



## ORIGINAL ARTICLE

# Effects of Vermicompost , Mycorrhizal Symbiosis and Biophosphate solubilizing Bacteria on seed yield and quality of Chickpea as Autumn Plantation in rain fed Conditions

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### ABSTRACT

With respect to the economical and environmental effects of organic and biological fertilization on crop production, and the importance of water efficiency under rainfed conditions the following experiment was performed. The effects of mycorrhization, phosphate solubilizing bacteria and vermicomposting on chickpea (*Cicer arietinum* L.) yield, nutrient uptake and proteins were investigated in the Experimental Research Institute of Khorram Abad, Lorestan, Iran, in 2009-2010. The experiment was a factorial on the basis of a completely randomized block design with four replications. Chickpea seeds were treated with inoculum (spore, hyphae and roots) of *Glomus intraradices*, (from Soil and Water Research Institute, Karaj, Iran), rock phosphate treated with *Pseudomonas striata* and vermicompost (0, 6, 12 T/ha, manure treated with *Eisenia foetida*, from Behsaman Co. Karaj, Iran). The results were statistically compared using Duncan's multiple range comparison. For the mycorrhizal treatment the results were like the following, grain yield (2497 kg/ha), grain nutrient concentrations of N (2.8g), P (0.28g), K (1.4g), and Fe (4.5 mg/100g), N uptake (69.4 kg/ha), grain protein percentage (17.2%), and grain protein amount (433.7 kg/ha). The corresponding values for the bacterial treatment were equal to the grain yield (2310.0 kg/ha), nutrient concentrations of N (2.8g), P (0.29g), K (1.3 g) and Fe (4.5mg/100g), N uptake (64.8 kg/ha), grain protein percentage (17.5%) and grain protein amount (405.1 kg/ha). The vermicompost treatment (12 T/ha) resulted in grain yield (2373.7 kg/ha), nutrient concentrations of N (2.9g), P (0.3g), K (1.4g), Fe (4.7mg/100g), N uptake (70.2 kg/ha), grain protein percentage (18.3%), and grain protein amount (438.7 kg/ha). Both mycorrhizal fungi and bacteria were effective on the production of chickpea, however their effects were more pronounced when combined with the vermicompost treatment. The right combination of biological and organic treatments can significantly increase chickpea production under rainfed conditions.

**Key words:** Chickpea (*Cicer arietinum* L.), mycorrhizal fungi, phosphorous solubilizing bacteria, N, P, K, and Fe

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### INTRODUCTION

Today the use of soil microbes as biological fertilization is considered as the most natural and favorite method to keep the soil biological properties active and alive. Supplying organic matter to the soil is among the most important benefits of such kind of fertilization. Moreover, providing nutrients with respect to the plant growth stage, improving the biological diversity, increasing biotic activities, and improving the quality and health of the environment are also among the important benefits of such fertilization [1-2-3].

Accordingly, the use of soil microbes including arbuscular mycorrhizal (AM) fungi, phosphorous (P) solubilizing bacteria, and vermicomposting increases both the population of soil beneficial microbes and enhances the availability of soil nutrients such as nitrogen (N), phosphorous (P), potassium (K) and micronutrients. This can be very useful for plant growth and crop production [4-5-6-7]. Ilbas and Sahin [8] indicated that the inoculation of soybean plants with mycorrhizal fungi improves grain uptake of N and P and increases grain yield. According to Ratti et al. [9] plant inoculation with P solubilizing bacteria and the insoluble tri-calcium phosphate significantly increased shoot P related to the control treatment.

Chickpea (*Cicer arietinum* L.) is an important source of protein for the use of human. With respect to the biological properties of chickpea it has been used as a source of biological fertilization to decrease the use of chemical fertilization as well as pesticides. Use of biological sources instead of chemical sources can be effective in improving the fertility of soil and microbial activities, increasing agricultural yield, and the health of ecosystem. Vermicomposting, increases soil organic matter, N availability, and microbial activities, improves the structure of soil as well as the cation exchange capacity of particles [10-11-12].

