FUNGICIDE EFFECT ON *Glomus intrarradices* IN DIFFERENT GENOTYPES OF BEANS (*Phaseolus vulgaris* L.), OAT (*Avena sativa* L.), AND WHEAT (*Triticum aestivum* L.) GROWTH CULTIVATED IN TWO SOIL TYPES UNDER GREENHOUSE CONDITIONS

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SUMMARY

The objective of this research was to evaluate the effect of fungicides on the association with *Glomus intraradices* and soil contamination on three genotypes of beans (*Phaseolus vulgaris* L.), one of oat (*Avena sativa* L.), and another one of wheat (*Triticum aestivum* 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 intraradices*. Metacaptan was used as a fungicide applied to half of the seeds. The pH of the soil was alkaline. Electric conductivity, and organic matter, nitric and ammoniac nitrogen, phosphorous, copper and nickel quantities were higher on the soils irrigated with sewage water. The soil contamination did not affect significantly plant responses in this study. It is concluded that endomycorrhiza inoculation (*Glomus intraradices*) gave better growth and yield, especially in beans. The application of fungicides improved plant growth.

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

INTRODUCTION

Common bean is the most important legume in Mexico. Almost 2 million hectares were planted in 2010 in this country. Oats and wheat are key cereals with high planted area (About 800 thousand, and 700 thousand hectares respectively in 2010). They are cultivated in the Mexican central highlands and in the northwest. Water is scarce in those areas. Sewage water has been used for irrigation there. Studies on its effect on plant growth and yield are needed.

Glomus intraradices has been used to improve plant growth under different conditions included contaminated soils. Several researchers consider that 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 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, 13, 14, 23].

Mycorrhiza can improve mineral nutrition uptake [17]. It can be an important strategy to reduce chemical fertilizer application. This is very important because the price of fertilizers have been increasing dramatically [18].

Glomus intraradices has increased bean yield 36% [19]. Novella *et al*. [22] had reported augmented corn and bean yield when they were cultivated together and were inoculated with a combination of *Rhizobium* and mycorhiza.

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 [5]. Mineral nitrogen improves biomass and root growth when mycorrhiza is present [26].

Fungicides are used routinely to protect seeds [6]. However, studies on their consequences on beneficial microorganisms as endomycorhiza are rare. The effect of the fungicides on mycorrhiza is variable. It depends at least, on the product used, its concentration, the fungus specie, and the crop host. In wheat, triazole derivates diminish infection and spore production. Tridemorph increase the infection level, and ethirimol do not have any adverse effect on the symbiosis [8]. Dehne [7] also found lower infection on *Glomus etunicatum* when fungicides and fumigants were used (benomyl, cycocel (chlormequat), CMPP [mecoprop], captan, ethephon, dazomet, fenaminosulf, halacrinate, methabenythiazuron, thiadiazuron) in cucumber, oats, and wheat. Those agrochemicals had a direct negative action on the fungus.

On the other hand, the use of metalaxyl incorporated in the soil significantly enhanced root colonization of *G. fasciculatum* associated with wheat. If the concentration of metalaxyl is increased, the mycorrhizal infection and seed yield of wheat are affected. Addition of urban compost slightly ameliorated the toxic effect of metalaxyl on VA mycorrhizal colonization, plant growth and yield of wheat compared with unamended soil [24].

The objective of this study was to investigate the effect of fungicides on *Glomus intraradices*, and on the growth and yield of beans, oats and wheat 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 2011. Two soils, from the Irrigation District 028 at Tulancingo, Hidalgo, 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.

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 3 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 [4].

The soil organic matter was determined using the Walkey and Black method, for phosphorus, Olsen was used. Interchangeable bases were measured with ammonium acetate 1 Normal (CH_3COONH_4) pH 7, and micronutrients with DTPA (dietilen-triamino-pentaacetic acid).

Three bean genotypes (Flor de Mayo M38, Flor de Durazno, and Bayo Azteca), one for oats (Turquesa), and one for wheat (Nana F2007) were planted. The seed was provided by the National Institute for Agriculture, Livestock and Forestry Research (INIFAP) of Mexico.

