

## Plantlets growth of *Piper mullesua* Buch.-Ham. ex D. Don. in organic manure amended soil inoculated with arbuscular mycorrhizal fungi

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### Abstract

Inoculation of *P. mullesua* plantlets with AMF at various levels of organic manure was found enhancing the biomass, shoot length, P and N content. It signifies that organic amendment supported increase in plant biomass and mycorrhizal fungal infection with *P. mullesua*. AM fungal species *Glomus versiforme* was found most effective for increasing the plant growth in all the three levels of FYM. The phosphatase enzyme activity in all the FYM levels was found higher which indicate that the application of FYM increased phosphatase activity of AMF. The correlation coefficient between plant biomass and phosphatase activity of AMF was highly significant ( $p > 0.001$ ) under FYM level I and III. Concentration of P and N in seedlings was found higher than that of non-mycorrhizal one. Mycorrhizal inoculants particularly *G. versiforme*, *G. aggregatum*, *G. claroidium* were better than other AMF in enhancing the growth of plantlets at all the three levels of FYM.

*Piper mullesua* Buch.-Ham. ex D. Don is a medicinal plant belonging to the family *Piperaceae*. Roots and fruiting spikes are used in treating diarrhoea, indigestion, jaundice, urticaria, and abdominal disorder, hoarseness of voice, asthma, cough, piles, malaria fever, vomiting, wheezing, chest congestion, throat infection, worms and sinusitis. Medicines prepared from this species also improve bioavailability of nutrients of the food and impart body resistance.

Mycorrhizal fungi constitute an important component of soil microflora and are

widely present in association with most of the plant species which is reported in more than 80% of the total plant families<sup>15</sup>. An efficient mycorrhizal root can increase absorption and translocation of nutrients to the plants<sup>25</sup> and can explore more soil volume than the non-mycorrhizal roots<sup>21</sup>. In addition, association of arbuscular-mycorrhizal fungi with organic matter<sup>39</sup> and the benefits of organic matter amendments to agricultural land are well documented<sup>24</sup>. The effect of organic manure amendments on AM fungi has often been attributed to the effect of organic matter on soil structure, water retention capacity,

microbial activity on to chemical exudates released from organic matter<sup>34</sup>. On reverse, addition of organic matter have a beneficial effect on the growth of indigenous AM fungi colonization in nutrient-limited soil<sup>8,12</sup> and on AM fungi on soil stabilization and plant establishment in eroded soils<sup>41</sup>.

During the process of decomposition and mineralization<sup>30</sup>, P can be released from decomposed tissues, directly increasing available P. on the other hand, organic anions produced during decomposition may compete with P for adsorption sites, also increasing P availability<sup>12,19</sup>. Addition of materials with a high P concentration results in net P mineralization and an increase in available P in soil, whereas those low in P result in a reduction in soil solution P through microbial immobilization<sup>29</sup>. Concentration of P  $< 2.5 \text{ g kg}^{-1}$  in organic matter has been considered to induce P immobilization<sup>31</sup>. In addition, Singh and Jones<sup>35</sup> found that organic materials with a P concentration  $> 2.2 \text{ g Kg}^{-1}$  reduce P sorption, while those with a P concentration  $< 2.2 \text{ g kg}^{-1}$  will increase P sorption. In phosphorus deficient soils, P became unavailable because of low native P content in soil and high P-fixation capacity of these soils. For this, large amount of P fertilizer are required to overcome limited P fixation.

Organic amendments enhance spore production<sup>10,20</sup>, extra radical proliferation of hyphae<sup>21,39</sup> and improve colonization of roots<sup>27</sup> and also enhance growth and spore formation even in eroded soil<sup>41</sup>. Giovanetti and Avio,<sup>14</sup> suggested that this beneficial effect might be related to increase pore volume in soil which has a beneficial effect on AM colonization, the

mycorrhizal growth response and AM spore numbers.

Present study deals on the efficiency of AM fungi with *P. mullesua* plantlets at different doses of organic manure.

#### *Study sites :*

Study was carried out in and around Doimukh area of Papum Pare District and Pasighat area of East Siang Districts of Arunachal Pradesh ( $26^{\circ} 30' \text{ N} - 29^{\circ} 30' \text{ N}$  Latitude and  $91^{\circ} 30' \text{ E} - 97^{\circ} 30' \text{ E}$  Longitude) having various altitudinal range from 100-600m asl. The average annual precipitation ranges from 1100mm – 1600mm and the day time temperature vary from a minimum of  $12^{\circ}\text{C}$  to a maximum of  $37^{\circ}\text{C}$  experiencing a humid tropical climate. 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.

