ORGANIC MATTER EFFECT ON *Glomus intrarradices* IN BEANS (*Phaseolus vulgaris* L.) GROWTH CULTIVATED IN SOILS WITH TWO SOURCES OF WATER UNDER GREENHOUSE CONDITIONS

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SUMMARY

The objective of this research was to evaluate the effect of organic matter on the association with *Glomus intrarradices* and soil contamination on beans (*Phaseolus vulgaris* L.). The study was done under greenhouse conditions at the Montecillo Campus of the Postgraduate College, Mexico. Two soils were used, one irrigated with sewage water and the other one with clean water from a well. Half of the plants were inoculated with *Glomus intrarradices*. Vermicompost was used as a source of organic matter. There were highly significant increases ($p \leq 0.05$) in all the variables recorded due to the application of organic matter, and to the inoculation with *Glomus intarradices*. The irrigation source of the soils used for this experiment only had a significant effect ($p \leq 0.05$) on pod number and nitrogen fixation. The best growth and grain yield occurred with inoculated plants and supplementary organic matter.

Key words: Soil contamination, vermicompost, sewage water, arbuscular endomycorrhiza, edible legumes.

INTRODUCTION

Common bean is the most important legume in Mexico. More than 1.5 million hectares were planted in 2011 in this country. This surface was reduced in 2012 due to severe drought conditions [24]. It is cultivated mainly in areas where water is scarce under rainfall conditions. Sewage water it is used for its irrigation in some regions. Studies on its effect on plant growth and yield are needed.

Farmers usually apply chemical fertilizer. Its cost has been increasing dramatically in the last years [18]. Organic matter sometimes is applied as fertilizer as a sole source or in combination with chemical products. However, its consequences on beneficial microorganisms as endomycorrhiza need to be understood in order to improve their usage.

Glomus intrarradices has been used to improve plant growth under different conditions, included contaminated soils. Several researchers consider that this kind of fungi as the most important organisms on earth interacting in agro environments. More than 80% of all terrestrial plants, among them most of the horticultural and crop plants have a symbiotic relationship with these fungi. The stimulation of plant growth can be attributed mainly to the improvement of phosphorus nutrition [1, 9, 12, 17, 23].

Glomus intrarradices has increased bean yield 36% [19]. Novella *et al*. [21] had reported augmented corn and bean yield when they were cultivated together and were inoculated with a combination of *Rhizobium* and mycorrhiza.

Glomus mosseae amplified shoot growth four times [27]. Mycorhizal development was better under no tillage conditions than using conventional one in an oat-wheat rotation [6]. Mineral nitrogen improves biomass and root growth when mycorrhiza is present [26].

The objective of this study was to investigate the effect of organic matter associated with *Glomus intrarradices*, on the growth and yield of beans, in two soils with different water source under greenhouse conditions.

MATERIALS AND METHODS

The study was done under greenhouse conditions at the Postgraduate College, Montecillo Campus, State of Mexico, in the spring and summer of 2012. Two soils, from Tocuila, Texcoco, Mexico, were used. One was irrigated with sewage water, and the other one with clean water from a well. Their characteristics are shown in Table 1.

Table 1 Soil analysis for the two types, one irrigated with sewage water and the other with clean one.

	pH	EC	OM	TN	NO_3	P	CEC
Soil sample	1:2	dm sec^{-1}	%	%	mg kg^{-1}	mg kg^{-1}	C mol (+) kg^{-1}
Soil depth 0-5	7.44	349	2.5	0.098	18	15	19
Residual water							
Soil depth 5-10	7.37	454	2.48	0.096	17	14	18
Residual water							
Soil depth 10-40	7.44	475	2.45	0.094	15	11	15
Residual water							
Soil depth 0-5	7.52	314	2.49	0.097	17	14	18
Clean water							
Soil depth 5-10	7.75	332	2.47	0.095	16	13	17
Clean water							
Soil depth 10-40	7.85	384	2.43	0.092	13	10	13
Clean water							

Soil sample	Ca	Mg	K	Na	Fe	Zn	Cu
	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Soil depth 0-5	1250	59	1180	400	5	3	0.5
Residual water							
Soil depth 5-10	1210	56	1220	640	5	3	0.5
Residual water							
Soil depth 10-40	1245	59	1400	800	4	3	0.4
Residual water							
Soil depth 0-5	1240	58	1120	360	5	3	0.5
Clean water							
Soil depth 5-10	1243	60	1110	640	3	3	0.4
Clean water							
Soil depth 10-40	1200	53	1140	720	4	2	0.4
Clean water							

Key: pH= Hydrogen potential, EC=Electrical conductivity, OM= Organic matter, TN= Total nitrogen, NO_3= Nitric nitrogen, CEC= Cation Exchange Capacity.