The inoculation was done during the planting, mixing 5 g of sand with sorghum roots with 78 % colonization of *Glomus intraradices* and 1050 spores per 100 g of inert material. Two levels of *Glomus* were applied, with and without *Glomus*.
The variables evaluated were plant height (PH, cm), stem diameter (SD, mm), biomass dry weight (BDW, g), grain dry weight (GDW, g), leaf area (LA, cm^2), root length (RL, cm), root volume (RV cm^3), root dry weight (RDW, g), pod number (PN), and nodule number (NN).

A factorial arrangement with 40 treatments (5x2x2x2) was used with a completely randomized block design using three replications. An analysis of variance for all variables registered was done and a Tukey mean comparison test for the significant variables.

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

Depth	Soil irrigation	pH	EC	Saturation point	OM	P
cm			dS m^{-1}	%	%	mg Kg^{-1}
0 - 5	Clean	8.05	0.209	41.0	2.60	57.84
5 - 10	Clean	8.03	0.252	42.0	2.43	78.10
10 - 40	Clean	8.01	0.179	39.0	1.86	38.86
0 - 5	Sewage	8.34	0.524	62.0	4.52	79.15
5 - 10	Sewage	8.38	0.506	52.0	3.28	73.91
10 - 40	Sewage	8.54	0.464	52.0	3.29	78.83

Depth	Soil irrigation	Ca	Mg	K	Na	$N\text{-}NO_3$	$N\text{-}NH_4$
cm		---------------- mg Kg^{-1} ------------------				----- mg Kg^{-1} -----	
0 - 5	Clean	1834.2	443.2	478	115.82	14.7	17.4
5 - 10	Clean	1948.4	457.6	572.6	110.38	19.9	21.8
10 - 40	Clean	1694.4	422.4	353.8	146.9	10.06	16.35
0 - 5	Sewage	2354.0	561	264.2	580	13.5	34.2
5 - 10	Sewage	2242.0	537.8	314.0	543.4	17.6	59.9
10 - 40	Sewage	2184.0	559.6	419.6	572.6	7.71	29.20

Depth	Soil irrigation	Cu	Mn	Fe	Zn	Ni	Cr
cm		-------------------------------------- mg Kg^{-1} ------------------------------------					
0 - 5	Clean	2.34	38.56	25.99	2.70	0.576	0.138
5 - 10	Clean	2.37	38.09	27.28	3.81	0.640	0.125
10 - 40	Clean	2.04	38.74	22.75	1.59	0.506	0.121
0 - 5	Sewage	2.75	16.60	38.74	4.78	1.601	0.113
5 - 10	Sewage	2.39	14.53	34.96	3.40	1.604	0.110
10 - 40	Sewage	2.08	14.42	38.15	3.07	1.542	0.109

Key: SP=Saturation point, EC=Electric conductivity, pH= Hydrogen potential, OM= Organic matter, N NO_3= Nitric nitrogen, N NH_4= Ammoniacal nitrogen.

RESULTS AND DISCUSSION

The pH of the soils was alkaline. The electric conductivity (EC), saturation point (SP), and organic matter (OM), calcium (Ca), magnesium (Mg), ammoniac nitrogen (N NH_4), phosphorous (P), iron (Fe), zinc (Zn), and nickel (Ni) quantities are higher in the soils that were irrigated with sewage water.

In both soils, the nitric nitrogen (N NO_3) and the ammoniac nitrogen (N NH_4) were higher in the 5-10 cm layer. The ammoniacal nitrogen (N NH_4) is higher on the two soils due to the organic matter decomposition. It explains why nitrogen is higher on the soils that were irrigated with sewage water that have higher organic matter [20].

The levels of Cu, Cr and Ni are below the threshold for considering them as contaminants [3], but Cu and Ni concentrations are increasing with the irrigation with sewage water. No Pb traces were found. The soils that were irrigated with clean water had higher K, Mn, and Cr concentration.

On beans, there were highly significant differences ($p \leq 0.01$) in at least one of the main factors in all the variables recorded. The nodule number was the only measurement significantly affected ($p \leq 0.05$) for the contamination of the soil with sewage water. Their higher content of ammoniacal nitrogen could reduce nodule number. It is well known the antagonistic effect between nitrogen content is soils and nitrogen fixation [13]. The lack of effect on growth and yield could be explained by the bean low N fixation [10, 21].

There were significant differences among treatments for all the variables recorded but plant height, due to the positive effect of inoculation with *Glomus intraradices* (Table 2). It 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*. [12, 13, 14, 15 and 16].