#### *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.* *Glomus 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.

*Experimental design and treatments :*

To evaluate the impact of organic manure three levels of farm yard manure amount (full, half and double of recommended dose) was used. Recommended FYM dose for piper plant is FYM 10 tons per ha. In treatment I ( $\frac{1}{2}$ FYM), 3.2g FYM was mixed with 3:1 sterilized sand and soil mixture and a healthy piper seedling was planted. In treatment II (1FYM), 6.4g FYM treatment was given to the plantlets. Again in treatment III (2FYM), 12.8g FYM was applied per 200g sterilized sand and soil mixture. 8 replicates for each treatment and control were taken. Pots were kept in Mist chamber and harvesting was done after 90 days of transplantation.

*Raising of Piper seedlings :*

Considering the requirement of large number of seedlings for various experiments during the study period piper plants were collected from different places. Seedlings of piper were raised through vegetative propagation from the stem cuttings for further experimental use. These seedlings were raised in sterilized 3:1 sand and soil mixture.

*Measurement of growth parameters :*

The plants were harvested after 90 days of transplantation. Shoot and root length was measured with the help of normal scale. Tap root as well as adventitious roots, both were taken in consideration for length measurement of roots. Biomass of plantlets was determined by drying shoot and root separately in hot air oven maintaining

temperature at 60° C for 48 hrs.

*AM root colonization :*

To study root colonization by mycorrhizal fungi randomly fine roots were taken from plantlets grown at different soil pH. Roots were washed in tap water and cleared with 2.5% KOH for 30min at 90°C. Then the root samples were acid soaked in 5N HCl for 1min, before being stained with Trypan blue (Phillips & Hayman, 1970). The percentage of the root colonized by VAM fungi were determined by using the formula as suggested by Brundrett *et al.*<sup>6</sup>.

*Determination of chlorophyll content of leaf :*

Fresh leaves of *Piper mullesua* were taken to determine the chlorophyll content of leaf. Leaves were ground to a fine pulp with addition of 20ml of 80% acetone and subsequently volume was made up to 100 ml. These solutions were then centrifuged at 5000 rpm following filtration. Finally the OD of filtrates was measured by spectrophotometer (at 645nm and 663nm) against 80% acetone as blank to determine chlorophyll content of the leaf.

*Determination of plant Nitrogen and Phosphorus concentration :*

The total Nitrogen content of plant material was determined by the Kjeldahl method<sup>2</sup>. Phosphorus concentration of plant material was determined by Vanadomolybdate method as suggested by Jackson (1971).

*Assay of Phosphatase activit :*

Fresh root of the plant were ground with citrate buffer (pH 5.3) to prepare an enzyme extract, following centrifugation at 10,000 rpm. The supernatant solution was than mixed with substrate solution consisting EDTA and p-nitrophenyl phosphate and after that it was incubated at 37 °C for 15 minutes. After incubation 0.085 N solution of NaOH was added to stop the reaction. Absorbance of solution was taken at 405 nm by spectrophotometer.

*Statistical analysis :*

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.

*Shoot length and root length :*

At FYM level I, maximum shoot length was found in the plantlets inoculated with *G. versiforme* (9.5cm) and *G. claroidium* (9.5cm), followed by *G. aggregatum* (9.22 cm). Minimum shoot length was recorded in the plantlets inoculated with *G. hoi* and *G. fasciculatum* (7.50 cm) but was higher than the non-mycorrhizal control plants (7.17 cm  $\pm$ 0.1). At FYM level II, shoot length of *Piper mullesua* plantlets was significantly higher (F=6.17,  $p>0.005$ ) than the non-Mycorrhizal control plants. *G. fasciculatum* recorded maximum shoot length of 13.83cm  $\pm$ 0.25, whereas in case of *G. claroidium* and *G. versiforme* it was 12.50 cm  $\pm$ 0.17 and 12.45

cm  $\pm$ 0.54 respectively. Plantlets inoculated with *G. macrocarpum* had lower shoot length of 9.08 cm  $\pm$ 0.17 which was still higher than the non-mycorrhizal control plants (8.25 cm  $\pm$ 0.08). At FYM level III, highest shoot length was found in the plantlets inoculated with *G. versiforme* (18.33 cm  $\pm$ 0.84) and *G. aggregatum* (18.23 cm  $\pm$ 0.29) and lowest was found in the plantlets inoculated with *G. fasciculatum* (12.95 cm  $\pm$ 0.56) which was slightly higher than the non-mycorrhizal control plant (12.5 cm  $\pm$ 0.17) (Table-1).

