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Effect of Phosphate Solubilizing Microorganisms and Phosphorus Chemical Fertilizer on Forage and Grain Quality 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 forage and grain quality 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. Three phosphorus fertilizer levels of 0 (control), 30 and 60 kg ha^{-1} 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. Applications of sole bacteria (accession 41) significantly decreased neutral detergent fibers (NDF) (57.4%) in barley forage. The maximum grain protein (11.37%) was obtained with application of sole Mycorrhiza. The same treatment also could increase Ash to an acceptable level (8.05%). NDF was significantly ($p<0.05$) decreased while WSC was increased by application of chemical phosphorous fertilizer. Sole and co-application of Mycorrhiza and bacteria had a significantly positive influence on increasing the percentage of grain protein and Ash and also decreasing the NDF value. The application of these inoculants could be considered as an appropriate substitute for chemical phosphorous fertilizer in organic and sustainable agricultural systems.

Key words: Phosphate solubilizing bacteria · Mycorrhiza · Phosphorus chemical fertilizer · Barley grain · Forage quality

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 the advantages of feeding the plants with phosphorus are to create deeper and more abundant roots. Phosphorus causes early ripening in plants, 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 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].

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

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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. Phosphate solubilizing bacteria not only release phosphorus but also produce other biological compounds like hormones such as auxin and gibberellic acid as well as vitamins. The increase in number and diversity of microorganisms and their interaction lead to increase in number and diversity of effective organic acids through the solubility process of insoluble phosphorus [4]. Yahya and Azawi [8] reported the highest population of Phosphate solubilizing bacteria in agricultural and rangeland soils. Kim *et al.* [9] 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 bacteria is of special importance as a biological fertilizer [9-10].

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 significantly environmental friendly way to help plant growth and improvement 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 biofertilizers. 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 evaluate the effect of different types of microbiological fertilizers (Phosphate solubilizing bacteria and Mycorrhiza) along with different quantities of chemical phosphorus fertilizer on forage and grain quality of barley (Karoon x Kavir cultivar).

MATERIALS AND METHODS

The experiment was conducted in Research field of College of Agriculture, University of Tehran, located between 35°, 48'N latitudes and 51°, 10'E longitude. The height of the experimental site from the sea level was 1312 m. The mean annual precipitation was 265.9 mm 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 experimental treatments were arranged as split plots 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 ha^{-1} and control (no chemical fertilizer) were allocated to the main plots and three levels of Phosphate solubilizing bacteria of 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 combination.

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 the 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 in Iran.

The inoculant 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. Micorrhriza 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 2 x 5 m in dimensions. The distance between cultivation lines in each plot was 25 cm. Weeds were removed by hand and plots were irrigated as required through the growing season.

The forage quality parameters of barley were measured when barely grains were at dough stage in March 2007, and the grain quality traits were measured when the grains reached physiological maturity in April 2007. In every harvest an area of 2 square meter from 5 cm above the ground was harvested. The harvested plants in March were dried in oven at 70°C for 3 days. The grinded samples of forage were prepared to measure the quality parameters by NIR (near infra red) set. The grain quality traits were also measured by another type of NIR (near infra red) set. The statistical analysis of the experimental data was done using SAS software.

RESULTS AND DISCUSSION

The result of statistical analysis of treatment effects on percent digestible dry matter (DMD), water soluble carbohydrates (WSC), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), minerals (ASH) and grain protein content as well as grain moisture are shown in Tables 1 and 2, respectively

The percentage of digestible dry matter (DMD): The analysis of variance did not show any significant ($p<0.05$) effect of phosphorus fertilizer, bacterial strains or Mycorrhiza and their interaction effects on the percentage of digestible dry matter (DMD) (Table1). Seed inoculation by bacteria strain 41, could increase the amount of DMD to an acceptable level of 66.34%.

