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The Effects of Chemical, Biological and Nano Fungicides on Mycorrhizal Colonization and Quality of Sunflower

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ABSTRACT

Application of fungicides interferes with plant-mycorrhiza symbiosis and inhibits root system development, absorption and growth. So this experiment was conducted in 2009 at the research field of Islamic Azad University, Karaj branch, Iran, to evaluate the effect of different fungicides on mycorrhizal symbiosis and quality of sunflower. The study was conducted in factorial in the form of a randomized complete block design with four replications and two factors. The first factor was fungicide (without, F_0; chemical, F_1; nano, F_2; biologic, F_3) and the second factor was mycorrhiza species (without, M_0; Glomus mosseae, M_1; G. etunicatum, M_2; G. intraradices, M_3). Results indicated that fungicide had only a significant effect on grain protein content, and mycorrhiza had a significant effect on grain oil content and root colonization. F_2M_1 was the best treatment with the highest effect which increased grain yield, protein yield and oil yield by 60.77, 60.93 and 69.90%, respectively, compared with the control (F_0M_0).

Keywords: *Glomus* spp., oil, nanosilver fungicide, protein.

INTRODUCTION

Sunflower is an important oil crop because of high oil content. Plant's oil content can be improved through different methods; one of them is the application of biofertilizers such as mycorrhiza. Mycorrhizal inoculation can significantly increase grain's oil content.

Vesicular Arbuscular Mycorrhizae (VAM) are the main group of mycorrhiza fungi which improve plant growth through different mechanisms such as phosphate solubilization and enhancement of water / nutrients absorption. This ability of mycorrhiza is mainly attributed to its hyphae. Hyphae are external root shaped organs which penetrate into soil pores and cracks, and make higher soil volume available to plant to be used as the source of water and nutrients [1-3]. Different experiments have reported the improvement of plant growth, yield and nutrients uptake as the function of mycorrhizal inoculation [4-7].

One of the factors that reduced the efficiency of plant-mycorrhiza symbiosis is the application of fungicides. Studies have revealed the inhibition of mycorrhiza growth and activity as the result of fungicides application. Fungicides affect mycorrhiza hyphae and spores; disturbing the activity of mycorrhiza and the subsequent benefits to plant [8]. In an experiment, the effect of chemical fungicides was evaluated on different species of mycorrhiza [9]. Results indicated that vitavax had the highest negative impact of maize growth and yield, and grain protein and phosphorus content; however, benomyl had the lowest negative impact on root colonization. In another experiment, the effect of benomyl, captan and PCNB fungicides was studied on three mycorrhiza species (*G. mosseae*, *G. rosea* and *G.*

etunicatum) in pea cultivation [10]. Results showed that *G. etunicatum* had the highest, and *G. rosea* had the lowest resistance against fungicides. Moreover, captan was safer for the fungi, compared with the two other fungicides.
Non chemical fungicides may cause lower damages to the beneficial microorganisms. Nanosilver, a non chemical fungicide, has antibacterial features. It reduces the activity of bacteria cell membrane protein and inhibits respiration by releasing silver ions [11]. Although the mechanisms of action of nanosilver is not still understood; however, it is clear that nanosilver release silver ions (Ag^+) slowly; these ions damage the cell structure of microorganisms [12]. Rostami and Shahsavar [12] reported that nanosilver fungicide eliminated microbial pollutions in transplanting medium without any risks to human or environment health. However, soaking the transplants in high concentration nanosilver solution damaged the plants. Finally, the objective of this experiment was to compare the effect of three types of fungicide on sunflower and mycorrhizal inoculation.

MATERIAL AND METHODS

This experiment was conducted in 2009 at the research field of Islamic Azad University, Karaj branch, Iran (50˚ 49' E, 35˚ 43' N, 1170 m above the sea level). The soil type at the test site was sandy loam. The study was conducted in factorial in the form of a randomized complete block design with four replications and two factors:

Fungicide. Without fungicide application (F_0), and chemical (F_1), nano (F_2) and biological (F_3) fungicides. The chemical fungicide was benomyl as 0.001 wetable powder. The biological fungicide was biosubtyl (0.002) which contained *Bacillus subtilis* (3% / L). The nano fungicide was nanosilver containing 4 g nano particles of silver / L, as 2000 L کلوئید with concentration of 60 ppm, which was applied on seeds.