At FYM level I, highest root length was observed in the plantlets inoculated with *G. occulatum* (39.5 cm  $\pm$ 1.09) whereas the root length of non-mycorrhizal control plant was 42.83 cm  $\pm$ 0.10. In case of FYM level II, highest root length was recorded in plantlets inoculated with *G. fasciculatum* (38.33 cm  $\pm$ 1.44) which was found lower than the non-mycorrhizal control plants (43.50 cm  $\pm$ 0.29). At FYM level III, it was found higher in the plantlets inoculated with *G. aurantium* (45.85 cm  $\pm$ 0.16) which was still lower than the non-mycorrhizal control plants (52.83 cm  $\pm$ 0.10) (Table-1).

*Biomass :*

In general biomass of the plantlets inoculated with AMF was significantly higher (F=2.778,  $p>0.01$ ) than the non-mycorrhizal one. At FYM level I, highest biomass was found in the plantlets inoculated with *G. versiforme* (0.97g) and *G. claroidium* (0.962g). *G. occulatum* inoculated plantlets had lower biomass of 0.741g, but was higher than the non-mycorrhizal control plants (0.647g). At FYM level II, *G. versiforme* inoculated plantlets produced the highest

Table-1. Shoot length, root length, biomass production, chlorophyll content, infection, phosphatase, plant P & plant N content of *P. mulliesia* plantlets after inoculation with AM fungi at Farm yard manure (FYM) level – I, II & III

Mycorrhizal isolates	Shoot Length (cm)			Root Length (cm)			Chlorophyll content			Infection (%)			Survivality (%)		
	FYM-I	FYM-II	FYM-III	FYM-I	FYM-II	FYM-III	FYM-I	FYM-II	FYM-III	FYM-I	FYM-II	FYM-III	FYM-I	FYM-II	FYM-III
Control	7.17 ±0.10	8.25 ±0.08	12.50 ±0.17	42.83 ±0.10	43.50 ±0.29	52.83 ±0.10	1.513 ±0.020	1.541 ±0.027	1.706 ±0.020	0.00 0.00	0.00 0.00	0.00 0.00	60	70	80
<i>Glomus etunicatum</i>	9.00 ±0.17	11.50 ±0.17	17.00 ±0.44	33.43 ±0.13	31.33 ±0.59	34.17 ±0.10	1.791 ±0.011	1.831 ±0.017	1.838 ±0.015	46.67 ±6.94	46.67 ±1.92	51.67 ±0.96	80	80	100
<i>G. versiforme</i>	9.50 ±1.00	12.45 ±0.54	18.33 ±0.84	34.67 ±0.69	27.33 ±0.75	34.00 ±0.58	1.775 ±0.006	1.803 ±0.011	1.861 ±0.008	33.33 ±5.09	53.33 ±5.09	61.67 ±5.36	80	90	80
<i>G. albidum</i>	8.07 ±0.13	10.70 ±0.27	13.33 ±0.25	36.00 ±0.76	38.00 ±1.53	42.67 ±0.63	1.872 ±0.024	1.766 ±0.021	1.764 ±0.005	35.00 ±5.00	46.67 ±3.85	50.00 ±6.67	70	80	90
<i>G. claroideum</i>	9.50 ±0.17	12.50 ±0.17	17.57 ±0.46	33.33 ±0.10	33.50 ±0.17	33.77 ±0.25	1.894 ±0.012	1.787 ±0.010	1.800 ±0.011	36.67 ±1.92	40.00 ±3.33	55.00 ±5.00	80	80	100
<i>G. occultum</i>	8.33 ±0.19	10.13 ±0.49	15.17 ±0.42	39.50 ±1.09	35.50 ±0.17	42.30 ±1.35	1.730 ±0.013	1.729 ±0.008	1.806 ±0.013	30.00 ±3.33	53.33 ±10.18	60.00 ±3.33	70	70	100
<i>G. macrocarpum</i>	8.67 ±0.19	9.08 ±0.17	15.50 ±0.73	38.33 ±1.50	36.00 ±.73	43.50 ±0.17	1.738 ±0.010	1.705 ±0.016	1.802 ±0.013	36.67 ±6.94	43.33 ±5.09	60.00 ±.33	70	90	75
<i>G. hoi</i>	7.50 ±0.17	9.17 ±0.10	14.00 ±0.17	36.83 ±1.64	34.17 ±0.10	42.90 ±1.43	1.707 ±0.008	1.731 ±0.010	1.796 ±0.008	31.67 ±6.74	40.00 ±6.67	56.67 ±.09	60	70	80
<i>G. aggregatum</i>	9.22 ±0.21	13.83 ±0.25	18.23 ±0.29	33.50 ±0.17	31.50 ±0.60	32.90 ±0.72	1.778 ±0.011	1.793 ±0.012	1.829 ±0.012	46.67 ±3.85	60.00 ±3.33	50.00 ±5.77	80	80	90
<i>G. fasciculatum</i>	7.50 ±0.17	10.50 ±0.93	12.95 ±0.56	37.17 ±1.70	38.33 ±1.44	40.50 ±0.76	1.623 ±0.007	1.831 ±0.012	1.828 ±0.019	43.33 ±5.09	43.33 ±5.09	63.33 ±5.09	60	80	100
<i>G. aurantium</i>	7.67 ±0.25	10.17 ±0.25	13.50 ±0.29	38.37 ±0.80	38.25 ±1.47	45.85 ±0.16	1.677 ±0.015	1.701 ±0.014	1.778 ±0.004	41.67 ±4.19	51.67 ±4.19	53.33 ±5.09	60	80	80