N availability is among the important properties of grain quality and protein. P is among the soil nutrients, which is required for plant growth, however its availability decreases significantly due to its reaction and fixation by mineral elements such as aluminum (Al), iron (Fe), calcium (Ca), and magnesium (Mg). Accordingly, the use of biological fertilization is very much recommendable to provide sufficient amounts of P for plant use [13-14].

Biological fertilization not only supplies plant required nutrients, it also enhances plant growth and crop production by decreasing the rate of pathogenic diseases, and improving soil structure. P solubilizing bacteria results in the release of P from the mineral sources and hence increase their availability to the plant influencing the quality and taste of agricultural crops [15-16].

It is important to produce and develop the right biological fertilization, which is usually a mixture of soil microbes and organic material such as vermicompost. Accordingly, a lot of experimental tests and research work must be conducted to find the right combination and formula of the fertilizer. Different research work has indicated that use of AM fungi can enhance the uptake of macro- and micro-nutrients by plant besides their other important effects on the properties of soil as well as plant growth and crop production [6-7-17-18]. Under arid and semi arid climatic conditions, the rate of soil organic matter decreases, continuously [19]. Hence, soil organic matter must be regularly supplied by using organic material such as vermicompost. Vermicompost is a great source of nutrients, vitamins, enzymes, and plant growth promoting hormones resulting in the enhanced growth of soil microbes and plant growth under the conditions of sustainable agriculture [10]. Zaller *et al.* [20] found that using vermicompost to produce tomato (*Lycopersicon esculentum* L.) significantly increased the concentration of N, P and K in the fruits and increased fruit yield related to the control treatment. Inoculation of chickpea plants with mycorrhizal fungi significantly increased the concentration of P and K [21]. Jat and Ahlawat [22] indicated that the combined use of biological fertilization, vermicompost and P solubilizing bacteria increased N and P concentration in chickpea grains. We were interested to test how the combination of AM fungi P solubilizing bacteria and vermicompost may affect chickpea yield, nutrient uptake and protein under rainfed conditions. This can be useful for the production of the right biological fertilization for chickpea production in the regions with such environmental and climatic properties.

## MATERIALS AND METHODS

The experiment was performed in the Agricultural Research Institute of Khorram Abad, Lorestan, Iran, located in the northern longitude of 33° and 29' and eastern latitude of 48° and 18', 175 m above the sea level in 2009-2010. The average yearly rainfall is 409.9 mm with the yearly average temperature of 17.5 °C. To analyse the soil and vermicompost properties, samples were collected and analysed using the standard laboratory methods [23-24] (Table 1).

Azad cultivar was used in the experiment, which is recommendable for the fall cropping in the region of Khorram Abad. The experiment was a three way factorial including the following treatments: mycorrhizal fungi (M1= control and M2= inoculated), P solubilizing bacteria (P1= control and P2= inoculated) and vermicompost (V1= control, V2= 6 and V3= 12 t/ha) on the basis of a completely randomized block design with four replications.

The species, *Glomus intraradices* from the Soil and Water Research Institute, Karaj, Iran, was used as the mycorrhizal treatment including the spore, hyphae and root propagules [23-24]. The biological P fertilizer has also been verified by the Soil and Water Research Institute, with rock phosphate and the strain of P solubilizing bacteria *Pseudomonas striata* including 10<sup>5</sup> active bacteria. The vermicompost treatment was supplied by the Behsaman Co. Karaj, Iran prepared from manure treated with the earthworm species of *Eisenia foetida*.

The experimental field had been uncultivated for years before performing the experiment. The field was prepared in the October of 2009, when the climatic conditions were suitable for cropping. The plots measuring 1.5 x 6 m with 5 cropping rows (30 cm apart) and the seed spacing of 6.5 cm were established. The plots and the replications were 1 and 2 m apart from each other, respectively. Chickpea seeds were planted after the fall precipitation, when the field was at suitable moisture. Vermicompost was broadcast on the field and mixed with the soil at the desired amounts. Before planting the seeds were treated with the fungal and the bacterial inoculum using glue. The plant density was 60 plants/m<sup>2</sup> planted in rows with 7 cm depth. The weeds were controlled mechanically and by hand. At harvest and to calculate the

amounts of grain yield, plant samples were collected from a 2.7 m<sup>2</sup> area for all the plots and were also analysed for the percentage of mineral nutrients for all treatments. The concentration of grain N, P, K, Fe, protein percentage and amount, N uptake and grain yield were determined [6-7]. Fe was measured using the atomic absorption spectrometry (Perkin Elmer Model 5000), P using the spectrophotometry method and K using flame photometer according to the AOAC [25] standards. Grain protein was determined using the Kjeldahl method [26]. To determine the protein percentage the Kjeldahl value (total N) was multiplied by 6.25 for each sample. Protein amount was calculated by multiplying grain yield by grain protein percentage and N uptake was determined by multiplication of N concentration by the grain yield.

