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Evaluation of Arbuscular Mycorrhizal Fungi of Orchard Soils for Growth Improvement in Maize under Protected Conditions

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A B S T R A C T

An experiment was conducted to determine the effect of arbuscular mycorrhiza fungal (AMF) isolates on various growth parameters and enzymatic activity in maize grown under protected conditions. One hundred and eight AMF isolates, collected from apple, pear and cherry rhizosphere, were chosen to assess their efficiency in improving growth of maize cv. "C6". The plant height, root biomass, shoot biomass, root mycorrhizal colonization and root and shoot phosphatase in AMF inoculated maize plants ranged from 16.0 to 27.9 cm, 2.75 to 4.60 g, 9.37 to 14.26 g, 17.78 to 65.15%, 3.59 to 40.66 m moles PNP/g fresh root weight and 3.20 to 30.73 m moles PNP/g soil, respectively, as compared 18.1 cm, 3.25 g, 10.74 g, 0%, 3.66 m moles PNP/g fresh weight and 3.33 m moles PNP/g soil in control. The AMF isolates collected from different hosts, revealed variable behaviour with respect to plant growth improvement and enzyme activities. The AMF isolates showed almost similar pattern with a little variation. The AMF isolates of cherry, apple and pear rhizosphere showed 23.2 to 65.2, 19.8 to 53.1 and 17.8 to 61.5% root colonization, respectively. On the basis of initial screening twenty most efficient isolates were compared statistically which showed significant increase of 7.0 to 54.6, 12.6 to 41.5 and 11.7 to 32.8% in plant height, root biomass and shoot biomass, respectively, in maize.

Keywords

Maize, Phosphatase activity, Root colonization, AMF fungi.

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Introduction

In natural ecosystems arbuscular mycorrhizal fungi (AMF) form regular component of rhizosphere microflora and are desirable for sustainable plant-soil system as they establish symbiotic associations with most of the terrestrial plants (Sharma *et al.*, 2009). AMF inhabit a variety of ecosystems including agriculture lands, forest, grasslands and many stressed environments. They colonize roots of most plants including bryophyte, pteridophyte, gymnosperms and angiosperms (Wang and Zao, 2008). The mycorrhizal association helps the host plants in overcoming several biotic and abiotic stresses and improves plant growth. Robinson *et al.*, (2014) reported increased plant height, root length, shoot length, root biomass and shoot biomass in berseem plants due to AMF inoculation. Several AM fungi namely *Glomus fasciculatum*, *G. mosseae*, *G. macrocarpum* and *Sclerocystis dussii* were screened by Dutt *et al.*, (2013) in apricot

(*Prunus armeniaca* L.) cv. 'New Castle' seedlings for vegetative growth performance in soil supplemented with 0, 25, 50 and 100 mg P kg^{-1} soil. *G. fasciculatum* inoculation gave significantly higher spore count and better root colonization and showed improvement in growth characteristics and leaf nutrient status over uninoculated control. The soil inoculation of *G. fasciculatum* + 50 mg P kg^{-1} application was found most effective with corresponding values (209, 59, 102.2, 86, 57.6, 70.8 and 131.5%) for higher spore count, root colonization, seedling height, stem diameter, dry weight of shoot and root and leaf area, respectively. Veerabhadraswamy and Garampalli (2011) found enhanced plant growth due to AM fungi (*Glomus fasciculatum*, *G. mosaea* and *Acaulospora laevis*). Several species of mycorrhizal fungi increased plant biomass in maize (Othira *et al.,* 2012). Li *et al.,* (2012) demonstrated that AMF communities increased plant height, shoot dry weight, root dry weight, and leaf area in cucumber. Mathew *et al.,* (2003) achieved significant increase in height of banana cultivars during all growth stages, reaching a final height of 16.76 cm which was 60.7% higher than uninoculated control. Sharma *et al.,* (2012a, b) found significant correlation between roots mycorrhizal root colonization and tree diameter/yield in apple cv. 'Royal Delicious'. AMF form their extended hyphal network which from nutrient-poor soils efficiently absorb and translocate water and mineral nutrients to its host (Ryan and Angus, 2003). AMF have positive effect on root longevity by increasing the lignification of plant cell walls which protects roots against infection by soil borne pathogens (Harrier and Watson, 2004). Besides, AMF induce higher photosynthetic efficiency and tolerance in plants against abiotic stresses such as drought, soil compaction salinity or cold, etc. (Auge, 2000; Hildebrandt *et al.,* 2007; Miransari, 2010; Wu *et al.,* 2013). Utkhede and Smith (2000) reported that *Glomus intraradices* enhanced plant growth and fruit yield in apple trees planted in soils conducive to replant disease. AMF are the main component of soil microbial population in most agroecosystems and form a close association with more than 80% plant species and render immobilized mineral nutrients available to the plants so as to sustain normal growth and reproduction (Bordoloi *et al.*, 2015). The present study was aimed to evaluate the efficacy of various AMF isolates, collected from apple, pear and cherry rhizosphere, in maize grown under protected conditions in Kashmir.