Mycorrhiza species. Without inoculation (M_0), *Glomus mosseae* (M_1), *G. etunicatum* (M_2) and *G. intraradices* (M_3). All mycorrhiza species contained 300-350 active fungus organs/m^2. 2 g of the inoculant for each seed was located in each seeding hole at the time of planting.

According to the results of soil analysis, 350 kg N/ha (as urea, split in two parts), 75 kg P/ha (as triple super phosphate) and 300 kg K/ha (as potassium sulfate) were applied in soil. Then, Sunflower was planted 60 cm × 20 cm on May 15th. Irrigation was repeated weekly and weeds were removed manually.

To measure the root colonization percentage, sampling was conducted at the flowering stage, which the colonization percentage is the highest. To do this, after irrigation and when the field was at FC, three plants were randomly harvested along with their root by digging a 30 cm profile around the root. Then, roots were washed; 10 g of the roots were randomly selected and colored. Colorized roots were cut to 1 cm slices and 50 slices were randomly located on a 1 cm netted petri dish, and root colonization percentage was measured based on the Gridline Intersect Method [13].

At the end of the growing season, harvest was conducted to obtain the final yield. To measure grain protein and oil percentage, 30 g of grains from each plot was randomly selected and grinded, and was subjected to NMR method by Inframatic 8620 instrument. Root dry weight was measured by the previously described method. Finally, data were analyzed using SAS and means were compared according to the Duncan's multiple rang test at $P \leq 0.05$.

RUSULTS AND DISCUSSION

Analysis of covariance indicated that fungicide had a significant effect only on grain protein content. Mycorrhizal inoculation significantly affected grain oil content and root colonization percentage. The interaction of fungicide × mycorrhiza had a significant effect on grain yield, protein yield, grain oil content and oil yield. Grain P content was not affected by any factor of the experiment (Table 1).

Table 1. Analysis of covariance of the measured traits

SOV	df	Mean Squares (MS)							
		Grain yield	Grain protein content	Protein yield	Grain oil content	Oil yield	Grain P content	Root colonization	Root dry weight
Block	3	**	**	**	ns	**	ns	ns	ns
Fungicide (F)	3	ns	*	ns	ns	ns	ns	ns	ns
Mycorrhiza (M)	3	ns	ns	ns	*	ns	ns	*	ns
FM	9	**	ns	**	**	**	ns	ns	ns
Error	44	120714.05	0.93	5580.39	4.55	27963.94	0.01	44.99	0.001
Covariance factor (X)	1	**	ns	**	*	**	ns	ns	Ns
CV (%)	-	19.10	4.75	20.18	4.56	19.54	14.08	18.51	21.20

*ns, nonsignificant; **, significant at $P \leq 0.01$; *, significant at $P \leq 0.05$.*

Grain yield. Results showed that grain yield was the highest in nanosilver × *G. mosseae* and the lowest in nanosilver × *G. etunicatum* (Figure 1).

