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Effect of *Mycorrhiza* and *Azotobacter* on Concentration of Macroelements and Root Colonization Percentage in Different Cultivars of Wheat (*Triticum aestivum* L.).

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ABSTRACT: To study effect of biofertilizers include *Mycorrhiza* and *Azotobacter* on concentration of nutrition elements and root colonization percentage in different cultivars of wheat (*Triticum aestivum* L.) An experiment was conducted in the crop years 2014-2015 in Lorestan Province, Iranas a factorial experiment in the form of a randomized complete block design with four replications. Effects of three factors were studied include Mycorrhiza, *Azotobacter* and Different Cultivars of Rainfed wheat. Four characteristics include percentage of root colonization, percentage of protein, nitrogen, phosphorus and potassium were studied during this experiment. Positive impact of biofertilizers was observed in most of the studied traits. The results showed that the effects of *Azotobacter* and cultivars was significant on all traits. Also interaction of *Azotobacter* × *Mycorrhiza* had significant effects on all studied traits. The results indicated effective function of bio-fertilizers in improving of plant growth characteristics and increasing of products quality of wheat cultivars under dry land conditions of Khorram Abad region.

Keywords: *Mycorrhiza, Azotobacter*, nutrient absorption, Wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important agricultural products which is most needed around the world (Smith *et al.* 2004). In order to increase agricultural production, unsustainable methods such as using of chemical fertilizers are using. As result of using these chemical inputs, environmental pollution especially soil and water pollution have increased during last decade which had catastrophic effects on human safety and respectively (AmirAbadi *et al.* 2009). Biofertilizers as a hopeful way in agricultural nutrition has been appeared in sustainable agriculture (Faheed and Abad-El Fatha 2008). Sustainable agriculture is an economical dynamic system which improve environmental protection and increase efficiency of using of natural resources for agricultural production. Also sustainable agriculture have important function in supplying of food sources and developing welfare in human society. One of the important key in sustainable agriculture is using of biofertilizers in agricultural systems which result to eliminate or reduction of applying chemical inputs (Sosana *et al.* 2006). Biofertilizers have significant effects on decreasing of plants Disease, development of soil structure, motivating of plant grow and increasing of quantity and quality of agricultural productions (Balemy *et al.* 2007).

Symbiosis of *Mycorrhiza* is one of the best known symbiotic systems in plants which is observable in most ecosystems and most of plants have at least one of types of *Mycorrhiza* (Ardekani *et al.* 2000). Using of *Mycorrhiza* symbiotic is an effective alternative method instead of applying chemical Phosphorus fertilizer because *Mycorrhiza* can increase the ability of plants to absorbing Phosphorus even in unavailable Phosphorus resources (Mukeriji and Chamola 2003). Results of a study about effects of Glomus fungus on wheat under water stress identified that this fungus increased Phosphorus, nitrogen, potassium and calcium in plants shoot and grain (Abo-Ghalia and Khalafallah2008). Also results showed higher amount of soluble proteins and total nitrogen of maize which were Inoculation with *Mycorrhiza*. Transaction of NO_3 via *Mycorrhiza* hyphae could increase of maize nitrogen components and activity of nitrogen stabilizer enzymes (Subramnian *et al.* 1997). A lot of types of soil bacteria and fungi have symbiotic life with plants which cause positive effects on ecosystems (Vessy 2003). *Mycorrhiza* have positive effects on plant ability for absorbing immobile nutrition elements special phosphorus. *Mycorrhiza* is a Multi-functional fungus in agricultural ecosystems which progress the physical quality of soil (with improvement of hyphae), the chemical quality of soil (with absorbing nutritional elements) and the biological quality of soil (with development of nutritional channels) (Cardose *et al.* 2006). Result showed which *Cymbopogon* (*Cymbopogon schoenanthus*) Inoculation with *Glomus* fungus had increasing effects on plant height, biological yield, root symbiotic percentage and phosphorus density in plant tissues (Ratii *et al.* 2001). Also reported that *Azotobacter* has ability to produce varies of side rophores and increasing of absorption capability of Zn, Fe and Mo and increasing of phosphorus Solubility from insoluble compounds which is one of the effective method to intensify nutrition mobility and absorption (Markovacki *et al.* 2001). *Mycorrhiza* Symbiosis with roots of *Mentha* have caused to intensify water and nutrition absorption important elements (K, P, N), increasing of photosynthesis and resulted positive effect on total yield. But most effect was observed on phosphorus absorbing. All of these improvement was due to contribution of large volume of external hyphae of *Mycorrhiza* which help to absorption of nutrition elements (Gupta *et al.* 2002). Glomus improved absorption of nitrogen, potassium, magnesium, copper and zinc in soils which were poor. *Mycorrhiza* increase depletion regions around roots which lead to increasing of root colonization and increasing of nutrition absorption (Smit and Read 2008).Increasing of root colonization can affect amount of soil moisture. One of the main reason of containing more moisture in the soil of farming systems with low chemical inputs is related to existence more colonization with their plants root (Gehring *et al.* 2006). The purpose of this experiment was to evaluate the concentration of important inorganic elements and the percentage of root colonization in different varieties of wheat effected by *Mycorrhiza* and *Azotobacter* inoculation.