The seeds were sterilized with 1% sodium hypochlorite during 4 minutes, and hydrated on filter paper in petri dishes for 48 hours. The seeds were sown in polyethylene bags that had been filled with three kg of two soil types. The treatments were: planting in soil irrigated with sewage water and the other one with clean water. Both soils were collected at three depths 0-5, 5-10, and 10-40 cm from plots of one hectare [5].

The soil organic matter was determined using the Walkey and Black method. For phosphorus, Olsen's method was utilized. Interchangeable bases were measured with ammonium acetate 1 Normal (CH_3COONH_4) pH 7, and micronutrients with DTPA (diethylene-triamine-pentaacetic acid).

Bayomex bean genotype was planted. The seed was provided by the National Institute for Agriculture, Livestock and Forestry Research (INIFAP).

The inoculation was done during the planting, mixing 5 g of sand with sorghum roots with 78 % colonization of *Glomus intrarradices* and 1050 spores per 100 g of inert material. Two levels of *Glomus* were applied, with and without *Glomus*.

Organic matter was applied as a vermicompost. It was prepared using 60 kg of bovine manure, 25 kg of melon waste, and 15 kg of wheat straw. The mixture was subjected four months to the action of earth worms. Four doses were applied. In every bag of three kg, 0, 28.86 g, 57.7 g, and 86.46 g of vermicompost were mixed. They are equivalent to 0, 25, 50, and 75 t ha^{-1} of organic matter.

The variables evaluated were: plant height (PH, cm), leaf area (LA, cm^2), pod number (PN), grain dry weight (GDW, g), root length (RL, cm), root volume (RV cm^3), root dry weight (RDW, g), pod dry weight (PDW, g), grain number (GN) biomass dry weight (BDW, g), white nodule number (WNN), red nodule number (RNN), and total nodule number (TNN).

A factorial arrangement with 16 treatments (4x2x2) was used with a completely randomized block design with three replications. An analysis of variance for all variables registered was done and a Tukey mean comparison test for the significant variables.

RESULTS AND DISCUSSION

The soil texture was sandy loam. The pH was alkaline. It was higher in the soils irrigated with clean water. The difference was greater in the 10-40 cm depth. The soil with clean water had a pH of 7.85, and that with sewage water, only 7.44 (Table 1). The electrical conductivity (EC), organic matter (OM), total nitrogen (TN), nitric nitrogen (NO_3), phosphorous (P), Cation Exchange Capacity (CEC), calcium (Ca), potassium (K), sodium (Na), iron (Fe), zinc (Zn), and copper (Cu) quantities are higher in the soils that were irrigated with sewage water.

In both soils, total nitrogen (TN), and the nitric nitrogen (NO_3) quantities were low. The distribution were higher in the 5-10 cm layer. No ammoniacal nitrogen (NH_4) was found in the two soils. It explains why total nitrogen is only slightly higher on the soils that were irrigated with sewage water that had a higher organic matter [20].

The levels of Cu were below the threshold for considering them as contaminants [5]. No Cr and Ni traces were found.

There were highly significant differences ($p \leq 0.01$) in all the variables recorded due to the application of organic matter, and to the inoculation with *Glomus intrarradices.*

The pod number, and the white, red, and total nodule number were significantly affected ($p \leq 0.05$) for the contamination from the soil with sewage water. Their higher content of nitric and total nitrogen could reduce the nodule number. It is well known the antagonistic effect between nitrogen content is soils and nitrogen fixation [7]. The lack of effect on growth and yield could be explained by the low N fixation [8, 22].

A positive effect of inoculation with *Glomus intrarradices* was found. The highly significant differences ($p \leq 0.01$) among treatments for all the variables recorded generated a beneficial effect on plant growth due to an improvement in the absorption of mineral nutrients required by the plants [2, 3]. This behavior was similar to that found by Gardezi *et al*. [8, 9, 12, 13 and 14].

Yield, root and shoot growth from plants with mycorrhiza were superior to those without inoculation (Table 2). The treatments with mycorrhiza were 54% taller, had 25% more leaf area (Figure 2), 48% heavier shoot dry weight, 47% longer roots, 56% greater root volume, and 48% more root dry weight[1] (Figure 3). This is an indication of a positive effect of mycorrhiza on plant growth originated by better mineral nutrient absorption required by the plant [1, 2]. Gardezi *et al.* [10, 11, 12] also found this beneficial effect in *Leucaena lecocephala* associated with endomycorrhiza and with *Rhizobium.* Positive responses to the inoculation with mycorrhiza were also found in a number of species [24], including beans [2].