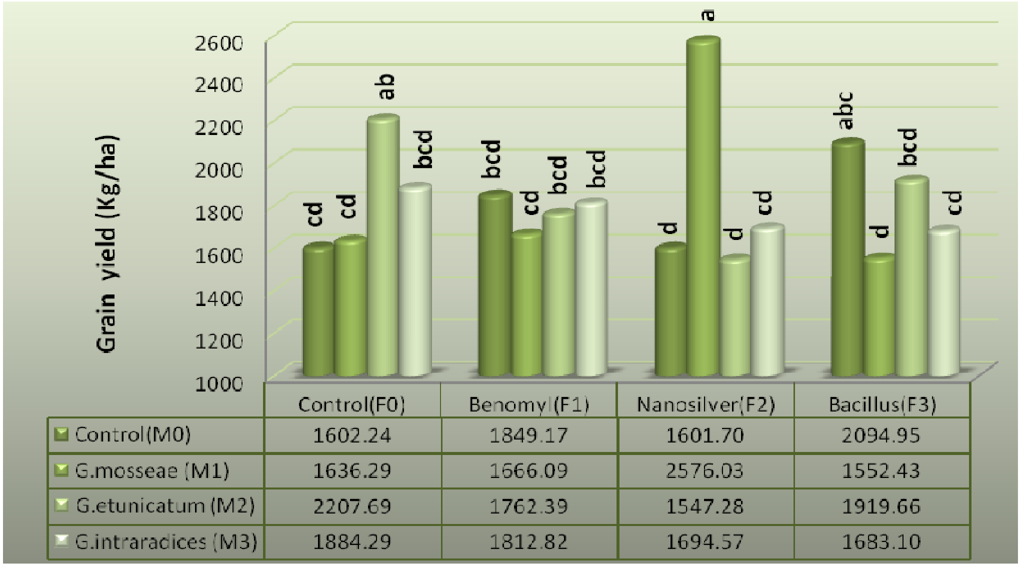


Fig 1. Effects of interaction of fungicide × mycorrhiza on grain yield.

Different mycorrhiza species increased grain yield compared with the control (F_0M_0); *G. etunicatum* was the most effective species which increased grain yield by 37.87%. This may be caused by better symbiosis of this species with the plant which results in the improvement of water and nutrient uptake, photosynthesis and plant growth [9, 14]. Moreover, higher efficiency of this species can be attributed to higher colonization percentage [15].

Different species of mycorrhiza reduced grain yield when applied along with benomyl fungicide [16]; the highest reduction was observed in *G. mosseae* (10.98%). This fungicide increased grain yield by 14.41% when applied individually [8]. Benomyl had the highest inhibitory effect on *G. etunicatum*. The fungicide inhibits mycorrhiza growth and development because of the activity of methyl 1, 2-benzimidazole carbamate which is a product of benomyl hydrolysis [1, 17]. Moreover, application of benomyl damages external hyphae of mycorrhiza and prevents root colonization; reducing water and nutrient uptake, photosynthesis and plant growth [15, 18].

Mycorrhiza species react differently to nanosilver fungicide, in the way that *G. etunicatum* decreased grain yield compared with F_2M_0 which is probably because of the sensitivity of this species to nanosilver. Silver's nano particles damage the proteins in cell membrane of external hyphae of mycorrhiza; preventing the growth and development of the hyphae. In addition, nanosilver inhibits the fungus cell respiration. *G. mosseae* along with nanosilver increased grain yield by 60.83% compared with F_2M_0. This species was not sensitive to nanosilver.

The individual application of nanosilver increased grain yield by 3%; however, application of *B. subtilis* along with mycorrhiza species decreased grain yield compared with F_3M_0; the highest reduction was observed in *G. mosseae* (34.94%). The sensitivity of the species to the fungicide can be attributed to the presence of antifungal and antibiotic lipopeptid compounds in the exudates of the bacterium. The activity of mycorrhiza external hyphae is related to succinate dehydrogenase enzyme which improves plant photosynthesis and P uptake; however, *B. subtilis* inhibits the activity of this enzyme by producing antifungal substances [19-20]. Results of this experiment indicated that the individual application of *B. subtilis* without mycorrhizal inoculation increased grain yield by 30.75% compared with F_0M_0.

Grain protein content. Results indicated that although benomyl and nanosilver were significantly the same as control (F_0); however, *B. subtilis* reduced grain protein content by 3.61% (Fig 2). This may be attributed to antibiotics exudation by the bacterium which damages plant root and reduces nitrogen absorption and protein synthesis [21].

