

Original Research Article

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## Efficiency of Mycorrhizal Fungi with Different Levels of Phosphatic Fertilizer on Growth of *Piper mullesua* Plantlets

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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 for the *Piper mullesua* plantlets at different levels of phosphatic fertilizer (Single Super Phosphate). In plants, P availability is considered the second most important limiting factor for growth after nitrogen. While P is generally abundant in soil, it is mostly present in insoluble and poorly mobile forms and therefore partly unavailable to plants. Mycorrhiza develop a dense hyphal network that extends far into the soil while still remaining connected to the root. This extraradical mycelium provides the plant with water and nutrients especially phosphorous that would otherwise remain inaccessible to roots. In the present study, *Piper mullesua* seedlings were infected with ten different strains of AM fungi and one without inoculation of AM fungi under three levels of phosphatic fertilizer (superphosphate). Shoot length, plant biomass, phosphatase enzyme activity, concentration of P and N of AM infected plants were found higher than those of non-mycorrhizal controls, which confirms the contribution of AM fungi. In our experiment it has been found that mycorrhizal fungal isolates *G. etinucatum* (0.887gm ±0.033), *G. claroidium* (0.861gm ±0.023) and *G. aggregatum* (0.859gm ±0.016) performed significantly ( $p>0.001$ , F=14.14) higher in improving plant biomass of *Piper mullesua* seedlings at lower dose of super phosphate which confirms higher efficiency of mycorrhizal fungi with reduced dose of phosphatic fertilizer.

### Keywords

Mycorrhizal fungi,  
Super phosphate,  
*Piper mullesua*,  
Plant biomass and  
phosphatase

### Article Info

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### 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).

Phosphorus (P) is one of the most important determinants of plant growth and in most soils it exists in forms that are largely unavailable for plant uptake (Zafar *et al.*, 2011). Phosphorus (P) serves various basic cellular functions in bioenergetics. P is the key player in plant metabolic processes, including energy transfer, signal transduction, biosynthesis of macromolecules, photosynthesis and respiration (Raghothama, 1999; Mengel and Kirkby, 2001). In plants, P availability is considered the second most important limiting factor for growth after nitrogen. While P is generally abundant in soil, it is mostly present in insoluble and poorly mobile forms and therefore partly unavailable to plants (Schachtman *et al.*, 1998). Like all other mineral nutrients, P enters the biosphere predominantly via the pedosphere through the root system of plants, where it is absorbed as inorganic orthophosphate (Pi), which is preferred form taken up by plants (Bucher, 2006). Phosphorus that exist in the environment as Pi, primarily evolved in inert complexes with cations such as iron phosphate (FePO<sub>4</sub>) and aluminium phosphate (AlPO<sub>4</sub>), and in organic molecules such as lecithin and phytate, the latter of which can account for up to 50% of total soil organic Pi

(Brinch-Pedersen *et al.*, 2002). Roots take up P as inorganic phosphate (Pi), and this leads to the creation of Pi depletion zones around them, a phenomenon that can lead to P deprivation. Crop plants are thus commonly supplied with chemical P fertilizers, which raises several major economic and environmental concerns related to energy use, freshwater pollution and mineral P resource scarcity (Cordell *et al.*, 2009; Gilbert, 2009).

Arbuscular mycorrhizal symbioses are arguably the most common underground symbiosis, with around 80% of terrestrial plant species (Smith and Read, 2008), forming the most widespread symbiosis on earth (Brachmann and Parniske, 2006). In this root endosymbiosis, the fungal partner colonizes the root cortex where it forms specialized structures called arbuscules that serve as an exchange interface. At the same time, the fungus develops a dense hyphal network that extends far into the soil while still remaining connected to the root. This extraradical mycelium provides the plant with water and nutrients that would otherwise remain inaccessible to roots. Among supplied nutrients, Pi is considered as quantitatively the most important (Smith and Read, 2008). AM fungi can obtain free Pi from the soil using high-affinity Pi transporters expressed in the mycelium (Harrison and van Buuren, 1995). Once taken up by the extraradical mycelium, P is translocated along the hyphae in the form of polyphosphates, which are then depolymerized so that Pi can be transferred to root cells in exchange for hexoses (Ohtomo and Saito, 2005). On the other hand, organic phosphorus in soil is made available to plants largely after its mineralization or when hydrolyzed into Pi. Mineralization and hydrolysis of Po is mediated by the enzymatic activity of phosphatase. AMF roots have greater enzymatic acid phosphatase (ACP) and alkaline phosphatase (ALP) activity compared to non AMF roots. It has been