The inoculation with *Glomus intrarradices* improved root and shoot growth. It also had a beneficial effect on the biological nitrogen fixation, and a superior absorption of nutrients [12]. Thus, it contributed to higher yield in beans, coinciding with other studies [2]. Inoculated plants had 38% heavier pods and yielded 116% more grain (Table 2, and Figure 1).

Table 2. Honest significant difference of the effect of *Glomus intrarradices* on common bean (*Phaseolus vulgaris* L.).

Glomus intraradices	Plant height (cm)	Dry weigth aerial part (g)	Leaf area (cm2)	Grain number (ln)	Grain dry weight (g)	Pod dry weight (g)	Pod number (ln)
Inoculated	124.542a	24.292a	448.79a	2.9806a	10.958a	3.0000a	2.1849a
No inoculated	100.667b	16.375b	357.92b	2.3845b	5.083b	2.1667b	1.8475b

Glomus intraradices	Root lenght (cm)	Root volume (cm3)	Dry weigth root (g)	White nodule number (ln)	Red nodule number (ln)	Total nodule number (ln)
Inoculated	28.708a	9.7917a	3.0833a	1.9818a	2.6397a	3.0620a
No inoculated	19.500b	6.2500b	2.0833b	1.2320b	1.9258b	2.2557b

ln=transformated to natural log. Means with the same letter in each column are not significantly different (Tukey α= 0.05)

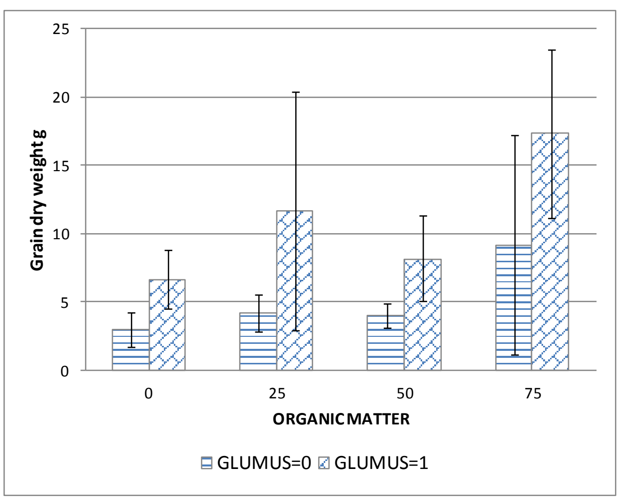

Figure 1. Effect of inoculation with *Glomus intrarradices* and different doses of organic matter (vermicompost) on soils with two types of irrigation in grain dry weight of common bean (*Phaseolus vulgaris* L.).
Key: Glumus=0: Noninoculated, Glumus=1: Inoculated with *Glomus intrarradices.* The vertical lines indicate standard error.

[1] Increase percentages are referred to the values found in bean plants inoculated with mycorrhiza compared to those without inoculation.

Plant growth was affected by the organic matter application (Table 3). It provided significantly higher ($p \leq 0.01$) plant height in all the treatments compared with the control. Aryal *et al* [3] found similar results. Only the higher quantities gave heavier dry weight of the aerial part (Figure 4). A similar situation was found in the leaf area (Figure 2).

Table 3. Honest significant difference of the effect of organic matter (vermicompost) on common bean (*Phaseolus vulgaris* L.).

Organic matter t*ha^{-1}	Plant height (cm)	Dry weigth aerial part (g)	Leaf area (cm2)	Grain number (ln)	Grain dry weight (g)	Pod dry weight (g)	Pod number (ln)
0	90.167b	15.333b	361.67b	2.289b	4.833b	2.083b	1.824b
25	115.000a	19.833ab	402.50ab	2.511ab	7.917ab	2.667a	1.949ab
50	123.417a	22.917a	415.00a	2.963a	6.083b	2.750a	2.131a
75	121.833a	23.250a	434.25a	2.968a	13.250a	2.833a	2.161a

