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Effect of Mycorrhizal Application on Plant Growth and Nutrient Uptake of *Piper mullesua* Plantlets under Sterilized, Unsterilized and Field Soil Condition

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ABSTRACT

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Mycorrhizal fungi occur in most of the soils and colonize roots of many plant species. A greenhouse experiment was carried out to study the efficiency of Arbuscular Mycorrhizal fungi indigenous to Arunachal Pradesh in uptaking plant nutrients for the *Piper mullesua* plantlets at different soil condition i.e., sterilized soil, unsterilized soil and field condition. As the sterilized soil condition is difficult to understand the performance of AM fungi, field experiments are necessary to understand the effect of mycorrhizal fungi on yield of crops in field condition. The experiment was done to determine the effectiveness of mycorrhizal fungal inoculated with *P. mullesua* plantlets assessing its effect on plant growth and plant nutrition when compared with non-mycorrhizal *P. mullesua* plantlets in sterilized, unsterilized and field condition. For this ten different mycorrhizal fungal species isolated from various land use systems such as forest area, jhum fields, home gardens as well as natural habitat of piper plants were inoculated with the plantlets of *P. mullesua* in three different set of experiment. In present study *G. claroidium* (2.238gm ±0.209), *G. aggregatum* (2.122gm ±0.057) and *G. versiforme* (2.109gm ±0.051) were found more capable in producing better growth by infecting *Piper mullesua* plantlets under field soil as compared to unsterilized and sterilized soil condition. Plant phosphatase content was significantly ($p>0.001$) higher in the seedlings grown in unsterilized soil, followed by in field condition and least in the sterilized soil. These inocula were efficient in establishing beneficial relationship with other native microorganisms of soil. As chemical fertilizers possess threat to the environment by polluting soil and environment, these efficient mycorrhizal species can work as potential biofertilizer for agriculturally important crops.

Introduction

Piper mullesua D. Don. (syn *P. brachystachyum* Wall ex Hook. f), an important medicinal plant belonging to the family *Piperaceae*. It is commonly known as Pipli, Pahari peepal, is indigenous to Arunachal Pradesh (India) and widely distributed in the Eastern Himalayan region at an altitude of about 600m to 1500m. Male and female flowers are found in separate

spikes of the plant. Male spikes are 3-6 cm long, erect, slender and cylindrical. Female spikes are globose, oblong erect.

Roots and fruiting spikes are used in treating diarrhea, indigestion, jaundice, urticaria, abdominal disorder, hoarseness of voice, asthma, cough, piles, malaria fever, vomiting, wheezing, chest congestion, throat infection, worms and sinusitis. *Piper mullesua* is also considered as a rejuvenating plant. Myristicin,

a 1,3-benzodioxole has been extracted from the hexane fraction of alcohol extract of fruit bearing inflorescence of *Piper mullesua* which has insecticidal properties (Srivastva *et al.*, 2001).

Mycorrhizal fungi occur in most of the soils and colonize roots of many plant species. Mycorrhiza is the structures resulting from the symbiosis between these fungi and plant roots, and are directly involved in plant mineral nutrition. The symbiotic root-fungal association increases the uptake of less mobile nutrients (Ortas *et al.*, 2001), essentially phosphorus (P) but also of micronutrients like zinc (Zn) and copper (Cu), the symbiosis has also been reported as influencing water uptake. AMF can also benefit plants by stimulating the production of growth regulating substances, increasing photosynthesis, improving osmotic adjustment under drought and salinity stresses and increasing resistance to pests and soil borne diseases (Al-Karaki, 2006).

The importance of mycorrhizae in plant growth and its role in ecosystem development has been overwhelmingly demonstrated in recent years. Establishment of ecologically adapted mycorrhizal fungi on plantlets before planting improve survival and growth rate of plants (Jha *et al.*, 1988). These points can all be taken as strong evidence for a mutualistic symbiosis. Differences in the effectiveness of mycorrhizal species were also recorded by various workers (Mosse, 1972; Bevege and Bowen, 1975; Caravaca *et al.*, 2006).