demonstrated that plants can receive up to 100% of their P via the mycorrhizal pathway, and from 4 to 20% of plant carbon can be transferred to the fungi (Cavagnaro, 2008). Considering the higher efficiency of mycorrhizal fungi in uptaking phosphate from soil, now days, application of AMF as a biofertilizers in crop production is recommended with aim of increasing productivity and reducing use of chemical fertilizers which are expensive, produce short term benefits and above all their use may create environmental pollution (Prasad *et al.*, 2012 and Kumar *et al.*, 2019).

Thus, the aim of the present study was to investigate how the AM symbiosis is effective in nutrient acquisition from soil at different levels of phosphatic fertilizer (Single Super Phosphate) for the growth of *Piper mullesua* plantlets under greenhouse condition.

### **Materials and Methods**

The study was carried out in and around Doimukh area of Papum Pare district and Pasighat area of East Siang 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.

A pot experiment was carried out with 11 treatments viz., non-mycorrhizal *P. mullesua* plantlets as control. Recommended dose of commercial phosphatic fertilizer for piper is 250kg single superphosphate (SSP) per ha. For this experiment we selected 3 treatments on the basis of recommended dose i.e. half, full and double of recommended dose. In treatment I, 0.089gm of SSP was mixed with 200gm of sterilized 3:1 sand and soil mixture and one seedling of piper plant was planted. Same treatment was given to each of the 3 replicates. In treatment II (100%), 0.1776gm of SSP was mixed with 200gm sterilized 3:1 sand and soil mixture. Same technique was followed in treatment III. Here the P fertilizer was taken 0.3552gm for each pot. 3 replicates were taken for each treatment. One set of control was also kept for comparison of data. Pots were kept in mist chamber and harvesting was done after 90 days after transplanting.

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 and root length

In the present investigation, it was found that mycorrhizal inoculation with low level of superphosphate fertilizer increased shoot and root length of the plantlets as compared to the control. At low level of superphosphate, shoot length of Piper seedling was observed highest in the seedlings inoculated with *G. claroidium* (8.83cm  $\pm$ 0.35) and least in the seedlings inoculated with *G. aurantium* (6.33cm  $\pm$ 0.1) which was significantly higher ( $p > 0.01$ ,  $F = 4.995$ ) than the non-mycorrhizal seedlings (5.63cm  $\pm$ 0.25) (Table 1). Increased in shoot length might be due to the enhanced nutrient uptake and greater rate of photosynthesis by mycorrhiza inoculated with the plantlets. This supports the results of Cooper (1984), Allen *et al.*, (1981) and Prasad *et al.*, (2012). In case of root length, AM inoculated roots were ranged 40.67cm  $\pm$ 0.48 to 32.63  $\pm$ 0.1 as compared to the non-mycorrhiza control seedlings (42.83cm  $\pm$ 0.1). Zhang *et al.*, (2019) also found mycorrhizal plants possessed better root hair growth as compared to the non mycorrhizal plants. It is evident from the table 1 that shoot length was found highest ( $p > 0.001$ ,  $F = 7.697$ ) in the seedlings of non-mycorrhizal control plants (8.25cm  $\pm$ 0.12) as compared to all AM inoculated seedlings under medium superphosphate and 8.22cm  $\pm$ 0.09 under high dose of superphosphate. Root length of the seedlings both inoculated and un-inoculated did not show any significant difference. Root length was highest in non-mycorrhizal plants (46.83cm  $\pm$ 2.36) and was least recorded in

seedlings inoculated with *G. etunicatum* (42cm  $\pm$ 0.95).

