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Influence of *Glomus etunicatum* and *Glomus intraradices* fungi inoculums and micronutrients deficiency on root colonization and dry weights of tomato and sorghum in perlite bed culture

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The objectives of this study were to determine the effects of micronutrient deficiency on root colonization by arbuscular mycorrhizal fungi (AMF), and to assess the role of AMF on growth of sorghum and tomato plants in perlite bed culture. In a pot culture experiment with sterile perlite, sorghum (*Sorghum bicolor* L.) and tomato (*Lycopersicon esculentum* Mill.) plants were inoculated with either *Glomus etunicatum* or *Glomus intraradices*, or left un-inoculated as control, and three levels of micronutrients (zero, half and full strength) in Rorison's nutrient solution were applied to the pots during vegetative growth period. In tomato plants, the mycorrhizal symbiosis was not observed. In addition, fungi treatments had no significant effect on dry weights of root and shoot of tomato plants. In sorghum plants, average root colonization for *G. etunicatum* and *G. intraradices* were 43 and 37%, respectively. Nevertheless, there were no significant differences in root colonization between *G. etunicatum* and *G. intraradices* fungi treatments with supplied levels of nutrient solution. In addition, three levels of supplied nutrient solution did not have significant effect on root colonization percent. Moreover, mycorrhizal symbiosis decreased dry weights of root and shoot of sorghum plants. It seems that, these results related to phosphorus concentration in Rorison's nutrient solution.

Key words: Mycorrhizal fungi, micronutrients, sorghum, tomato, perlite, colonization, dry weight.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are soil microorganisms that have establish mutual symbiosis with majority of higher plants roots, such as sorghum and tomato plants that have high colonization potential with AMF (Lendzemo and Kuyper, 2001; Mwangi et al., 2011). It has widely been accepted that AMF have increasing affect on their host plants growth through nutrient uptake enhancement. Smith et al. (1986) reported that the AMF can stimulate plant growth especially in soils with low fertility mainly due to improved phosphorous absorption. In addition, Manoharan et al. (2008) reported that the nitrogen, phosphorus and potassium content increased in vesicular-arbuscular mycorrhizal (VAM) fungus treated seedlings compared with non-mycorrhizal tree seedlings. Also, Caris et al. (1998) reported that the Fe concentration in shoots were significantly higher in mycorrhizal than non-mycorrhizal sorghum plants. Nevertheless, AMF utilize 10 to 20% of net photosynthate

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Abbreviations: **AMF**, Arbuscular mycorrhizal fungi; **VAM**, vasicular-arbuscular mycorrhizal.

in exchange for the transfer of nutrients of the host to lead a symbiotic life (Allen, 1991). In addition to nutrient uptake, the AMF has other beneficial effects on plant growth. For example, AMF have been reported to protect plant roots from some root infecting fungi (Caron, 1989). Aroca et al. (2008) reported that the AM plants regulate their abscisic acid levels better and faster than non-AM plants, allowing a more adequate balance between leaf transpiration and root water movement during drought and recovery. Bhosale and Shinde (2011) reported that the amount of chlorophyll content was found to be decreased due to increase in water stress however the chlorophyll contents in mycorrhizal plants recorded more than non mycorrhizal plants. Also, it has been reported that the chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, protein, content increased in VAM fungus treated tree seedlings (Manoharan et al., 2008). Moreover, Çekiç et al. (2012) reported that plants inoculated with *Glomus intraradices* had less lipid peroxidation, and therefore it can be said that these plants have an advantage under salt stress. Anyways, there were many reports of beneficial effects of mycorrhizal symbiosis on plants cultivated in soils. However, there were no found studies for that effect on plants in hydroponic growth mediums.