Table-2. Correlation coefficient between Biomass and Percentage infection, Phosphatase activity, Phosphorus and Plant Nitrogen of *P. mullesua* seedling grown under various levels of FYM.

Fertilizer Level	Infection	P-ase	P	N
FYM-I	0.617 <i>p</i> >0.05	0.933 <i>p</i> >0.001	0.787 <i>p</i> >0.005	0.943 <i>p</i> >0.001
FYM-II	0.573 <i>p</i> >0.05	0.767 <i>p</i> >0.005	0.818 <i>p</i> >0.001	0.901 <i>p</i> >0.001
FYM-III	0.451 ns	0.807 <i>p</i> >0.001	0.805 <i>p</i> >0.001	0.672 <i>p</i> >0.05

‘ns’ not significant

biomass (1.46g) compared to the other AMF inoculated plantlets. Non-mycorrhizal control plants produced biomass of 0.747g which was lesser than the AMF infected plants. At FYM level III, *G. versiforme* had the best by producing 1.743g while minimum biomass was recorded in the plantlets inoculated with *G. albidum* (1.101g). Non-mycorrhizal control plants (1.047g) produced less biomass as compared to the AMF infected plants (Fig. 6.1).

#### Chlorophyll content :

Chlorophyll content at FYM level I was found highest in the plantlets inoculated with *G. claroideum* (1.894 mg/g). Under this treatment chlorophyll content variation was found between 1.894 mg/g to 1.623 mg/g which was significantly higher ( $F=21.11$ ,  $p>0.001$ ) than the non-mycorrhizal plants (1.513 mg/g). At FYM level II, highest chlorophyll content was found in the plantlets inoculated with *G. etunicatum* and *G. fasciculatum* (1.831mg/g) and lowest was recorded in the plantlets inoculated with *G. aurantium* (1.701 mg/g). In Non-mycorrhizal plants chlorophyll content