Increased shoot and root length might be the result of enhanced inorganic nutrient uptake and and greater rate of photosynthesis. This results were supported by the findings of Tanwar *et al.*, (2012), Cooper, 1984) and (Allen *et al.*, (1981). The results are also in close conformity with Gaur *et al.*, (2000) who found increase in vegetative growth of *Petunia hybrida*, *Callistephus chinensis* and *Impatiens balsamina* while inoculating with AM fungi and recommended dose of chemical fertilizers. El-Khateeb *et al.*, (2010) also observed increase in height of *Chamedora elegans* by AM fungi and NPK. The effectiveness of lower concentration of superphosphate in increasing the root length may be due to the direct effect of superphosphate fertilizers or indirectly through the microbial propagation activation. AMF enhanced nutrient uptake by increasing surface area of roots with the development of an extensive extra- radical hyphae network (Smith and Read, 1997).

### Biomass

Both AM colonization and P nutrition caused an increase in plant biomass as compared to the non-mycorrhizal control plants at low level of superphosphate, where half of the recommended dose of P-fertilizer was applied. This increase was evident at the lower P level of nutrition in AM colonizes plants, where fungal isolates *G. etinucatum* (0.887gm  $\pm$ 0.033), *G. claroidium* (0.861gm  $\pm$ 0.023) and *G. aggregatum* (0.859gm  $\pm$ 0.016), performed significantly ( $p > 0.001$ ,  $F = 14.14$ ) higher in improving plant biomass of *Piper mullesua* seedlings relative to control plants (0.454gm  $\pm$ 0.02). At medium level of P, same trend of performance of the fungal isolates as low level of P has been shown where plant biomass of *Piper mullesua*

seedlings ranged between 0.553gm  $\pm$ 0.006 to 549gm  $\pm$ 0.006. At this level, non-mycorrhizal control seedlings produced significantly ( $p > 0.001$ ,  $F = 8.403$ ) higher biomass (0.530gm  $\pm$ 0.034) as compared to some seedlings inoculated with AM fungi. At higher P level, Plant biomass produced by non-mycorrhizal control *P. mullesua* seedlings (0.541gm  $\pm$ 0.001) was significantly ( $p > 0.001$ ,  $F = 15.16$ ) higher than the AM inoculated plants and least biomass was produced by *G. aurantium* (0.414gm  $\pm$ 0.003) (Figure 1).

Mycorrhizal inoculation increased plant biomass in the plants compared to the non-mycorrhizal control plants at low and medium level of P, supports the results of Subramanian *et al.*, (2008) suggested that the growth enhancement in AM fungus-inoculated plants may be associated with the availability of soil nutrients. This shows that the recommended fertilizer dose can be reduced in combination with efficient mycorrhizal strain. This finding is in line with Cebalos *et al.*, (2013), Aliyu *et al.*, (2019) and Singh *et al.*, (2019). Higher concentration of phosphatic fertilizer markedly reduced plant biomass as compared to medium and low concentration of phosphatic fertilizer. Prasad *et al.*, (2012), noted similar findings while studying the impact of different levels of superphosphate on mycorrhizal fungi. Reduced biomass at higher level of P might be due to lesser efficiency of mycorrhizal fungi in presence of sufficient amount of water soluble phosphorus.

### Chlorophyll content

Low concentration of superphosphate was found to be more effective in high chlorophyll content. Inoculation with *G. claroidium* and low level of superphosphate significantly increased the chlorophyll content of the Piper mullesua plantlets (1.902mg/gm  $\pm$ 0.02) which was significantly ( $p > 0.005$ ,  $F = 3.442$ ) higher

than the non-mycorrhizal control plants. Similar result was found in medium level of superphosphate which was significantly ( $p > 0.005$ ,  $F = 3.442$ ) higher in *G. macrocarpum* (1.802mg/gm  $\pm$ 0.009) than the non-mycorrhizal control plants. At high superphosphorus level, non mycorrhizal control plants showed highest chlorophyll content which was not significant (Table 2).

