Caspian J. Env. Sci. 2006, Vol. 4 No.1 pp. 53~58

CJES
Caspian Journal of Environmental Sciences

[Article]

Effects of Arbuscular Mycorrhizal Fungi and Phosphorus Supplement on Leaf P, Zn, Cu and Fe Concentrations of Tea Seedlings

E. Kahneh[*1]. H. RamezanPour[2], M. R. Haghparast Tanha[2], A. Shirinfekr[3]
1- Guilan Research Center of Agriculture and Natural Resource, Rasht-Iran 2- Dep. of Soil Sci., Guilan University, Rasht- Iran 3- Tea Research Center, Lahidjan-Iran. [*]Corresponding author Email: kahneh_ehsan@yahoo.com

ABSTRACT
Influence of arbuscular mycorrhizal fungi (AMF) (*Glomus etunicatum, G. intraradices and Glomus versiforme*) and external phosphorus supplement (8 and 35 mg P Kg^{-1} soil) on leaf P, Zn, Cu and Fe nutrition of 4-month-old tea(*Camellia sinensis*) was studied in an glass house pot experiment. The highest leaf P concentration (up to 59.43% increase) was found in *G. versiforme* inoculated seedlings grown in the zero mg P Kg^{-1} soil, compared to the control seedlings. Higher level of Zn, Cu, and Fe concentrations were found in plants inoculated with *G. intraradices* as compared to other treatments. Our results showed that inoculation with *G. intraradices* had greater effect on uptaking of P, Zn, Cu, and Fe by tea seedlings than inoculation with either *G. etunicatum* or *G. versiforme*.

Keywords: *Arbuscular mycorrhiza, Camellia sinensis, Glomus etunicatum, G. intraradices, G. versiforme, leaf nutrient concentrations Tea*

INTRODUCTION

Tea (*Camellia sinensis* (L.) O. kuntze) is consumed by over two thirds of the world's population. It is the cheapest hot beverage (Menon, 2002). After water, tea is the most popular beverage consumed anywhere in the world. Historically and therapeutically, tea plant is one of the most fascinating of all medicinal herbs. The Chinese used tea as a medicinal drink as early as 3000 B.S. and by the end of the 6-century as a beverage (Menon, 2002). The tea plant is an evergreen shrub or tree, tea is made from the shoots of tea plant (Sana, 1987).

A scientifically managed system of soil-mycorrhiza-bacteria plant association is useful in conserving energy by reducing fertilizer requirement of crops and meeting production targets in nutritionally deficient soils. In the past decades, AM fungi have emerged as potential bio fertilizers, a cheap, environmentally friendly alternative to expensive chemical fertilizers (Srivastava *et al.,* 1996).

The principle effect of AM fungi in agricultural production systems and natural ecosystems in most cases involves the capability to supply the host plant with mineral nutrients that are relatively immobile in the soil, particularly phosphorus and trace elements (Li *et al.,* 1991; Jakobsen *et al.,* 1994) as compared to non-mycorrhizal controls. Inoculation with AM fungi has been shown to increase dry matter accumulation in shoot, nutrient uptake by plant and soil fertility status (Champawat, 1990). There has been considerable interest in the potential use of AM fungi in agricultural systems (Chen *et al.,* 2001).

The productivity of many plants is dependent on the formation of AM fungi, however, little is known about their potential to enhance the productivity of tea plant. There have only been a few attempts to study the impact of AM inoculation on the growth and nutrient uptake in tea plant. Sieverding and Toro (1987) observed the impact of different isolates of AM fungi on tea and found a three–fold increase in growth parameters compared to non- mycorrhizal plants. Study by Roy *et al.* (2002) reported that a good correlation could be established between AM fungi population, root colonization, and plant growth for the tea varieties with 6 years old. In addition, Zhi (1993) has reported the positive impact of AM fungi on tea plant nutrition (P, K, Fe, and Zn). There fore, an experiment was carried out to study inoculation responses of three species of AM fungi and phosphorus on mineral nutrition of tea (*Camellia sinensis*) seedlings under green house conditions.