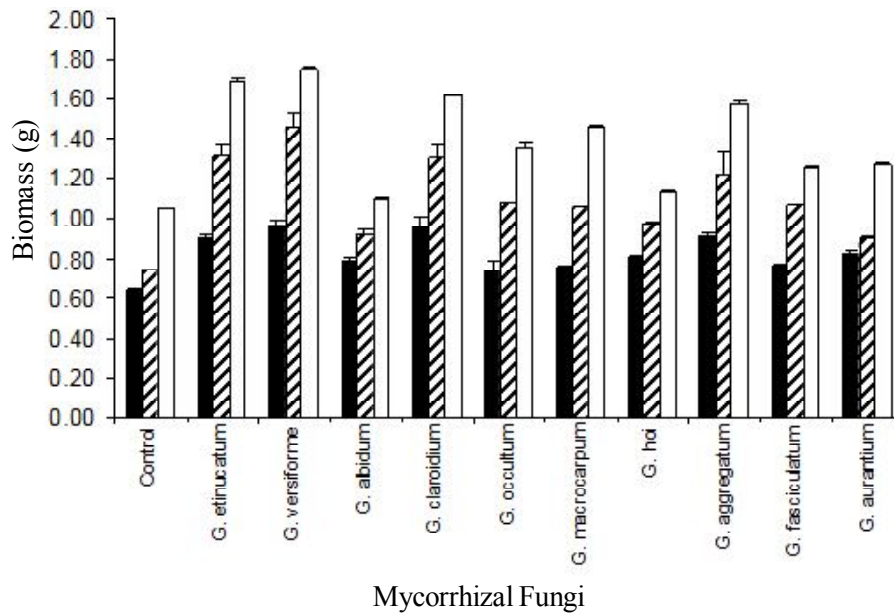
was 1.541 mg/g. At FYM level III, plantlets inoculated with *G. versiforme* (1.861 mg/g) consisted highest chlorophyll content and lowest was recorded in the plantlets inoculated with *G. albidum* (1.764 mg/g) which was also higher than the non-mycorrhizal plants (1.706 mg/g).

#### Percent infection :

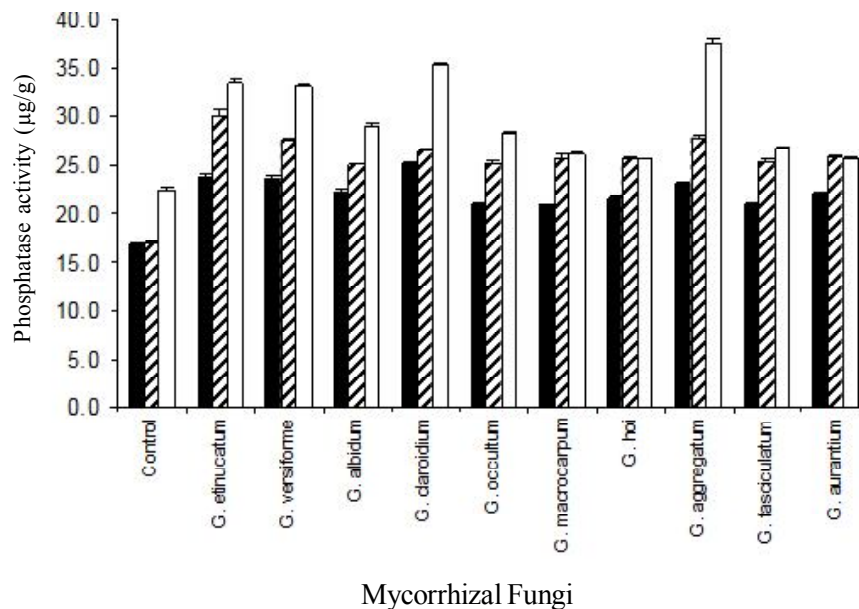
Colonization of *P. mullesua* plant roots by inoculated AMF was measured as percent infection and is given in table 6.1. Percent infection at FYM level I, was found highest in the plantlets inoculated with *G. aggregatum* and *G. etunicatum* (46.67%) and at FYM level II, highest infection was observed in the plantlets inoculated with *G. aggregatum* (60.00%). In case of FYM level III, highest percent infection was in the plantlets those were inoculated with *G. fasciculatum* (63.33%). Non-mycorrhizal control plants in all the experiments were remained infection free (Table-1).