Materials and Methods

Arbuscular mycorrhizae were isolated during active growth period from apple, pear and cherry orchard soils of Kashmir valley as per wet sieving and decantation method (Gerdman and Nicolson, 1963). These AMF isolates were identified and multiplied on maize plants under polyhouse conditions. For raising maize plants, first growth medium was prepared by mixing sterilized soil and sand in 2:1 (w/w) ratio (Bhardwaj *et al.*, 2000). The soil and sand was sterilized at 1.5 kg/m^3 for one hour for three consecutive days. The growth medium was filled in polybags of 1.5 kg capacity. Apparently healthy maize seeds cv. 'C-6' were surface sterilized with 0.5% sodium hypochlorite and germinated on petri-plates under aseptic conditions. Two seeds were sown per polybag and inoculated with 50 spores of each AMF isolate individually at a soil depth of 5 cm. Uninoculated control polybags were also maintained. For each isolate three replications were maintained and kept in a greenhouse at 30±1ºC in a completely randomized design. The maize plants in polybags were irrigated as and when required. The plants were uprooted 45 days after sowing and checked microscopically for the presence of AMF spores. The spores of AMF isolates were characterized on the basis of their morphological characters i.e. shape, size, colour and ornamentation, type of hyphal attachment, spore wall characters, etc. Such examinations were made under a compound microscope (Olympus U-CTR-30-2). Spore identification was done according to Schenck and Perez (1987) and also by comparison with the authentic literature/standard monographs (INVAM, 2005; Schenck and Perez, 1990; Morton and Redecker, 2001; Schubler and Walker, 2010).

AM fungal isolates were evaluated on the basis of plant growth improvement and soil and root phosphatase activity. Maize plants cv. 'C-6' were grown in polybags (1.5 kg capacity) maintained under polyhouse conditions and inoculated with test AMF fungal spores. Four replications were maintained for each isolate in a completely randomized design. Various plant growth parameters *viz.*, dry root and shoot biomass, plant height and root colonization and enzyme activity of roots and soil (acid phosphatase) were recorded 45 days after the inoculation. For assessing mycorrhizal colonization, fresh maize roots were collected and thoroughly washed with distilled water to remove the adhering soil. The washed roots were cut into small bits of 1 cm size and placed in 10% KOH. Later the roots were rinsed with dilute HCl (2%) and boiled for 5 minutes in cotton blue stain followed by overnight destaining in lactophenol. The roots were examined under stereomicroscope (Olympus SZ-61) and infectivity with AM fungi proved by observing the presence of arbuscules, vesicles, Hartig net and hyphae of endophytes on maize roots, if any. Root segments were mounted on glass slide with lactophenol and observed under compound microscope (Olympus U-CTR-30-2). A minimum of 150 segments for each sample were observed for the assessment of mycorrhizal infection as per the formula of Biermann and Lindermann (1981).

$$\text{Per cent mycorrhizal infection} = \frac{\text{Number of infected root segments}}{\text{Total number of segments examined}} \times 100$$

Acid phosphatase activity of roots and soil was estimated as per method of Tabatabai and Bremner (1969) and expressed in terms of milli moles PNP/g fresh wt. root and milli moles PNP/g soil respectively.

Statistical analysis

The data was analyzed by using analysis of variance technique as per Gomez and Gomez (1983). Wherever necessary the data prior to analysis was appropriately transformed using arc-sine transformation to fulfill the assumption of normality.

