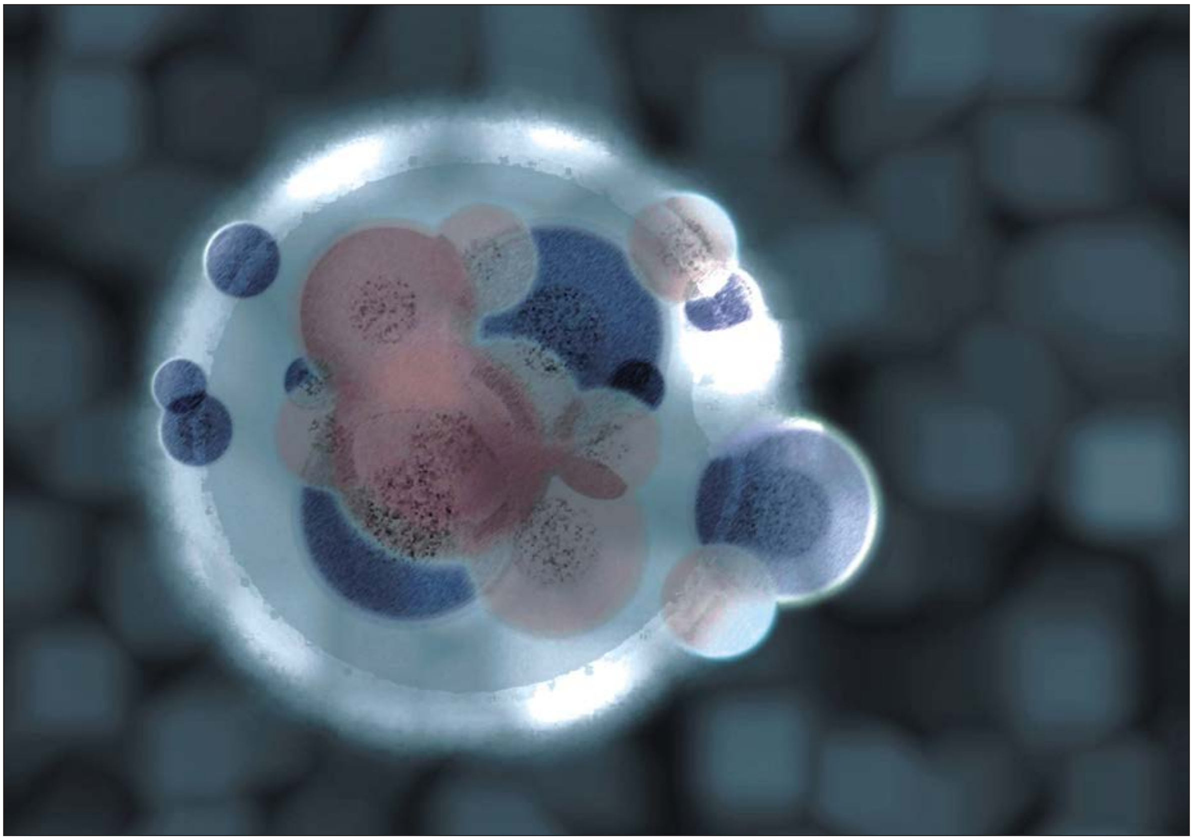

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The Combined Effects of Fungicides and Arbuscular Mycorrhiza on Corn (*Zea mays* L.) Growth and Yield under Field Conditions

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Abstract: With respect to the significance of the combined effects of fungicides application and Arbuscular Mycorrhizal fungi on the growth and yield of different crop plants such as corn (*Zea mays* L.) a field experiment was conducted. The objectives were to determine: (1) the combined effects of different fungicides and different arbuscular mycorrhiza (AM) species on the growth and yield of corn and (2) the efficiency of different AM species in symbiosis with corn plants, treated by different fungicides, under field conditions. Four AM treatments including control (M_0), *Glomus mosseae* (M_1), *G. etunicatum* (M_2) and *G. intraradices* (M_3) and four fungicide treatments including control (F_0), benomyl (F_1), vitavax (F_2) and captan (F_3) were tested in a factorial fashion on the basis of a completely randomized block design in three replicates in 2006. Different species of AM significantly affected corn growth and yield when subjected to different fungicides treatments. *G. mosseae* and corn plants established the most efficient symbiosis. In addition, fungicide benomyl had the least unfavorable effects on the colonization of corn roots by AM species. Determination of the appropriate rates of fungicides for treating seeds to alleviate the unfavorable effects of fungicides on plant growth, especially when in symbiosis with AM species, is of great significance.