#### Survivality percentage :

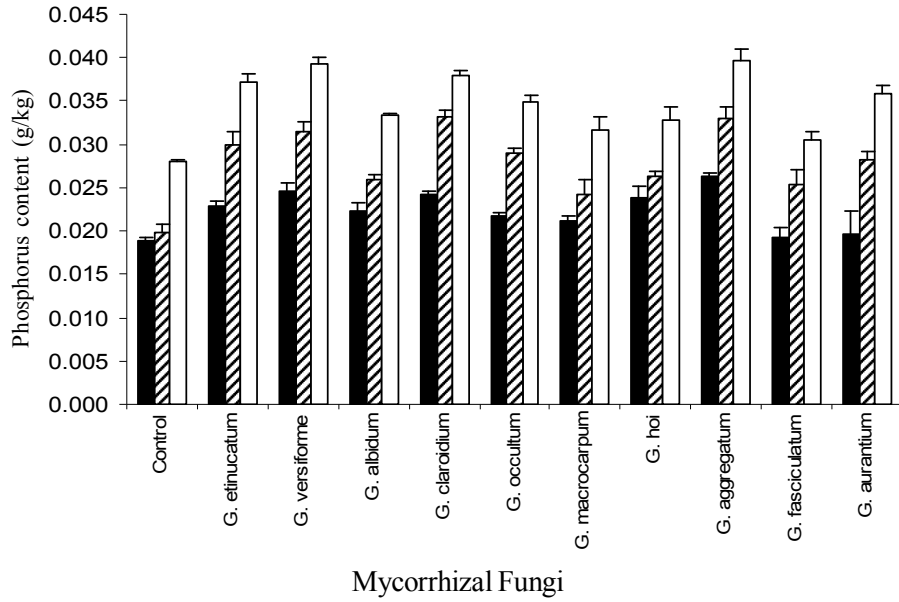
In general survivality of plantlets was



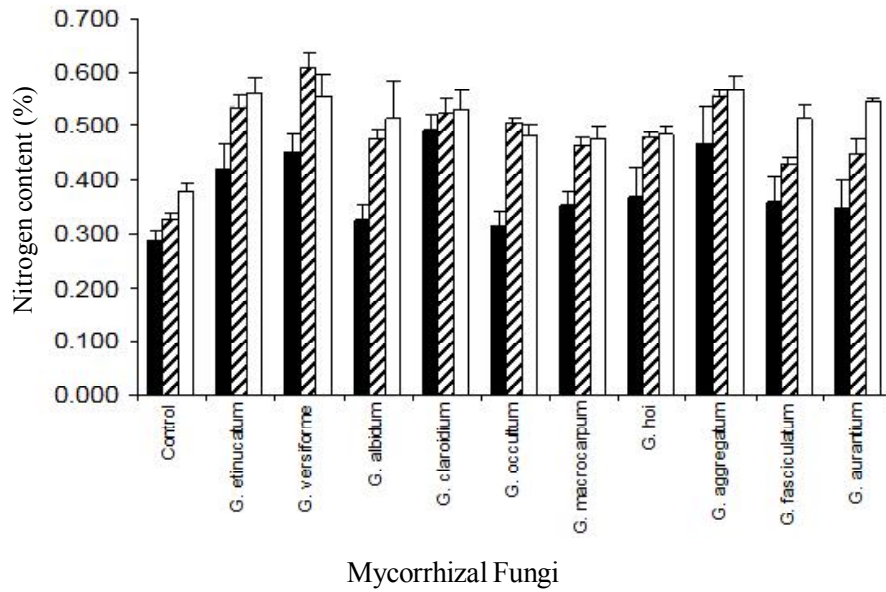
**Figure 1** Total Biomass (g) of *P. mullesua* plantlets inoculated with different mycorrhizal fungi and non-mycorrhizal one in FYM level-I ■, level-II ▨ and level-III □



**Figure 2.** Phosphatase activity (µg/g) of *P. mullesua* plantlets inoculated with different mycorrhizal fungi and non-mycorrhizal one in FYM level-I ■, level-II ▨ and level-III □



**Figure 3** Phosphorus content (g/kg) of *P. mullesua* plantlets inoculated with different mycorrhizal fungi and non-mycorrhizal one in FYM level-I ■ level-II ▨ and level-III □



**Figure 4** Nitrogen content (%) of *P. mullesua* plantlets inoculated with different mycorrhizal fungi and non-mycorrhizal one in FYM level-I ■ level-II ▨ and level-III □



maximum at higher dose of FYM in all the AMF inoculated plantlets as well as in control one. At FYM level I, survivality ranges from 60-80% and at FYM level II, it ranges from 70-90%, while at FYM level III, survivality % was found 70-100%. However, survivality percent of nonmycorrhizal plantlets was lower at all the three doses of FYM compare to mycorrhizal one.