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

Glomus intraradices	Grain dry weight (g)	Shoot dry weight (g)	Pod number	Pod dry weight (g)	Leaf area (cm^2)	Plant height (cm)
Inoculated	10.37a	23.32a	10.89a	2.03a	955.56a	56.11a
Non inoculated	5.86b	14.754b	5.83b	1.36b	586.92b	54.50a
Glomus intraradices	**Stem diameter (cm)**	**Root length (cm)**	**Root volume (cm3)**	**Root dry weight (g)**	**Small nodule number**	**Total nodule number**
Inoculated	0,24a	31.75a	7.08a	2.25a	3.24a	3.56a
Non inoculated	0,20b	27.64b	5.58b	1.58b	2.07b	2.54b

Means with the same letter in each column are not significantly different (Tukey $\alpha = 0.05$)

The fungicide application improved vegetative growth (Table 3). It provided significantly higher ($p \leq 0.01$) plant height, and leaf area. This treatment also increased the number of small nodules and the total nodule number. The increase in leaf area could contribute to better N fixation.

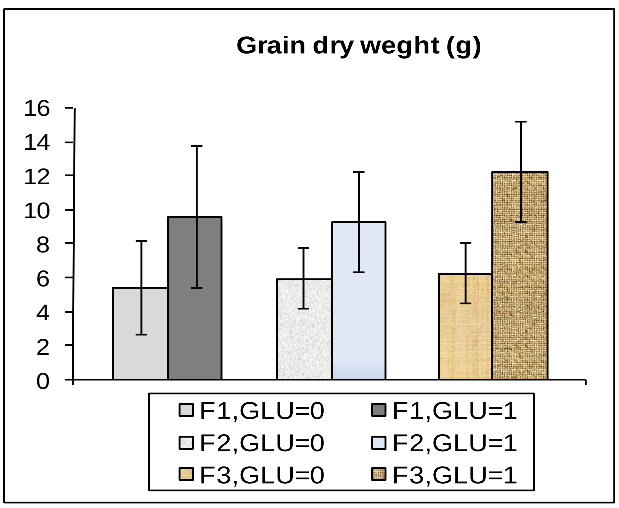

Figure 1. Effect of inoculation with *Glomus intraradices* on soils with two types of irrigation in grain dry weight of three cultivars common bean (*Phaseolus vulgaris*).
Key: Cultivars: F1: Flor de Durazno bean, F2: Bayo Azteca bean, F3: Flor de Mayo M 38 bean. Glu=0: Noninoculated, Glu=1: Inoculated with *Glomus intraradices.* The vertical lines indicate standard error.

Table 3. Honest significant difference of the effect of fungicide on common bean (*Phaseolus vulgaris*).

Fungicide (Metacaptan)	Grain dry weight (g)	Shoot dry weight (g)	Pod number	Pod dry weight (g)	Leaf area (cm^2)	Plant height (cm)
Not applied	8.44a	18.96a	8.39a	1.78a	729.64b	53.42b
Applied	7.79a	19.11a	8.33a	1.60a	812.83a	57.19a
Fungicide (Metacaptan)	**Stem diameter (cm)**	**Root length (cm)**	**Root volume (cm3)**	**Root dry weight (g)**	**Small nodule number**	**Total nodule number**
Not applied	0.23a	29.36a	6.31a	1.90a	2.57b	2.97a
Applied	0.21a	30.03a	6.36a	1.92a	2.74a	3.13a

Means with the same letter in each column are not significantly different (Tukey $\alpha = 0.05$)