Key words: Mycorrhizal symbiosis, stress alleviation, interaction effects, chemical treatments

INTRODUCTION

Treating seeds with different chemicals such as fungicides to ensure their high germination is a common practice before planting (Miransari and Smith, 2007, 2008). However, such treatments may influence soil microbial population including arbuscular mycorrhizal fungi developing a beneficial symbiotic association with most of the terrestrial plants including crop plants (Allen, 1991; Rillig, 2004).

Consideration of the actions and interactions between plants and soil microorganisms including Arbuscular Mycorrhiza (AM), influenced by different parameters such as chemicals in the field, can be very useful for the development of favorable strategies, which can enhance the efficiency of agricultural production. Although, using different chemicals including fungicides for seeds treatments, is a very useful practice for increasing seed germination and hence yield production, however, such kind of treatments may affect AM activities, which are of great significance for the production and health of ecosystems (Schreiner and Bethlenfalvay, 1997; Chiocchio *et al*., 2000; Salem *et al*., 2003; Zhang *et al*., 2006).

Accordingly, it can be very pertinent to evaluate how different fungicides may affect the activities of AM in the soil and how AM can interactively adjust the unfavorable effects of fungicides on soil microbial populations. This is because AM have some very great abilities, including alleviating the effects of different soil stresses on plant growth (Miransari *et al*., 2006-2009) and also controlling the adverse effects of soil pathogens (Schreiner and Bethlenfalvay, 1997).

Corn is among the most important crop plants feeding a large number of people in the world and is able to develop symbiotic association with AM. Hence, evaluation of its responses under different situations, including treating seeds with different fungicide products can be very beneficial agronomically and environmentally. Accordingly, it seems rational to use different soil microbes including AM as biofertilizers to ensure the high productivity of agricultural production (Miransari *et al*., 2006-2009).

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AM species can contribute to enhanced corn growth and metabolism, differently. For example, Cliquet and Stewart (1993) indicated that the activity of glutamine synthetase, which is a necessary enzyme for the incorporation of nitrogen into the organic compounds such as proteins, was increased in mycorrhizal plants, relative to non-mycorrhizal plants. Accordingly, the amount of soluble proteins in the plants shoots increases, resulting in enhanced plant resistance to different stresses.

Relative to vitavax, benomyl and captan are much more common fungicides (Salem *et al*., 2003; Sudisha *et al*., 2006; López-Herrera and Zea-Bonilla, 2007). Scientists have stated that fungicides such as captan affect the symbiosis of AM with the host plant in different manners including negatively, neutrally and positively. The concentration of fungicide may also affect the symbiosis as higher concentrations adversely affected the symbiosis of *Glomus etunicatum* with the host plant and decreased the colonization ratio (Nemec, 1980; Smith *et al*., 2000; Zhang *et al*., 2006).

Since, data related to the combined effects of different fungicides and AM on corn plants, particularly under field conditions is rare and with respect to the ecological and environmental significance of such evaluations, we performed this research work hypothesizing that AM are able to alleviate the unfavorable effects of fungicide products, necessary for high seed germination, on corn growth. The objectives were to determine: (1) the combined effects of different fungicides and Arbuscular Mycorrhizal species on the growth and yield of corn and (2) the efficiency of different AM species in symbiosis with corn plants, treated by different fungicides, under field conditions.

MATERIALS AND METHODS

Experimental procedure: The research was conducted in the spring of 2006 in the Research Field (with a loamy sand texture) of Islamic Azad University, Karaj, Iran. The experiment was a factorial on the basis of completely randomized design including two factors and three replicates. The first factor was different treatments of AM species including control (M_0), *Glomus mosseae* (M_1), *G. etunicatum* (M_2) and *G. intraradices* (M_3). The second factor was different treatments of fungicide including control (M_0), benomyl (F_1), vitavax (F_2) and captan (F_3). Seeds were treated with the fungicides concentrations of 0.1-15% for benomyl, 0.15-0.2% for vitavax and 0.2-0.5% for captan. Hence, 16 experimental treatments were tested in each replicate making the total of 48 plots.