Materials and Methods

Soil Preparation

Surface soil (0-30 cm), which did not receive any phosphate fertilizer for two years, was collected from the Tea Research Station of Rezvanshahr at north of Iran. These samples were air-dried and processed. The properties of the soil after sterilization was 21% Sand, 35.7% Silt, 43.3% Clay; 3.9% organic matter (Walkley-Black procedure); 5.47 pH (Soil: 0.01 M $CaCl_2$ 1:2.5); 0.498 dS m^{-1} electrical conductivity (EC; Soil: Water 1: 5); 7.9 P (Bray-1 extractable); 8.5 Fe, 1.1 Zn; 1.8 Cu (5 mM DTPA extractable) in mg kg^{-1} soil. Methods of analysis for the various elements are described in Page *et al.* (1982). The soil was sterilized twice with autoclave (121 ^{o}C) at an interval of 48 h and dried. There after the soil was mixed with sterile (autoclaved) acid-washed sand in a 1:1 v/v ratio to aid drainage and aeration, and then put into 36 plastic pots of 7-kg capacity. Aliquots of the soil/sand mixture were amended with three levels of phosphorus viz., zero, eight and 35 mg P kg^{-1} soil as KH_2PO_4 solution. Nitrogen and potassium were applied to all pots. K_2SO_4 solution was also applied at the appropriate rates to balance the quantity of K added to each treatment.

Experimental Design

The experiment was conducted as a completely randomized block factorial with two factors: 1- AM inoculation [un-inoculated (F_0), *Glomus etunicatum* Becker and Gerdemann (F_1), *Glomus intraradices* Schneck and Smith (F_2), and *Glomus versiforme* (Karesten) Birch (F_3), singly] and 2- Phosphorus; zero (P_1), 8 (P_2) and 35(P_3) mg P kg^{-1} soil. Each treatment had three replications.

Mycorrhizal Inocula

The procured cultures were multiplied on maize plants in a soil sand mixture. The six-month-old cultures of three AM fungal species viz. *Glomus etunicatum*, *Glomus intraradices* and *Glomus versiforme* were employed for the study.

Glasshouse Experiment

Tea (*Camellia sinensis*) rootstock (Colon 100 Iran) was micro propagated from buds. Buds were sampled from surface sterilized young shoots and were cultured for 180 days in a sterile sand medium. After rooting, plants were transplanted into pots containing 5-Kg soil sand mixture. The roots of tea were washed under tap water, surface sterilized with 50% alcohol for 1 min., and rinsed thrice with sterile water (Gupta *et al.,* 2002). At transplant, plants were inoculated with the AM fungi. Ninety to hundred grams of inoculums were placed in each planting hole about 1 cm below the roots. Non-inoculated plants received 100g of the autoclaved inoculums.

The pots were irrigated manually with tap water as needed (60% F.C) during the experiment. Leaching from pots did not occur, and plants did not experience water deficit. Two-cm layer of sterilized quartz sand was used to cover the upper surface of the soil (Tarafdar, 1995). Supplementary humidity was used to maintain a minimum humidity of 75%. The glass house had additional light from mercury vapor lamps to provide light at 200-400 μ mol m^{-2} s^{-1} photon flux densities at plant height during cloudy days. Throughout the experiment, the temperature did not vary more than 5^{o}C from 26^{o}C in the light period and 21^{o}C in the dark period.

The plants were harvested at the end of 16 weeks, shoots and roots were recovered separately. The roots were recovered by washing with de-ionized water. Shoots were weighed after oven drying at 70^{o}C overnight.

Chemical Analysis of Plant Material

Dried shoot samples were ground to pass a 0.5-mm screen, mixed thoroughly and 200-mg samples were digested in hydrochloric acid (2 M) for the analysis of mineral elements. The concentration of phosphorus in the digested sample was estimated according to Murphy and Riley (1962). Estimated of Cu, Fe and Zn were made by using Atomic Absorption Spectrophotometer (Varian Model AA 220).