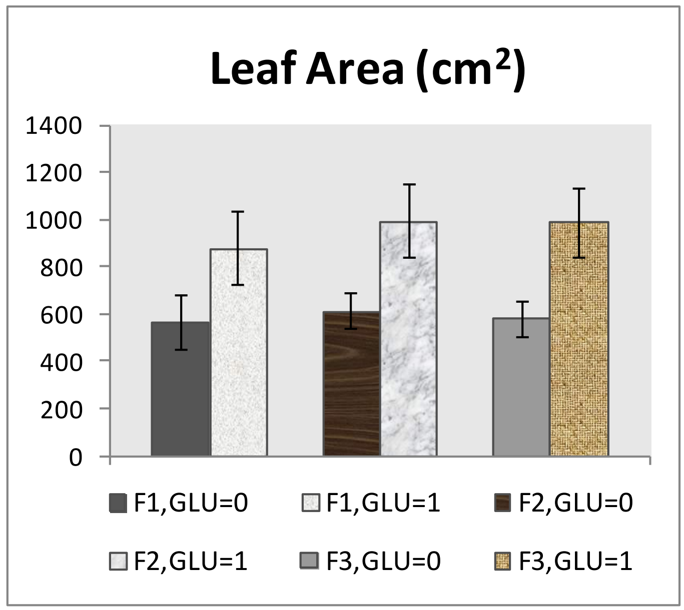

Figure 2. Effect of inoculation with *Glomus intraradices* on soils with two types of irrigation on leaf area of three cultivars common bean (*Phaseolus vulgaris*).
Key: Cultivars: F1: Flor de Durazno bean, F2: Bayo Azteca bean, F3: Flor de Mayo M 38 bean. Glu=0: Noninoculated, Glu=1: Inoculated with *Glomus intraradices*. The vertical lines indicate standard error.

Yield, root and shoot growth were superior to those of plants without inoculation (Table 2). The treatments with mycorrhiza had 63% more leaf area (Figure 2), 58% heavier shoot dry weight, 15% longer roots, 27% greater root volume, and 40% more nodules[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.* [11, 14] 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, including those studied in this experiment [25], and in beans [2].

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

The inoculation with *Glomus intraradices* improved root and shoot growth and also had a beneficial effect on the biological nitrogen fixation, and a higher absorption of nutrients [16], contributing to higher yield in beans, coinciding with other studies [2]. Inoculated plants yielded 87% more pods and 77% more grain (Table 2 and Figure 1).

Bean genotypes were only significantly different in plant height ($p \leq 0.01$). This can be explained for the growth habit. Flor de Durazno genotype had a lower height because is determinate. The other two varieties (Bayo Azteca and Flor de Mayo M38) have indeterminate growth (Figure 4). However, other vegetative growth variables and yield were similar.

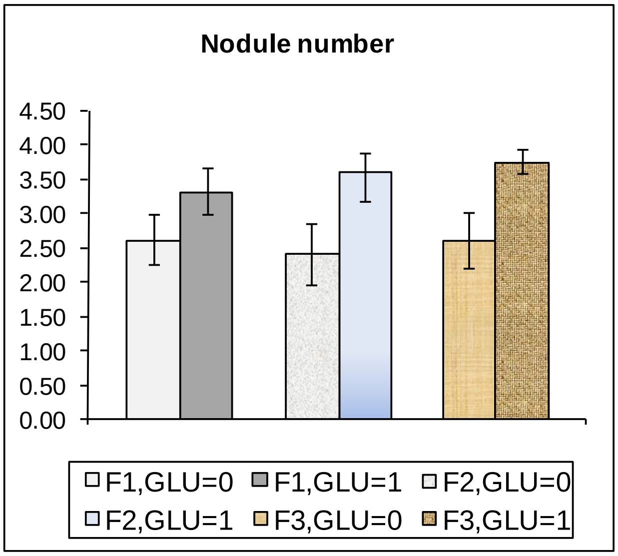

Figure 3. Effect of inoculation with *Glomus intraradices* on soils with two types of irrigation on the number of nodules of three cultivars common bean (*Phaseolus vulgaris*).
Key: Cultivars: F1: Flor de Durazno bean, F2: Bayo Azteca bean, F3: Flor de Mayo M 38 bean. Glu=0: Noninoculated, Glu=1: Inoculated with *Glomus intraradices*. The vertical lines indicate standard error.