MATERIAL AND METHODS

This experiment was conducted in the crop years 2014-2015 at the experimental field of Deh Bagher village near city of Khoram Abadin Lorestan Province, Iran (48° 19' E, 33° 29' N and 1170m above the sea level). The experimental design was a factorial experiment in form of randomized complete block design with four replications. Before conducting the experiment to determine the physical and chemical properties of soil samples were collected from 0-30 and 30-60 cm depth of soil (Table 1). During this experiment effects of three factors were studied: 1. Inoculation with *Mycorrhiza* in tow levels (M1= inoculation, M2= no inoculation), 2. Inoculation with *Azotobacter* in tow levels (A1= inoculation, A2= no inoculation) and 3. Different Cultivars of Rain fed wheat in three levels (V1: Sardari, V2: Kouhdasht, V3: Karim). The seeds were inoculated with mentioned biological compounds before culturing. The physiological and chemical characteristics include Root colonization percentage, seed protein content (%), seed nitrogen content (%), seed phosphorus content and seed potassium content were measured.

Table 1: Chemical features of soil at the experimental field.

Sample	PH	Ec(ds/m)	Oc (%)	N (%)	P(mg/kg)	K(mg/kg)	Mn(mg/kg)	Fe(mg/kg)
depth 0-30 cm	7.5	5.60	0.56	0.07	6.2	332	7.9	10
Depth 30-60 cm	7.2	0.54	0.57	0.08	5.9	301	7.01	7.6

RESULTS AND DISCUSSION

A. Root colonization percentage

Results of variance analysis (Table 2) showed treatments include *Mycorrhiza, Azotobacter*, Cultivars and also interaction of *Azotobacter* × *Mycorrhiza* had significant effects on percentage of root colonization ($P \leq 0.01$).Comparison of Means showed *Azotobacter* inoculation had highest colonization (27.31%) which is about 32% more than no inoculation treatment (20.61%). Also *Mycorrhiza* inoculation caused more root colonization (26.78%) in compare with free of *Mycorrhiza* samples (21.14%) which is about 26% highest. Maximum root colonization percentage was belonged to the Karim cultivar (30.35%) which was 23% and 77% highest that Kouhdasht (24.27%) and Sardari (17.06%) respectively (Table 3). The interaction between *Azotobacter* × *Mycorrhiza* had highest root colonization (29%) which showed 74%superiority rather than no inoculation treatment (16.41%) (Table 4). A high percentage of root colonization have two important aspects. First for accomplishment of maximum efficiency of potential of symbiosis systems it is necessary to make adequate strain of microbial fertilizer in soil rhizosphere region along with increasing of contamination between plant roots and symbiotic microbial to intensify biofertilizer efficiency. Also adequate amount of colonization is necessary for efficient interaction between fungus and plant especially when plant is in growing stage (Singh *et al.* 2000).This study showed that an appropriate percentage of root colonization of biofertilizers along with Karimcultivar have become a critical factor for nutrition absorption. The results also showed that organic fertilizers used in these tests have high potential to occupied cortex parts of host plant roots.

