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Influence of AM Fungi Inoculation in the Efficiency of *Piper mullesua* Plantlets in Organic Amended Soil

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ABSTRACT

A greenhouse experiment was carried out to study the efficiency of arbuscular mycorrhizal fungi indigenous to Arunachal Pradesh in uptaking plant nutrients from applied organic matter in *Piper mullesua* plantlets. Application of organic amendment to *Piper mullesua*plantlets inoculated with arbuscular mycorrhizal fungi (AMF) enhances plant biomass, phosphatase activity, P and N content. Organic amended soil increases the percentage of mycorrhizal fungal infection as well as other growth parameters in *P. mullesua* plantlets. The plant biomass, phosphatase enzyme activity, concentration of P and N was significantly higher in *P. mullesua* plantlets inoculated with AM fungal species viz *Glomus versiforme, G. etunicatum*and *G. aggregatum.* This study shows that mycorrhizal inoculants were efficient in utilizing and mobilizing inorganic nutrients available in organic amendment. The correlation coefficient between plant biomass and phosphatase activity was highly significant ($p>0.001$) on application of organic amendment in those AM fungi inoculated plantlets.

Key words: Organic amendment, AM fungi, Infection, Plant biomass, Phosphatase

Mycorrhizal fungi constitute an important component of soil microflora and are widely present in association with most of the plant species which are reported in more than 80% of the plant families (Giovannetti and Sbrana 1998). An efficient mycorrhizal root can increase absorption and translocation of nutrients to the plants and can explore more soil volume than the non-mycorrhizal roots (Joner and Jackobsen 1995). Association of arbuscular-mycorrhizal fungi with organic matter benefits the agricultural land by manipulating the soil structure, water retention capacity, microbial activity on to chemical exudates released from organic matter. In reverse, addition of organic matter have a beneficial effect on the growth of indigenous AM fungi colonization in nutrient-limited soil (Gaur and Adholeya 2002) and on soil stabilization and plant establishment in eroded soils (Vaidya *et al.* 2007). On the other hand organic amendments enhance spore production, extra radical proliferation of hyphae and improve colonization of roots and also enhance growth and spore formation of AMF even in eroded soil (Vaidya *et al.* 2008). This beneficial effect might be related to increase pore volume in soil resulting in AM colonization and mycorrhizal growth response. For the present study, the *Piper mullesua*, belonging to the family *Piperaceae* has been selected which is a medicinally important plant species. The roots and fruiting spikes are used in treating diarrhea, indigestion, jaundice, urticacia, and abdominal disorder, horseness of voice, asthama, cough, piles, malaria fever, vomiting, wheezing, chest conjestion, throat infection, worms and sinusitis (Gajurel *et al*. 2008). Medicines prepared from this species also improve bioavailability of nutrients of the food and impart body resistance. The present study deals on the efficiency of different AM fungi with *P. mullesua* plantlets amended with organic manure.

MATERIALS AND METHODS

Study was carried out in and around Doimukh area of Papum Pare District and Pasighat area of East Siang Districts of Arunachal Pradesh (26º 30′ N - 29º 30′ N Latitude and 91º 30′ E - 97º 30′ E Longitude; altitude100-600 m asl). The region experiences a humid tropical climate (Rainfall 110-160 cm; annual temperature 12°C-37°C). The vegetation type corresponds to tropical semi-evergreen forest. The soil texture of area ranges from sandy loam to loamy sand and pH ranges from 4.9-6.7.

Raising of piper seedlings

Seedlings of piper were raised through stem cuttings. The seedlings were raised in sterilized sand and soil mixture (3:1).

