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EVALUATING THE EFFECTS OF BIOFERTILIZERS ON GROWTH AND EMERGENCE OF BARLEY (*HORDEUM VULGARE*)

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ABSRACT

In order to study the effects of some biological fertilizers on growth parameters of three barley cultivars, a factorial experiments with three replications were performed in pots (using natural field soil, pH=8) as completely randomized design at the Research Greenhouse of Fars Science and Research Branch of Islamic Azad University. Three barley cultivars namely; Yousef, Nosrat and Reihan, and six different biological fertilizers: Phosphotidic Barvar 2, Barvar 3, Nitrokara, Nitroxin, Biophosphorus, EM and control (non-inoculated seeds) were used. The investigated traits, including morphological characters: percentage of emergence, shoot length, volume of seminal roots, and wet and dry weight. Results showed that the application of biological fertilizers had significant effects on all traits except for shoot length. The highest averages for these traits were obtained from Nosrat cultivar and the most effective biological fertilizers with respect to these traits were Barvar 2. It seems that, in practice, biological fertilizers have the required potential to replace the chemical (synthetic) fertilizers.

Keywords: Biological Fertilizers, Morphological Characters, Barley Cultivars

INTRODUCTION

Biological fertilizers have long been used in agriculture; however, the scientific exploitation of this type of resources doesn't have a long history. Although the application of these fertilizers has been decreased in the last few decades, today, they has restated their use in agriculture in respect to problems which have resulted from excess consumption of chemical fertilizers (Chen et al., 2005). Moreover, it is being tried to use organic materials and soil organisms in order to maximize the production regarding soil quality and environmental safety and hygiene (Mo'alem and Eshghi Zadeh, 2007).

Today, biological fertilizers are accounted as a substitute for chemical fertilizers by purpose of increasing soil fertility and product yield in stable agriculture (Wu et al., 2005). Using biological fertilizers leads to not only increase in population and activities of beneficial soil micro-organisms, but also provides plants required food elements such as nitrogen and phosphorus, and causes improvement in growth and yield of products (Arancon et al., 2004).

In an experiment, Darzy et al. (2006) reported that bio-phosphate fertilizer effects significantly on fennel biological yield and height, and also there are significant interaction effects between *Mycorrhiza* and *bisphosphonate* on seed weight. Sharifi and Hagh Nia (2007) stated that biological Nitroxin fertilizer effects on wheat yield and yield components of Sabalan cultivar, so that this fertilizer has a positive effect on seed and straw yield, plant height, spike length, number of seeds in spike, and number of spikes in square-meter (m^2).

The set of bacteria existing in biological fertilizer of nitrogen with properties such as dissolution of phosphorus in soil, secretion of various types of growth stimulating hormones, natural enzymes, different types of anti-biotic, and compounds like Siderophores and volatile gases causes root growth, plant aerial parts development, tolerance against pathogens, and nematods attacks (Astaraei and Kochaki, 1996; Mohammadian, 2003). Hussain et al, (2010) believes the effect of EM fertilizer on wheat growth is positive and it is more effective if it is accompanied by green manure. Certain researchers such as Daly and Stewart (1999), Yau and Xu (2002), Javid (2006) and Khaligh et al (2006) reported that consuming EM fertilizer causes an increase in growth and yield of crop plants, however, other researchers such as Jawid et al (2008) and Bajwa et al (1999) stated that consuming EM fertilizer has no/even negative effect on growth and yield of crop plants. Hence, since some researchers don't believe in the positive effect of biological

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fertilizers on crop plant growth, and on the other hand, there is little research on the effects of biological fertilizers on barley plant, this experimental study the effects of several types of biological fertilizers of some morphological characters of the barley plant.

MATERIALS AND METHODS

This study was conducted in Islamic Azad University research greenhouse, Fars Sciences and Research branch located in Iran. Measured traits included morphological parameters such as: emergence percentage, shoot length, volume of seminal root, wet and dry weight. The type of trial design was a factorial test based on a completely random design. The test parameters included various types of seed (in three levels) and fertilizers (in 7 levels, one as a control) which were performed in three replications. Collecting data via Excel & SAS software, it was analyzed; the comparison of means was done by Duncan's test in 1& 5 percent significance level.