Results of the present study confirmed other reports in this subjects. Research reports have shown that the combined use of both biological fertilizers *Azotobacter* and *Mycorrhiza* on corn, increased percentage of root colonization 43.3% (Amir Abadi *et al.* 2009).

Also Shirzadi *et al.* (2013) reported that the effect of *Mycorrhiza* inoculation and *Azotobacter* on mint root colonization was significant ($P \leq 0.05$) (Shirzadi *et al.* 2013).The results of some investigation have also confirmed the relationship between the increasing of bio-fertilizers amount and increasing of root symbiotic efficiency (Hazarika *et al.* 2000; Ratti *et al.* 2001). The simultaneously inoculation of *Azotobacter* and *Mycorrhiza* in safflower Increased colonization of this plant and it seems using of different strains of bacteria along with Glomus fungus increased percentage of root colonization in safflower (Omidi *et al.* 2012).

B. Seed protein content

Based on analysis of variance (Table 2) effects of *Azotobacter* and interaction between Mycorrhiza× Cultivars were significant ($P \leq 0.01$) for seed protein percentage. Duncan analysis (mean comparison) identified inoculation with *Azotobacter* resulted to maximum seed protein percentage (13.91%) which is 47% more in comparison with no inoculation (9.40%). Also inoculation with *Mycorrhiza* resulted to 8% more of seed protein content (12.12%) rather than control sample (11.19%). Results about studied cultivars also revealed that Karim had maximum seed protein percentage (12.74%) which was 9% and 20% more than Kouhdasht (11.61%) and Sardari (10.61%) respectively (Table 3). Another experiments also showed that using of *Azotobacter* increased protein content in seeds (Carlelli 2002). Amiri *et al.* (2006) reported that application of *Azotobacter* in wheat have caused to a significant increasing in the percentage of protein contain of seeds rather than other treatments. This results is similar of Rajaee *et al.* (2007) which showed *Azotobacter* inoculation on wheat increased seed protein content.

C. Seed nitrogen content

The analysis of variances (Table 2) revealed that treatments include Mycorrhiza, *Azotobacter* and Cultivars had significant effects on seed nitrogen percentage ($P \leq 0.01$). Comparison of Means showed that inoculation with *Azotobacter* had highest nitrogen content (2.38%) which is about 47% more than no inoculation treatment (1.61%). Also *Mycorrhiza* inoculation resulted more seed nitrogen content (2.08%) in compare with control samples (1.92%) which is about 8% highest. Maximum seed nitrogen percentage was belonged to the Karim cultivar (2.18%) which was 11% and 19% highest rather than Kouhdasht (1.96%) and Sardari (1.82%) cultivars respectively (Table 3).

Table 2: Variance of analysis results for three studied factors.

S.O.V	**d.f**	**Root colonization** (%)	**Seed protein content** (%)	**Seed nitrogen content** (%)	**Seed phosphorus content** (%)	**Seed potassium content** (%)
R	3	2.20	4.14**	0.12**	0.003	0.003
A	1	538.27**	2243.72**	7.20**	0.02*	0.12
M	1	381.71**	10.32**	0.31**	0.01*	0.005**
V	2	709.41**	18.08**	0.53**	0.006**	0.006
A×M	1	91.74**	1.47	0.04	0.006**	0.008
A×V	2	28.66	1.71	0.05	0.01**	0.01
M ×V	2	5.57	0.21	0.007	0.005**	0.005
A×M×V	2	22.33	0.54	0.01	0.005**	0.005
E	33	9.07	0.63	0.01	0.0007	0.0007
Total	47	-	-	-	-	-
C.V	-	12.56	6.83	6.87	11.42	5.32

*, Significant at $P \leq 0.05$; **, Significant at $P \leq 0.01$. A: *Azotobacte*r, M: *Mycorrhiza*, V: Cultivar.