Table 1: Analysis of variance of measured parameters on barley quality

Variables	Df	ASH	NDF	ADF	CP	WSC	DMD	Grain protein	Grain moisture
Block	2	0.46	50.97	54.79	22.48	6.60	69.69	3.31	7.67
Phosphorus Fertilizer	2	0.57^{ns}	32.21**	62.42^{ns}	0.85^{ns}	11.58**	43.72^{ns}	3.65^{ns}	15.19^{ns}
Error a	4	0.11	7.58	24.68	4.44	1.65	26.96	1.62	6.99
Bacteria Accessions	2	0.30^{ns}	18.91ns	1.95^{ns}	0.32^{ns}	1.26^{ns}	1.48^{ns}	4.02^{ns}	0.41^{ns}
Phosphorus × Accessions of Bacteria	4	0.19	59.84**	14.63^{ns}	1.16^{ns}	6.50**	5.34^{ns}	0.86^{ns}	2.86^{ns}
Mycorrhiza	1	0.60**	105.01**	19.76^{ns}	0.35^{ns}	0.43^{ns}	2.40^{ns}	1.92^{ns}	2.86^{ns}
Phosphorus Fertilizer x Mycorrhiza	2	0.28^{ns}	2.66^{ns}	0.75^{ns}	0.79^{ns}	0.78^{ns}	1.65^{ns}	6.07**	9.61^{ns}
Bacteria Accessions x Mycorrhiza	2	0.42**	37.23**	48.20^{ns}	3.16^{ns}	8.64^{ns}	25.90^{ns}	0.48^{ns}	0.44^{ns}
Phosphorus Fertilizer × Bacteria Accesations × Mycorrhiza	4	0.001**	23.63^{ns}	7.78^{ns}	3.35^{ns}	4.61^{ns}	7.26^{ns}	7.09**	7.41^{ns}
Error b	30	0.20	18.61	19.70	2.09	3.76	14.48	2.57	7.90
Coefficient of Variation		5.64	7.15	12.67	10.98	12.88	5.86	15.56	28.43

Table 2: Mean comparison of the main factors effects on barely quality

Treatments	DMD	ASH	NDF	ADF	CP	WSC	Grain protein	Grain moisture
P0	65.36^{a}	8.01^{a}	61.62^{a}	35.07^{a}	13.39^{a}	14.87^{b}	10.60^{a}	10.46^{a}
P30	63.27^{a}	7.92^{a}	60.54^{ab}	36.88^{a}	13.19^{a}	14.42^{ab}	10.4^{a}	8.82^{a}
P60	66.31^{a}	7.90^{a}	58.92^{b}	33.15^{a}	12.96^{a}	15.96^{a}	9.81^{a}	10.37^{a}
S0	65.31^{a}	7.99^{a}	61.03^{a}	34.66^{a}	13.17^{a}	14.96^{a}	10.06^{a}	9.87^{a}
S9	64.84^{a}	7.80^{a}	60.88^{a}	35.20^{a}	13.32^{a}	14.84^{a}	10.04^{a}	9.74^{a}
S41	64.77^{a}	8.04^{a}	59.18^{a}	35.25^{a}	13.05^{a}	15.35^{a}	9.77^{a}	10.44^{a}
M0	65.19^{a}	7.84^{b}	58.97^{b}	34.43^{a}	13.26^{a}	15.14^{a}	10.10^{a}	9.65^{a}
M1	64.77^{a}	8.05^{a}	61.74^{a}	35.64^{a}	13.10^{a}	14.96^{a}	10.40^{a}	10.11^{a}

Table 3: Mean comparison of the interaction effects of phosphorus and bacteria treatments on WSC, NDF

Levels of phosphorus fertilizer	Accessions of bacteria	NDF	WSC
P0	S0	62.90^{ab}	14.34^{ab}
P0	S9	64.58^{a}	13.69^{ab}
P0	S41	58.39^{b}	16.31^{ab}
P30	S0	62.93^{ab}	14.16^{ab}
P30	S9	59.84^{ab}	14.55^{ab}
P30	S41	58.86^{ab}	14.56^{ab}
P60	S0	57.26^{b}	16.40^{a}
P60	S9	58.21^{b}	16.29^{ab}
P60	S41	61.30^{ab}	15.18^{ab}