It is seen that higher dose of phosphatic fertilizer does not play an important role in enhancement of chlorophyll and ultimately the photosynthesis process in mycorrhizal inoculated plantlets. But in the mycorrhizal plantlets when applied with low dose of recommended dose and recommended superphosphate showed very good relation with mycorrhizal infection and total chlorophyll content. Increased chlorophyll content on inoculation with mycorrhizal fungi was also reported by Tanwar *et al.*, (2013).

### Percent infection

After 90 days of inoculation, percent mycorrhizal root colonization increased in all the treated plantlets over the control (Table 2). maximum percent root colonization was present in the plantlets inoculated with *G. macrocarpum* (73.33%  $\pm$ 6.94) at low level of P and least in the plantlets at higher P level inoculated with *G. aurantium* (6.7%  $\pm$ 1.9). From the results, it has been found that *G. macrocarpum* not only performed best in lower P level, it also produced better infection in medium P level (36.65 $\pm$ 5.09) and also in higher P level (18.3 $\pm$ 4.2) where *G. versiforme* (20%  $\pm$ 3.3) produced highest infection (Table 2). In our experiment it has been found that percent infection in the plantlets at medium level of P was lesser than the infection produced by same strain at lower level of P fertilizer. It was found least in presence of higher level of phosphatic fertilizer. Lower concentration (half of the

recommended dose of P fertilizer) showed better results than recommended and double of recommended dose. It may be high soil phosphate level determines the reduction of hyphal growth of mycorrhizal fungi. Results are in accordance of Guillemain *et al.*, (1985), Linderman and Davis (2004), Prasad *et al.*, (2012) and Tiamtanong *et al.*, (2014). Balzergue *et al.*, (2013), supports these findings explaining that a high level of P fertilization inhibits AM symbiosis predominantly by acting on the plant itself rather than on the content of its root exudates or on the fungal partner. Aliyu *et al.*, (2019) also reported that colonization of the cassava roots by AMF decreased with increasing P application.

### **Survivality**

Effect of mycorrhizal fungi on survivality of inoculated mycorrhizal fungi was contrasting. The percent survivality in P level I was found in the seedlings inoculated with AM fungi *G. claroidium* (90%) and least survivality was found in the non-mycorrhizal seedlings. In case of P level-II, highest survivality was observed in case of non-mycorrhizal seedling (80%). At P level III, highest survivality was found in non-mycorrhizal *P. mullesua* seedlings (Table 2.1). This parameter did not show any stimulatory effect on mycorrhizal plantlets at different level of superphosphate (Table 2).

### **Phosphatase content of roots**

After 90 days of inoculation, maximum phosphatase activity was observed in the plantlets at low level of P inoculated with AM fungi *G. claroidium* (27.28 $\mu$ g/gm  $\pm$ 0.192), where half of recommended dose of phosphatic fertilizer were used. In this experiment Phosphorus content of root was always significantly ( $p > 0.001$ ,  $F = 14.83$ ) higher than the control plants. The activity of phosphatase also followed the same pattern in

medium level of P as plantlets inoculated with *G. claroidium* (23.77  $\mu$ g/gm  $\pm$ 0.504), followed by *G. etunicatum* (23.490  $\mu$ g/gm  $\pm$ 1.285). However higher concentration of P did not show any significant result (Figure 2).