Table 3: Mean comparisons of main effects of characters.

Treatment	**Root colonization** (%)	**Seed protein content** (%)	**Seed nitrogen content** (%)	**Seed phosphorus content** (%)	**Seed potassium content** (%)
A_1	27.31 ^{a}	13.91 ^{a}	2.38 ^{a}	0.33 ^{a}	0.57 ^{a}
A_2	20.61 ^{b}	9.40 ^{b}	1.61 ^{b}	0.28 ^{b}	0.47 ^{b}
M_1	26.78 ^{a}	12.12 ^{a}	2.08 ^{a}	0.34 ^{a}	0.56 ^{a}
M_2	21.14 ^{b}	11.19 ^{b}	1.92 ^{b}	0.31 ^{b}	0.51 ^{b}
V_1	17.06 ^{c}	10.61 ^{c}	1.82 ^{c}	0.28 ^{b}	0.50 ^{c}
V_2	24.47 ^{b}	11.61 ^{b}	1.96 ^{b}	0.30^{ab}0.32 ^{a}	0.52 ^{b}
V_3	30.35 ^{a}	12.74 ^{a}	2.18 ^{a}		0.54 ^{a}

Means in a column followed by the same letter are not significantly different at 5% level. A: *Azotobacter*, M: *Mycorrhiza*, V: Cultivar.

Results of another experiment have shown which Individual application of *Azotobacter* have increased nitrogen concentration (35%) in shoot parts of wheat in compare with control (Khan *et al.* 2007). Another research showed *Azotobacter* consumption had positive impact on root growing and resulted 18% increasing of yield, along with 20%conserving of nitrogen (Kader *et al.* 2002). Increasing in seed nitrogen content in effect of *Azotobacter* is probably because of developing of roots absorption through the influence of fungus mycelium on the beneath of soil layers.

D. Seed phosphorus content

Based on variance analysis (Table 2) effects of *Azotobacter*, *Mycorrhiza*, Cultivar and triple action between *Azotobacter* × *Mycorrhiza* × Cultivars were observed significant (P?0.01) for seed phosphorus content. Results of mean comparison identified *Azotobacter* inoculation caused maximumseed phosphorus percentage (0.33%) which is 17% more in comparison with no inoculation (0.28%). Also inoculation with *Mycorrhiza* caused 14% more of seed phosphorus content (0.34%) rather than control sample (0.31%). Results about studied cultivars also revealed that Karim cultivar had maximum seed phosphorus percentage (0.32%) which was 6 and 14 percentage more than Kouhdasht (0.30%) and Sardari (0.28%) respectively (Table 3). Interaction between *Azotobacter* × *Mycorrhiza* had identified significant effects on seed phosphorus content as interaction treatments resulted 33% further phosphorus content (0.36%) while non-inoculated samples had less (0.27%) (Table 4). Also result of mean comparison of interaction between *Azotobacter* × *Mycorrhiza* × Cultivars revealed highest seed phosphorus percentage for interaction treatment with Karim cultivar (0.42%) which is 44% more than control (0.29%). Reports showed that application of *Mycorrhiza* fungi alone or together with bacteria significantly increased amount of phosphorus in a cultivar of strawberry (Reddy *et al.* 2003). Results of same study about two species of fennel (*G. marcrocarpum, G. fasiculatum*) showed that phosphorus content was significantly increased (15/1%) in treated samples with *Mycorrhiza* in comparison with non-inoculated treatments. This results suggested that *Mycorrhiza* symbiosis improving phosphorus in plant seeds by physical increasing of fungi hyphae in the soil pores. (Ratti *et al.* 2001; Kapoor *et al.* 2004). Also about coriander described that symbiosis with *Mycorrhiza* have increased nutrient elements especially phosphorus, which confirmed the results of this study (AliabAbadi *et al*, 2008).