No significant ($p<0.05$) difference among means with the same letter in the column

The percentage of water soluble carbohydrates (WSC): The effect of phosphorous fertilizer on WSC was significant ($p<0.05$) (Table1). The maximum WSC (15.96%) was obtained, using 60 kg ha^{-1} phosphorous fertilizer (Table 2). The interaction effect of phosphorus and bacteria on WSC was also significant ($p<0.05$) (Table 3). The maximum amount of WSC (16.40%) was obtained using 60 kg ha^{-1} of sole chemical phosphorous fertilizer (Table 2), which was not significantly different from treatment using sole bacterial strain 41. The maximum WSC obtained from 60 kg ha^{-1} of sole phosphorous fertilizer application was not significantly different from application of sole bacterial strain 9 treatment. The co-application of bacteria strain 9 and 30 kg ha^{-1} phosphorus treatment was also significantly increased the amount of WSC compared to control. Recent studies show that based on eco-physiological characteristics, Phosphate solubilizing bacteria have a better efficiency to provide with available phosphorous in poor and unfertilized soils. Salehrastin [5] also reported that the considerable increase in yield as a result of application of Phosphate solubilizing bacteria in maize, soybean and wheat. It seems that the sole application of bacteria strain 41 can provide the best conditions in terms of increased WSC in barley (cultivar Karoon x Kavir) in absence of any chemical phosphorous application.

Crude protein (CP): The percent of crude protein was not affected either by phosphorous fertilizer, bacterial strains or their interaction effects (Table 1). However, the maximum amount of crude protein (14.41%) was obtained by co-application of 30 kg ha^{-1} of triple super phosphate fertilizer, bacterial strain 41 and Mycorrhiza. These results can indicate the synergetic effect between bacterial strains 41; Mycorrhiza and chemical phosphorus fertilizer (up to 30 kg ha^{-1} level).

Neutral Detergent Fibers (NDF): The effect of chemical phosphorus fertilizer on NDF was significant ($p<0.05$) (Table 1). The minimum amount of NDF was obtained using 60 kg ha^{-1} triple supper phosphate fertilizer which was not significantly different from 30 kg ha^{-1} phosphorus fertilizer level (Table 2). The NDF was also significantly affected by interaction between phosphorus and bacteria treatments (Table 3). The minimum NDF value was obtained (57.27%) by sole application of 60 kg ha^{-1} supper phosphate triple which its effect was not significantly different from sole application of bacterial strain 41. These results are in line with those findings which showed the application of Phosphate solubilizing bacterial leads to increase in phosphorus uptake and digestibility of the forage. Considering the destructive results of over application of chemical fertilizers on environment, it appears that bacterial strain 41 could substitute chemical phosphorous fertilizers to provide the best conditions for decreasing NDF in barley Karoon x Kavir cultivar and increase the quality of forage. The above results indicate that there is an antagonistic effect between chemical phosphorus fertilizer and bacteria activity in regard to NDF value in barley forage.

Acidic Detergents Fibers (ADF): The interaction effect between bacteria and Mycorrhiza on ADF was observed. The co-application of bacteria strain 41 and Mycorrhiza

Table 4: Mean comparisons of the interaction effects of phosphorus chemical fertilizer and Mycorrhiza on Grain protein of barely

Levels of phosphorus fertilizer	Mycorrhiza	Grain protein (%)
P0	M0	10.02[ab]
P0	M1	11.37[a]
P30	M0	10.60[ab]
P30	M1	10.76[ab]
P60	M0	10.27[ab]
P60	M1	9.35[b]

Table 5: Mean comparison of interaction effects of bacteria and Mycorrhiza on total ASH

Bacteria Accessions	Mycorrhiza	ASH (%)
S0	M0	7.86[ab]
S0	M1	8.12[a]
S9	M0	7.55[b]
S9	M1	8.04[a]
S41	M0	8.10[a]
S41	M1	7.98[ab]

Table 6: Mean comparison of interaction effects of bacteria and Mycorrhiza on grain protein