Results and Discussion

The AMF isolates from four districts varied in their impact on maize plant growth, mycorrhizal colonization and phosphatase activities in root and soil (Table 1). District-wise AMF isolates showed almost similar trend in improving the plant growth. The plant height in AMF inoculated plants, in general, ranged from 16.0 to 27.9 cm as compared to 18.1 cm in uninoculated control. The root biomass varied from 2.75 to 4.60 g as compared to 3.25 g in control. The shoot biomass ranged from 9.37 to 14.26 g in comparison to 10.74 g in control. The root mycorrhizal colonization in inoculated plants varied from 17.78 to 65.15%. The root phosphatase in AMF inoculated plants was in the range of 3.59 to 40.66 m moles PNP/g fresh root weight in comparison to 3.66 m moles PNP/g fresh weight in control. The shoot phosphatase in AMF inoculated plants varied from 3.20 to 30.73 m moles PNP/g soil as compared to 3.33 m moles PNP/g soil in control.

The AMF isolated from different host *viz.*, apple, pear and cherry rhizosphere, and evaluated on maize revealed variable behaviour with respect to plant growth improvement and enzyme activities (Table 2). Crop-wise AMF isolates showed almost similar pattern with a little variation. The plant height, in general, was in the range of 15.50-27.93 cm as compared to 18.06 cm in control. The root biomass varied from 2.75-4.60 g in AMF inoculated plants as compared to 3.25 g in uninoculated control.

The shoot biomass ranged from 9.37 to 14.26 g in AMF inoculated plants as compared to 10.74 g in uninoculated control. Mycorrhizal root colonization in inoculated plants was in the range of 17.78 to 65.15%. The root colonization by AMF isolates from cherry, apple and pear rhizosphere was 23.2-65.2, 19.8-53.1 and 17.8-61.5%, respectively. The root phosphatase was in the range of 3.30-40.66 m mol PNP/g fresh root weight as compared to 3.66 m mol PNP/g fresh root weight in control. The soil phosphatase activity varied from 3.23-30.73 m mole PNP /g soil in AMF inoculated plants as compared to uninoculated control.

On the basis of initial screening, twenty most efficient isolates were compared statistically (Table 3). The efficient isolates identified were *Glomus geosporum*, *G. diaphanum*, *G. versiforme*, *G. boreale*, *G. glomerulatum*, *G. sinuosum*, *G. macrocarpum*, *G. heterosporum*, *Acaulospora rehmii*, *A. bireticulata*, *A. laevis*, *A. longula*, *Acaulospora* spp., *Septoglomus deserticola*, *Gigaspora decipiens*, *Funneliformis verrucolosum* and *Scutellospora armeniaca.*

Of these, the most efficient 20 AMF isolates compared statistically revealed significant increase of 7.0 to 54.65, 12.6 to 41.5 and 11.7 to 32.8% in plant height, root biomass and shoot biomass, respectively, in maize. Further, mycorrhizal root colonization and root and soil phosphatase activities in AMF inoculated plants ranged from 23.7 to 65.2%, 3.6 to 40.7 m moles PNP/g fresh root weight and 5.7 to 30.7 m moles PNP/g soil, respectively.

Table.1 Effectiveness of AMF isolates isolated from various districts on growth and enzyme activity of maize

District	Plant height (cm)	Mycorrhizal colonization (%)	Root biomass (g)	Shoot biomass (g)	Root phosphatase (milli moles PNP/g fresh wt. root)	Soil phosphatase (milli moles PNP/g soil)
Srinagar	16.26-27.93	17.78-65.15	2.75-4.60	9.37-14.26	3.59-40.66	3.20-30.73
Ganderbal	16.03-26.33	20.83-61.53	2.80-4.53	9.89-14.22	3.73-40.60	6.30-30.65
Shopian	16.00-25.20	19.84-50.84	2.77-4.25	9.64-14.16	6.34-38.50	3.46-27.66
Baramulla	16.33-25.20	19.84-50.73	2.76-4.27	9.77-14.18	3.66-39.53	3.23-27.86
Control	18.06 ±0.03	0.00 ± 0.00	3.25±0.01	10.74±0.01	3.66 ± 0.33	3.33 ± 0.33

Table.2 Effectiveness of AMF isolates isolated from the rhizosphere of various horticultural crops on growth and enzyme activity of maize