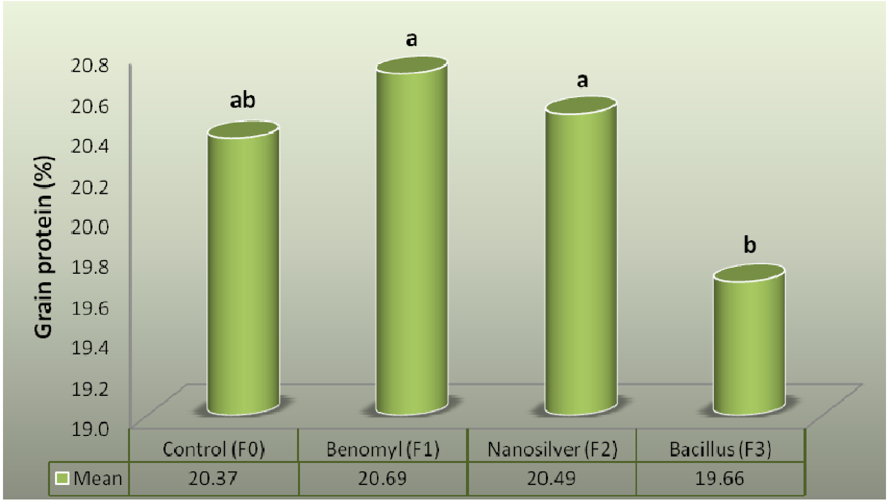
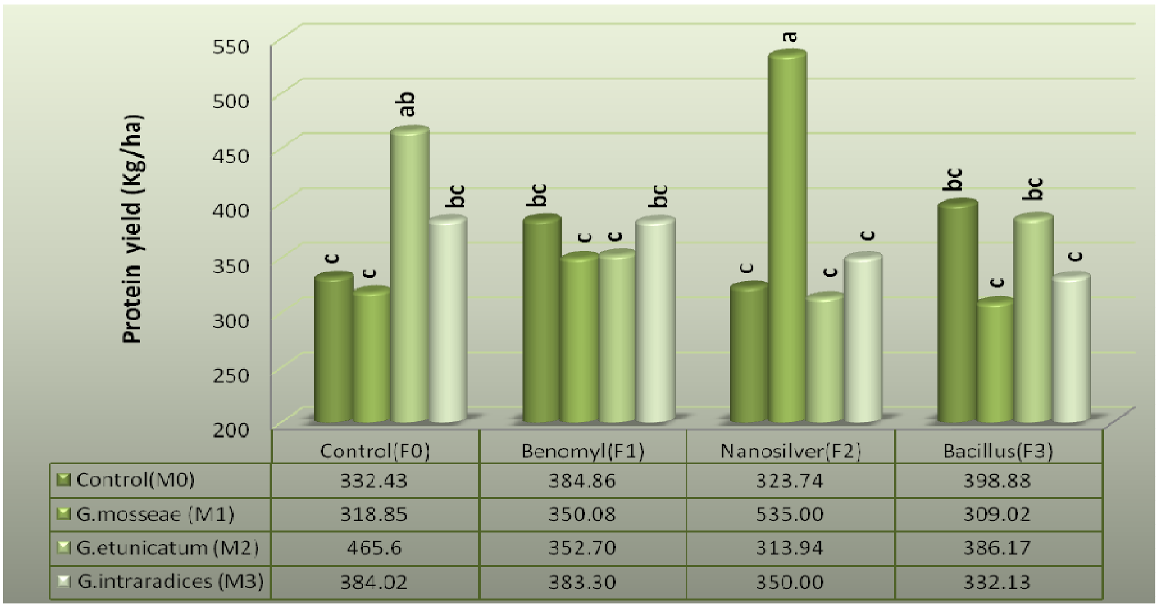


Fig 2. Effects of fungicides on grain protein content.

Protein yield. Application of nanosilver × *G. mosseae* resulted in the highest, and B. subtilis × *G. mosseae* resulted in the lowest protein yield (Figure 3).

Inoculation of *G. etunicatum* and *G. intraradices* increased protein yield; however, *G. mosseae* decreased protein yield compared with F_0M_0. *G. etunicatum* was the best treatment which increased protein yield by about 40%.

When benomyl was applied, all mycorrhiza species reduced protein yield compared with F_1M_0; the highest reduction was related to *G. mosseae* (9.93%). *G. mosseae* and *G. intraradices* increased; however, *G. etunicatum* decreased protein yield compared with F_2M_0, when applied along with nanosilver. *G. mosseae* increased protein yield by 65.25% compared with F_2M_0. Nanosilver application without mycorrhizal inoculation reduced protein yield by 2.68% compared with the control (F_0M_0). Different genotypes of mycorrhiza reduced protein yield when applied along with *B. subtilis*; the highest reduction was observed in *G. mosseae* (29.07% compared with F_3M_0). The variations in protein yield are attributed to the variations in grain yield.



Grain oil content. The highest grain oil content was achieved in F_3M_3 and the lowest in F_1M_1 (Figure 4).