Some species such as the fine endophyte *Glomus tenue*, rarely produce growth responses, even in infertile soil (Powell, 1979), yet this is often the most abundant mycorrhizal fungus in natural soils. Almost all work on mycorrhizal effects was carried out in sterilized soil. As the sterilized soil condition is difficult to understand the

performance of AM fungi, field experiments are necessary to understand the effect of mycorrhizal fungi on yield of crops in field condition. Pot experiments often examined the response of single plants, and in arable crops the individuals were typically well-spaced. In grasslands and most non-agricultural vegetation, however, the root systems of adjacent individuals overlap to a great extent. Mycorrhizal mycelium is known to be capable of linking plants physically, and transfer of P from one plant to another by mycorrhizal hyphae has been demonstrated (Whittingham and Read, 1982).

In recent years there has been considerable interest on plant growth promoting rhizobacteria (PGPR), which improve plant growth by providing growth promoting substances and suppressing root pathogens (Goswami *et al.*, 2016; Olanrewaju *et al.*, 2017). Synergistic interaction between AMF and PGPR benefitting the growth of plants compared to single inoculation with either of them has been reported by earlier workers (Cely *et al.*, 2016, Divyananda *et al.*, 2006). AMF and PGPR in soil and plant tissues mutually cooperate with each other in benefitting plant growth through increased nutrition, hyphal permeability in plant roots, bacterial survival and protection against biotic and abiotic stresses. Communication through signaling molecules, such as flavonoids, strigolactones and sesquiterpenes, is important for regulation of these interactions. Strigolactones released in low concentrations by rhizosphere microorganisms is known to facilitate colonization of plants by AMF (Nanjundappa *et al.*, 2019).

The AMF inoculation in field conditions was been evaluated by some authors as Romero and Bago (2010), Pellegrino *et al.*, (2011, 2012), and Ortas (2012) showing a high potential to increase crops yields. However, the success of AMF inoculation in

agricultural soils can be determined by many factors such as species compatibility, habitat niche availability for AMF and competition with native fungi (Verbruggen *et al.*, 2013), these aspects need to be evaluated under local conditions for a more appropriate assessment of the viability of AMF use as biofertilizer in crops.

The objective of the work was to determine the effectiveness of mycorrhizal fungal inoculated with *P. mullesua* plantlets assessing its effect on plant growth and plant nutrition when compared with non-mycorrhizal *P. mullesua* plantlets in sterilized, unsterilized and field condition.

Materials and Methods

The study was carried out in and around Doimukh area of Papum Pare district of Arunachal Pradesh (26°30' N-29 °30' N Latitude and 91 °30'E-97 °30'E Longitude; altitude 100-600m 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. Plantlets of piper were raised through stem cuttings. The plantlets were raised in sterilized sand and soil mixture (3:1). 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 as forest area, jhum fields, home gardens 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. etunicatum*, *G. versiforme*, *G. albidum*, *G. claroidium*, *G. occulatum*, *G. macrocarpum*, *G. hoi*, *G. aggregatum*, *G. fasciculatum*, *G. aurantium*

were selected to carry out the experiment. To evaluate the efficiency of mycorrhizal fungi a set of plantlets was transplanted in pots filled with sterilized soil (free from any contamination) and plants were inoculated with ten different species of AMF maintaining three replicates for each treatment. Another set of plantlets was transplanted in pots containing unsterilized garden soil with numerous microorganisms under natural condition and plants were inoculated with ten different strains of mycorrhizal fungi maintaining three replicates for each treatment. One set of three replicates was also maintained as control (without inoculating any AMF). Pots were kept in Mist chamber and were harvested after 3 months.

To evaluate the efficiency of mycorrhizal fungi under field condition *P. mullesua* plantlets were initially inoculated with ten AMF and after that transplanted in field on a hill slope. Ten replicates were maintained for each inoculant. A control set with same number of replicates was also maintained without inoculation of AMF initially. Harvestings of plants was done after 3 months of transplantation.