Nutrient availability can have major effect on arbuscular mycorrhizal colonization. It is well known that high P levels in soil inhibit mycorrhizal development and root colonization (Abbott and Robson, 1984). Valentine et al. (2001) reported that the AM infection depend on both P supply and the availability of other nutrients, and plants grown at low P with high concentrations of other nutrients had the highest AM infection, and a higher biomass due to an enhanced maximum net photosynthetic rate. Variation in the amount of extraradical hyphal development is more directly related to enhanced plant growth response of highly responsive plants under low or deficient soil P conditions (Graham et al., 1982; Yao et al., 2001). However, the majority of studies have focused on the significance of phosphorus and there were few published results on the effects of micronutrients on mycorrhizal development. Results of some studies indicated that high concentrations of micronutrients in soil reduced root colonization. For example, a negative correlation between Zn or Cu concentration and AMF root colonization was found for plants grown in soil to which sludge had been applied (Boyle and Paul, 1988; Gildon and Tinker, 1983). Reduced root colonization was also observed for mycorrhizal plants grown close to an old copper mine (Griffioen et al., 1994). However, there were not found published results on the effect of micronutrients deficiency on mycorrhizal development in the hydroponic growth mediums.

In a pot culture experiment with sterile perlite, we investigated the effect of micronutrient deficiency on root colonization of tomato and sorghum plants by *Glomus etunicatum* and *G. intraradices* fungi, and the effect of mycorrhizal symbiosis on growth of these plants. The objectives of this study were to determine the effects of micronutrient deficiency in Rorison's nutrient solution on root colonization by AMF, and to assess the role of AMF on growth of sorghum and tomato plants in perlite bed culture.

MATERIALS AND METHODS

Mycorrhizal inoculum production

Two species of arbuscular mycorrhizal fungi, *G. etunicatum* W.N. Becker & Gerd (GE) and *G. intraradices* N.C. Schenck & G.S. Sm. (GI) were propagated with sorghum plants in 7 L pots containing sterile sandy loam soil. Rorison's nutrient solution, 20 mM $Ca(NO_3)_2.4H_2O$; 10 mM $MgSO_4.7H_2O$; 10 mM $K_2HPO_4.3H_2O$; 0.5 mM FeNaEDTA; 0.1 mM $MnSO_4.4H_2O$; 0.5 mM H_3BO_3; 0.01 mM $(NH_4)_6$ $Mo_7O_{24}.4H_2O$; 0.02 mM $ZnSO_4.7H_2O$ and 0.015 mM $CuSO_4.5H_2O$ in deionized water (Merryweather and Fitter, 1991) with 1/2 strength of phosphorus were added to the pots twice a week to bring the soil moisture to field capacity. Pots were kept in growth room with 28/20 ±2°C day/night temperatures and 16 h photoperiod. After four months, top plants were cut off and pot materials containing soil, mycorrhizal roots, hyphae and spores were thoroughly mixed and used as fungal inoculum. Root colonization percentage (Giovanetti and Mosse, 1980) and number of spores per 10 g soil (Gerdemann and Nicolson, 1963) were assessed to determine inoculum potential. Both inocula had an average of 65% root colonization and ~150 spores per 10 g soil.

Plant culture

Tomato (*Lycopersicon esculentum* Miller) and Sorghum (*Sorghum bicolor* L.) seeds were surface sterilized with 0.5% sodium hypochlorite for 15 min, and 10 seeds were sown in pots containing 2.8 L acid washed and sterilized perlite. Fungal inocula were rinsed three times with distilled water to minimize their micronutrients content. Each pot received 60 g mycorrhizal inoculum as a layer of 0.5 cm thickness, 5 cm below the seeds. Control pots (non-mycorrhizal) received 60 g autoclaved inoculum. Two weeks after sowing, tomato and sorghum plants were thinned to 1 and 3 plants per pot, respectively. Rorison's nutrient solution with three levels of zero, half and full strength (N_0, $N_{0.5}$, N_1, respectively) of micronutrients was applied to the pots twice a week during total growth period of 85 days. Pots were kept in growth room with 28/20 ±2°C day/night temperatures and 16 h photoperiod.

