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ORIGINAL ARTICLE

Effects of Vermicompost , Mycorrhizal Symbiosis and Biophosphate Soulbilizing Bacteria on some Characteristics related to Chickpea Root Growth under Autumn in the Dryland Condition

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ABSTRACT
Use of biological fertilization is a useful method to feed plants under different conditions including stress. However, the method must be tested to increase its efficiency. Accordingly, a field experiment was performed in 2009-2010 in the Agricultural Research Station of Lorestan province to evaluate the effects of biological fertilization on the root and yield properties of chickpea (Cicer arietinum L.) under rainfed conditions. The experimental treatments of mycorrhizal fungi (Glomus intraradices), biological phosphorous (P) (Pseudomonas striata) and vermicompost (0, 6 and 12 T/ha) were tested. Different plant parameters including root properties (main and root hairs) and grain yield were significantly affected by the treatments. The combined use of the fungi and the bacteria with vermicompost resulted in the highest grain yield (3105 and 2810.7 kg/ha, respectively) and improved root properties. Accordingly, it may be likely to produce plants with favorable architecture using the biological fertilization treatments. For example, if longer main roots are desirable (under rainfed conditions) use of mycorrhizal fungi may be more recommendable. However, if plants with higher number of lateral roots are favorable use of P solubilizing bacteria may be more suitable.
***Key words**: biological phosphorous, chickpea (Cicer arietinum L.) root architecture, grain yield, mycorrhizal fungi, vermicompost*

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INTRODUCTION

The use of biological fertilization is a safe and economical method to provide plants with different nutrients. Although chemical fertilization is a fast way of supplying plants with nutrients, at high rates, it may not be beneficial environmentally and economically. Accordingly, the use of biological fertilization including the use of soil microbes and organic medium such as manure and compost has been suggested as a useful method of fertilization [1-2-3-4].

Biological fertilization, which is the use of soil microbes, can significantly affect plant growth including roots. It is not usually easy to study root growth, because such kind of research is difficult and expensive, however, evaluating some root traits such as root length, area and volume is required [5] to determine plant response to the stress. Nevertheless, there has been some interesting field and greenhouse research related to the growth and activity of plant roots under different conditions. There are environmental and genetic parameters controlling root growth and development [6-7]. The important role of plant roots to alleviate drought stress has been indicated. During the growing season the soil surface with a higher root density may be dry, however, the plant is able to survive due to the deeper plant roots, which are able to absorb water for plant growth [8].

Under stressful conditions including drought, plant allocates more photosynthate (carbon) to the roots to increase root to shoot ratio and hence plant ability to absorb more water and handle the stress [9-10-11-6]. Accordingly, some morphological changes are resulted and root length may increase [12-10-6]. Plants with larger root length, higher number of root hairs, denser root and higher root to shoot ratio are more tolerant to stress [5].

Mycorrhizal fungi, which are symbiotic to most plant species, are able to significantly increase plant ability to absorb water and nutrients by their extensive hyphal network. This may result in higher root dry weight, and hence plant growth, which can be very advantageous under stress [13]. The alleviating effects of mycorrhizal fungi and some of the related mechanisms under stress have been indicated by researchers [14-15]. Increased root growth of mycorrhizal plants, including the main and the lateral roots have been shown under stress. Miransari et al. [9-10] indicated that the mycorrhizal fungi enhanced the growth of corn main roots under stress.

Plant growth promoting rhizobacteria (PGPR) including rhizobium are also able to increase plant growth under different conditions including stress by the following mechanisms: 1) increased plant nutrient uptake by production of different enzymes, 2) production of plant hormones, 3) increased root growth, 4) alleviating stress by production of enzymes such as ACC deaminase, 5) controlling the pathogens, etc. [16-17-18-19].

The use of PGPR as a source of biological fertilization has been proved to be very effective on the growth of plant. Among the important mechanisms, utilized by PGPR to increase plant growth is the production of different enzymes and organic acids, increasing the solubility of insoluble nutrients such as phosphorus (P) [20-18-21]. Hence, the increasing the presence of PGPR such as *Pseudomonas* sp. in plant rhizosphere can be a useful tool to enhance plant ability to absorb higher rate of nutrients. Accordingly, we examined the effects of *Pseudomonas striata* on the growth of chickpea under rainfed conditions.