Measurement of the phosphatase activity provided a good index of mycorrhizal effect on nutrient uptake, especially phosphorus. The ability of mycorrhizae to produce phosphatase enzymes actually depends upon the availability of phosphorus in the soil. In the P cycle, enzyme activities are inversely related to P availability (Tadano *et al.*, 1993) which also supports the findings that decrease the available phosphate cause an overall increase in the phosphatase activity (Azcón and Barea, 1997). Tiamtanong *et al.*, (2015) concluded that at low phosphorus availability, P demand increases that resulting increase the phosphatase activity which supports our results. High soil superphosphate concentration resulted in the reduction of hyphal growth and spore production of arbuscular mycorrhizal fungi that ultimately reduced the phosphatase secretion which are actually responsible for the conversion of bound P into available form and hence lesser P-uptake in high superphosphate concentration (Prasad *et al.*, 2012).

### **Plant phosphorus content and Plant Nitrogen content**

The amount of plant phosphorus significantly increased ( $p > 0.001$ ,  $F = 12.133$ ) in inoculated plantlets at low level of phosphorus as compared to the control plantlets after 90 days of inoculation. At this level, highest plant P content was found in the plantlets inoculated with AM fungi *G. aggrgatum* (0.0284gm/kg  $\pm$ 0.0004). In the medium concentration, there was no significant difference between the results of plant phosphorus contents among the mycorrhizal and non-mycorrhizal plants.

**Table.1** Shoot length, root length, of *P. mullesua* plants after inoculation with AM fungi at P-fertilizer level – I, II and III (P-I, P-II, P-III)

Mycorrhizal isolates	Shoot Length (cm)			Root Length (cm)		
	P-I	P-II	P-III	P-I	P-II	P-III
<b>Control</b>	5.67	8.25	8.22	42.83	46.17	46.83
	±0.25	±0.12	±0.09	±0.10	±0.82	±2.36
<b><i>G. etinucatum</i></b>	8.5	7.77	5.43	33.33	34.73	42
	±0.33	±0.13	±0.13	±0.51	±0.52	±0.95
<b><i>G. versiforme</i></b>	8.33	7.1	5.67	32.67	39.67	43.67
	±0.25	±0.12	±0.25	±0.10	±1.02	±0.10
<b><i>G. albidum</i></b>	6.5	6.2	5.1	39.5	41	44.33
	±0.17	±0.12	±0.32	±0.50	±0.76	±0.19
<b><i>G. claroidium</i></b>	8.83	7.33	6.17	33.5	35.17	45.17
	±0.35	±0.25	±0.42	±0.29	±0.75	±0.25
<b><i>G. occultum</i></b>	6.17	6.15	5.33	39.17	40.9	43.33
	±0.10	±0.19	±0.14	±0.92	±1.18	±0.25
<b><i>G. macrocarpum</i></b>	7.67	6.6	5.23	40.67	39	44
	±0.38	±0.06	±0.13	±0.48	±1.36	±0.17
<b><i>G. hoi</i></b>	7.17	6.13	5.33	40.33	41.27	42.67
	±0.25	±0.20	±0.25	±0.54	±0.85	±0.10
<b><i>G. aggregatum</i></b>	7.5	6.37	5.37	36.02	34	43.17
	±0.29	±0.17	±0.11	±0.73	±0.33	±0.10
<b><i>G. fasciculatum</i></b>	6.5	5.77	5.17	36.03	38.33	42.83
	±0.33	±0.08	±0.42	±0.45	±0.35	±0.25
<b><i>G. aurantium</i></b>	6.33	6	5	36.97	39	42.5
	±0.10	±0.17	±0.29	±0.62	±0.60	±0.17

± SE, n=3

**Table.2** Chlorophyll, infection and survivility of *P. mullesua* plants after inoculation with AM fungi at P-fertilizer level – I, II and III (P-I, P-II, P-III)

