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

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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 Ca Cl_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).

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

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)

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.

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