Plants dry weights and root colonization

85 days after sowing, plants were harvested and whole root system was washed. Fine feeding roots (0.5 g fresh weight) were sub-sampled, cleared in 10% KOH and stained with trypan blue. Root mycorrhizal colonization percentage was determined by gridline intersects method (Giovanetti and Mosse, 1980). In addition, plants were divided to two parts (root and shoot) and dried in oven, and then the shoot and root dry weights were recorded.

Statistical analysis

A factorial randomized in complete blocks design was used with two factors of mycorrhizal fungi with three variations (*G. etunicatum*, *G. intraradices* and non-mycorrhizal) as well as nutrient solution with

Table 1. Effect of mycorrhizal fungi on tomato (roots and shoots) dry weight (g) in variable regimes of micronutrients.

Parameter	N_0	$N_{0.5}$	N_1
Roots			
NM	2.23[b]*	3.10[a]	2.77[a]b
GE	2.57[ab]	2.50[ab]	3.00[ab]
GI	2.23[b]	2.63[ab]	2.70[ab]
Shoots			
NM	16.27[abc]*	16.93[a]	14.83[c]
GE	15.33[abc]	16.50[ab]	15.97[abc]
GI	15.30[abc]	16.40[abc]	14.97[bc]

NM, Non-mycorrhizal; GE, *Glomus etunicatum*; GI, *Glomus intraradices*. N_0, $N_{0.5}$ and N_1 are Rorison's nutrient solution with zero, half and full strength of micronutrients, respectively. *Means in each column and row followed by same letter are not significantly different ($p<0.05$).

Table 2. Effect of variable mycorrhizal fungi inoculums and regimes of micronutrients on sorghum root colonization (%).

Parameter	N_0	$N_{0.5}$	N_1
NM	0[b]*	0[b]	0[b]
GE	46[a]	41[a]	42[a]
GI	40[a]	35[a]	37[a]

NM, Non-mycorrhizal; GE, *Glomus etunicatum*; GI, *Glomus intraradices*. N_0, $N_{0.5}$ and N_1 are Rorison's nutrient solution with zero, half and full strength of micronutrients, respectively. *Means in each column and row followed by same letter are not significantly different ($p<0.05$).

three concentrations of micronutrients (N_0, $N_{0.5}$ and N_1), with three replications per treatment. Analysis of variance and mean comparison by Duncan's multiple range test were carried out using MSTATC software.

RESULTS

Tomato root colonization with AMF

The experiment was repeated with two varieties of tomato seeds in the same condition. Nevertheless, results were not different at the end of experiment, and the mycorrhizal symbiosis was not observed in tomato plants.

Tomato root and shoot dry weight

There were no significant differences between fungi treatments in each level of nutrient solution. In addition, there were no significant differences between three levels of nutrient solution in each one of the *G. etunicatum* and *G. intraradices* fungi treatments (Table 1).

Sorghum root colonization with AMF

Root colonization did not appear in the non-mycorrhizal treatments. In addition, there were no significant differences between root colonization with *G. etunicatum* and *G. intraradices* in the levels of nutrient solution supplied. In each mycorrhizal treatment (*G. etunicatum* or *G. intraradices*), supplied, the three levels of nutrient solution did not have significant differences (Table 2).

Sorghum root dry weight

In this experiment, between mycorrhizal treatments (*G. etunicatum* and *G. intraradices*), there were no significant differences. However, mycorrhizal treatments in comparison with non-mycorrhizal treatment had low root dry weight. In non-mycorrhizal treatment, N_0 level of nutrient solution in comparison with level of N_1, significantly reduced root dry weight (Table 3).

Sorghum shoot dry weight

Between mycorrhizal treatments in each levels of nutrient solution, there were no significant differences. In each three levels of nutrient solution supplied, mycorrhizal treatments in comparison with non-mycorrhizal treatments reduced shoot dry weight (Table 3 and Figure 1). In the non-mycorrhizal treatment, N_0 level of nutrient solution in comparison with levels of $N_{0.5}$ and N_1,

Table 3. Effect of mycorrhizal fungi on sorghum dry weight (g) in variable regimes of micronutrients.