Mycorrhizal isolates	Chlorophyll			Infection			Survivility		
	(mg)				%			%	
	P-I	P-II	P-III	P-I	P-II	P-III	P-I	P-II	P-III
<b>Control</b>	1.603	1.727	1.738	-	-	-	40	80	70
	±0.028	±0.015	±0.002						
<b><i>G. etinucatum</i></b>	1.76	1.747	1.721	50	33.33	10	60	60	40
	±0.034	±0.008	±0.005	±3.33	±1.92	±5.8			
<b><i>G. versiforme</i></b>	1.717	1.745	1.693	53.33	33.33	20	70	50	40
	±0.026	±0.004	±0.009	±3.85	±5.09	±3.3			
<b><i>G. albidum</i></b>	1.764	1.715	1.699	56.67	43.33	16.7	80	60	50
	±0.024	±0.003	±0.014	±1.92	±3.85	±5.1			
<b><i>G. claroidium</i></b>	1.902	1.768	1.705	70	36.67	8.3	90	60	60
	±0.020	±0.006	±0.011	±3.33	±1.92	±2.5			
<b><i>G. occultum</i></b>	1.861	1.732	1.68	46.67	23.33	6.7	60	50	60
	±0.025	±0.006	±0.006	±1.92	±1.92	±2.5			
<b><i>G. macrocarpum</i></b>	1.802	1.744	1.699	73.33	36.67	18.3	80	40	40
	±0.009	±0.009	±0.007	±6.94	±5.09	±4.2			
<b><i>G. hoi</i></b>	1.753	1.708	1.667	66.67	33.33	15	60	70	40
	±0.020	±0.011	±0.004	±1.92	±1.92	±1.7			
<b><i>G. aggregatum</i></b>	1.724	1.737	1.659	43.33	26.67	11.7	80	50	55
	±0.007	±0.010	±0.011	±1.92	±3.85	±2.5			
<b><i>G. fasciculatum</i></b>	1.722	1.718	1.633	60	40	13.3	70	50	40
	±0.017	±0.006	±0.007	±3.33	±3.33	±1.0			
<b><i>G. aurantium</i></b>	1.733	1.724	1.637	66.67	26.67	6.7	60	60	40
	±0.040	±.002	±0.004	±1.92	±1.92	±1.9			
<b>± SE, n=3</b>									

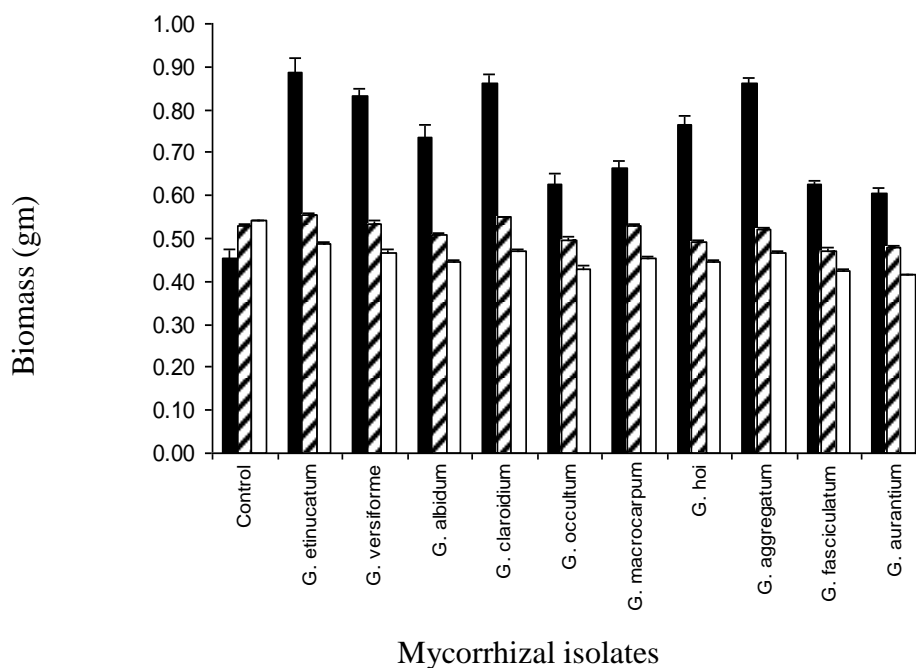


**Table.3** Correlation coefficient between Biomass and Percentage infection, Phosphatase content, Phosphorus and Plant Nitrogen in *P. mullesua* seedling grown in Phosphorus level I, II and III