Crop	Plant height (cm)	Mycorrhizal colonization (%)	Root biomass (g)	Shoot biomass (g)	Root phosphatase (milli molesPNP/g fresh wt. root)	Soil phosphatase (milli moles PNP/g soil)
Apple	15.50-26.90	19.84-53.14	2.75-4.54	9.37-14.26	3.60-40.63	3.52-29.66
Pear	16.26-26.33	17.78-61.53	2.77-4.41	10.37-14.21	7.76-40.60	3.73-28.83
Cherry	17.03-27.93	23.19-65.15	2.76-4.60	10.20-14.24	3.62-40.66	3.23-30.73
Control (uninoculated)	18.06 ±0.03	0.00 ± 0.00	3.25±0.01	10.74±0.01	3.66 ± 0.33	3.33 ± 0.33

Table.3 Most efficient AMF isolates showing improvement in growth parameters and soil phosphatase activity on maize

S. No.	AMF species/genera	Plant height (cm)	Mycorrhizal colonization (%)	Root biomass (g)	Shoot biomass (g)	Root phosphatase (millimoles PNP/g fresh wt. of root	Soil phosphatase (millimoles PNP/g soil)
0.	Control	18.06	0.00	3.25	10.74	3.66	3.33
1.	*Glomus geosporum*	27.93	65.15	4.56	14.24	40.66	30.73
2.	*Acaulospora rehmii*	27.33	53.67	4.60	14.23	40.65	29.76
3.	*Glomus diaphanum*	26.90	53.14	4.54	14.26	40.63	29.66
4.	*Gegaspora decipiens*	26.33	61.53	4.41	14.21	40.6	28.83
5.	*Acaulospora bireticulata*	25.23	52.19	4.53	14.22	40.57	30.66
6.	*Septoglomus deserticola*	25.23	38.26	2.85	10.57	39.53	27.86
7.	*Glomus versiforme*	25.23	23.19	2.99	14.20	3.59	9.33
8.	*Glomus boreale*	25.20	38.84	4.23	14.15	38.46	15.69
9.	*Acaulospora* spp.	25.20	34.99	3.29	12.54	24.07	14.33
10.	*G. glomerulatum*	24.23	31.95	3.13	11.60	16.53	8.66
11.	*Glomus macrocarpum*	24.23	49.92	4.18	14.10	25.1	16.26
12.	*Acaulospora* spp.	24.23	50.12	3.65	13.96	26.12	16.16
13.	*Glomus heterosporum*	24.20	33.34	3.93	9.48	30.6	21.27
14.	*Acaulospora laevis*	24.03	41.13	3.80	13.78	7.76	19.75
15.	*Acaulospora* spp.	23.40	29.74	4.10	11.43	34.79	5.73
16.	*Glomus sinuosum*	23.40	40.84	2.99	14.01	10.34	6.66
17.	*Funneliformis verrucolosum*	23.73	49.11	4.12	13.63	30.56	23.86
18.	*Scutellospora armeniaca*	23.76	49.31	4.13	11.99	30.44	24.66
19.	*Acaulospora longula*	23.10	27.25	2.93	10.85	3.62	9.66
20.	*Glomus boreale*	22.86	23.75	2.84	13.60	17.7	8.46
CD ($p \leq 0.05$)		**0.35**	**2.89**	**0.16**	**0.36**	**3.12**	**2.12**

Plate.1 (I) Intraradical AMF fungal hyphae and spores in host (maize) root tissue; (II) Extramatrical AMF fungal hyphae

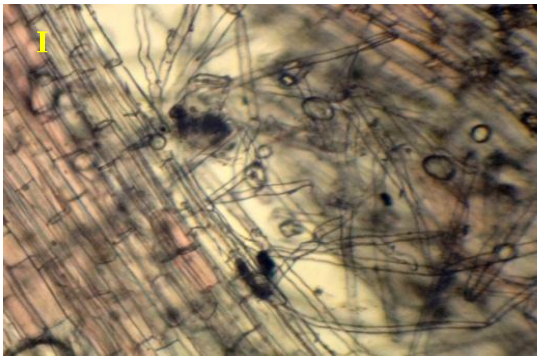
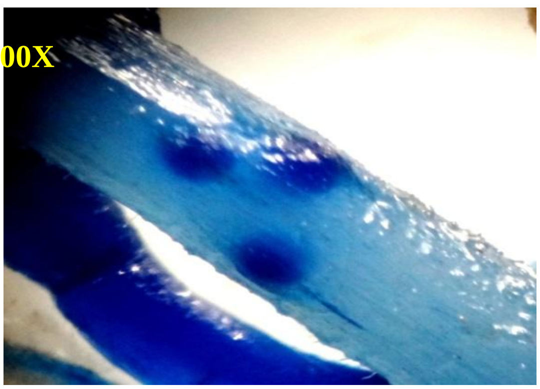
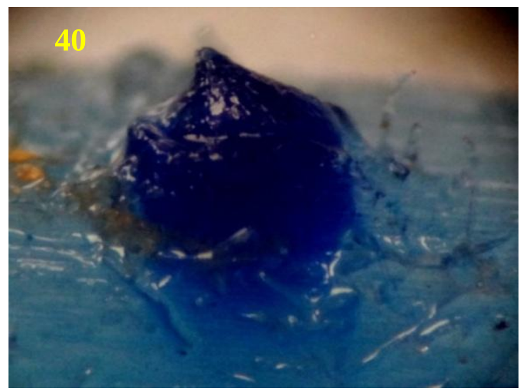