E. Seed potassium content

The analysis of variances (Table 2) discovered *Mycorrhiza* treatment had significant effect on seed potassium percentage (P≤0.01). Comparison of Means showed that inoculation with *Azotobacter* had highest potassium content (0.56%) which is about 9% more than no inoculation treatment (0.51%) (Table 3). Arrigada *et al.* (2007) studied effect on 2 types of *Mycorrhiza* (*Glomus desrticola*, *Glomus mosseae*) on Eucalyptus which was cultured with soybean. Results of this study showed that symbiosis between roots of *Eucalyptus* and *Glomus desrticola* resulted more seed potassium content (1.52%) in comparison with free *Mycorrhiza* samples. In another experiment on an American native Clover (*Desmodium paniculatum*) was identified that Root inoculation with *Mycorrhiza* (*Glomus intradices*) caused increasing of seed potassium percentage (1.78%) in compare with non-inoculation samples (0.8%).

Table 4: Mean comparison of interacation effect of characters.

Treatment	**Root colonization** (%)	**Seed protein content** (%)	**Seed nitrogen content** (%)	**Seed phosphorus content** (%)	**Seed potassium content** (%)
A1 M1	29.00 a	14.19 a	2.44 a	0.36 a	0.59 a
A1 M2	25.87 b	13.62 a	2.33 b	0.30 b	0.55 b
A2 M1	24.81 b	10.04 b	1.72 a	0.29 b	0.47 c
A2 M2	16.41 c	8.76 c	1.50 d	0.27 c	0.47 c
A1 V1	18.87 c	12.49 c	2.14 c	0.31 b	0.58 b
A1 V2	28.45 b	14.02 b	2.41 b	0.31 b	0.55 b
A1 V3	34.61 a	15.21 a	2.61 a	0.36 a	0.58 a
A2 V1	15.25 d	8.74 e	1.50 e	0.25 c	0.42 d
A2 V2	20.50 c	9.20 e	1.58 e	0.30 b	0.49 c
A2 V3	26.08 b	10.27 d	1.76 d	0.29 b	0.50 c
M1 V1	19.25 c	10.96 c	1.88 c	0.29 bc	0.53 ab
M1 V2	27.29 b	12.19 b	2.10 b	0.32 ab	0.52 b
M1 V3	33.70 a	13.21 a	2.26 a	0.35 a	0.55 a
M2 V1	14.87 d	10.27 c	1.76 d	0.27 c	0.47 c
M2 V2	21.56 c	11.03 c	1.89 c	0.28 c	0.52 b
M2 V3	26.99 b	11.27 b	2.10 b	0.30 bc	0.54 ab

Means in a column followed by the same letter are not significantly different at 5% level. A: *Azotobacter*, M: *Mycorrhiza*, V: Cultivar.

Researchers explained which the increasing of seed potassium content in Clover is related to improvement of symbiotic relation between plant and *Mycorrhiza* which caused growing of fungus hyphae in the soil and increasing of nutrition absorption (Shockley *et al.* 2004).

CONCLUSION

Positive impact of biofertilizers was observed in most of the studied traits. All studied characteristics include root colonization percentage, percentage of protein, nitrogen, phosphorus and potassium increased after using of biofertilizers. Results of this study identified that applying of *Mycorrhiza* and *Azotobacter* lead to aggregation of organic material in soil, plant root development and more access to nutrient elements. In order to the present study it concluded that the application of *Azotobacter* of and *Mycorrhiza* had positive effect on absorption nutrient elements by plant. Due to its ease of use as well as the low prices and positive effects on yield, application of biofertilizers is one of the best sustainable methods in agriculture.

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