Growth parameters like shoot and root length as well as plant biomass was determined by drying them separately in hot air oven at 60 °C for 48 hours. The percentage of the root colonized by VAM fungi were determined by using the formula as suggested by Brundrett *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 Kjehldahl method and Vanadomolybdate method respectively (Juo, 1982). The activity of Phosphatase was estimated 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. 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

Shoot length

The study on the effect of arbuscular mycorrhizal fungi on the shoot length of *Piper mullesua* seedlings in sterilized soil showed that it was highest in *G. versiforme* (9.83cm \pm 0.096) infected one which is significantly ($p>0.005$) higher than the other mycorrhizal and non-mycorrhizal seedlings (Table 1). A similar result was also observed in unsterilized soil ($p>0.05$) and in field condition. In field condition the shoot length production was highest in *G. etinucatum* (12.83cm \pm 0.585) and *G. aggregatum* (12.83cm \pm 0.385); followed by *G. versiforme* (12.33cm \pm 0.255), the difference is however insignificant.

The seedlings of *P. mullesua* infected with *G. versiforme* (18.1cm \pm 0.271), *G. aggregatum* (17.3cm \pm 0.45) and *G. etinucatum* (17.0cm \pm 0.503) showed better shoot growth than the other mycorrhizal isolates in the field condition (Table 1). However the value was lowest in the non-mycorrhizal seedlings in all the three cases (6.23cm, 8.66cm and 13.5cm respectively in sterilized soil, unsterilized soil and field condition).

Root length

The root length on the other hand was higher in the non-mycorrhizal seedling (44.3cm \pm 0.556) and was almost uniform in all the mycorrhizal seedlings having insignificant differences within the isolates in sterilized soil condition. The values were rather lower than the unsterilized soil and in field condition (Table 1).

In the unsterilized soil, the non mycorrhizal seedling produces higher root length (48.167cm \pm 0.096) which is significant ($p>0.001$) than the mycorrhizal seedlings. A nearer value was obtained in the seedlings infected with *G. fasciculatum* (45.83cm \pm 0.255), *G. occultum* (45.33cm \pm 0.694) and *G. macrocarpum* (45.167cm \pm 0.419). However in field condition, *G. hoi* produced highest root length (68.5cm \pm 0.354) followed by non-mycorrhizal seedlings (67.5cm \pm 1.768). A uniform value was recorded in the seedlings infected with different mycorrhizal isolates which ranges from 60.5cm – 63cm. Three of the mycorrhizal isolates perform significantly ($p>0.001$) poor result (*i.e.*, value less than 60cm) (Table 1).

Total biomass

The effect of mycorrhizal fungi on biomass production of *P. mullesua* seedlings in sterilized soil condition showed that *G. etinucatum* (0.932gm \pm 0.033), *G. versiforme* (0.926gm \pm 0.019), *G. claroidium* (0.939gm \pm 0.051) and *G. aggregatum* (0.934gm \pm 0.023) produces significantly higher biomass ($p>0.001$). It was least in the non-mycorrhizal seedling (0.506gm \pm 0.042). In unsterilized soil condition *G. versiforme* produced highest biomass (1.394gm \pm 0.242) followed by *G. aggregatum* (1.377gm \pm 0.031) and *G. claroidium* (1.239gm \pm 0.022) which are higher than the biomass produced by mycorrhizal plantlets under sterilized condition.

Least total biomass was produced by the non-mycorrhizal seedlings (0.688gm \pm 0.043). Biomass production in field condition was produced highest by *G. claroidium* (2.238gm \pm 0.209) followed by *G. aggregatum* (2.122gm \pm 0.057) and *G. versiforme* (2.109gm \pm 0.051) were much more than biomass produced under sterilized and unsterilized condition.

However the difference between different mycorrhizal isolates and non-mycorrhizal seedlings was not significant. It was also observed that the biomass production by non mycorrhizal plantlets in field condition was higher than the biomass produced by mycorrhizal plantlets in sterilized and unsterilized condition (Figure 1).