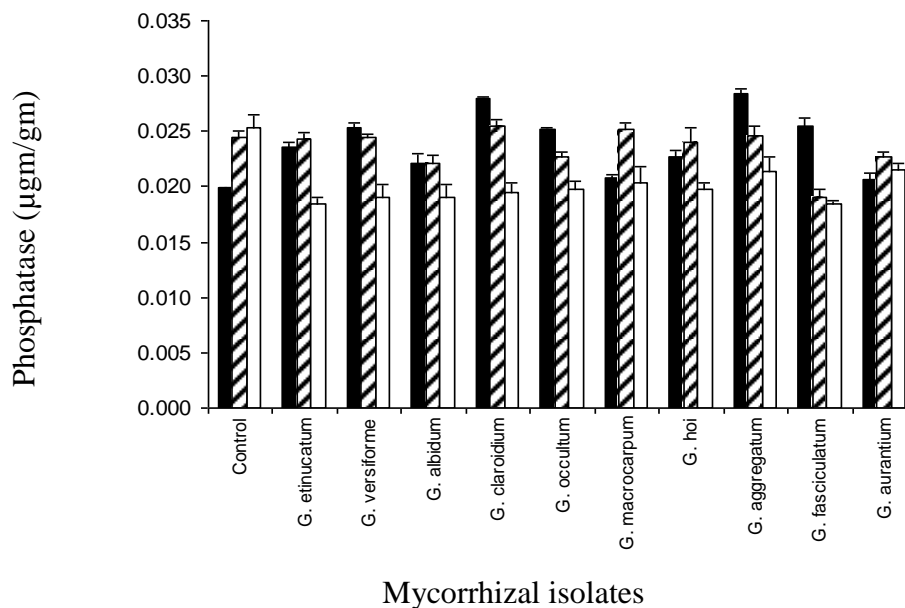
P-Level	Infection	Phosphatase	Phosphorus	Nitrogen
P-I	-0.295*	0.816	0.652	0.712
	ns	$p>0.001$	$p>0.05$	$p>0.005$
P-II	0.107*	0.765	0.780	0.609
	ns	$p>0.005$	$p>0.005$	$p>0.05$
P-III	0.233*	0.731	0.585	0.448
	ns	$p>0.005$	$p>0.05$	ns

\*  $df=9$ ,  $df=10$ , ns= Not significant

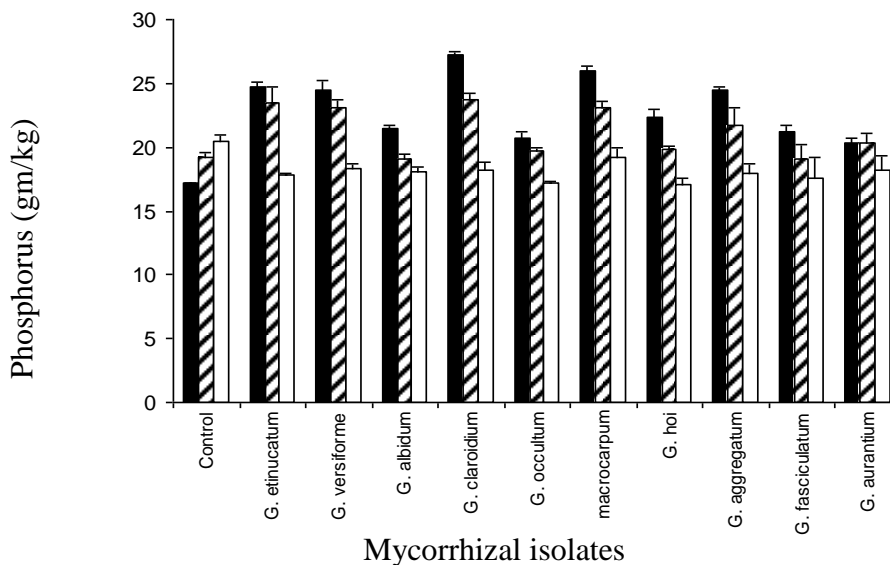
**Figure.1** Total Biomass (gm) produced by *P. mullesua* seedlings infected with different mycorrhizal isolates and non-mycorrhizal one in phosphorus level ■ P-I, ▨ P-II and □ P-III