The required barley seed for this test was prepared from Fars-Zarghan agriculture research center, and three modified barley varieties with names of Yousef, Reihan and Nosrat were provided. Biological fertilizers (6 types) which were provided by these fertilizers manufacture centers, including: Phosphotidic Bavaria 2, Bavaria 3, Nitrokara, Nitroxin, Biophosphorus, and EM. The method of applying biological fertilizers in this test was seed-spreading (this method was practiced based on the guide). The required soil for pot culture was prepared from farms and fields around the university (Table 1).

After inoculation of seeds with fertilizers, ten seeds were planted in each pot in 2 cm depth. After emergence of sprouts, five strong sprouts were maintained and then the rests were discarded. Pot irrigation was adjusted so that plant neither encounter drought stress nor water immersion. For this reason, soil moisture was maintained based on limit of field capacity (F. C). To determine emergence percentage, the pots were controlled after 48 hours and emerged plants were numbered at certain times (every 18h) and the percentage of emergence was counted. For other morphological characters, plants were harvested and transferred to the laboratory, after 21 days. A mm Ruler, a 250 cc Erlen Dish, and an Analog Digital Scale by 0.001 g accuracy (A & D, Made in Japan) were applied to measure the short length, root volume, wet and dry weights of samples, respectively. After measuring wet weight, samples were put in an oven at 75°C for 48 hours, and then dry weight was measured.

RESULT AND DISCUSSION

1- Emergence percentage

Variance analysis of emergence percentage and also means comparison with Duncan's test (in significant level of 1 & 5%) showed that there wasn't any significant interaction effect between cultivar and fertilizer, although a significant effect had been observed in 1% level among fertilizers and cultivars (table 2). The study of considered characters, the highest average of seed cultivar and biological fertilizer related to Nosrat and Barvar are 2, respectively (Tables 7 & 8). In other research, the results of Rezvani Moghadam et al. (2010), suggested that application of biological fertilizers in wheat, didn't result in any significant effect on emergence percentage, but cultivar type and interaction effects of fertilizer and cultivar on this character showed significant effects. Krishna et al. (2008) reported that the biological fertilizers affect on the rate of pharmaceutical plant emergence such as *Somniferum and Ocimum sanctum* to be positive, and they also considered the use of Azospirillum biological fertilizers, phosphate-dissolution bacteria, Azetobacter of Nitrogen-fixing bacteria, and their compounds in *Withania somniferum* and *Ocimum sanctum* plants to be effective to improve percentage and rate of germination and root and shoot length. DE Freitas and Germid (1989) found that two strains of growth-additive bacteria in Fertility soil increased the wheat emergence significantly. Siqueira et al. (2009) found that there wss a positive effect of EM fertilizer on seed germination of plants such as tomato, pea, cucumber, carrot, bean and corn.

2- Volume of Seminal root

Variance analysis of volume of seminal root and also comparison with Duncan's test (i 1 & 5% significance level) demonstrated that the interaction effect of cultivar and fertilizer and also among fertilizers was 1% of significance level, but any significant effect among seed cultivars has not been observed (Table 3). In the study of considered parameters, the highest

mean of seed cultivar and biological fertilizer is related to Reihan cultivar and Nitrokara, respectively (Table 7 & 8). In other research, Bashan et al. (1989) showed that inoculation of cereal with Azospirillum caused an increase in volume and number of roots which was related to increase in growth hormones and also proton secretion. The results of Rezvani Moghadam et al. (2010) also demonstrated that application of biological fertilizer in wheat resulted in a significant effect on the volume of seminal roots and there wasn't observed any significant effect among cultivars.

Joshi et al. (2007), in a study of plate pharmaceutical plants (*Integrifolia scutellaria*), stated that inoculation of this plant root was effective, especially in root growth and also increased the plant's ability to grow in marginal soils which encountered with lack of phosphorus. The investigation of Hazarika et al. (2000), which was conducted on a tea plant (*Camellia sinensis*) in farmland conditions showed that application of one strain of phosphate-dissolution bacteria in the presence of mineral phosphate stone caused an increase in biomass and root colonization percentage.