**Statistical analyses**

To analyse the data, SAS, SPSS and MSTATC software were used and the analysis of variance was determined. Means were compared using Duncan's multiple range comparison.

**RESULTS**

According to the analysis of variance, the effects of AM fungi, bacteria, vermicompost, and their two way interactions on grain yield were significant. For grain N just the experimental parameters and the two way interactions of AM fungi and bacteria had significant effects, and for K just the experimental parameters and not their interactions. AM fungi, and its interaction with bacteria and vermicompost significantly affected grain Fe. N uptake and protein amounts were significantly affected by the experimental parameters, as well as the two way interactions of AM and bacteria with vermicompost. For grain protein percentage, the experimental parameters and the two way interaction of AM fungi and bacteria were significantly effective (Table 2).

According to Table 3 the effects of mycorrhization, bacteria and vermicompost on grain yield were significantly different from the control treatments, with the AM fungal treatment being the most effective (2497 kg/ha) followed by 12 T/ha vermicompost (2373.7 kg/ha) and bacteria (2310.4 kg/ha). Interestingly, while AM fungi and bacteria significantly increased grain N, the effects of vermicomposting was negative. However, this was not the case for P, K, N uptake, and protein percentage as all the experimental parameters including vermicomposting significantly increased them related to the control treatments. For Fe just the effects of AM fungi and vermicompost was significant (Table 3).

The table of two way interactions (Table 4) indicated that mycorrhization was the most effective treatment and resulted in the highest amounts of grain yield when combined with bacteria (2779.3 kg/ha) and vermicompost (3105.8 kg/ha). The two way interaction effect of bacteria and vermicompost (12 T/ha) was also significantly different from their other two way interactions. For grain N, P and K as well as N uptake, and grain protein percentage and amount the two way interactions of both AM fungi and bacteria with vermicompost were the most effective experimental treatments related to the combination of AM fungi and bacteria. However, the highest effect on Fe was related to the two way interaction of bacteria and vermicompost (4.78 mg/100g) (Table 4).

Table 1. The chemical analyses of soil and vermicompost

Sample	pH	EC (dS/m)	O.C. (%)	N(%)	P(ppm)	K(ppm)	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