Plate.2 Maize roots showing arbuscules and vesicles





Perusal of data on root biomass revealed that *A. rehmii*, *G. geosporum*, *G. diaphanum* and *A. bireticulata* were statistically at par with one another and significantly superior over all other species evaluated. Maximum and minimum root biomass 4.60 and of 2.84 g was observed in *A. rehmii* and *G. boreale*, respectively (Table 3). Perusal of Table 1 revealed maximum and minimum shoot biomass of 14.26 and 9.48 g in *G. diaphanum* and *G. heterosporum*, respectively. *G. diaphanum*), *G. geosporum*, *A. rehmii*, *Gigaspora decipiens*, *A. bireticulata*, *G. versiforme*, *G. boreale* and *G. macrocarpum* were statistically at par with one another but superior over other isolates (Table 3). Maximum root phosphatase activity was observed in *G. geosporum* (40.66 m moles PNP/g fresh root weight) but was at par with *A. rehmii*, *G. diaphanum*, *G. decipiens*, *A. bireticulata*, *S. desrticola* and *G. boreale*. *G. versiforme* showed a minimum root phosphatase activity of 3.59 m moles PNP/g fresh root weight though at par with *Acaulospora longula* (Table 3). Maximum soil phosphatase activity of 30.73 m moles PNP/g soil was observed in *G. geosporum* which was statistically at par with *Acaulospora rehmii*, *G. diaphanum*, *G. decipiens* and *A. bireticulata*. *G. sinuosum* showed minimum soil phosphatase activity of 6.66 m moles PNP/g soil (Table 3).

The plant height, root biomass, shoot biomass, mycorrhizal colonization, root phosphatase and shoot phosphatase in AMF inoculated maize plants generally was higher in comparison to the uninoculated control. District-wise AMF isolates showed almost similar trend in improving the plant growth.

Trindade *et al.,* (2006) noticed 33 AMF fungal species in the agrosystems of coffee, fruits and annual crops in Brazil and reported *Acaulospora* species as the most frequently encountered species in the soils having pH 6.5 or less. Further, they observed that certain species like *Gigaspora margarita* exhibited highest frequencies in aluminium rich soils. Similarly, Cuenca and Meneze (1996) during the survey of cacao plantations and nurseries in Venezuela observed 15 AMF fungal species and found species richness and diversity inversely correlated with available soil phosphorus.