3- Wet and Dry weight

Variance analysis of wet weight and also means comparison with Duncan's test (1 & 5% level) showed that there wasn't any significant interaction effect between cultivar and fertilizer, although a significant interaction effect has been observed at significance level of 1% among fertilizers and cultivars (Table 4). In the study of considered characters, the highest average of seed cultivar and biological fertilizer were related to Nosrat cultivar and Barvar-2 fertilizer, respectively (Tables 7 & 8). Also, any significant interaction effect between cultivar and fertilizer for dry weight has been observed, although a significant effect at 1 and 5 percent has been observed among fertilizers and seed cultivars, respectively (Table 5). In the study of considered characters, the highest mean of seed cultivar and biological fertilizer was related to Nosrat and Barvar-2, respectively. In other research, in a study which was conducted on barley plant, it was determined that application of compost caused a significant improvement in biologic yield (Kumawat et al., 2006). The results of Rezvani Moghadam et al. (2010) also demonstrated that application of biological fertilizer in wheat, didn't result in any significant effect on dry weight, but it had a significant effect on cultivar type and an interaction effect between fertilizer and cultivar. Ammo Aghaei et al. (2003) stated that contamination of wheat with Azospirillum had enhanced dry weight of root and stem as compared with control. The investigations showed that inoculation of pharmaceutical plant of lemon grass (*Cymbopogon martini*) with Mycorrhiza Fungus species (*Glomus aggregatum*) caused a significant increase in biological yield and root symbiosis percentage (Ratti et al., 2001). Nanda et al. (1995) stated that inoculation of corn seeds with Azospirillum biological fertilizers and Azetobacter caused a significant increase in forage yield. In a research in India, Mahshavari et al. (2000) concluded that phosphorus chemical fertilizer and biological fertilizers have no significant effect on yield and yield components in pharmaceutical fleawort plants. Valverde et al. (2006) demonstrated that the compound of Rhizobia modulating chickpea [C- 212] in the chickpea plant with phosphate solubilizing bacterial caused a decrease in dry weight as compared with use of only strain c2/2, and no significant effect on plant grow was observed in inoculated with *Pseudomonas Jensen* (ps06) bacteria. Mikhailouskaya and Bogdevitch (2009) showed that the effects of biological fertilizers (Azotobacter) caused an increase in protein and Amino-Acid improvement in barley seeds. Mehrvarz and Chaichi (2008) reported that applying phosphate solubilizeing bacterial caused a decrease in Neutral detergent fibers= NDF such as lignin cellulose and hemi-cellulose in barley plant. Canbolate et al. (2006) showed that inoculation of barley seeds with Rhizobacteria PGPR (Plant Growth Promoting) caused 9-12% increase in root weight and 29-43% increase in stem weight as compared with control.

4- Shoot length

Variance analysis of shoot length and also mean comparison with Duncan's test (i 1 & 5% level) shows that there isn't any significant interaction effect between cultivar and fertilizer and also among fertilizers, although a significant effect has been observed in 5 percent level among cultivars, statistically. In the study of considered characters, the highest mean of seed cultivar and biological fertilizer were related to Nosrat cultivar and biophosphorus fertilizer, respectively (Tables 7

& 8). In another research, the results of Rezvani Moghadam et al. (2010) also demonstrated that biological fertilizer application in wheat resulted a significant effect on root and shoot length, but no significant effect was observed among cultivar types. In a study, which was conducted by Khoram Del et al. (2008), it was shown that inoculation of Nigella seeds with biological fertilizer caused a significant increase in plant height, leaf area index, maximum dry matter accumulation and plant growth rate as compared with control.

Kader et al. (2002) reported a beneficial effect of Azospirillum on shoot length, which was attributed to production of growth stimulating hormones such as auxin, gibberellin and cytokinin. In the study of biological fertilizer inoculation and various amounts of chemical fertilizers on yield and yield components of dry farming lentil production, the results showed that except for plant height, there were significant effects among other studied characters in 1% level (Asghar Zadeh, 2008). Generally, the experimental results showed that application of biological fertilizers resulted in a significant effect between biological fertilizers and control in measured traits except for shoot length. The highest average for most of the studied traits was related to Nosrat cultivar and the most effective fertilizer was Barvar-2 fertilizer. With respect to this point that in this study the method of applying fertilizer was seed- spreading, it is suggested the study be replicated considering the effect of these fertilizers as the spray solution to plant and soil in various plant growth stages and in farm conditions. Generally, it can be hoped to the positive effects of these fertilizers and their replacement with chemical fertilizers via more investigations in future.