Vermicompost can also be used as a source of organic fertilization to increase plant growth. Vermicompost is usually produced by treating organic matter with earthworm species. As a result a suitable source of nutrients with important benefits on plant growth is produced. According to Roy and Singh [22] addition of vermicompost to the soil increased the availability of nutrients and improved soil properties and hence increased plant growth and yield.

In this research work we examined the effects of different biological and organic fertilization and their combination on the growth and yield of chickpea under rainfed conditions. The effects of *Glomus intraradices*, *Pseudomonas striata* and vermicompost were tested and grain yield and root morphological parameters of chickpea were determined. We were looking to quantify root response under stress to understand how the use of biological fertilization may improve plant growth under such conditions.

MATERIALS AND METHODS

The experiment was performed in the 2009-2010 growing season in the Agricultural Research Station of Lorestan province, Iran, located in the northern latitude of 33^o and 29' and eastern altitude of 48^o and 18', 1175m above the sea level. The average rainfall and temperature in the region is 409.9 mm 17.5 ^{o}C, respectively. Using standard laboratory methods, properties of soil and vermicompost samples were determined (Table 1) [9-11].

Chickpea (*Cicer arietinum* L.) Azad variety, which is recommendable for fall planting in the region, was used. The experiment was a three way factorial (2x2x3) on the basis of a completely randomized block design with four replications. The experimental treatments including mycorrhizal fungi (M1= control and M2= inoculated) and biological phosphorous (P1= control and P2= inoculated) and vermicompost (V1= control, V2= 6 and V3= 12 T/ha) were tested.

The mycorrhizal inoculum of *Glomus intraradices* including the active organs of spore, hyphae and roots, supplied by Soil and Water Research Institute (Karaj, Iran), was used for the experiment. The P fertilization treatment verified by Soil and Water Research Institute was a rock phosphate treated by the P solubilizing bacteria, *Pseudomonas striata*. The vermicompost treatment used in the experiment was manure fertilizer treated with the earthworm species of *Eisenia foetida*, and was supplied by Behsaman Co., Karaj, Iran. The experimental field had been uncultivated for years.

The experimental plots (1m apart), measuring 1.5 x 6 m with 5 rows (30 cm apart), and replications, which were 1m apart were used for the experiment. The seeds were planted after the autumn rainfall when the field moisture was suitable. Vermicompost was broadcast on the soil surface and was mixed with the soil thoroughly. Before seeding chick pea seeds were treaded with mycorrhizal inoculum and P solubilizing bacteria using glue. The plant density of 60 plant /m^2 was used in 7-cm deep furrows. Weeds were controlled twice during the season using hand and mechanically. To determine the properties of plant roots, for each sample plant, two samples were used, one collected from the rows and one collected from between the rows using a cylinder with 5cm diameter and 20 cm height.

Plant root was measured using a ruler. To determine root total length (cm), root weight (g) was multiplied by 0.89. Root total surface area was measured using the following formula:

Root total surface area (cm^2) = 2 x (root volume x (3.14) x root total length (cm)). Because the highest number of nodules is produced at the onset of flowering, 5 plants were collected from each plot and after washing the roots with water the number of nodules/plant were determined.

After measuring root dry weight, root volume was determined by immersing the roots in a cylinder of water with a certain volume. Root density was calculated using the ratio of root total length to the soil volume of a cylinder with 5 cm diameter and 20 cm height. At maturity a length of 3m from the middle of each row with a 2.7 m^2 area was harvested and after drying, and weighing, grain yield was determined. Data were anlaysed using MSTATC, SAS and SPSS and means were compared using Duncan's multiple range comparison.

RESULTS

The effect of mycorrhizal fungi was significant on grain yield (P= 0.01), number of nodule (P= 0.05) and root length (P= 0.01). Grain yield (P= 0.05), root volume (P= 0.05), root wet weight (P= 0.05), root surface area (P= 0.01) and density (P= 0.05) were significantly affected by P solubilizing bacteria. The interaction effect of fungi and bacteria was only significant on grain yield (P= 0.05). Interestingly, vermicompost significantly affected all the experimental parameters. Grain yield was the only parameter, which was significantly affected by the interaction effect of fungi and bacteria with vermicompost (Table 1).