Chlorophyll content

The chlorophyll content varied insignificantly among the seedlings infected with different mycorrhizal isolates in different sterilized and unsterilized condition. However it was highest in the seedlings infected by *G. claroidium* (1.895 μ gm/gm \pm 0.019) followed by *G. macrocarpum* and *G. aggregatum* in sterilized soil condition rather than unsterilized soil. Seedlings grown in field condition showed significantly ($p > 0.001$, $F = 16.364$) higher chlorophyll content (Table 2).

Percent infection and seedling survivality

In sterilized soil condition, no infection was observed in controlled one. On the other hand highest infection percentage was observed in the seedlings grown in field condition and then in unsterilized soil. The highest percentage of infection was observed in the seedlings infected with *G. versiforme* (90% \pm 2.357) followed by *G. fasciculatum* and *G. aurantium* (80%) in field condition. Similarly the value was highest in the seedlings infected by *G. albidum* (88.33% \pm 2.546) followed by *G. aurantium* (78.3% \pm 2.546) in unsterilized soil. There is a significant ($p > 0.001$) variation in the percentage of infection among different mycorrhizal isolates.

The percent of survivality of *P. mullesua* seedling was higher in unsterilized soil and in field condition which ranges from 60 – 100%. However, seedlings of sterilized soil showed

poor percentage of survivality (50 – 90%). No strong correlation was observed between percent infection and seedling survivality (Table 2).

Plant phosphatase content

Plant phosphatase content was significantly ($p > 0.001$) higher in the seedlings grown in unsterilized soil, followed by in field condition and least in the sterilized soil. In unsterilized soil condition an equally higher value was exhibited by the species *G. etinucatum* (64.33 μ gm/gm \pm 0.694), *G. versiforme* (48.5 μ gm/gm \pm 0.5), *G. claroidium* (44.83 μ gm/gm \pm 0.585.) and *G. aggregatum* (45.00 μ gm/gm \pm 0.333). In field condition higher phosphatase content was recorded in the seedlings infected with *G. claroidium* (47.8 μ gm/gm \pm 0.684) and *G. aggregatum* (46.5 μ gm/gm \pm 0.601). A significantly lower value of phosphatase content was recorded in the non-mycorrhizal seedlings (Figure 2).

Plant phosphorus content

The effect of soil condition and mycorrhizal isolates in phosphorus uptake was significant ($p > 0.001$). The plant phosphorus content was higher in the *P. mullesua* seedlings infected with *G. versiformi* (0.048gm/kg \pm 0.0012), *G. claroidium* (0.43gm/kg \pm 0.0009) and *G. etinucatum* (0.042gm/kg \pm 0.0009.) and *G. aggregatum* (0.041gm/kg \pm 0.0014) in field condition and *G. versiformi* (0.048gm/kg \pm 0.0005), *G. aggregatum* (0.046gm/kg \pm 0.0002), *G. claroidium* (0.44gm/kg \pm 0.0005) and *G. etinucatum* (0.0436gm/kg \pm 0.0009).

However there is a significant difference in phosphorus content among different mycorrhizal isolates. Here also the plant seedlings grown in sterilized soil produces significantly ($p > 0.05$) lower phosphorus than the other two soil condition (Figure 3).

Plant nitrogen content

The plant nitrogen content was recorded highest in the seedlings grown in unsterilized soil followed by seedlings grown in field condition and sterilized soil. In sterilized soil condition, *G. aggregatum* ($0.51\% \pm 0.071$) shows highest nitrogen concentration followed by *G. claroidium* ($0.47\% \pm 0.027$). In unsterilized soil condition *G. versiforme* ($0.67\% \pm 0.027$) and *G. claroidium* ($0.65\% \pm 0.027$) shows higher nitrogen concentration followed by *G. etinucatum* ($0.61\% \pm 0.027$).

Here *G. hoi* ($0.42\% \pm 0.047$) produces least nitrogen which is non-significant. And in field condition *G. aggregatum* ($0.65\% \pm 0.077$) and *G. claroidium* ($0.65\% \pm 0.030$) produces highest nitrogen concentration (Figure 4).