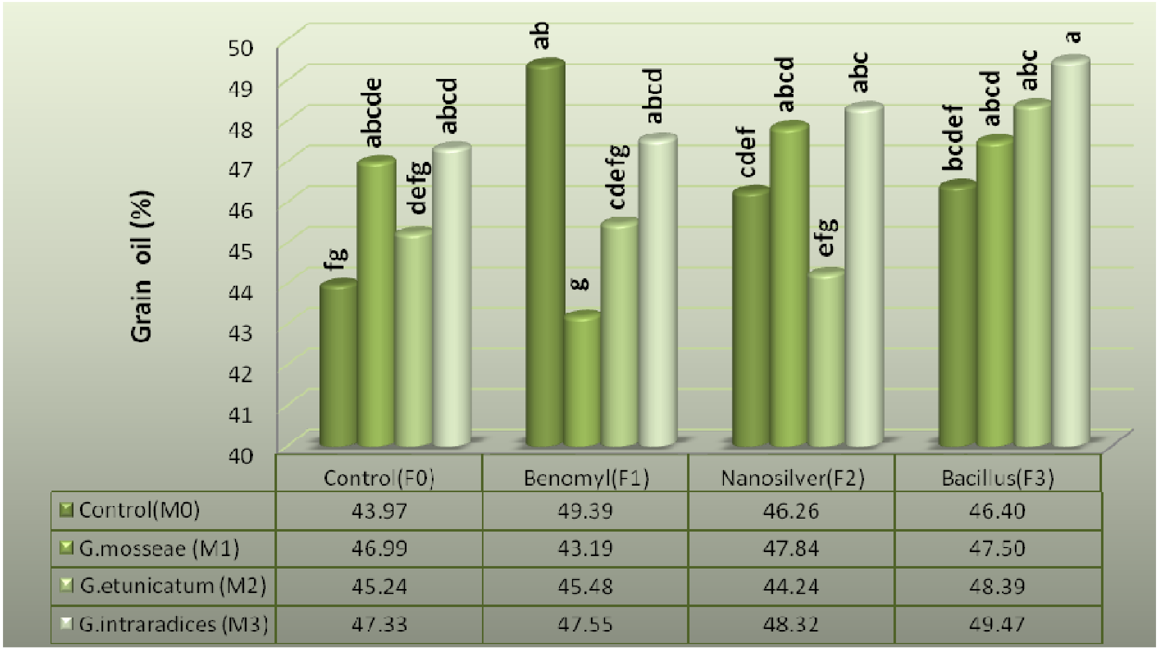


Fig 4. Effects of interaction of fungicide × mycorrhiza on grain oil content.

Inoculating the plant with the three mycorrhiza species increased grain oil content. *G. intraradices* was the most effective treatment and increased this trait by 7.64%. Mycorrhiza increases plant nutrients uptake, especially micronutrients such as sulfur, and consequently increases plant oil content [22].

Different mycorrhiza species reduced oil content compared with F_1M_0 when applied along with benomyl fungicide; the highest reduction was related to *G. mosseae* (14.35%). There are evidences representing that vesicular arbuscular mycorrhiza stimulates lipids synthesis; however, benomyl fungicide inhibits the formation and development of mycorrhiza arbuscules and reduces the efficiency of the inoculation and formation of lipids [23-26].

Grain oil content increased when *G. mosseae* or *G. intraradices* were applied along with nanosilver fungicide; however, oil content reduced in the co-application of *G. etunicatum* compared with F_2M_0. This reduction may be attributed to the effect of nanosilver on proteins of cell membrane and cellular respiration of mycorrhiza [11]. Applying nanosilver without mycorrhizal inoculation increased oil content by 5.20% compared with the control (F_0M_0).

