimum ear weight was gained in control plots (no phosphorus fertilizer) (Table1). Like the 1000-grain weight, the ear weight increased, as phosphorus fertilizer increased to 60 kg ha^{-1}. It means that phosphorus fertilizer plays an important role in barley generative growth and therefore to make a significant increase in ear weight, it is recommended to apply phosphorus fertilizer at 60 kg ha^{-1}.

The Number of Grains per Ear: The number of grains per ear was significantly affected by bacterial strains (Table 2). The maximum number of grains in ear (41.66) was observed in S2 treatment (bacterial strain 41) and the minimum number in S1 treatment (bacterial strain 9).

The interaction effect of phosphorus and bacterial on the number of grains in ear was also significant (Table 4). The maximum number of grains in ear of 43.24 grains was obtained using 30 kg ha^{-1} phosphorus fertilizer and seed inoculation with bacterial strain 41 treatment. The minimum number of grains per ear was gained using 30 kg ha^{-1} of phosphorus fertilizer and inoculation by bacterial strain 9. Unlike bacterial strain 9, which relies on a minimum amount of phosphorus fertilizer to be active, application of sole bacterial strain 41 can increase significantly the number of grains in Karoon x Kavir cultivar.

The triple interaction effect of phosphorus fertilizer and bacterial and Mycorrhiza on the number of grains in ear was also significant (Table 2). The maximum number of grains in ear (45.83 grains) was obtained using 60 kg ha^{-1} phosphorus fertilizer and inoculation of seed with bacteria strain 41 without Mycorrhiza, and the minimum number was gained in control plots (without any treatments). A synergic interaction effect between chemical phosphorus fertilizer and bacteria was observed on number of grains per ear. It seems that to increase the number of grains in barley ear (Karoon x Kavir cultivar), application of 30 kg ha^{-1} phosphorus fertilizer and inoculation of seed with bacterial strain 41 will lead to an excellent result.

One Thousand Grain Weight (1000-grain Weight): The effect of phosphorus fertilizer on 1000-grain weight was significant (Table 2). The maximum 1000-grain weight of 39.28 g was observed using 60 kg ha^{-1} phosphorus fertilizer and the minimums 1000-grain weight of 35.50 g in control plots (no chemical fertilizer application), which was not significantly different from 30 kg ha^{-1} phosphorus fertilizer (Table 3). 1000-grain weight was significantly affected by bacterial strains (Table 2). The maximum 1000-grain weight of 38.10 g was observed in control treatment (no bacteria inoculation) which was not significantly different from bacterial strain 9, and the minimum 1000-grain weight was observed in S2 treatment (accession 41) (Table 3).

The triple interaction effect of phosphorus fertilizer, bacteria and Mycorrhiza on 1000-grain weight was also significant (Table 2). The best results in the application of Mycorrhiza treatment was obtained when applied along with bacterial strain 9 and no phosphorus fertilizer application. On the some result of experiment the use of phosphorus solubilizing bacteria leads to an increase in grain dry weight and phosphorus uptake by bean plant. It is notable that the maximum 1000-grain weight was obtained in sole application of bacterial strain 9, which was not significantly different from co-application of bacteria strain 41 and 60 kg ha^{-1} phosphorous fertilizer level. Sole application of bacteria S1 (strain 9) seems to be able to provide the highest 1000 grain weight in barley (Karoon x Kavir cultivar). Also, according to the positive effect of Mycorrhiza on 1000-grain weight, it can be concluded that to increase 1000-grain weight, the use of biological fertilizers is preferred to the application of chemical ones. This can partially encourage farming with the mere use of biological fertilizers (organic systems).

Grain Yield: Grain yield was not significantly affected by any of experimental treatments (Table 2). But the maximum grain yield (4462.5 kg ha^{-1}) obtained using 60kg ha^{-1} phosphorus fertilizer, bacterial strain 9 and Mycorrhiza. This indicates the presence of a synergetic interaction among bacteria, Mycorrhiza and phosphorus fertilizer. Tohidi-Moghaddam *et al.* [10] reported that phosphorus solubilizing bacteria increase the available nitrogen and phosphorus in the soil which could enhance the crop production.