The main hypotheses that growing of AM infected seedlings in unsterilized and field condition performed better than the AM infected seedlings planted in sterilized soil was confirmed in our experiment. From the results it is confirmed that Piper seedlings planted in field condition achieve better environmental factors producing greater plant biomass than the seedlings planted in unsterilized and sterilized soil. The findings agrees with that of Gryndler *et al.*, (2006), suggested that along with AM fungi other groups soil microorganisms also take part in supplying nutrients in unsterilized and field condition.

This result was supported by the findings of Harishkumar *et al.*, 2019, who suggested that the combined effect of biofertilizers and VAM improves the plant growth and productivity. Akyol *et al.*, 2019 also supported this concept by large-scale study to investigate interactions between AM fungal inoculation and indigenous root microbial communities in agricultural fields.

From the results it is found that three mycorrhizal species viz., *G. versiforme*, *G. claroidium* and *G. aggregatum* were capable of producing higher biomass by acquisition of more nutrients from soil solution than the other species of mycorrhiza. It was also noticed that the species *G. etinucatum* worked better only in sterilized condition. This may be the inability of the species to compete with other microorganisms present in unsterilized soil and field soil.

Other studies have shown that AM fungi and free-living soil biota can inhibit one another (Bukovska *et al.*, 2018, Leigh *et al.*, 2011) and do not consistently enhance plant nutrient acquisition from organic matter. Berruti *et al.*, (2016) revealed that soil inoculation with AM fungi increased root colonization rates, and increased root colonization rates led in turn to increased root and shoot biomass, improved plant nutrition, and higher crop yields under diverse experimental conditions.

The present observation further indicates that though mycorrhizal infection percentage is not directly related to the plant biomass (Smith *et al.*, 2003), increased development of percent infection was observed in the field experiment. Such increased development of mycorrhizal infection may be due to the organic matter naturally present in field soil which increases the soil biological activities, where mycorrhizal fungi may benefit from the release of growth stimulating substances.

While carbon in mycorrhizal mycelium proliferating organic matter rich field condition most likely originates from plant photosynthates (Gavito and Olsson, 2003), mycelial growth of AMF may benefit from the release of other nutrients such as N from the organic matter present soil, as suggested by Ravnskov *et al.*, (1999). These results were supported by Fiscus and Markhart, 1979 and Wang and Jiang, 2015 stated that

Funelliformis mosseae and *Acaulospora laevis* have a different magnitude of root colonization because the extent of absorption of water and minerals might differ among treatments. If the level of absorbed minerals is different, that could lead to a variation in plant growth parameters (Fageria and Moreira, 2011) which was observed in our study.

Similar results were obtained by Saini *et al.*, 2019 reported that the AMF root colonization (%) and AMF spore number were significantly more developed in the treated plants as compared to the control. In the experiments, mycorrhizal colonization in control plant was around 50% indicating that the agricultural soils support an active indigenous AMF community (Cely *et al.*, 2016).

Piper mullesua plants infected with AM fungi at field condition were survived the most than the laboratory condition. This may be because of the organic matter present in the field and unsterilized condition whereas no organic matter is present in sterilized condition.

In our experiment, phosphatase content was found highest in soils of unsterilized and field condition. This support the same reason that soil microorganisms provide mutualistic relationship with mycorrhizal fungi of *P. mullesua* seedlings in both unsterilized and field condition. But, in unsterilized condition phosphatase was found significantly ($p>0.001$) higher than field condition as growth parameters under field condition may be influenced by the organic matter present.

It is certain that mycorrhizal phosphate and nitrogen transport to root occurred in field condition also. The plant phosphorus and plant nitrogen content of *P. mullesua* seedlings in unsterilized and field condition were higher than that of sterilized soil. This result agrees with the findings of Hayman and

Mosse (1979) found that mycorrhizal infection greatly increased P uptake in unsterilized clover without increasing the yield.

This increase in P in plants in both unsterilized soil condition may be due to the presents of natural organic matter and soil microorganisms. Gryndler *et al.*, (2009), observed that fungi, bacteria, or protozoa of soil are important for the formation of 3, 4, 5-substituted benzyl in soil organic matter and this may indirectly affect the growth of AM fungi. These results in the line with previous studies carried out by various workers.