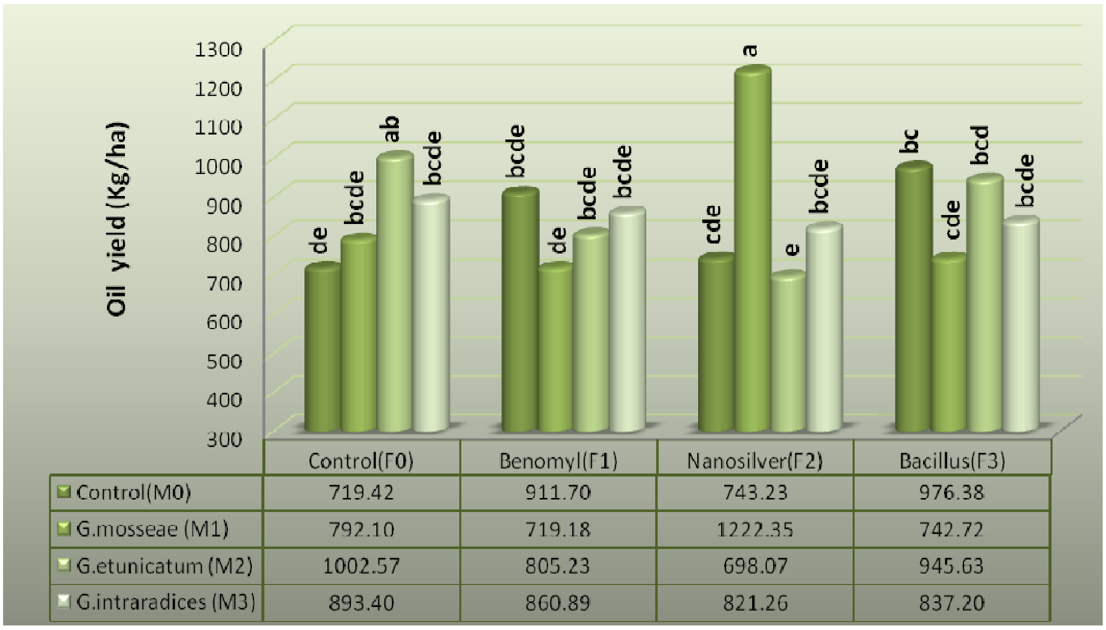


Fig 5. Effects of interaction of fungicide × mycorrhiza on oil yield.

All mycorrhiza species increased oil content when applied along with *B. subtilis*; the most effective species was *G. intraradices* which increased this trait by 6.61% compared with the control. Results indicated that *B. subtilis* reduced protein content. There is a negative correlation between protein and oil content.

Oil yield. Results indicated that the highest oil yield was achieved in F_2M_1 and the lowest was achieved in F_2M_2 (Figure 5).

Mycorrhiza species increased oil yield compared with F_0M_0; *G. etunicatum* was the most effective one which increased oil yield by 39.35%.

When benomyl was applied, inoculation of the three mycorrhiza species reduced oil yield; *G. mosseae* reduced it the most (by 26.76%). Oil yield increased when *G. mosseae* or *G. intraradices* were applied along with nanosilver; however, oil yield decreased when *G. etunicatum* was applied along with the fungicide. All mycorrhiza species showed sensitivity to *B. subtilis* fungicide and reduced oil yield compared with F_3M_0.

Root colonization. Results showed that the three mycorrhiza species increased root colonization percentage compared with M_0; however, there was no significant difference between the species (Figure 6). The highest root colonization was observed in *G. etunicatum* which was about 21% higher than M_0. Higher root colonization percentage contributes to higher grain and biomass production because of higher nutrients supply.

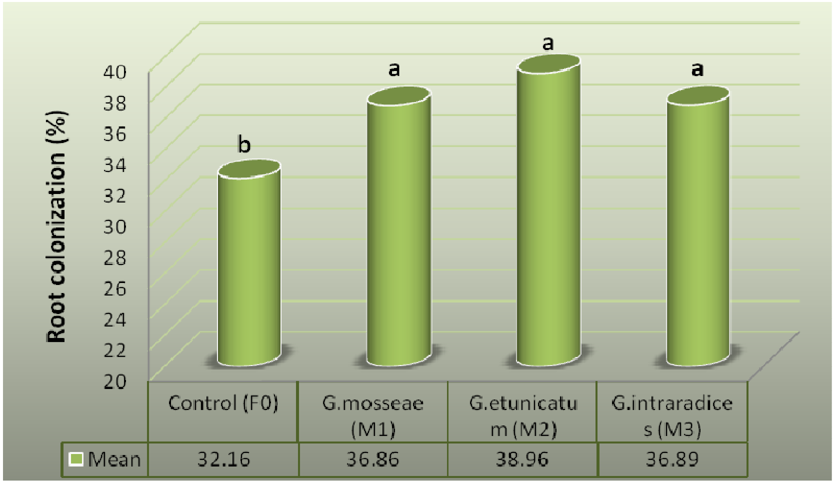


Fig 6. Effects of interaction of mycorrhiza on root colonization.

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