Mean comparison indicated that there was a significant difference between the control (1803.4 kg/ha) and mycorrhization (2497 kg/ha) treatment on the amount of yield. The bacteria and vermicompost (V3) treatments had a significant effect on grain yield by a 16 and 19% increase, respectively related to the control. A maximum number of 20.6 and 21.7 nodule number was resulted by the fungi and vermicompost (V3) significantly different from the control at 17.2 and 17.3, respectively (Table 3).

While the fungi did not affect root volume and wet weight, both the bacteria and V3 had significant effect on these parameters. Root length was significantly increased by the fungi and V3 with a significant increase of 16.4 and 24%, respectively. Root surface showed a significant response to the bacterial (35% higher) and vermicompost (V3, 71% higher) treatments compared with control. Just V3 significantly increased root density by 38% related to the control treatment (Table 3).

Table of interactions indicated that the combined use of mycorrhizal fungi and vermicompost treatment had the highest effect on chickpea grain yield as the amount of 3105.8 kg/ha was resulted significantly different from the control treatment. It was followed by the interaction effects of bacteria and vermicompost (2810.7 kg/ha) and fungi and bacteria (2779.3 kg/ha). However, the highest number of root nodule was related to the interaction effect of bacteria and vermicompost (23) followed by the interaction effect of fungi and vermicompost (21.8) and fungi and bacteria (21.4) (Table 4).

Root volume (5.9 cm^3) was the highest by the interaction effect of fungi and vermicompost, however not significantly different from the interaction effect of bacteria and vermicompost (5.8 cm^3) and fungi and bacteria (5.3 cm^3). The highest root wet weight was resulted by the combined use of bacteria and vermicompost (8.7 g), followed by the interaction effect of fungi and vermicompost (7.3 g) and fungi and bacteria (7.1g) (Table 4).

Root length was the highest by the interaction of fungi and vermicompost (24.3 cm) significantly different from the interaction of bacteria and vermicompost (20.9 cm). The combined use of bacteria and vermicompost resulted in the highest root surface area at 144.9 cm^2, followed by the interaction of fungi and vermicompost (120.1 cm^2) and fungi and bacteria (106.1 cm^2). A similar trend was also resulted for root density (Table 4).

Table 1. Soil and vermicompost chemical properties

Sample	pH	EC (dS/m)	O.C. (%)	N (%)	P (mg/kg)	K (mg/kg)	Mn (mg/kg)
Soil	7.7	0.82	1.03	0.09	8.2	340	-
Vermicompost	7.8	5.7	7.2	1.64	0.81	0.7	424

Table 2. Analyses of variance presenting the effects of biological fertilization on chickpea yield and root properties.

S.O.V	d.f.	Grain yield	Number of root nodule	Root volume	Root wet weight	Root length	Root surface area	Root length density
Rep	3	1011280.1ns	60.5ns	0.556ns	5.65ns	44.5ns	1924.6ns	0.102ns
M	1	577300.8**	143.4*	0.333 ns	1.25 ns	111.02**	438.9 ns	0.069 ns
P	1	1232787.1*	100.6ns	6.75*	9.55*	13.02ns	9282.9**	0.215*
M x P	1	714514.7*	25.96ns	2.08ns	0.008ns	0.52ns	519.7ns	0.005ns
V	2	653605.4*	95.2*	5.81**	18.7**	85.1**	12687.6**	0.413**
M x V	2	1861259.3**	34.99ns	0.27ns	5.08ns	0.646ns	776.2ns	0.094ns
P x V	2	1861259.3**	34.99ns	0.27ns	5.08ns	0.646ns	776.2ns	0.094ns
M x P x V	2	569924.2ns	77.24ns	0.27ns	1.6ns	5.02ns	319.2ns	0.025ns
error	33	204301.3	30.91	1.01	2.21	11.57	917.4	0.044

Rep: replication, M: mycorrhizal inoculation, P: P bacterial inoculation, V: vermicompost Ns, *, **: significant at 5 and 1% level of probability.

Table 3. The single effects of biological fertilization treatments on chickpea yield and root.