At planting seeds were planted in the rows and inoculated with 2 g of AM inoculum at the 5 cm depth (Miransari *et al*., 2006-2009). Seeds of single cross corn (cv. 302) were planted on the 15th of May 2006. Two to three corn seeds were planted on the rows at 10 cm spacing. The field was fertilized according to soil test analysis at 250 kg ha^{-1} urea (50% at planting and the rest at 4-6 leaf and flowering stages) and 30 kg ha^{-1} super phosphate, which were mixed with the soil. The first irrigation was performed on the 16th of May and to facilitate seed germination the second irrigation was conducted on the 23rd of May. Plants were thinned in the 4-5 and 7-8 leaf stages. To inhibit the unfavorable effects of herbicides, no herbicides were used in the experiment, however weeding was conducted manually.

Inoculum preparation of arbuscular mycorrhizal species: To produce the inoculum of arbuscular mycorrhiza, species including *Glomus mosseae*, *G. etunicatum* and *G. intraradices* from the collection of Soil and Water Research Institute, Tehran, Iran were used to inoculate sorghum plants in a four-month period under greenhouse conditions using sterilized sand (Miransari *et al*., 2007, 2008). The daily and nightly temperature of 28 and 25°C, respectively, the humidity of 60% and the daily and nightly light length of 16 and 8 h were the conditions in the greenhouse. Accordingly, the nutrient requirements of plants were supplied. Plants were irrigated until flowering and after two weeks plants shoots were harvested from the soil surface.

The mixture of plant roots and sand were used as the inoculum. Using MPN method the inoculation potential of different isolates were tested (Mahaveer, 2000; Feldman and Idczak, 1992). Hence, using sterilized soil, concentrations of 0.1, 0.01 and 0.001 of AM (g AM per g of sand) were used for sorghum plantation, which were grown for one month. The plants were then harvested and the inoculation percentage on the sorghum roots was determined (Mahaveer, 2000).

Statistical analysis: Using SAS (SAS Institute Inc., 1988) data were subjected to analysis of variance. Mean comparison was conducted using Dunkan's Multivarite Range Test (Steel and Torrie, 1980).

RESULTS

The effects of AM, fungicides and their interaction effects on corn growth and yield was significant (Table 1). Interestingly, according to our hypothesis and objectives the combined or actually the interaction effects of AM and fungicide treatments on almost all determined corn

Table 1: Analysis of variance, for the effects of different experimental parameters on corn growth and yield

		Sum of squares										
SOV	df	CL	CBR	CBD	WTG	CW	GY	Biomass	SD	PH	GP	GPR
Rep.	2	101.91	199.50	88.87	1858.72	4219.62	50.35	460.14	0.23	4630.03	31.00	23.88
AM	3	4.26	47.71	47.00	3047.28	479.57	15.25	27.33	0.04	345.64	93.68	3.21
F	3	19.42	36.15	4.20	4532.16	2264.37	5.89	22.41	0.09	406.81	72.02	20.50
AM*F	9	4.87	19.26	1.75	1653.25	520.21	3.60	23.09	0.02	334.05	15.87	3.12
Error	30	1.34	8.29	0.32	440.62	165.31	1.68	6.10	0.01	94.18	2.51	0.36
CV	-	9.38	10.31	3.14	9.57	15.28	23.67	10.86	8.17	6.23	5.08	6.16

SOV: Source of variation, Rep: Replicate, AM: Arbuscular Mycorrhiza, F: Fungicide, CV: Coefficient of variation. CL: Cob length, CBR: No. of cob grains on a row, CBD: No. of cob grains, diagonally, WTG: Weight of thousand grains, CW: Cob weight, GY: Grain yield, SD: Stem diameter, PH: Plant height, GP: Grain phosphorous, GPR: Grain protein

Table 2: The effects of different arbuscular mycorrhiza (AM) species, averaged across different fungicides, on corn growth and yield