Levels of phosphorus	Accessions of bacteria	Mycorrhiza	Grain protein (%)
P0	S0	M0	10.03[abc]
P0	S0	M1	11.57[ab]
P0	S9	M0	10.33[abc]
P0	S9	M1	11.80[a]
P0	S41	M0	9.70[abc]
P0	S41	M1	10.73[abc]
P30	S0	M0	11.13[abc]
P30	S0	M1	11.40[b]
P30	S9	M0	9.23[abc]
P30	S9	M1	11.47[ab]
P30	S41	M0	9.80[abc]
P30	S41	M1	9.40[abc]
P60	S0	M0	10.30[bc]
P60	S0	M1	9.63[abc]
P60	S9	M0	11.90[a]
P60	S9	M1	8.03[c]
P60	S41	M0	8.60[bc]
P60	S41	M1	10.40[abc]

No significant ($p<0.05$) difference among means with the same letter in the column

had a positive effect on decreasing the ADF value. This phenomenon indicates the synergic effect between bacteria and fungus in this regard. These results are supported by other research findings in which *Glumes* fungus and phosphate solubilizing bacteria were used as inoculants [9].

Total Ash (ASH): The effect of phosphorous fertilizer and bacteria on total ash was not significant while there was a significant effect of Mycorrhiza on total ash of barely forage ($p<0.05$) (Table 1). The maximum total ash of 8.05% was obtained by Mycorrhiza inoculation and the minimum total ash of 7.84% was found in control (Table 2). The amount of ash was also significantly affected by the interaction effect between bacteria and Mycorrhiza (Table 5). The maximum amount of ash of 8.12% was obtained when sole Mycorrhiza was applied which was not significantly different from treatments of co-application of Mycorrhiza and bacteria strain 9 and sole application of bacterial strain. It is concluded that the application of Mycorrhiza can provide favorable conditions for more phosphorus uptake. In phosphorous deficient soils, the effect of Mycorrhiza in providing phosphorous to the plants is equal to the role of *Rhizobium* sp. in supplying nitrogen for legume family [11].

Percent of Grain Moisture (GRAIN MOIUSTURE): The percentage of grain moisture was not affected by any experimental treatments (Table 1).

Grain Protein Percent: The effect of phosphorus fertilizer, bacteria and Mycorrhiza on grain protein was not significant ($p<0.05$) (Table 1). However, the percentage of grain protein was significantly affected by interaction between phosphorus fertilizer and Mycorrhiza treatments (Table 4). The maximum grain protein of 11.37% was obtained by sole application of Mycorrhiza (Table 2) which was not significantly different from co-application of Mycorrhiza and 30 kg ha^{-1} phosphorous fertilizer treatment. It seems that to increase the amount of grain protein under organic system, phosphorous fertilizer could be substituted by Mycorrhiza. Some agricultural practices such as over application of chemical fertilizers, herbicides, fungicides and pesticides have an adverse effect on Mycorrhiza fungus life and development in the soil [12].

The interaction effect between phosphorus, bacteria and Mycorrhiza on grain protein was also significant ($p<0.05$) (Table 6). The most grain protein up to 11.90% was obtained by co-application of 60 kg ha^{-1} phosphorus fertilizer and bacterial strain 9, which was not significantly different from co-application of bacterial strain 9 and Mycorrhiza treatment. Thus, it seems that to achieve an acceptable increase in grain protein, application of bio-fertilizer is preferred to chemical ones [13]. Reported that Phosphate solubilizing bacteria increase the amount of soil nitrogen and phosphorus. Geneva *et al*. [14] reported the positive effect of Mycorrhiza fungus on crop yield and quality, due to the increase in nutrient uptake, particularly phosphorus.

CONCLUSION

The obtained data showed that the application of sole bacteria strain 41 or its co-application with Mycorrhiza fungus significantly increased the forage and grain quality of barely even in absence of any chemical phosphorous fertilizer. 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 bio- 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 bio- fertilizers are one of the basic components of sustainable agriculture.

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