EC: Electrical conductivity, O.C.: organic carbon

Table 2. Analysis of variance for the experimental treatments and parameters

S.V.	d.f.	Mean of sum squares							
		Grain yield	Grain N	P	Grain K	Grain Fe	Protein percentage	Grain N uptake	Protein amount
Rep.	3	1011280.1 <sup>ns</sup>	43956.8 <sup>ns</sup>	45.9 <sup>ns</sup>	14065.7 <sup>ns</sup>	0.046 <sup>ns</sup>	1.71 <sup>ns</sup>	655.8 <sup>ns</sup>	25617.5 <sup>ns</sup>
AM (a)	1	5773005.8 <sup>**</sup>	124603.3 <sup>*</sup>	985.5 <sup>*</sup>	326403.1 <sup>**</sup>	0.151 <sup>**</sup>	4.86 <sup>*</sup>	5536.2 <sup>**</sup>	216256.8 <sup>**</sup>
SB (b)	1	1232787.1 <sup>*</sup>	505448.6 <sup>**</sup>	2512.8 <sup>**</sup>	65564.08 <sup>**</sup>	0.014 <sup>ns</sup>	19.74 <sup>**</sup>	1816.3 <sup>**</sup>	70950.1 <sup>**</sup>
a x b	1	714541.7 <sup>*</sup>	468786.3 <sup>**</sup>	744.9 <sup>*</sup>	4088.5 <sup>ns</sup>	0.109 <sup>**</sup>	18.3 <sup>**</sup>	154.1 <sup>ns</sup>	6019.8 <sup>ns</sup>
Vermi-compost (c)	2	653605.4 <sup>*</sup>	889576.1 <sup>**</sup>	5269.8 <sup>**</sup>	145151.4 <sup>**</sup>	0.969 <sup>**</sup>	34.74 <sup>**</sup>	1842.4 <sup>**</sup>	71969.4 <sup>**</sup>
a x c	2	1861259.3 <sup>**</sup>	62251.8 <sup>ns</sup>	145.3 <sup>ns</sup>	525.1 <sup>ns</sup>	0.002 <sup>ns</sup>	2.43 <sup>ns</sup>	1883.8 <sup>**</sup>	73584.7 <sup>**</sup>
b x c	2	1308707.4 <sup>**</sup>	3739.2 <sup>ns</sup>	30.6 <sup>ns</sup>	3003.4 <sup>ns</sup>	0.019 <sup>ns</sup>	0.146 <sup>ns</sup>	1109.5 <sup>**</sup>	43341.1 <sup>**</sup>
a x b x c	2	569924.2 <sup>ns</sup>	52268.9 <sup>ns</sup>	10.75 <sup>ns</sup>	2477.7 <sup>ns</sup>	0.023 <sup>ns</sup>	2.04 <sup>ns</sup>	297.7 <sup>ns</sup>	11630.7 <sup>ns</sup>
Experimental error	33	204301.3	35020.1	133.2	3798.57	0.011	1.36	199.8	7806.5

S.V.: source of variation, ns, \* and \*\*: not significant, significant at 5 and 1% level of probability, respectively.

AM: mycorrhizal fungi, SB: P solubilizing bacteria

Table 3. Pea related parameters as affected by different experimental parameters.

Treatment	Grain yield (kg/ha)	Grain N (mg/100g)	Grain P (mg/100g)	Grain K (mg/100g)	Grain Fe (mg/100g)	Grain N uptake (kg/ha)	Grain protein (%)	Protein amount (kg/ha)
M1	1803.4b	2653.5b	275.2b	1276.6b	4.42b	47.9b	16.6b	299.5b
M2	a2497	2755.4a	284.2a	1441.5a	4.53a	69.4a	17.2a	433.7a
P1	1989.9b	2601.8b	272.5b	1322.1a	4.45a	52.5b	16.3b	328.2b
P2	2310.4a	2807.1a	286.9a	1396a	4.5a	64.8a	17.5a	405.1a
V1	1980b	3462.7c	261.9c	13263.4c	4.24c	48.9b	15.4c	328.2b
V2	2096.6ab	2716.9b	279.1b	1359.9b	4.44b	56.8b	16.9b	405.1a
V3	2373.7a	2933.9a	298.2a	1453.8a	4.73a	70.2a	18.3a	438.7a

M1: control, M2: mycorrhizal treatment, P1: control, P2: bacterial treatment, V1: control, V2: vermicompost treatment (6t/ha), V3: vermicompost treatment (12t/ha). Means followed by the same letter in the same column are not statistically different at P= 0.05.

Table 4. The interactive effects of different experimental parameters on pea yield, protein and nutrient uptake.