Treatment	Grain yield (kg/ha)	Number of root nodule	Root volume (cm^3)	Root wet weight (g)	Root length (cm)	Root total area (cm^2)	Root length density (cm/cm^3)
M1	1803.4a	17.2b	4.9a	6.3a	18.8b	89.4a	0.014a
M2	2497a	20.6a	5.1a	6.7a	21.9a	95.4a	0.015a
P1	1989.1b	17.4a	4.6b	6b	20.8a	78.5b	0.014a
P2	2310.4a	20.4a	5.3a	6.9a	20.03a	106.3a	0.016a
V1	1980.2b	17.3b	4.5b	5.7b	18.5b	72.9b	0.013b
V2	2096.6ab	17.7b	4.8b	6.02b	19.6b	79.6b	0.014b
V3	2373.7a	21.7a	5.7a	7.4a	22.9a	124.8a	0.018a

M1: control , M2: mycorrhizal treatment, P1: control, P2: P bacterial treatment, V1: control,
V2: 6 T/ha vermicompost, V3: 12 T/ha vermicompost
Means followed by the same letter in the same column are not statistically different

Table 4. Mean interactions of different treatments of biological fertilization on chickpea yield and root.

Treatment	**Grain yield (kg/ha)**	**Number of root nodule**	**Root volume (cm^3)**	**Root wet weight (g)**	**Root length (cm)**	**Root total area (cm^2)**	**Root length density (cm/cm^3)**
M1P1	1765.1c	14.9b	4.3b	5.9b	19.2b	72.2b	0.013a
M1P2	1841.6bc	19.3ab	5.5a	6.7ab	18.4b	106.6a	0.015a
M2P1	2214.7b	19.9a	4.9ab	6.2b	22.5a	84.8ab	0.014a
M2P2	2779.3a	21.4a	5.3a	7.1ab	21.3ab	106.1a	0.016a
M1V1	1755.4bc	14.5b	4.6b	5.2c	16.7c	68b	0.012a
M1V2	2013.1bc	15.3b	4.63b	5.5c	18.2bc	70.8b	0.013a
M1V3	1641.6c	21.7a	5.5ab	8.2a	21.5ab	129.4a	0.019a
M2V1	2205.1b	20.1ab	4.5b	6.2bc	20.3bc	77.9b	0.014a
M2V2	2180.1b	20ab	4.9ab	6.5bc	21ab	88.4b	0.015a
M2V3	3105.8a	21.8a	5.9a	7.3ab	24.3a	120.1a	0.016a
P1V1	1802.3b	16.3b	4c	5.7b	18.7b	62.7c	0.013a
P1V2	2230.8b	15.7b	4.4bc	5.6b	18.9b	68.4c	0.013a
P1V3	1936.7b	20.4ab	5.5a	6.8b	25a	104.5b	0.016a
P2V1	2158.2b	18.3ab	5.1ab	5.8b	18.3b	83.2bc	0.013a
P2V2	1962.5b	19.7ab	5.1ab	6.4b	20.4b	90.8bc	0.014a
P2V3	2810.7a	23a	5.8a	8.7a	20.9b	144.9a	0.20a

M1: control , M2: mycorrhizal treatment, P1: control, P2: P bacterial treatment, V1: control,
V2: 6 T/ha vermicompost, V3: 12 T/ha vermicompost
Means followed by the same letter in the same column are not statistically different

DISCUSSION

The effects of biological fertilization on chickpea root and yield properties were investigated in this research work. Some interesting results have been obtained, which clearly show the enhancing effects of different biological treatments on the root and yield of chickpea under rainfed conditions. Such results can be useful for the production and use of biological fertilization under different conditions including stress.

The results of this research work indicated that both the fungi and the bacteria are effective treatments for use under rainfed conditions. However, they may be used under different conditions if they are used singly. If they are used combined their appropriate rate must be determined [23]. According to the results the fungi were able to significantly increase the length of the main root, which is in agreement with the result of Miransari et al. [9-10]. According to Millet et al. [24] *G. intraradices* is able to produce lipochitooligosaccharides (Myc factors) acting as signal molecule during the establishment of symbiosis between the fungi and the host plant. Such molecules are able to induce morphological and physiological changes in the host plant including the increase of root length and number.