#### *Phosphatase activity :*

Generally phosphatase activity was significantly higher ( $F=41.027$ ,  $p>0.001$ ) in AMF inoculated plants than the non-mycorrhizal control plants at FYM level I. Maximum activity was recorded in the plantlets inoculated with *G. etinucatum* (23.73  $\mu\text{g/g}$ ) and *G. versiforme* (23.62  $\mu\text{g/g} \pm 0.2$ ) and minimum was in the plantlets infected by *G. macrocarpum* (20.77  $\mu\text{g/g}$ ). At FYM level II, *G. etinucatum* inoculated plantlets has the highest activity of phosphatase (30.04  $\mu\text{g/g}$ ), followed by *G. aggregatum* (27.85  $\mu\text{g/g}$ ). Lowest activity was recorded in the plantlets inoculated with *G. albidum* (25.08  $\mu\text{g/g}$ ) and *G. occulatum* (25.05  $\mu\text{g/g}$ ) but was higher than the non-mycorrhizal control plantlets (17.03  $\mu\text{g/g}$ ). At FYM level III, highest phosphatase activity was observed in the roots of plantlets inoculated with *G. aggregatum* (37.37  $\mu\text{g/g}$ ), followed by *G. claroideum* (35.29  $\mu\text{g/g}$ ) and lowest activity was recorded in the plantlets inoculated with *G. hoi* (25.55  $\mu\text{g/g}$ ). Phosphatase activity of non-mycorrhizal control plant was found less than the AMF inoculated plants (22.30  $\mu\text{g/g}$ ) (Fig. 2).

#### *Plant Phosphorous :*

At FYM level I, plant phosphorous

was found highest in the plantlets inoculated with *G. aggregatum* (0.0264 g/kg), followed by *G. versiforme* (0.0246 g/kg) which were significantly higher ( $F=21.03$ ,  $p>0.001$ ) than the non-mycorrhizal (0.0188 g/kg) control plants. At FYM level II, maximum amount of plant phosphorous was found in the plantlets inoculated with *G. aggregatum* (0.0329 g/kg), followed by *G. versiforme* (0.01325 g/kg). At same level of FYM in non-mycorrhizal control plants, Phosphorous content was found 0.0198 g/kg. At FYM level III, higher plant phosphorous was found in the plantlets inoculated with *G. aggregatum* (0.0398 g/kg) and *G. versiforme* (0.393 g/kg). Lower plant phosphorous was found in the plantlets inoculated with *G. fasciculatum* (0.0306 g/kg). Plant phosphorous of non-mycorrhizal control plant was recorded 0.028 g/kg (Fig. 3).

#### *Nitrogen content :*

Nitrogen content in general was found higher in AMF inoculated plantlets compare to non-mycorrhizal one. In present study, at FYM level I, plant nitrogen content was found highest in the plantlets inoculated with *G. claroideum* (0.49%), *G. aggregatum* (0.47%) and *G. versiforme* (0.45%). Lowest plant nitrogen was found in the plantlets inoculated with *G. occulatum* (0.33%) whereas in non-mycorrhizal control plants it was recorded 0.29%. In case of FYM level II, highest nitrogen content was found in the plantlets inoculated with *G. versiforme* (0.607%) and lowest in the plantlets inoculated with *G. fasciculatum* (0.43%). Plant nitrogen content of non-mycorrhizal control plant was recorded 0.327% only. At FYM level III, maximum plant nitrogen was found in the plantlets inoculated with *G. aggregatum* (0.57%) and minimum

with *G. macrocarpum* (0.47%) but that was higher than the non-mycorrhiza plants (0.38%) (Fig. 4).