Table 1. Analysis of soil

Moisture (%)	Silt (%)	Gravel (%)	Clay (%)	Texture
46	56	17	27	Silty loam
Potassium & phosphorus		Lime, EC & pH		
K mg/lit	P mg/lit	Lime (%)	EC ds/m	pH
424.2	11	33.5	0.58	8.11

Table 2. Variance analysis of emergence percentage

F	S^2	SS	df	Variation
5.17 **	641.778	1283.556	2	Cultivar
3.87 **	480.444	2882.667	6	Bio fertilizer
0.63 ^{ns}	78.667	944.000	12	Interaction
	124.190	5216.000	42	Error
		10326.223	62	Total

Table 3. Variance analysis of seminal root volume

F	S^2	SS	df	Variation
1.70 ^{ns}	0.007	0.014	2	Cultivar
3.69 **	0.016	0.094	6	Bio fertilizer
3.31 **	0.014	0.169	12	Interaction
	0.004	0.179	42	Error
		0.456	62	Total

Table 4. Variance analysis of wet weight

F	S^2	SS	df	Variation
7.02 **	6.379	12.758	2	Cultivar
6.30 **	5.726	34.361	6	Bio fertilizer
1.57 ns	1.429	17.152	12	Interaction
	0.908	38.150	42	Error
			62	Total

Table 5. Variance analysis of dry weight

F	S^2	SS	df	Variation
3.47 *	0.040	0.080	2	Cultivar
5.77 **	0.067	0.402	6	Bio fertilizer
1.42 ns	0.017	0.198	12	Interaction
	0.012	0.198	42	Error
		1.168	62	Total

Table 6. Variance analysis of shoot length

F	S^2	SS	df	Variation
4.18 *	26.464	52.928	2	Cultivar
1.78 ns	11.296	67.776	6	Bio fertilizer
0.98 ns	6.186	74.236	12	Interaction
	6.332	265.933	42	Error
		460.874	62	Total

Table 7. Comparing traits mean in response to barley cultivar (Duncan's test)

Traits Cultivar	Emergence (%)	Seminal root volume (cm^3)	Wet Weight (gr)	Dry weight (gr)	Shoot length (cm)
Yousef	51.143 ab	0.29048 a	4.3650 b	0.32905 a	1.23476 a
Nosrat	56.286 a	0.30381 a	5.2420 a	0.41257 a	1.27619 a
Reihan	45.238 b	0.32714 a	4.2311 b	0.34819 a	1.23190 a

Table 8. Comparing trait mean in response to bio fertilizer (Duncan's test)

Trait Bio fertilizer	Emergence (%)	Shoot length (cm)	Seminal root volume (cm^3)	Wet weight (gr)	Dry weight (gr)
Control	56.667 a	29.900 a	0.318 ab	4.994 a	0.326 bc
Barvar 2	61.556 a	29.956 a	0.298 b	5.189 a	0.489 a
Barvar 3	51.778 ab	31.322 a	0.318 ab	4.959 a	0.373 ab
Nitrokara	38.667 b	28.522 a	0.389 a	2.971 b	0.211 c
Nitroxin	46.444 ab	30.100 a	0.292 b	4.147 ab	0.342 abc
Bio p.	51.778 ab	31.689 a	0.277 b	5.032 a	0.419 a
EM	49.333 ab	31.378 a	0.259 b	4.977 a	0.381 ab

**, *: 1&5 Significant Level, Means followed by the same letters in each column is not significantly different (Duncan's Multiple Range test).
ns: Not Significant, df: degree of freedom, SS: sum of square, S^2: variance, F: Treatment variance/Error variance
Bio P.: Biophosphorus, EM: Effective Microorganism

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