Biological Yield: The interaction effect of phosphorus and Mycorrhiza on biological yield was significant (Table 2). The maximum biological yield of 11660 kg ha^{-1} was obtained in control treatment (no application of Mycorrhiza and phosphorus fertilizer) which was not significantly different from the use of 30 and 60 kg ha^{-1} of phosphorus fertilizer (Table 5). The interaction effect of bacteria and Mycorrhiza on biological yield was also significant in which the maximum biological yield of 11630 kg ha^{-1} was obtained in sole application of S2 treatment (bacterial strain 9) without Mycorrhiza and the minimum biological yield of 10250 kg ha^{-1} was gained in sole application of Mycorrhiza treatment (without bacteria) (Table 6). The obtained results conclude that the sole application of bacteria can considerably increase biological yield. Salehrastin [5] reported the considerable increase in maize, soybean and wheat yield as a result of the use of bacterial fertilizer.

Harvest Index: The interaction effects of phosphorus, bacteria and Mycorrhiza on harvest index was significant (Table 2). The maximum harvest index of 41.45 was obtained by co-application of 60 kg ha^{-1} phosphorus fertilizers and Mycorrhiza which was not significantly different from sole application of bacterial strain 9. It should be mentioned that the maximum harvest index obtained by sole application of 60 kg ha^{-1} fertilizer level was not significantly different from control treatment (0 kg ha^{-1} phosphorus fertilizer levels) and co-application of bacterial strain 9 and Mycorrhiza. It seems that the co-application of Mycorrhiza, and bacterial S1 (strain 9) can provide the best desirable conditions to increase the harvest index in barley (Karoon x Kavir cultivar). Koide [9] reported the positive effect of Mycorrhiza fungus on crop yield due to the increase of nutrients uptake (particularly phosphorus and zinc).

The application of Mycorrhiza does not significantly affect the biological yield as well as 1000-grain weight in absence or limited presence of phosphorus. Only in presence of high rates of phosphorus (phosphorus 60 levels), some Mycorrhizal effects are observed. It means that in this case Mycorrhiza plays a catalyzor role to release phosphorus from dissolvable resources and provide it to the plant. This occurs only when the level of soil phosphorus is adequately high. It could be concluded that under the conditions of the present experiment, to increase the harvest index, it is not recommended to use Mycorrhiza along with chemical phosphorus fertilizer.

The positive effect of the Mycorrhiza application was observed only on harvest index, 1000 grain weight and to some extent on grain yield in this experiment. This could explain the probable incidence of some environmental stresses (the presence of some toxins in soil because of previous cultivations) which adversely affected the efficiency of Mycorrhiza. This could also adversely affect the activity of bacteria in co-inoculation with Mycorrhiza. However, Mycorrhiza could play a better role when applied along with bacteria. It seems that Mycorrhiza is less affected by environmental stresses in the presence of bacteria and indeed, bacteria acts as a supporter of Mycorrhiza. In terms of increasing the phosphorus uptake, the obtained results imply the superiority of continuous application of biological fertilizers (organic systems) over the use of them after chemical fertilizer (current systems). In fact, the advantageous effects of biological fertilizers on soil conditions to be noticeable, more than just one farming season is necessary.

The Bio-fertilizers are considered as the most favorable natural compounds to enhance the micro-organism activities in the soil. The highest privilege of application of these fertilizers in Iran is providing with organic matter in desperately needed arid and semi-arid soils. Also providing with the nutrients in accordance to natural abilities of plant uptake potential, enhancing and improving the soil biodiversity, developing the biological activities, increasing the environmental hygiene, conservation and supporting the natural and non-renewable resources are among the most important reasons to increase the utilization of biological fertilizers. Soil fertility management by biological fertilizers is one of the basic components of sustainable agriculture.

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