Organic matter t*ha^{-1}	Root lenght (cm)	Root volume (cm3)	Dry weigth root (g)	White nodule number (ln)	Red nodule number (ln)	Total nodule number (ln)
0	19.583b	5.917b	2.000b	1.125b	1.744b	2.070b
25	24.833ab	7.667ab	2.750a	1.637ab	2.564a	2.919a
50	25.917a	9.083a	3.000a	1.682ab	2.337ab	2.692a
75	26.083a	9.417a	2.583a	1.983a	2.487a	2.954a

ln=transformated to natural log. Means with the same letter in each column are not significantly different (Tukey α= 0.05)

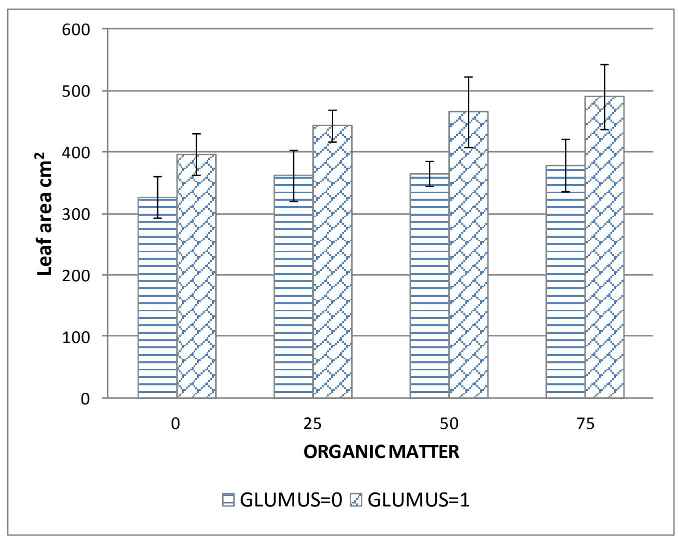

Figure 2. Effect of inoculation with *Glomus intrarradices* on soils with two types of irrigation on leaf area of three cultivars common bean (*Phaseolus vulgaris* L.).
Key: Glumus=0: Noninoculated, Glumus=1: Inoculated with *Glomus intrarradices.* The vertical lines indicate standard error.

Root length and root volume (Figure 5) were also significantly greater ($p \leq 0.05$) only with the two higher applications of vermicompost. However, all the doses of organic matter gave heavier roots.

Organic matter also promoted nitrogen fixation. All the vermicompost applications had a significantly higher total nodule number. However, only the elevated dose was related with a greater white and red nodule number.

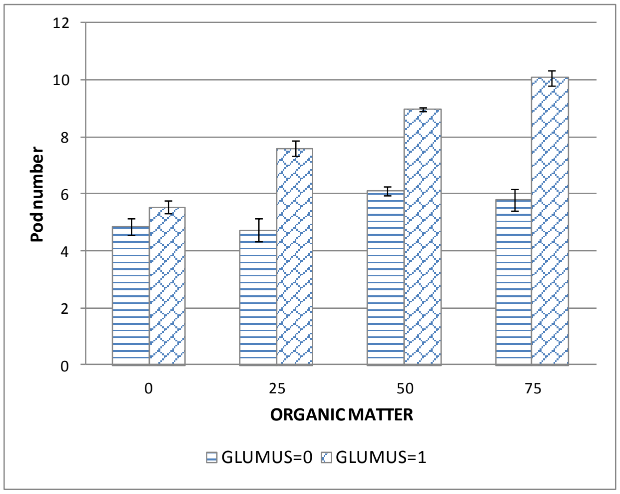

Figure 3. Effect of inoculation with *Glomus intrarradices* and organic matter application (vermicompost) in soils with two types of irrigation on pod number of common bean (*Phaseolus vulgaris* L.).
Key: Glumus=0: Noninoculated, Glumus=1: Inoculated with *Glomus intrarradices.* The vertical lines indicate standard error.

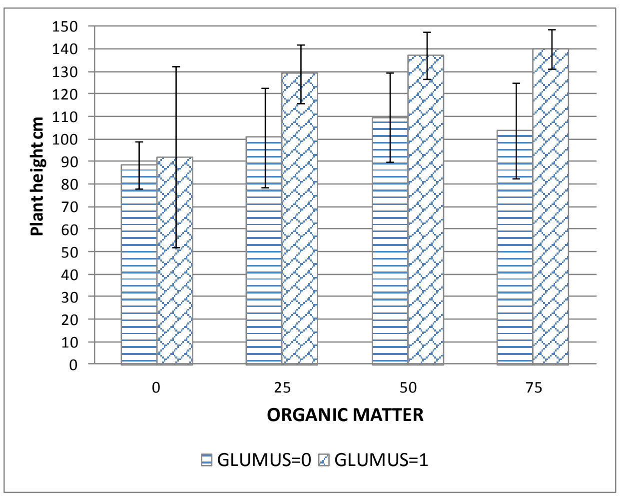

Figure 4. Effect of inoculation with *Glomus intrarradices* and organic matter application (vermicompost) in soils with two types of irrigation on plant height of common bean (*Phaseolus vulgaris* L.).
Key: Glumus=0: Noninoculated, Glumus=1: Inoculated with *Glomus intrarradices.* The vertical lines indicate standard error.