In the case of shoot and root P concentration, a mycorrhizal effect was evident, because the AMF-associated roots produced some acid phosphatases and hydrolase enzymes that increased phosphate availability in the rhizosphere (Miller *et al.*, 2001; Renella *et al.*, 2006). Also increased activity of phosphatase enzyme, which results in mineralization of inorganic phosphorus from organic compounds (Amaya- Carpio *et al.*, 2009).

Same results was also found in case of plant N. similar type of results was also found by Caravaca *et al.*, (2006) who agrees that plant N increased with the application of organic matter in AM infected plants Vaidya *et al.*, (2007), found that N within the organic amendment have a beneficial effect on the growth of AM fungi.

The increased plant N content found in the mycorrhizal plants may be due to the ability of AM fungi to enhance N capture from soil to increase P uptake, which strongly promotes biological N₂- fixation (Azcon and Barea, 1992). Several studies have explained that AMF have the ability to absorb and transfer N to the nearby plants or host plants (Hodge and Storer, 2015; Battini *et al.*, 2017; Turrini *et al.*, 2018).

Table.1 Shoot length, root length, of *P. mullesua* plants after inoculation with AM fungi at sterilized soil, unsterilized soil and field soil

VAM fungal	Shoot Length (cm)			Root length (cm)		
Species	Sterilized	Unsterilized	Field	Sterilized	Unsterilized	Field
Control (NM)	6.23	8.66	13.50	44.30	48.17	67.50
	±0.360	±0.694	±0.347	±0.555	±0.096	±1.768
<i>G. etinucatum</i>	9.33	12.83	17.00	33.66	36.67	60.50
	±0.347	±0.585	±0.503	±0.192	±0.192	±0.354
<i>G. versiforme</i>	9.83	12.33	18.10	34.83	38.50	53.50
	±0.096	±0.255	±0.271	±0.481	±1.893	±1.061
<i>G. albidum</i>	8.00	11.50	16.80	36.00	39.83	63.00
	±0.167	±0.441	±0.590	±0.167	±0.255	±0.707
<i>G. claroidium</i>	9.67	12.16	16.40	36.00	38.00	53.50
	±0.096	±0.419	±0.384	±0.333	±0.667	±1.061
<i>G. occultum</i>	7.00	9.66	16.50	35.00	45.33	62.00
	±0.167	±0.419	±0.835	±0.333	±0.694	±0.707
<i>G. macrocarpum</i>	7.50	9.83	15.15	35.17	45.17	61.50
	±0.441	±0.255	±0.532	±0.347	±0.419	±1.768
<i>G. hoi</i>	7.83	10.83	16.20	36.17	40.33	68.50
	±0.255	±0.419	±1.086	±0.255	±0.192	±0.354
<i>G. aggregatum</i>	8.10	12.83	17.30	36.13	34.67	54.50
	±0.176	±0.385	±0.450	±0.077	±0.419	±0.354
<i>G. fasciculatum</i>	7.33	10.00	16.50	36.67	45.83	61.50
	±0.255	±0.167	±0.800	±0.385	±0.255	±0.354
<i>G. aurantium</i>	6.67	10.50	16.20	34.67	40.67	59.70
	±0.255	±0.726	±0.371	±0.192	±0.385	±0.839
±SE, n=3						

Table.2 Chlorophyll, infection and survivality of *P. mullesua* plants after inoculation with AM fungi at sterilized soil, unsterilized soil and field soil (S-I, S-II, S-III)