Parameter	N_0	$N_{0.5}$	N_1
Roots			
NM	1.44^{b}*	2.11^{ab}	3.05^{a}
GE	0.13^{c}	0.23^{c}	0.29^{c}
GI	0.22^{c}	0.19^{c}	0.19^{c}
Shoots			
NM	6.42^{b}*	9.24^{a}	10.34^{a}
GE	0.69^{d}	1.17^{cd}	2.01^{c}
GI	0.50^{d}	1.00^{cd}	1.26^{cd}

NM, non-mycorrhizal; GE, Glomus etunicatum; GI, Glomus intraradices. N0, N0.5 and N1 are Rorison's nutrient solution with zero, half and full strength of micronutrients, respectively. *Means in each column and row followed by same letter are not significantly different ($p<0.05$).

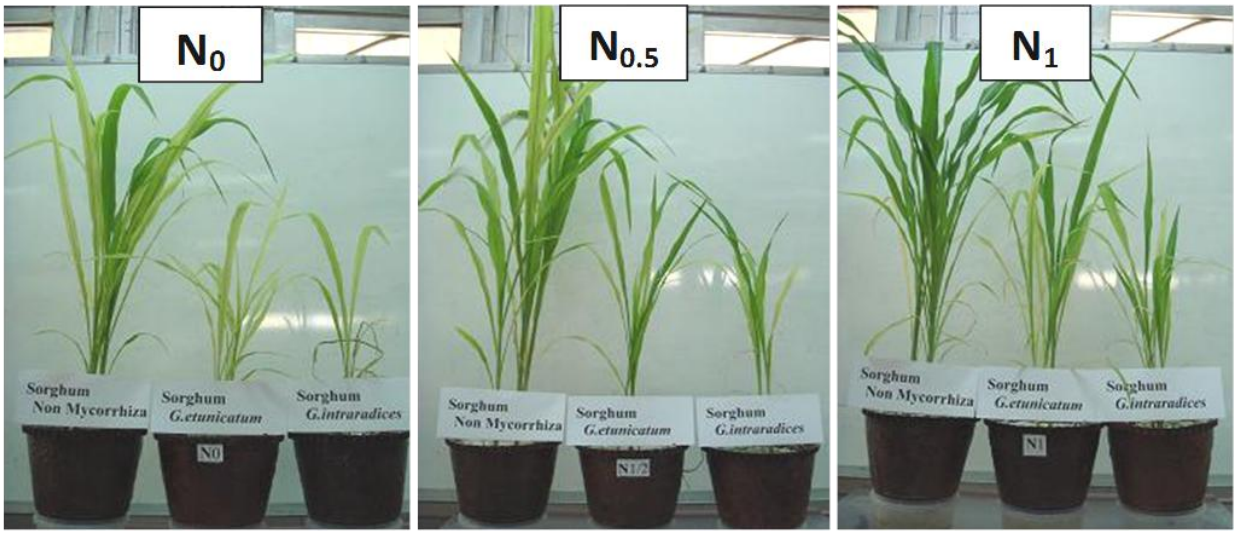

Figure 1. Sorghum plants, 85 days after sowing. Each one of the photos contains non-mycorrhizal plant and plants inoculated with *Glomus etunicatum* and *Glomus intraradices* (respectively left to right). N_0, $N_{0.5}$ and N_1 are Rorison's nutrient solution with three levels of zero, half and full strength of micronutrients supplied for plants nutrition.

significantly reduced shoot dry weight. In addition, in the *G. etunicatum* fungus treatment, N_0 level of nutrient solution in comparison with level of N_1, significantly reduced shoot dry weight. However, in the *G. intraradices* fungus treatment, between the three levels of nutrient solution, there were no significant differences in this respect (Table 3 and Figure 2).