In an analogous way with mycorrhizal inoculation, the organic matter promoted better root and shoot growth. Therefore, as a result, photosynthetic production increased. Pod number was higher with organic matter (Figure 3). Grain yield was 174% enhanced with the highest dose of organic matter compared with the control (Figure 1, Table 3).

Thus, the poorest growth and grain yield occurred with uninoculated plants that lacked supplementary organic matter.

The irrigation source of the soils used for this experiment only had a significant effect ($p \leq 0.05$) on pod number and nitrogen fixation. The higher content of organic matter and nitrogen found in the soils watered with sewage water generated a greater pod number. However, this effect was not present in grain yield (Table 4).

Table 4. Honest significant difference of the effect of the irrigation source on common bean (*Phaseolus vulgaris* L.).

Irrigation Source	Plant height (cm)	Dry weigth aerial part (g)	Leaf area (cm2)	Grain number (ln)	Grain dry weight (g)	Pod dry weight (g)	Pod number (ln)
Clean water	109.17a	19.38a	400.88a	2.64a	8.46a	2.58a	1.93b
Sewage water	115.29a	21.29a	405.83a	2.74a	7.58a	2.58a	2.10a

Irrigation Source	Root lenght (cm)	Root volume (cm3)	Dry weigth root (g)	White nodule number (ln)	Red nodule number (ln)	Total nodule number (ln)
Clean water	23.46a	7.50a	2.46a	2.31a	2.71a	3.23a
Sewage water	24.75a	8.54a	2.71a	0.91b	1.85b	2.09b

ln=transformated to natural log.Means with the same letter in each column are not significantly different (Tukey α= 0.05)

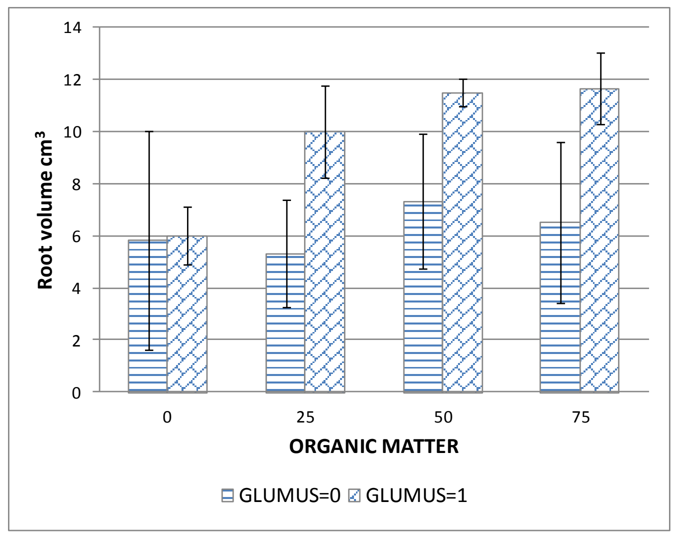

Figure 5. Effect of inoculation with *Glomus intrarradices* and organic matter application (vermicompost) in soils with two types of irrigation on root volume of common bean (*Phaseolus vulgaris* L.).
Key: Glumus=0: Noninoculated, Glumus=1: Inoculated with *Glomus intrarradices.* The vertical lines indicate standard error.

The nitrogen fixation, measured as the nodule number (white, red, and total) was higher in the soils irrigated with clean water (Table 4). A plausible explanation for this finding is the antagonistic effect between nitrogen content is soils and nitrogen fixation [7]. The soils watered with sewage water had higher total and nitric nitrogen (Table 1). Gardezi *et al.* [15, 16] found similar results in previous experiments.

CONCLUSIONS

Mycorrhizal inoculation and nitrogen fixation provided higher bean root and shoot growth and therefore, better yields. Previous evidence with legumes showed that they have benefited with this symbiosis because the treatments with this fungus produce the highest values for all evaluated variables. The application of organic matter, as vermicompost, improved plant growth, and grain yield. The contamination from the soil by sewage water did not affect plant growth or yield. It only affected nitrogen fixation.

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