VAM fungal species	Chlorophyll			Infection			Survivality		
	mg/gm			(%)					
	S-I	S-II	S-III	S-I	S-II	S-III	S-I	S-II	S-III
Control (NM)	1.553	1.35	1.23	-	13.33	65.00	50	80	70
	±0.053	±0.041	±0.023		±1.925	±2.357			
<i>G. etinucatum</i>	1.771	1.49	1.71	53.3	68.33	75.00	90	90	95
	±0.020	±0.014	±0.027	±3.85	±2.546	±2.357			
<i>G. versiforme</i>	1.761	1.23	1.50	46.7	31.67	90.00	80	100	100
	±0.018	±0.034	±0.008	±1.92	±6.310	±2.357			
<i>G. albidum</i>	1.725	1.38	1.71	36.7	88.33	70.00	70	100	100
	±0.012	±0.063	±0.004	±3.85	±2.546	±2.357			
<i>G. claroidium</i>	1.895	1.39	1.59	46.7	73.33	65.00	80	80	100
	±0.019	±0.004	±0.012	±3.85	±1.925	±2.357			
<i>G. occultum</i>	1.749	1.77	1.61	23.3	26.67	65.00	70	60	90
	±0.057	±0.179	±0.012	±3.85	±1.925	±4.714			
<i>G. macrocarpum</i>	1.793	1.63	1.42	46.7	23.33	50.00	50	60	70
	±0.035	±0.017	±0.035	±5.09	±5.092	±7.071			
<i>G. hoi</i>	1.731	1.30	1.64	43.3	71.67	65.00	60	100	80
	±0.028	±0.044	±0.003	±5.09	±0.962	±2.357			
<i>G. aggregatum</i>	1.785	1.61	1.54	36.7	33.33	75.00	85	95	60
	±0.043	±0.032	±0.039	±1.92	±1.925	±4.714			
<i>G. fasciculatum</i>	1.663	1.57	1.54	26.7	55.00	80.00	70	100	80
	±0.041	±0.035	±0.029	±1.92	±1.667	±2.357			
<i>G. aurantium</i>	1.675	1.64	1.78	55	78.33	80.00	70	80	100
	±0.066	±0.112	±0.006	±5.00	±2.546	±4.714			
±SE, n=3									

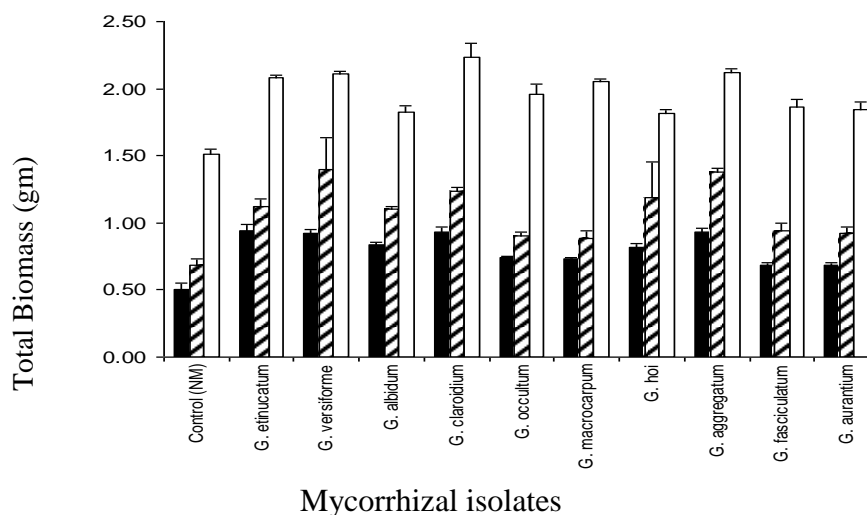
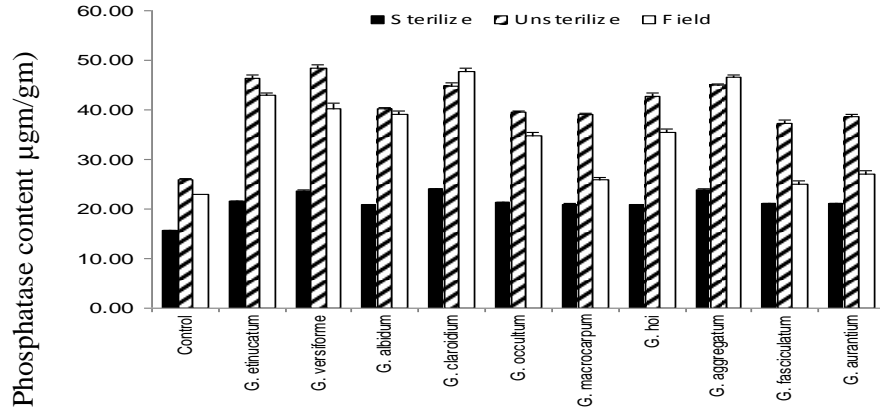
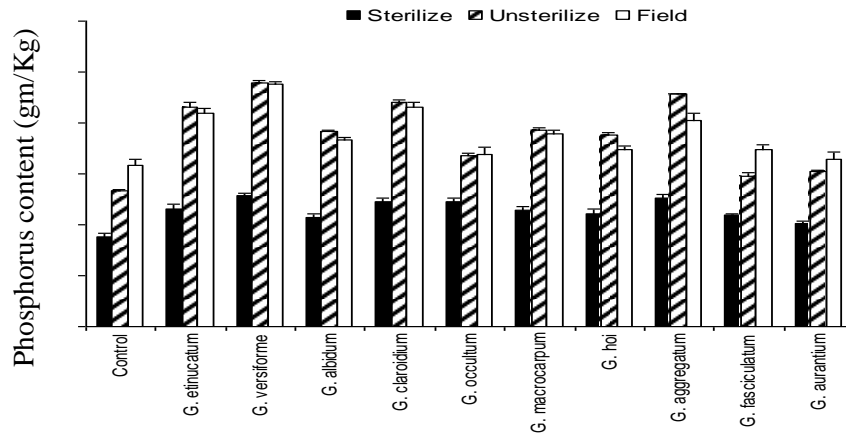