The soil microbes tested in this research work may significantly increase P uptake under different conditions including stress. Plant may utilize different morphological strategies to alleviate P deficiency under stress including the alteration of root hair, number, root elongation, increased root/shoot ratio, branching of roots, enhanced root density and root cluster growth [25-5-7-26]. There is a set of complicated interactions between plant morphology and physiology to alleviate P deficiency under different conditions including stress. Root architecture is hence a very important factor determining plant response to P stress [27-28]. Usually shallower and denser plant root architecture (higher surface area) may be more efficient to absorb P from the surface soil related to a deeper root [29]. However, a deeper root can be more efficient under stresses such as nutrient and water stress, which is a very important parameter under rainfed conditions.

The production of the plant hormone strigolactones can influence the behavior of root hairs in the host plant. The hormone is able to regulate plant response, root elongation and number and hence its symbiosis with the host plant under different levels of P [30-31]. According to Kapulnik et al. [32] the hormone is more able to affect root elongation rather than root number and hence regulate root growth under different conditions.
In the case of bacteria the root volume, wet weight, root surface area and root density increased indicating that P solubilizing bacteria are able to increase the growth and length of the lateral roots. This can be of interesting implications, because it indicates how the two microbes may be used under different conditions. It is accordingly, likely to plan the efficient plant architecture under different conditions. Wang et al. [33] investigated the effects of co inoculation with mycorrhizal fungi and rhizobium on different soybean varieties with different root architectures. They indicated that mycorrhizal symbiosis and root architecture were positively related and the deep rooted soybean had a higher rate of colonization related to the shallow rooted soybean, especially at low level of P. Co inoculation with the fungi and bacteria significantly increased the uptake of P and N by soybean in both varieties. The effects of co inoculation were also more pronounced on the deep rooted soybean related to the shallow rooted soybean. The increased number of nodules by the biological treatments tested in this experiment indicated that the use of biological fertilization can also be effective on the uptake of nitrogen by plant [34].
Although mycorrhizal fungi can increase plant root growth, according to the results the fungi are more effective on the main roots, related to the P solubilizing bacteria. Hence, if a plant with a deep rooting part is favorable, which is especially the case under drought stress (rainfed conditions) the use of the fungi may be more favorable. If a plant with more lateral surface root is desired, then the use of the bacteria may be more recommendable. However, for a greater efficiency the combined use of the fungi and the bacteria may be more effective. The important point is to determine what may be the most effective rate under different conditions including stress. More experiments must be performed so that such a rate can be determined with respect to the morphological and physiological properties of plant, when treated with different biological treatments. According to the analyses of variance (Table 2) the single use of mycorrhizal fungi significantly affected chickpea grain yield, root nodule number and root length. The single use of bacteria had significant effects on chickpea grain yield, root volume, root wet weight, root surface area and root length density. However, their combined use just significantly affected grain yield. Such results indicate that there are some adverse interactions between the fungi and the bacteria, as predicted [35]. This is especially related to the effects of both microbes on the behavior of P. By the production of different enzymes both microbes are able to enhance P availability in the soil. However, high rate of P production can adversely affect the activity of both [36]. The results also clearly show the significant effect of vermicompotsing on different experimental parameters, which are higher in almost all cases than the values related to the fungi and bacteria. When used with the fungi and/or bacteria vermicompost just significantly affected chickpea grain yield. However, the highest root and yield values were resulted indicating the positive effect of vermicompost on the growth and activity of the fungi and bacteria. This may indicate that both the single and the combined use of vermicompost with the tested microbes may be recommendable.

CONCLUSION

Some important finding has been resulted. The effects of biological treatments including *G. intraradices*, *P. striata* and vermicompost were tested on the root and yield properties of chickpea under rainfed conditions. All treatments indicated significant effects on the experimental parameters. Mycorrhizal fungi greatly effected grain yield, nodule number and root length of chickpea. The bacteria, however, considerably influenced the properties of root hairs including their number, wet weight, surface area, and density. Vermicompost was also a very effective treatment with significant effects on all the experimental parameters. With respect to the desired plant architecture (higher responsiveness and efficiency) and environmental conditions the single or combined use of the biological treatments, tested in this research work, may be planed.

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