DISCUSSION

The results of the experiment with sorghum plant shows that the three levels (zero, half and full strength) of the micronutrient concentration had no effect on root colonization by AMF. Even in tomato plants, the mycorrhizal symbiosis was not observed. It has been reported that the AMF colonization and extra radical hyphae growth were suppressed when plants were grown with the high level of micronutrients but this level of elements was not toxic to the plants and do not suppressed growth (Liu et al., 2000). In addition, Val et al. (1999) reported that the mycorrhizal colonization are strongly inhibited by Zn and Cu in contaminated soils. Moreover, AMF colonization depends on both P supply and availability of other nutrients, and plant grown in low P and sufficient other nutrient elements had the highest AMF colonization (Schreiner, 2007; Valentine et al., 2001). In this process appeared a fact that micronutrient reduced AMF colonization when the availability of this elements are very high such as supply of soil and water polluted with these elements but moderate or lower concentration of these metal elements do not affect root

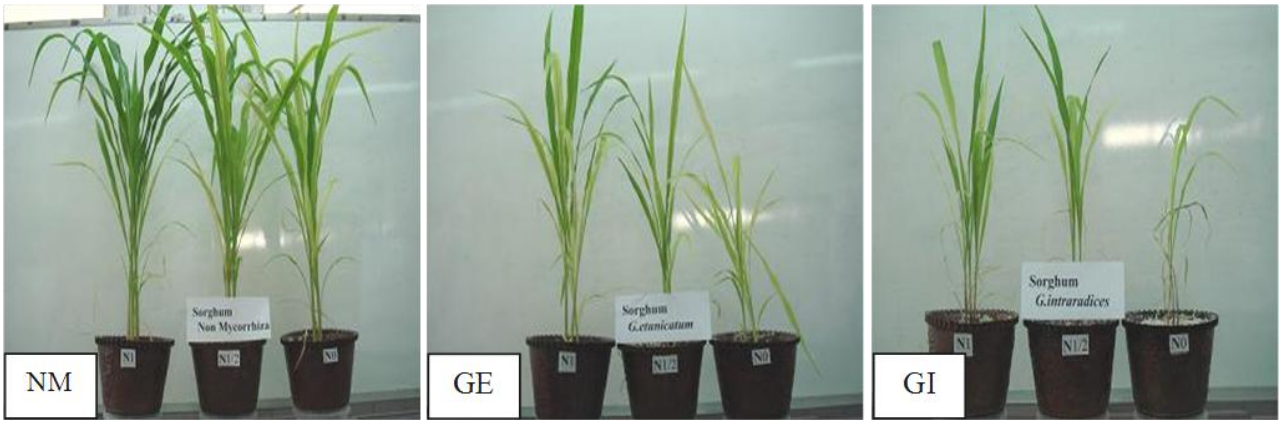

Figure 2. Sorghum plants, 85 days after sowing. Each one of photos contains three pots with sorghum plants inoculated with same inoculum, respectively: left to right contains non-mycorrhizal (NM) plants, plants inoculated with *Glomus etunicatum* (GE) and *Glomus intraradices* (GI). Each photo shows, Rorison's nutrient solution with full, half and zero strength of micronutrients (respectively left to right) supplied for plants nutrition.

colonization by AMF. In addition, it seems that root colonization was more affected by availability of P element in bed culture. The results of Liu et al. (2000) confirm this assumption. In the present study, P concentration supplied was equal in all of treatment compounds. It seems that, this agent caused sorghum root colonization did not have significant difference. Maybe, P concentration in nutrient solution with other conditions of the present experiment caused the mycorrhizal symbiosis not to be organized with tomato plants.