Figure.1 Graph showing the Total Biomass content (gm) in *P. mullesua* seedling in ■ sterilized, ▨ unsterilized and □ field soil



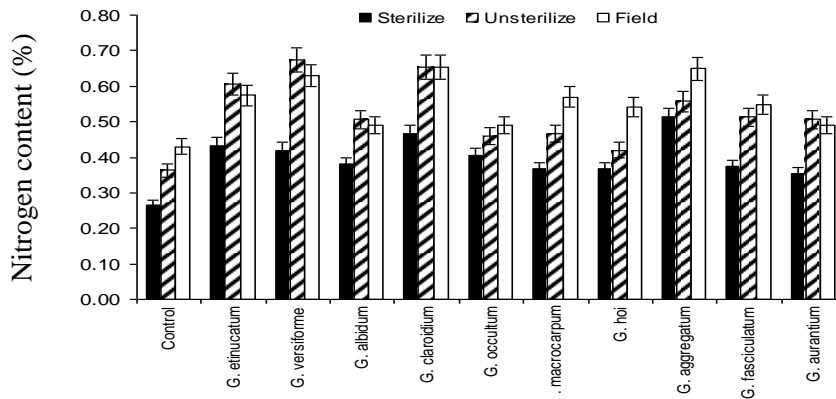
Mycorrhizal isolates

Figure.2 Graph showing the phosphatase content (µgm/gm) in *P. mulliesua* seedling in ■ sterilized, ▨ unsterilized and □ field soil



Mycorrhizal isolates

Figure.3 Graph showing the phosphorus content (gm/kg) in *P. mulliesua* seedling in ■ sterilized, ▨ unsterilized and □ field soil



Mycorrhizal isolates

Figure.4 Graph showing the Nitrogen content (%) in *P. mulliesua* seedling in ■ sterilized, ▨ unsterilized and □ field soil

The present study conclude that *G. versiforme*, *G. claroidium* and *G. aggregatum* were more capable in producing better growth by infecting *Piper mullesua* plantlets under unsterilized soil and field soil as well. These inocula were efficient in establishing beneficial relationship with other native microorganisms of soil. As chemical fertilizers possess threat to the environment by polluting soil and environment, efficient mycorrhizal species can work as potential biofertilizer for agriculturally important crops.

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