Statistically Analysis

The data were subjected to analysis of variance using the ANOVA procedures of the SAS program (SAS Institute, 1999). Statistical significance was determined at P= 0.01. The Duncan's multiple range tests following a significant F test compared means. When interactions between factors were significant, the means of combinations of each level of these factors were compared.

Results and Discussions

Total dry weight

Our results indicated that AM fungal inoculation and phosphorus supplemental don't have significant effect on total dry weight of tea seedlings (Table 1).

Table1. Effects of AMF inoculation and P levels on the mean total dry weight ± SE (gr) of tea seedlings

	P_1	P_2	P_3
Non-inoculated	15.70(1.7)	17.51(1.1)	14.70(2.0)
G.etunicatum	18.90(1.2)	18.24(2.3)	15.20(2.9)
G.intraradices	16.12(1.2)	19.43(1.2)	18.62(3.5)
G.versiforme	18.20(2.4)	15.36(2.1)	19.65(1.8)

Foliar P Concentrations

At P_1 level, mycorrhizal plants (except *Glomus etunicatum*) had higher foliar P concentrations than non- mycorrhizal controls (Table2). Our results are in agreement with Call and Davies (1988) who reported that grass species inoculated with AM fungi had higher concentrations of N and P when grown in lignite overburden soil substrate compared to un-inoculated plants. The improved P uptake by the mycorrhizal plants is emphasized (Schubert and Lubraco., 2000 Vaast *et al.,* 1996).

In the present study the highest P concentration was found in the foliar of *Glomus versiforme* inoculated plants grown in the zero mg P kg^{-1} soil, and recorded 59.43% increase over the control plant (Table 2). Aikio and Ruotsalainen (2002) reported that when nutrient availability is constant and below the threshold levels for growth of the non-mycorrhizal plant, the non- mycorrhizal plant has zero RGR (Relative Growth Rate) while the mycorrhizal plant has a positive RGR. No significant differences were observed in the leaf P concentration between the different treatments at 8-mg P kg^{-1} soil level. Addition of phosphorus fertilizer to the poor soil probably enhanced root proliferation hence increased P uptake by the fertilized tree seedling (Valentine *et al.,* 2001).

With the increased P rates, P concentrations decreased in foliar of *Glomus versiforme* inoculated plants, whilst for *Glomus intrara-*

Table 2. Effects of AMF inoculation and P levels on the leaf nutrient concentrations (mg kg^{-1}) of tea seedlings

Element		**P**			**Zn**	
Add P(mg Kg^{-1} soil)	0	8	15	0	8	15
Non-inoculated	115.39(1.04)d	156.15(1.10)bc	151.58(1.01)bc	44.57(0.51)c	54.83(0.52)bc	101.30(1.02)a
G.etunicatum	129.44(1.05)cd	156.08(1.11)bc	161.71(1.14)ab	51.05(0.63)bc	66.41(0.57)b	99.09(1.01)a
G.intraradices	132.88(1.02)cd	149.40(1.05)bc	154.05(1.11)bc	87.06(0.74)a	45.04(0.52)c	43.90(0.51)c
G.versiforme	183.95(1.12)a	144.89(1.05)bc	152.83(1.08)bc	65.30(0.57)b	41.66(0.47)c	63.07(0.73)b
Significant F						
Inoculum's Type		N.S			*	
P rate		N.S			**	
Inoculum's Type*P rate		**			**	

Table 2. Continued

Element		**Cu**			**Fe**	
Add P(mg Kg^{-1} soil)	0	8	15	0	8	15
Non-inoculated	26.90(0.41)f	40.35(0.57)c	9.98(0.67)h	6.20(0.71)bc	6.63(0.56)c	6.20(0.47)bc
G.etunicatum	29.30(0.47)f	32.93(0.49)de	25.73(0.72)fg	9.70(0.78)a	8.29(0.62)ab	9.70(0.69)a
G.intraradices	63.40(0.65)a	52.82(0.54)b	35.73(0.76)cd	4.18(0.37)d	8.99(0.63)a	4.18(0.41)d
G.versiforme	31.30(0.52)def	20.90(0.41)g	31.00(0.68)def	6.30(0.52)c	6.54(0.49)c	6.97(0.62)c
Significant F difference due to Inoculum's Type		**			**	
P rate		**			**	
Inoculum's Type*P rate		**			**	