In present study, the AMF inoculated plants, except a few AMF species, showed remarkable improvement in shoot biomass as well as in plant height as compared to uninoculated control. Of these, the most efficient 20 AMF isolates compared statistically revealed significant increase of 7.0 to 54.65, 12.6 to 41.5 and 11.7 to 32.8% in plant height, root biomass and shoot biomass, respectively, in maize. Further, mycorrhizal root colonization and root and soil phosphatase activities in AMF inoculated plants ranged from 23.7 to 65.2%, 3.6 to 40.7 m moles PNP/g fresh root weight and 5.7 to 30.7 m moles PNP/g soil, respectively. Our results are in conformity with Mehboob and Vyas (2013) who reported 52–78 % increase in mycorrhizal colonization in *Trigonella foenumgreacum* crop by inoculation of different AMF species. The gains in plant growth and development appear due to better root colonization and improvement in rhizosphere activities. The results are in agreement with Veerabhadraswamy and Garampalli (2011) who reported significant increase in dry weight and plant height in mycorrhizal maize plants due to high degree of fungal colonization in treated plants as compared to non-mycorrhizal ones. High degree of mycorrhizal colonization reflects better proliferation and wide hyphal network in rhizosphere which, in turn, ensures better nutrient acquisition and transport to host plant thereby resulting in significant stimulation of aerial growth (Hamza *et al.,* 2010). Kabir and Koide (2000) also observed higher shoot dry weight of maize plants due to higher mycorrhizal colonization and higher phosphorus content. Our findings are in agreement with Tabasum *et al.,* (2012) who reported improved growth of leguminous plants by AM fungi due to enhancement of phosphate uptake by mycorrhizal plants. In present study, the percentage of root colonization was higher in mycorrhizal than non-mycorrhizal plants. *Glomus geosporum* proved significantly superior in increasing mycorrhizal colonization ability of isolates over all other species tested showing 65.15% colonization, followed by *Gigaspora decipiens* with 61.53% colonization. Tanvir *et al.,* (2011) reported variations in AMF root colonization in various members of Lamiaceae. Different edapho-climatic factors like soil type, nutritional status, pH, organic matter, moisture, rain fall, temperature, etc. may be responsible for variations in root colonization and spore population (Sharma *et al.,* 1986). Our findings are favoured by various workers who reported that root colonization of AMF is genetically controlled (Raju *et al.,* 1990). The effects of colonization by mycorrhizal fungi on growth and nutrient contents have also been reported for many other plants, such as basil [*Ocimum basilicum*] (Copetta *et al.,* 2006; Rasouli-Sadaghiani *et al.,* 2010). Extramatrical hyphae produced by AM fungi act as extensions of roots and increase the surface area of root system, making it more efficient for the absorption of water and diffusion of limited nutrients (Bagyaraj and Reddy, 2000). *G. etunicatum* inoculated mint and oregano (*Origanum vulgare*) plants had increased growth and nutrient contents (Karagiannidis *et al.,* 2012). The positive effect of AMF root colonization by *G. mosseae* on shoot biomass of oregano plants raised in greenhouse has also been reported by Khaosaad *et al.,* (2006). Sharma *et al.,* (1998) found highest root colonization of 16% in apple crop in Manali and lowest in Kotgarh region.

Initial screening revealed *Glomus geosporum, Acaulospora rehmii, Glomus diaphanum, Gigaspora decipiens* and *A. bireticulata* to be significantly superior in improving maize plant growth and soil nutrient availability *viz-a-viz* phosphatase enzyme activity. Othira *et al.,* (2012) reported increased plant height and biomass in maize due to the inoculation of several species of mycorrhizal fungi and found *Glomus* spp. as the most efficient isolates. Further, they attributed the variable growth efficacy of AMF isolates to the differences in their effectiveness to colonize plant roots and their ability to grow in soil, thereby enhancing nutrient mobilization and uptake. Increase in root and shoot weight have also been reported in *Cucurbita pepo* (Colla *et al.,* 2007), *Lotus glaber* (Sannazzaro *et al.,* 2006) and soybean (Sharifi *et al.,* 2007). Our work is also in line with Feng *et al.,* (2002) and (Tian *et al.,* 2004) who reported significant increase in shoot biomass in cotton plants inoculated due to *G. mosseae.* Mycorrhization allows the plant to have a high root: shoot ratio causing better hydro-mineral nutrition and thereby reinforcing the capacity to resist stress (Caravaca *et al.,* 2003). Acid phosphatases are considered to be important in the phosphate metabolism of the fungus. The results of present study revealed that AMF inoculated plants show higher enzyme activity than uninoculated plants. Maximum root phosphatase activity (40.66 m moles PNP/g fresh root weight) and soil phosphatase activity (30.73 m moles PNP/g soil) was observed in *G. geosporum* inoculation treatments. Our findings are in agreement with Song *et al.,* (2001) who observed that AMF increased soil acid phosphatase activities in rhizosphere and found phosphorus sources influenced soil phosphatase activity. Ramakrishnaiah and Vijaya (2013) have reported that acid phosphatases of AMF inoculated soils are significantly influenced by the presence of AM fungi in rhizosphere of *Stevia rebaudiana.* The phosphatase enzyme activity is a physiological characteristic related to plant efficiency in relation to phosphorus acquisition and utilization, and is genetically variable (Tadano *et al.,* 1993). Plants usually secrete root acid phosphatases when phosphorus availability is low; however, plant species differ in secretion ability and enzyme activity (Yan *et al.,* 2001).

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