Inoculation of *P. mullesua* plantlets with AMF under various levels of FYM was found enhancing the biomass, shoot length, P and N content. It indicates that organic amendment supported the high plant biomass and AM fungal infection. This statement agrees with the results of Noyd *et al.*<sup>28</sup>, who reported that enhanced plant cover and biomass, mycorrhizal infectivity and spore population by addition of composted yard waste. Douds *et al.*<sup>10</sup> found that the soil under low-input sustainable agriculture involving animal and green manure had a greater capacity for AM fungal colonization. In present study plant growth was higher in AM inoculated plants in comparison to the non-mycorrhizal plants in all the three levels of FYM. Similar results have been reported on application of organic matter in AM fungi inoculated fodder crops by other workers<sup>1,17,22,27</sup>. It is generally recognized that the benefits of organic manures are not only due to the supply of the nutrient elements, but also to the improvement of soil physical characters<sup>26</sup>. On the other hand, the mycorrhizal inoculation treatments showed different levels of effectiveness with respect to improving the performance of plantlets. In this experiment *G. versiforme* was found most effective for increasing the plant growth in all the three levels of FYM. Carvaca *et al.*<sup>9</sup>, found that *G. mosseae* was the most effective for increasing the crop growth as compared to the other fungal isolates. It is important to note that *G. versiforme* performed good result in sterilized soil condition in our previous experiment and on application of FYM it showed better

performance.

Though mycorrhizal infection percentage is not directly related to the plant biomass<sup>38</sup>, but in this study, roots of inoculated *Piper mullesua* plants were well colonized with mycorrhizal fungi, though the percent infection was sometimes non-significant. It was true for all the three levels of FYM. This result agrees with the findings of Perner *et al.*<sup>32</sup>. Bearden and Petersen<sup>5</sup> found that the percentage of colonized root length in plants inoculated with *G. mosseae* was significantly higher than non-inoculated plants which increased the stability of the soil aggregates. Recent studies have also indicated that AM fungi produce a glycoprotein, glomalin that acts as an insoluble glue to stabilize aggregates<sup>42</sup>. Under present study statistical analysis of data shows a positive correlation ( $r=0.617$ ,  $p>0.05$ ;  $r=0.573$ ,  $p>0.05$ ) with plant biomass in both FYM level I and II.

As evident from the results that phosphatase enzyme activity in all the FYM levels was higher than our previous experiments. This suggested that on application of FYM, increased activity of AMF help in enhancing nutrient uptake<sup>36,40</sup> and as a result phosphatase activity increased. The correlation coefficient between plant biomass and phosphatase activity was highly significant ( $p>0.001$ ) in FYM level I and III (Table-2).

In general, the concentration of P was higher in AMF inoculated plants as compared to the non-mycorrhizal plants. This agrees with the results of Palm<sup>30</sup> who suggested that P released from the tissues supplied as organic matter during decomposition and mineralization,

directly increases available P. An increase in mineral uptake by mycorrhizal plants in various crops has been reported by various workers<sup>23</sup> Gaur and Adholeya<sup>13</sup>, found that P uptake in fodder crops was higher at 30 days and decreased thereafter. Similarly, Buwalda *et al.*<sup>7</sup>, found that mycorrhiza increase the concentration of P in the plant mainly at the early stage of growth and normally declines with time. Raju *et al.*<sup>33</sup> reported enhanced mineral nutrient uptake in plants inoculated with AM fungi in soils deficient in P.

In this study, percent N was found higher than that of non-mycorrhizal plants. Mycorrhizal inoculants particularly *G. versiforme*, *G. aggregatum*, *G. claroidium* were found more effective in up taking of N at all the three levels of FYM. Experiments by Hodge *et al.*<sup>18</sup>, demonstrated that the AM fungus *Glomus hoi* was able to enhance decomposition and increase plant N capture from grass leaves. Similarly, Azcon and Barea<sup>4</sup> described that increased plant N content found in the mycorrhizal plants may be due to the ability of AM fungi to enhance N capture from soil and to increase P uptake. However, Ames *et al.*<sup>3</sup>, reported that uptake of N by *A. graveolens* was related to root length colonized by *G. mosseae*. Performance of AMF *G. versiforme*, *G. aggregatum* and *G. claroidium* was better than other AMF in enhancing the growth of plantlets at different doses of FYM.

Inoculation of Arbuscular mycorrhizal fungi improves the ability of *Piper mullesua* plantlets to uptake plant nutrients from organic amendments, which promote better plant biomass, plant phosphorus, plant nitrogen and

phosphatase activity of roots. In addition, organic amendment influences infectivity of AMF and percentage survivality of *Piper mullesua* plantlets. At the present study, recommended organic amendment had a positive effect on AMF inoculated in *Piper mullesua* plantlets. Due to enhancement of phosphatase activity and thus available phosphorus, *G. versiforme* elevated P & N uptake from organic amendment and thus resulted in better plant growth.

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