N.S: not significant   * P< 0.05   ** P< 0.01

Means (±SE) followed by the same letter for each element is not significantly different

dices inoculated plants, foliar P concentration was not significantly affected by soil P (Table 2). In contrast, within the *Glomus etunicatum* treatment, foliar P concentration increased significantly at the highest soil P level. These results are in agreement with Vaast *et al.* (1996) who reported that within the *Glomus clarum* treatment, foliar P concentration was not significantly affected by soil P availability. In contrast, within the *Acaulospora mellea* treatment, foliar P concentration increased significantly at the two highest P availabilities. It has been well documented that fungal hyphae attached to the host plant roots can extend beyond the zone of P-depletion (Hayman, 1983), resulting in enhanced P uptake by AMF- inoculated plants.

Foliar Zn Concentrations

The highest Zn foliar concentrations were found in the non- inoculated control plants, grown in the application of zero mg P kg^{-1} soil (Table 1). Liu *et al.* (2000) found that soil P and micronutrients levels significantly influenced the mycorrhizal contribution to Zn, Cu, and Mn and Fe uptake by maize.

Foliar Zn concentration was not significantly affected by soil P availability in plants inoculated with *Glomus versiforme* (Table 1). For plants inoculated with *Glomus etunicatum* and un-inoculated controls, foliar Zn concentration (Table 1) decreased with increasing soil P and were significantly lower at the highest soil P availability. Soil P availability affected Zn nutrition through its influence on AM symbiosis. On the other hand, foliar Zn status of the plants inoculated with *Glomus intraradices* tend to increase with increasing soil P levels and was significantly higher at the highest soil P availability. Kothari *et al.* (1991) showed that AM hyphae have the ability to absorb and translocation Zn to host roots, thereby contributing up to 25% of host-plant Zn acquisition.

Foliar Cu Concentrations

Our results indicated that AM fungal inoculation (except *Glomus versiforme*) significantly influenced the Cu concentration of plants, as compared to non-inoculated plants (Table 2). These results are in agreement with the findings of Chen *et al.* (2001), Rajan *et al.* (2000), Rao and Tak (2001). According Buerkert and Robson (1994), extra radical hyphae can absorb and transport Cu and Zn to their host plants, when no micronutrients are added to soil by enlarging the root absorption area and reducing Cu and Zn diffusion distance.

In the present study, significantly higher concentrations of Cu were found in foliar of *Glomus intraradices* inoculated plants with increasing P levels as compared to other treatments (Table 2). The highest Cu concentrations were found in the foliar of *Glomus intraradices* inoculated plants grown in the 35 mg P kg^{-1} soil.

Uptake of micronutrients by roots is diffusion limited (Tisdal *et al.*, 1993) and mycorrhizal plants could take up more metal nutrients via extra radical hyphae. The extraradical hyphae provide larger surface areas than the roots alone and reduce the distance for diffusion, there by enhancing the absorption of immobile metal nutrient (Jakobsen *et al.*, 1992).

Foliar Fe Concentrations

Results showed that in *Glomus versiforme* inoculated plants and non-inoculated controls; foliar Fe concentration was not significantly affected by soil P level. These results are similar to those reported for Fe (Kothari *et al.*, 1991) but are in contrast to the finding of Clark and Zeto (1996).

Since, for plants inoculated with *Glomus etunicatum* the Fe concentration increased with increasing P levels and the highest level was recorded at 35 mg P kg^{-1} soil. While for plants inoculated with *Glomus intraradices* highest level was recorded with 8 mg P kg^{-1} soil (Table 2). These results are in agreement with those of Purakayasta *et al.* (1998) who found that Fe^{+2} in the leaves and Fe uptake by the curd and straw were highest when broccoli was grown on inoculated soil amended with NPK plus pyrite. This may have been due to the acquisition of more Fe solubilised by sidrophorus produced by AM.