AM species	CL (cm)	CBR	CBD	WTG (g)	Biomass (t ha^{-1})	SD (cm)	PH (cm)	GP (%)	GPR (%)
M_0	11.54b	25.36b	17.82b	196.03b	19.22b	1.22b	151.18b	9.28b	27.16b
M_1	12.58a	30.49a	18.38a	225.24a	24.26a	1.33ab	163.24a	10.48a	33.16a
M_2	12.44ab	27.68ab	18.07ab	223.59a	23.71a	1.33ab	152.69b	9.84ab	33.00a
M_3	12.94a	27.90ab	17.93ab	232.31a	21.88ab	1.37a	155.8ab	9.52b	31.41a

M_0: Control, M_1: *Glomus mosseae*, M_2: *G. etunicatum,* M_3: *G. intraradices.* CL: Cob length, CBR: No. of cob grains in a row, CBD: No. of cob grains, diagonally, WTG: weight of thousand grain, SD: stem diameter, PH: Plant height, GP: Grain phosphorous, GPR: Grain protein. Values within the same column with diffrent letter(s) are significantly different

Table 3: The effects of different fungicides, averaged across different AM species, on corn growth and yield

Fungicides	CL (cm)	CBR	CBD	WTG (g)	Biomass (t ha^{-1})	SD (cm)	PH (cm)	GP (%)
F_0	14.27a	30.35a	18.91a	246.34a	23.97a	1.44a	164.28a	11.69a
F_1	11.97b	27.51ab	17.89b	200.10b	21.60ab	1.27b	153.96ab	9.51b
F_2	11.56b	26.23b	17.56b	216.29b	19.66b	1.23b	151.21b	8.78c
F_3	11.71b	27.61ab	17.84b	214.35b	23.85a	1.32b	153.46ab	9.14bc

F_0: Control F_1: Benomyl, F_2: Vitavax, F_3: Captan. CL: Cob length, CBR: No. of cob grains in a row, CBD: No. of cob grains, diagonally, SD: stem diameter, PH: Plant height, GP: Grain phosphorous, GPR: Grain protein. Values within the same column with different letter(s) are significantly different

Table 4: Effects of different species of Arbuscular Mycorrhiza and fungicides on cob weight (g), grain yield (t ha^{-1}) and grain protein (%)

	AM species				Fungicides			
Parameters	M_0	M_1	M_2	M_3	F_0	F_1	F_2	F_3
Cob weight (g)	76.66b	90.00a	81.19ab	88.71a				
Grain yield (t ha^{-1})	4.00a	6.56a	5.27ab	6.14a				
Grain protein (%)					11.69a	9.51b	8.78c	9.14bc
Grain yield (t ha^{-1})					6.45a	5.55ab	4.99b	4.98b

M_0: Control, M_1: *Glomus mosseae*, M_2: *G. etunicatum*, M_3: *G. intraradices*, F_0: Control, F_1: Benomyl, F_2: Vitavax, F_3: Captan

growth and yield parameters were significant. While, the effects of M_1 and M_3 on enhancing cob only M1 increased the number of row and diagonal cob grains, relative to the control. Weight of thousand grain was significantly enhanced by all species of arbuscular mycorrhiza. The results also indicate that plant biomass was significantly enhanced by species M_1 and M_2. There were significant increases in stem diameter and plant height by species M_3 and M_1, respectively. Relative to the control while M1 significantly increased grain phosphorous, all AM species enhanced grain protein significantly (Table 2). Species M_1 and M_3 significantly increased cob weight (Table 4). Relative to the control treatment inoculation of seeds with AM species resulted in significant increase in corn grain yield (Table 4).

Fungicide addition significantly decreased cob length, diagonal number of cob grain, weight of thousand grains, stem diameter and grain P. Fungicide vitavax significantly decreased the number of cob grains in a row and grain P, related to the control. Fungicide addition resulted in substantial decrease in plant height, which in the case of fungicide vitavax the decrease was significant, relative to the control treatment. Grain P was significantly decreased by fungicides vitavax and captan. According to the results vitavax was the most unfavorable fungicide on corn growth and yield, relative to the other fungicides (Table 3). Fungicide addition significantly decreased grain protein and vitavax resulted in the highest decrease (Table 4). Grain yield was significantly decreased by fungicides vitavax and captan (Table 4).