In sorghum plants, the mycorrhizal (*G. etunicatum* and *G. intraradices* fungi) treatments in comparison with non-mycorrhizal treatments reduced root and shoot dry weight. This shows that AMF cheating is difficult because it benefits the host plant in the wide range, and their cheating occur at specific times or under certain environmental condition or stress. However, in some experiments, AMF symbiosis reduced weight of plants (Citterio et al., 2005; Fidelibus et al., 2000; Valentine et al., 2001; Walling and Zabinski, 2006). In many cases, reduction of growth in mycorrhizal plant was observed when the P availability of soil was high (Graham et al., 1996; Peng et al., 1993; Schreiner, 2007). Valentine et al. (2001) reported that with high P and high concentrations of the other nutrients, a growth depression was found with mycorrhizal plants. Also Fidelibus et al. (2000) suggested that P limited condition might enhance AMF benefit. Furthermore, suppression of growth may be due to increasing metabolic activities of AMF and resulting increase carbon costs to the host plant (Fidelibus et al., 2000; Graham et al., 1996; Peng et al., 1993; Smith and Smith, 2012; Walling and Zabinski, 2006). From the latter reasons, it seems that existence of full strength of P concentration in the supplied nutrient solution caused reduction in AMF benefits and increased carbon costs for plants and consequently reduced plant growth in the present study.

Conclusion

Micronutrient concentration in the range supplied did not affect tomato and sorghum roots colonization with AMF and probably, P concentration in the supplied nutrient solution (that were in full strength) is the main agent in reducing mycorrhizal sorghum dry weight and preventing initiation of mycorrhizal symbiosis in tomato plants. Maybe, for growing mycorrhizal plants in hydroponic growth mediums, nutrient elements (especially P) concentration in nutrient solution should be reduce. It seems that any advantage or disadvantage associated with AMF was affected by three factors: kind of plants, kind of AMF and genetic characters of this symbiosis. Environmental condition (such as elements availability, pH, light etc) can express or silence some genes in the host plant or AMF, and consequently cause different behavior to appear from this symbiosis.

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REFERENCES

Abbott LK, Robson AD (1984). The effect of VA mycorrhizae on plant growth. In: Powell CL, Bagyaraj DJ (eds) VA mycorrhiza. CRC, Boca Raton, Fla. pp. 113-130.

Allen MF (1991). The Ecology of Mycorrhizae. Cambridge University Press, Cambridge, England.

Aroca R, Vernieri P, Lozano JMR (2008). Mycorrhizal and non-mycorrhizal *Lactuca sativa* plants exhibit contrasting responses to