Treatment	Grain yield (kg/ha)	Grain N (mg/100g)	Grain P (mg/100g)	Grain K (mg/100g)	Grain Fe (mg/100g)	Grain protein (%)	N uptake (kg/ha)	Protein amount (kg/ha)
M1P1	1765.1c	2452.1b	264b	1230.4d	4.45b	15.3b	43.5c	272.2c
M1P2	1841.6bc	2855a	286.3a	1322.8c	4.38b	17.8a	52.2bc	326.7bc
M2P1	2214.7b	2751.7a	280.9a	1413.8b	4.46b	17.2a	61.4b	384.1b
M2P2	2779.3a	2759.3a	287.5a	1469.3a	4.59a	17.2a	77.3a	483.4a
M1V1	1755.4bc	2351.3d	254.6d	1187.3e	4.19d	14.6d	41.5c	259.4c
M1V2	2013.1bc	2730bc	274.1bc	1272.9d	4.38c	17.1bc	54.9bc	343.2bc
M1V3	1641.6c	2879.4ab	296.8a	1369.5c	4.68a	17.9ab	47.3bc	295.9bc
M2V1	2205.1b	2574.3c	269.2c	1339.4c	4.29cd	16.1c	56.4bc	352.8bc
M2V2	2180.1b	2703.8bc	284.1b	1447b	4.51b	16.9bc	58.6b	366.8b
M2V3	3105.8a	2988.4a	299.5a	1538.2a	4.48a	18.6a	93.1a	581.6a
P1V1	1802.3b	2358.9d	253.6e	1210.9d	4.23d	14.7d	42.8c	267.8c
P1V2	2230.8b	2630.1bc	273.4cd	1327.8c	4.46c	16.4bc	58.9b	268.5b
P1V3	1936.7b	2816.6b	290.4b	1427.5ab	4.68b	17.6b	55.7bc	348.2bc
P2V1	2158.2b	2566.6c	270.2d	1315.8c	4.25d	16c	55.1bc	344.4bc
P2V2	1962.5b	2803.6b	284.7bc	13912.1b	4.43c	17.5b	54.6bc	341.5bc
P2V3	2810.7a	3051.1a	305.9a	1480.2a	4.78a	19.1a	84.6a	529.3a

M1: control, M2: mycorrhizal treatment, P1: control, P2: bacterial treatment, V1: control, V2: vermicompost treatment (6t/ha), V3: vermicompost treatment (12t/ha). Means followed by the same letter in the same column are not statistically different at P= 0.05.

## DISCUSSION

There are some interesting details resulted by this research work, which can be very useful for the production of biological fertilization. Biological fertilizer may be produced with respect to the following parameters. 1) The interactions between different microbes and organic material, 2) the climatic and environmental parameters, 3) plant species, 4) the kind of nutrient, and 5) the plant properties, which are the objective of enhancement by fertilization [27-1-2-3].

All such parameters must be considered when designing and producing biological fertilizer. The parameters tested in this experiment include the following: chickpea as the experimental plant with the ability to fix atmospheric N and grow under rainfed conditions, and a temper climate with the yearly rainfall of more than 400 mm. It was also aimed to increase, chickpea grain yield, nutrient uptake and protein. The results clearly indicated that in most cases the right combination was selected and tested with the exception of fungal and bacterial treatments as mentioned earlier and was predictable. Vermicomposting is also an effective tool and must be considered for biological fertilization. Use of biological fertilization can be of the following importance: 1) economical and environmental benefits, 2) less use of chemical fertilization, 3) production of more healthy and productive plant species with higher grain yield, and 4) enhancing the biological activities of soil [28]. It is important to use the right combination of microbial and organic products when producing biological fertilizer. Such interactions must be tested and the most appropriate combination be selected. The results of this experiment indicated that AM fungi are the most effective treatment and when combined with the other experimental treatments, especially vermicompost their effectiveness can increase.

However, as predicted, the combination of AM fungi and P solubilizing bacteria is not as effective as the combination of fungi and bacteria with vermicompost. It is because, AM fungi can be the most effective

when soil P is present at low or medium amounts, the bacteria is able to increase the amount of P from the rock phosphate by the production of different products such as organic acids [29-30]. The experimental results indicate that if the right combination of soil microbes and vermicompost as the organic material is selected it is possible to increase chickpea yield, nutrient uptake, and grain protein under rainfed conditions. However, it is also important to indicate the N fixation ability of chickpea under such conditions so that the consideration of biological fertilization can be done more precisely and more efficiently. These results are some important contribution to our knowledge regarding the production of chickpea under rainfed conditions using biological fertilization.

## CONCLUSION

Some important details related to the use of biological fertilization as a combination of soil microbes and vermicompost are presented. Such details can be very useful for the production of biological fertilization with respect to the properties of plant, climate, environment, and economy. Mycorrhizal fungi indicated to be the most effective treatment on the production of chickpea; however its enhancing potentials increased when combined with vermicompost. P solubilizing bacteria can also significantly influence the production of chickpea plant under rainfed conditions and can be more effective if used with vermicompost. However, the combined use of mycorrhizal fungi and P solubilizing bacteria, as predicted, was not as effective as the other treatments.

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