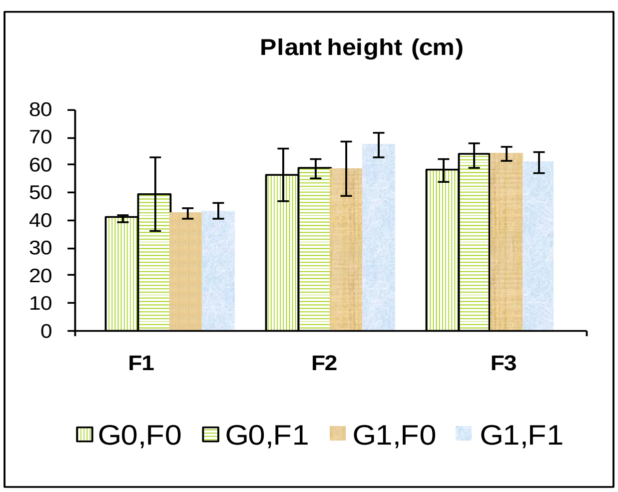

Figure 4. Effect of inoculation with *Glomus intraradices* and fungicide application in soils with two types of irrigation on plant height of three cultivars common bean (*Phaseolus vulgaris*).
Key: Cultivars: F1: Flor de Durazno bean, F2: Bayo Azteca bean, F3: Flor de Mayo M 38 bean. Glo0= Noninoculated, Glo1= Inoculated with *Glomus intraradices*. Fungicide: F0: No application, F1: Metacaptan application. The vertical lines indicate standard error.

In a similar way to beans, oat and wheat plants inoculated with *Glomus intraradices* had an enhanced root growth ($p \leq 0.05$) expressed as greater root length (110%[2]), greater root volume (130.5%), and heavier root dry weight (127.7%); as well as a larger photosynthetic apparatus ($p \leq 0.05$), expressed in biomass dry weight (184.7%) and leaf area (127.3%).

Table 4. Honest significant difference of the effect of *Glomus intraradices* on oat (*Avena sativa* L.), and wheat (*Triticum aestivum* L.).

Glomus intraradices	Grain dry weight (g)	Shoot dry weight (g)	Grain number	Leaf area (cm^2)	Plant height (cm)
Inoculated	3.65a	12.19a	4.35a	293.7a	67.71a
Non inoculated	2.84b	6.60b	4.04b	230.71b	59.21a

Glomus intraradices	Stem diameter (cm)	Root length (cm)	Root volume (cm3)	Root dry weight (g)
Inoculated	0.17a	24.50a	4.79a	1.89a
Non inoculated	0.12b	22.21a	3.67b	1.48b

Means with the same letter in each column are not significantly different (Tukey α= 0.05)

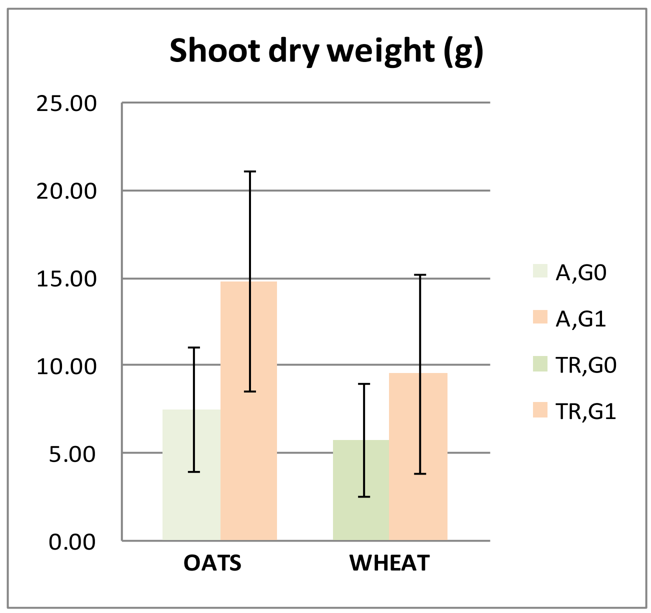

Figure 5. Effect of inoculation with *Glomus intraradices* on shoot dry weight (g) of oats (*Avena sativa*) and wheat (*Triticum aestivum*).
Key: Cultivars: A: Oats, TR: Wheat. G0= Noninoculated, G1= Inoculated with *Glomus intraradices*. The vertical lines indicate standard error.

Better shoot and root development due to the improvement in mineral nutrition for inoculation with *Glomus intraradices* increased photosynthetic production and augmented grain yield (128.5%).

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[2] Increase rate are referred to the values found in oat and wheat plants inoculated with mycorrhiza compared to those without inoculation.

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. In oats and wheat, inoculation with *Glomus intraradices* leads also to superior root and shoot growth, and better yields. The contamination of the soil by sewage water did not affect plant growth or yield. The application of fungicide improved plant growth, but it did not change grain yield.

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