The availability of Mn and Fe in soil depends on soil pH value and soil oxidation- reduction potential (Liu *et al.*, 2000). Arbuscular mycorrhizal fungi were found to increase the number of Mn-oxidizing bacteria in the rhizosphere, there by indirectly reducing oxidation-reduction potential and availability of Mn and Fe in the mycorhizosphere (Liu *et al.*, 2000).

The results of this study suggest that AMF inoculation could significantly increase the nutrient concentrations of tea (*Camellia sinensis*) seedlings. This study also indicates differences in tolerance to soil P levels, between AMF species. In addition, under the same experimental condition, *Glomus intraradices* has better ability to increased mineral nutrition as compared *Glomus etunicatum* and *Glomus versiforme* respectively.

Acknowledgments

The authors are grateful to Dr. A. Forghani Dr. N. Aliasgharzadeh for providing original inoculums, Mr. A. Mirhosseini (Tea Research Center, Lahidjan, Iran) and Mr. R. Ansari (Soil Sci. Lab., Guilan University) for their help. Tea Research institute provided financial support for this study and University Grant Commission, Guilan (Rasht) - Iran.

References

Aikio, S. and Ruotsalainen, A. L. (2002) The modeled growth of mycorrhizal and non- mycorrhizal plants under constant versus variable soil nutrient concentration. *Mycorrhiza.* **12,** 257- 261.

Buerkert, B. and Robson, A. D. (1994) ^{65}Zn Uptake in subterranean clover (*Trifolium subterraneum* L.) by three vesicular-arbuscular mycorrhizal fungi in a root-free sandy soil. *Soil Biol. Biochem.* **26,** 1117-1124.

Call, C. A. and Davies, F. T. (1988) Effect of vesicular- arbuscular mycorrhizae on survival and growth of perennial grasses in lignite overburden in Texas. *Agri. Eco. Env.* **24,** 395- 405.

Champawat, R. S. (1990) Current trends in mycorrhizal research, Proc. Nat. Conf. Mycorrhiza, HAU, Hisar, Feb. 14-16. P. 141.

Chen, B., Christie, P. and Li, X. (2001) A modified glass bead compartment cultivation system for studies on nutrient and trace metal uptake by arbuscular mycorrhiza. *Chemosphere.***42**, 185- 192.

Clark, R. B. and Zeto, S. K. (1996) Iron acquisition by mycorrhizal maize grown on alkaline soil. *J. plant Nut.* **19**, 247- 264.

Gupta, M. L., Prasad, A., Ram, M. and Kumar, S. (2002) Effect of the Vesicular-Arbuscular Mycorrhizal (VAM) Fungus *Glomus fasiculatum* on the essential oil yield related characters and nutrient acquisition in the crops of different cultivars of Menthol Mint (*Mentha Orvensis*) under field conditions. *Biores. Tech.* **81,** 71- 79.

Hayman, D. S. (1983) The physiology of vesicular arbuscular endomycorrhizal symbiosis. *Canadian J. Botany.* **61,** 944-963.

Jakobsen, I., Abbott, L. K. and Robson, A. D. (1992) External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *trifolium subterraneum L.*, I. spread of hyphae and phosphorus inflow into roots. *New Phytologist.* **120,** 371- 380.

Jakobsen, I., Joner, E. J. and Larsen, J. (1994) Hyphal phosphorus transport, a keystone to mycorrhizal enhancement of plant growth, In: Gianinazzi, S., Schiiepp, H. (eds.), Impact of arbuscular mycorrhizas on sustainable agriculture, pp. 133-146.

Kothari, S. K., Marschner, H. and Romheld, V. (1991) Contribution of the VA mycorrhizal hyphae in acquisition of phosphorus and zinc by maize grown in a calcareous soil. *Plant Soil.* **131,** 177-185.

Li, X. L., George, E., Marschner, H. (1991) Phosphorus depletion and pH decrease at the root-soil and hyphae- soil interfaces of VA mycorrhizal white clover fertilized with ammonium. *New phytologist.* **119,** 397- 404.