Isolation and collection of mycorrhizal fungi

Soil samples were collected from different locations in Arunachal Pradesh for isolation of VAM fungal spores. Samples were taken from depth of 0-15 cm under various land use systems such forest area, *jhum* fields, home gardens etc as well as natural habitat of piper plants. Mycorrhizal fungal spores were isolated from soil by the method as suggested by Gerdmann and Nicholson (1963). Ten AM fungal species i e *G. etinucatum, G. versiforme*, *G. albidum*, *G. claroidium, G. occultum, G. macrocarpum*, *G. hoi*, *G. aggregatum, G. fasciculatum*, *G. aurantium* were selected to carry out the experiment.

Pot experiment

A pot experiment was carried out with 11 treatments viz non-mycorrhizal *P. mullesua* plantlets as control and plantlets inoculated with the above mentioned ten inoculants. About 6.4g of organic amendment was applied per 200g of sterilized sand and soil mixture (3:1) uniformly in all the treatments. A healthy piper plantlet was planted in each pot inoculating with 50 gm of AM fungi cultured soil. Eight replicates of *P. mullesua* plantlet were taken per treatment. Pots were kept in mist chamber and harvesting was done after 90 days of transplantation.

Laboratory analysis

Growth parameters like shoot and root length as well as plant biomass was determined by drying them separately in hot air oven at 60º C for 48 hours. The percentage of the root colonized by VAM fungi were determined by using the formula as suggested by Brundreett *et al*. (1996). The chlorophyll content of leaf of *P. mullesua* was estimated by the method of Witham *et al*. (1971). The total Nitrogen and Phosphorus content of plant material was determined by the Kjeldahl method and Vanadomolybdate method respectively Juo (1982). The activity of Phosphatase was determined by method suggested by Tabatabai and Bremner (1969).

The data was subjected to one-way analysis of variance (ANOVA) to determine the effect of treatments using computer software SYSTAT 10. Correlation coefficient was calculated to evaluate the strength of the relationship of total plant biomass with the other parameters considered in the study.

RESULTS AND DISCUSSION

After harvesting the *P. mullesua* plantlets inoculated with AMF, shoot length, root length (Table 1), chlorophyll content, percent infection and survivality percentage (Fig 1) were recorded. In the present study, mycorrhizal infection has increased the growth rate in terms of biomass (Fig 2), phosphatase activity (Fig 3), plant phosphorous (Fig 4) and nitrogen content (Fig 5). The greater shoot length and root length as well as higher plant biomass in the treatments having mycorrhizal association agrees with the previous studies (Gryndler *et al.* 2009) which is due to organic amendment and enhanced uptake of plant nutrient by inoculated AM fungi (Medina and Azcon 2010). Organic amendment also increases nodule number and hyphal length in roots (Medina *et al.* 2007) of the inoculated plants. Our findings supports the statement by observing better plant growth in mycorrhizal plantlets rather than the control plants treated only with organic amendment. In this experiment *G. versiforme* was found to be most effective for increasing the plant biomass in presence of organic amendment. Similar results was obtained by Caravaca *et al.* (2006) which indicated that *G. mosseae* was the most effective for increasing the crop growth as compared to the other fungal isolates. Though mycorrhizal infection percentage is not directly related to the plant biomass, but in this study, roots of inoculated *P.mullesua* plants were well colonized with mycorrhizal fungi, though the percent infection was not significant among the treatment which also agrees with the findings of Perner *et al*. (2007). Recent studies have also indicated that AM fungi produce a glycoprotein, glomalin that acts as an insoluble glue to stabilize aggregates (Wright and Anderson 2000).

Table 1 Shoot and root length of AM fungi inoculated *P. mullesua* seedlings on application of organic amendment

Treatments	Shoot length (cm)	Root length (cm)
Control	8.25 ± 0.08	43.5 ± 0.29
G. etinucatum	11.5 ± 0.17	31.33 ± 0.59
G. versiforme	12.45 ± 0.54	27.33 ± 0.75
G. albidum	10.7 ± 0.27	38 ± 1.53
G. claroidium	12.5 ± 0.17	33.5 ± 0.17
G. occultum	10.13 ± 0.49	35.5 ± 0.17
G. macrocarpum	9.08 ± 0.17	36.0 ± 0.73
G. hoi	9.17 ± 0.10	34.17 ± 0.10
G. aggregatum	13.83 ± 0.25	31.5 ± 0.60
G. fasciculatum	10.5 ± 0.93	38.33 ± 1.44
G. aurantium	10.17 ± 0.25	38.25 ± 1.47