DISCUSSION

Both of the experimental factors and their interaction significantly affected corn growth and yield indicating that both factors are of great influence in corn plantation.

Their significant interaction indicates that the behavior of AM species differs when subjected to different fungicides and some combination of AM and fungicide may be more efficient under certain circumstances. While, it is necessary to apply fungicide to avoid fungal activity and hence increase seed germination in the field (Murillo-Williams and Pedersen, 2008) the activities of soil microbes are also of great significance as they can very much affect the sustainability of agriculture and environment (Smith *et al*., 2000 ; Schweiger *et al*., 2001).

It should also be mentioned that this is the nature of such experiments with interactive effects, meaning that recognition of the individual means is not likely since there is always the mixed effects of the parameters tested in the experiment and this is the reality of these experiments in the field. Although, interactively affecting the growth and yield of corn, however the trend of the two parameters tested in this experiment can very much help predict the response of corn to such parameters under similar conditions.

Soil microbes are the very important component of the ecosystem and can very much affect the activities of other components through activities such as nutrient cycling (Smith *et al*., 2000), by mineralizing soil organic matter, affecting the health of the environment. Thus it is can be very important and also very applicable to test the effect of different fungideids and even indicate their optimum concentration (Murillo-Williams and Pedersen, 2008) at which there is an acceptable balance in the soil microbial activities. Recommending the fungicide that has the least unfavorable effects on the activity of soil microorganisms and also the highest impact to inhibit the activity of pathogenic fungi can very much contribute to the stability of agricultural systems.

Since, only *G. mosseae* resulted in significantly enhanced grain P it may be the most effective fungal species for seed inoculation, when they are treated with fungicides (Zhang *et al*., 2006). However, the significant enhancing effect of all fungal species on grain protein indicate that all the species are very much effective on enhancing N uptake by the host plant and hence the assimilation and tranlocation of organic N compounds into the shoots (Cliquet and Stewart, 1993) even when the seeds are treated by fungicides.

These all indicate that how using chemicals, among their other unfavorable effects, particularly on the environment, can influence the nutritional quality of the grain. Such statements illustrate the great significance of recommending the more favorable fungicide with the least unfavorable effects on plant growth and on the environment even more clearly.

According to the results, AM inoculation of seeds can significantly alleviate the unfavorable effects of fungicides on corn productivity and yield. This means that they are able to be a very efficient symbiont to their host plant even at the presence of chemicals such as fungicides in the soil (Zhang *et al*., 2006). This can be very advantageous as in the one hand fungicides application guarantees the high germination of seeds and on the other hand AM symbiosis can also contribute to enhanced plant growth and yield under such conditions.

Fungicide addition significantly decreased corn growth and yield indicating that they may affect either the activity of the host plant or soil microbes or both (Schweiger *et al*., 2001). Chemicals are not very favorable to the environment and can adversely influence plant growth and microbial activities in the soil (Chiocchio *et al*., 2000; Kjoller and Rosendahl, 2000). The results also indicate that fungicide vitavax is the most unfavorable fungicide and hence, must be used less frequently for treating seeds. There are different mechanisms by which fungicides adversely affect plant growth and yield, some of which can affect plant growth, microbial activities in the soil and the symbiosis between the symbiotic microbes and the host plant (Chiocchio *et al*., 2000; Kjoller and Rosendahl, 2000; Schweiger *et al*., 2001).

CONCLUSION

The results indicate that while treating seed with fungicides can adversely affect the growth and yield of corn to some degrees, depending on the kind of fungicide used, AM inoculation of seeds can alleviate such unfavorable effects on corn growth and yield. AM species are affected differently by fungicides as according to the results *G. mosseae* was the most efficient species in symbiosis with corn. The fungicides also influenced the nutritional quality of corn grains through affecting AM symbiosis with the corn plants. While, determination of appropriate rates of fungicides, which can inhibit fungal pathogenic effects on seed germination and in the meanwhile keep the balanced microbial activities in the soil is very important, illustration of the molecular pathways by which the AM species can allevaite the unfavorable effects of fungicide on plant growth and yield is also of great significance.

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