exogenous ABA during drought stress and recovery. J. Exp. Bot. 59(8):2029-2041.
Bhosale KS, Shinde BP (2011). Influence of arbuscular mycorrhizal fungi on proline and chlorophyll content in *Zingiber officinale* Rosc grown under water stress. Ind. J. Fund. Appl. Life Sci. 1(3):172-176.
Boyle M, Paul EA (1988). Vesicular-arbuscular mycorrhizal associations with barley on sewage-amended plots. Soil Biol. Biochem. 20:945-948.
Caris C, Wolfgang H, Heidi JH, Volker R, Eckhaed G (1998). Studies of iron transport by arbuscular mycorrhizal hyphae from soil to peanut and sorghum plants. Mycorrhiza 8:35-39.
Caron M (1989). Potential use of mycorrhizae in soil borne diseases. Can. J. Plant Pathol. 11:177-179.
Çekiç FÖ, Ünyayar S, Ortaş I (2012). Effects of arbuscular mycorrhizal inoculation on biochemical parameters in *Capsicum annuum* grown under long term salt stress. Turk. J. Bot. 36:63-72.
Citterio S, Prato N, Fumagalli P, Aina R, Massa N, Santagostino A, Sgorbati S, Berta G (2005). The arbuscular mycorrhizal fungus *Glomus mosseae* induces growth and metal accumulation changes in *Cannabis sativa* L. Chemosphere 59(1):21-29.
Fidelibus MW, Martin CA, Wright GC, Stutz JC (2000). Effect of arbuscular mycorrhizal (AM) fungal communities on growth of 'Volkamer' lemon in continually moist or periodically dry soil. Sci. Hortic. 84:127-140.
Gerdemann JW, Nicolson TH (1963). Spores of edomycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Trans. Br. Mycol. Soc. 46:235-244.
Gildon A, Tinker PB (1983). Interactions of vesicular-arbuscular mycorrhizal infection and heavy metals in plants. 1. The effects of heavy metals on the development of vesicular-arbuscular mycorrhizas. New Phytol. 95:247-261.
Giovanetti M, Mosse B (1980). An evaluation of techniques for measuring vesicular-arbuscular infections in roots. New Phytol. 84(3):489-500.
Graham JH, Drouillard DL, Hodge NC (1996). Carbon economy of sour orange in response to different *Glomus spp*. Tree Physiol. 16:1023-1029.
Graham JH, Linderman RG, Menge JA (1982). Development of external hyphae by different isolates of mycorrhizal *Glomus spp*. in relation to colonization and growth of Troyer citrange. New Phytol. 91(2):183-189.
Griffioen WAJ, Jetswaart JH, Ernst WHO (1994). Mycorrhizal infection of an *Agrostis capillaris* population on a copper contaminated soil. Plant Soil 158(1):83-89.
Lendzemo VW, Kuyper TW (2001). Effects of arbuscular mycorrhizal fungi on damage by *Striga hermonthica* on two contrasting cultivars of sorghum, *Sorghum bicolor*. Agric. Ecosyst. Environ. 87(1):29-35.
Liu A, Hamel C, Hamilton RI, Ma BL, Smith DL (2000). Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays* L.) growing in soil at different P and micronutrient levels. Mycorrhiza 9:331-336.
Manoharan PT, Pandi M, Shanmugaiah V, Gomathinayagam S, Balasubramanian N (2008). Effect of vesicular arbuscular mycorrhizal fungus on the physiological and biochemical changes of five different tree seedlings grown under nursery conditions. Afr. J. Biotechnol. 7(19):3431-3436.
Merryweather JW, Fitter AH (1991). Techniques in Arbuscular Mycorrhiza Research. York Mycorrhiza Research Group, UK.
Mwangi MW, Monda EO, Okoth SA, Jefwa JM (2011). Inoculation of tomato seedlings with *Trichoderma Harzianum* and Arbuscular Mycorrhizal Fungi and their effect on growth and control of wilt in tomato seedlings. Braz. J. Microbiol. 42(2):508-513.
Peng S, Eissenstate DM, Graham JH, Williams K, Hodge NC (1993). Growth depression in mycorrhizal citrus at high phosphorus supply. Plant Physiol. 101:1063-1071.
Schreiner PR (2007). Effect of native and non native arbuscular mycorrhizal fungi on growth and nutrient uptake of 'Pinot noir' (*Vitis vinifera* L.) in two soils with contrasting levels of phosphorus. Appl. Soil Ecol. 36:205-215.
Smith SE, John JB, Smith FA, Bromley JL (1986). Effect of mycorrhizal infection on plant growth, nitrogen and phosphorous nutrition in glass house grown *Allium cepa* L. New Phytol. 103(2):359-373.
Smith SE, Smith FA (2012). Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. Mycologia 104(1):1-13.
Val CD, Barea JM, Aguilar CA (1999). Assessing the tolerance to heavy metals of arbuscular mycorrhizal fungi isolated from sewage sludge-contaminated soils. Appl. Soil Ecol. 11:261-269.
Valentine AJ, Osbarne BA, Mitchell DT (2001). Interactions between phosphorus supply and total nutrient availability on mycorrhizal colonization, growth and photosynthesis of cucumber. Sci. Hortic. 88:177-189.
Walling SZ, Zabinski CA (2006). Defoliation effect on arbuscular mycorrhizae and plant growth of two native bunchgrasses and on invasive forb. Appl. Soil Ecol. 32:111-117.
Yao Q, Li X, Feng G, Christie P (2001). Influence of extramatrical hyphae on mycorrhizal dependency of wheat genotypes. Commun. Soil Sci. Plant Anal. 32:3307-3317.