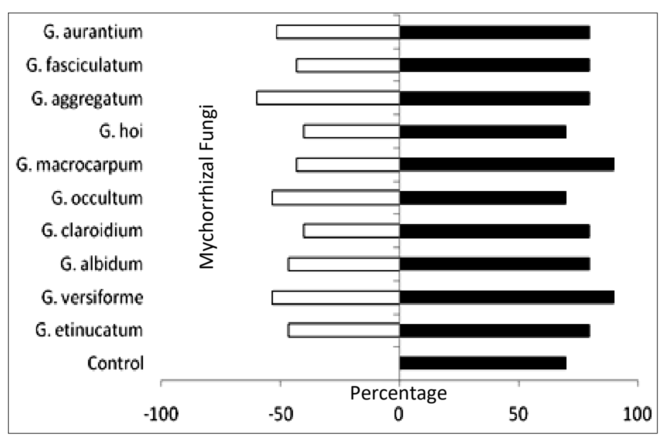

Fig 1 Survivality %● ○% infection of *P. mullesua* plantlets after inoculation with AM fungi on application of organic amendment

Table 2 Correlation coefficient between biomass and percentage infection, phosphatase activity, phosphorus and plant nitrogen of *P. mullesua* plantlets grown under various levels of organic amendment

Infection	P-ase	Plant P	Plant N
0.573	0.767	0.818	0.901
p>0.05	p>0.05	p>0.001	p>0.001

The phosphatase enzyme activity is generally an important parameter when evaluating responses in mycorrhizal fungal associations. In the present study, phosphatase enzyme activity increased in all the treatments (r = 0.767, p>0.005), while the phosphatase activity found significantly low in non-mycorrhizal plantlets. It has been well demonstrated that the enhancement of phosphatase activity is one of the physiological and biochemical mechanism involved in the mycorrhization effect on plant nutrition (Hue *et al*. 2010). Compared to the non-mycorrhizal plantlets, although the mycorrhizal plantlets performed better result, it is important to select appropriate AM fungal species that can enhance the phosphatase activity of the *P. mullesua* plantlets. Nevertheless, different AM fungal species play different role in this plant- fungal symbiosis (Hue *et al.* 2013), *G. versiforme* was the most efficient to enhance the phosphatase activity in our current study.

Our results supported the hypothesis that concentration of phosphorus is higher in mycorrhizal inoculants plants than non-mychorrhizal one. As compared to the non-mycorrhizal plantlets, mycorrhiza inoculated *P. mullesua* plantlets showed significantly higher plant P (r = 0.818, p>0.001) in presence of organic amendment. The increase of plant P in plantlets on application of organic amendment may be due to ability of AM fungi to uptake more available P from decomposed organic matter. This result was similar to the study made by Palm (1995), who indicated that P released from the tissues supplied as organic matter during decomposition and mineralization, directly increases available P. In the present study, *G. versiforme* inoculated *P. mullesua* plantlets were found efficient in enhancing phophatase activity as well as in uptaking plant P from applied organic amendment as compared to other AM fungi (Fig 4).