Liu, A., Hamel, C., Hamilton, R. I., Ma, B. L. and Smith, D.L. (2000) Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea maize L.*)grown in soil at different P and micronutrient levels. *Mycorrhiza* **9,** 331- 336.

Menon, K. K. G.(2002) The tea industry in India, How to redesign a native, In: Mulky, M. J., V. S. Sharma (eds.) Tea culture processing and marketing, Oxford and IBH publishing Co. PVt. Ltd. New Delhi, pp. 3-10.

Murphy, J., J. P. Riley (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta.* **27,** 31-36.

Page, A. L., Miller, R. H., Keeney, D. R. (1982) Methods of soil analysis. American Society of Agronomy. Madison. WI.1158pp.

Purakayasta, T. J., Singh, C. S. and Chhonkar, P. K. (1998) Growth and iron nutrition of broccoli (*Brassica oleracea L. var. italica plenck*) growth in a typic ustochrept, as influenced by vesicular-arbuscular mycorrhizal fungi in the present of pyrite and farmyard manure. *Biol. Fert. Soil.* **27,** 35- 38.

Rajan, S. K., Reddy, B. J. D. and Bagyaraj, D. J. (2000) Screening of arbuscular mycorrhizal fungi for their symbiotic efficiency with *Tectona grandis*. *Forest Ecol. Manag.* **126**, 91-95.

Rao, A. V., and Tak, R. (2001) Influence of mycorrhizal fungi on the growth of different tree species and their nutrient uptake in gypsum mine spoil in India. *App. Soil Eco.* **17,** 279- 284.

Roy, A. K., Kumari, R., Chakraborty, B. N. and Chakraborty, U. (2002) VA mycorrhizae in relation to growth of different tea varieties. *Mycorrhiza News* **14**, 9-11.

Sana, D. L. (1987) Tea science, ASHRAFIA, B, I Ghar, Bangladesh.

SAS Institute Inc (1999) The SAS system for windows, version 8. SAS Institute, Cary, N.C.

Schubert, A., Lubraco, G. (2000) Mycorrhizal inoculation enhances growth and nutrient uptake of micro propagated apple rootstocks during weaning in commercial substrates of high nutrient availability. *App. Soil Eco.* **15**, 113-118.

Sieverding, E. and Toro, S.T. (1987) Growth of Coffee and tea plants in nurseries inoculated with different VAM fungal species. In proceedings of the seventh North American conference on mycorrhiza, Sylvia, D.M., Hung, L. L. and Graham, J. H. (eds.) Florida, University of Florida. 364 pp.

Srivastava, D., Kapoor, R., Srivastava, A. K. and Mukerji, K. G. (1996) vesicular arbuscular mycorrhiza, an overview, in: Mukerj, k. G. (ed.) concepts in mycorrhizal research, Kluwer, Dordrecht, pp. 1-34.

Tarafdar, J.C. (1995) Role of VA mycorrhizal fungus on growth and water relations in wheat in presence of organic and inorganic phosphates. *J. Indian Soc.Soil Sci.* **43**, 200-204.

Tisdal, S. L., Nelson, W. L., Beaton, J. D. and Havlin, J. L. (eds.) (1993) Soil fertility and fertilizers. Macmillan, New York.

Vaast, Ph., Zasoki, R. J. and Bledose, C. S. (1996) Effects of vesicular-arbuscular mycorrhizal inoculation at different soil P availabilities on growth and nutrient uptake of invitro propagated coffee (*Cofea arabica L.*) plants. *Mycorrhiza* **6**, 493- 497.

Valentine, A. J., Osborne, B. A., Mitchell, D. T. (2001) Interaction between phosphorous supply and total nutrient availability on mycorrizal colonization, growth and photosynthesis of cucumber.*Sci. Hort.* **88**, 177- 189.

Zhi, L. (1993) Effect of VA mycorrhiza on growth and mineral nutrition of tea plant. *J. Tea Sci.* **13**, 15-20.

(Received: Nov. 10, Accepted Oct. 2, 2005)