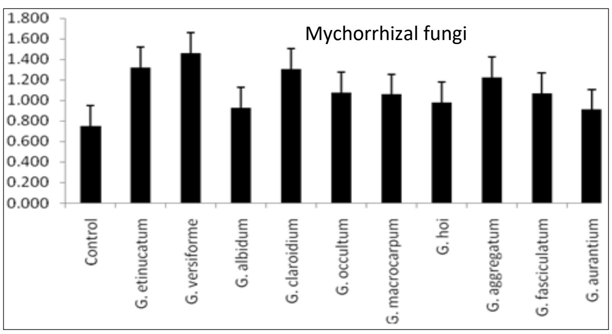

Fig 2 Total biomass (g) of *P. mullesua* plantlets inoculated with different mycorrhizal fungi and non-mycorrhizal control on application of organic amendment

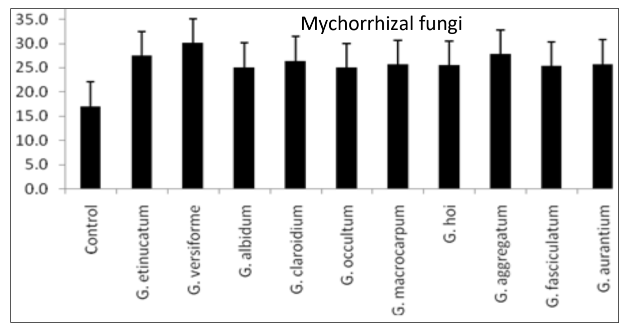

Fig 3 Phosphatase activity (µg/g) of *P. mullesua* plantlets inoculated with different mycorrhizal fungi and non-mycorrhizal control plant on application of organic amendment

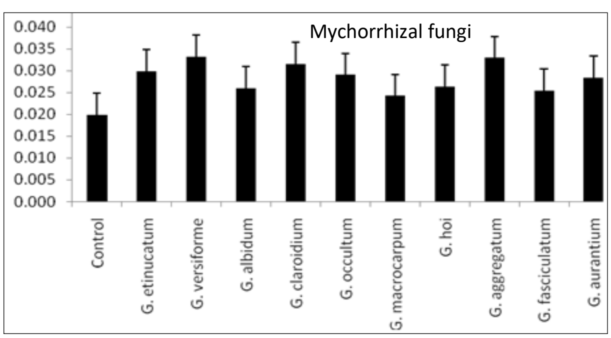

Fig 4 Phosphorus content (g/kg) of *P. mullesua* plantlets inoculated with different mycorrhizal fungi and non-mycorrhizal control plant on application of organic amendment

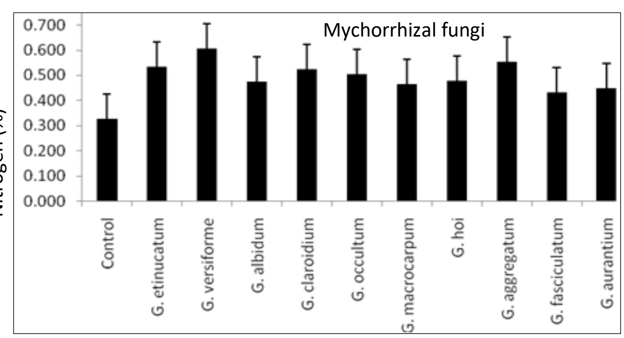

Fig 5 Nitrogen content (%) of *P. mullesua* plantlets inoculated with different mycorrhizal fungi and non-mycorrhizal control plant on application of organic amendment

Mycorrhizal inoculants particularly *Glomus versiforme*, found more effective in up taking inorganic N from applied organic amendment, which signifies the ability of the inoculants to utilize nutrients from organic amendment. This result agrees with Hodge *et al*. (2001) demonstrated that the arbuscular mycorrhizal fungus *Glomus hoi* was able to enhance decomposition and increase plant N capture from grass leaves. Similarly, Azcon and Barea (1992) described that increased in plant N content in the mycorrhizal plants may be due to the ability of arbuscular mycorrhizal fungi to enhance N capture from soil and to increase P uptake.

Application of organic amendment to *Piper mullesua* plantlets inoculated with arbuscular mycorrhizal fungi enhances plant biomass, phosphatase activity, P and N content also increases the percentage of mycorrhizal fungal infection as well as other growth parameters. Mycorrhizal inoculants were efficient in utilizing and mobilizing inorganic nutrients available in organic amendment.

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