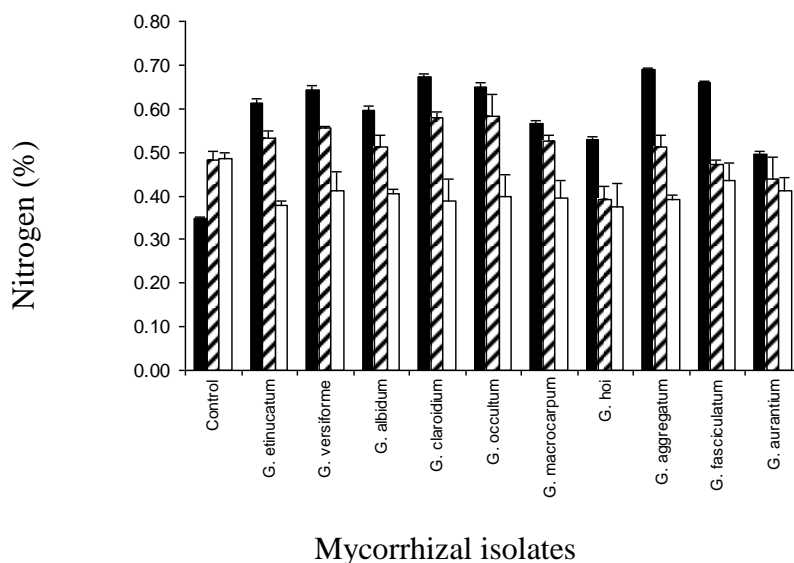
**Figure.2** Total Phosphatase ( $\mu\text{gm}/\text{gm}$ ) produced by *P. mullesua* seedlings infected with different mycorrhizal isolates and non-mycorrhizal one in phosphorus level ■ P-I, ▨ P-II and □ P-III



**Figure.3** Total Phosphorus ( $\text{gm}/\text{kg}$ ) produced by *P. mullesua* seedlings infected with different mycorrhizal isolates and non-mycorrhizal one in phosphorus level ■ P-I, ▨ P-II and □ P-III



**Figure.4** Total Nitrogen (%) produced by *P. mullesua* seedlings infected with different mycorrhizal isolates and non-mycorrhizal one in phosphorus level ■ P-I, ▨ P-II and □ P-III



Highest plant phosphorus content was found in the plantlets inoculated with *G. macrocarpum* (0.025gm/kg  $\pm$ 0.0007) and least was found in *G. fasciculatum* (0.019gm/kg  $\pm$ 0.0007), whereas P content of non-mycorrhizal control plant was 0.024gm/kg  $\pm$ 0.0006. At higher level of P, plant phosphorus content was significantly less than the non-mycorrhizal control plants (0.0254gm/kg  $\pm$ 0.0012) whereas AM fungi inoculated plants were ranged 0.0214gm/kg  $\pm$ 0.0013 to 0.0184gm/kg  $\pm$ 0.0005 (Figure 3).

In case of low level P, significantly ( $p > 0.001$ ,  $F = 27.133$ ) higher Nitrogen was found in the plantlets inoculated with *G. aggregatum* (0.69%  $\pm$ 0.003) and least was found in *G. aurantium* (0.5%  $\pm$ 0.007). Plant Nitrogen content was found in non-mycorrhizal control plant was found 0.35%  $\pm$ 0.005. At medium P level, plant Nitrogen was found highest in the seedlings inoculated with *G. occulatum* (0.58%  $\pm$ 0.049) and least was found in *G. hoi* (0.39%  $\pm$ 0.033). Nitrogen content was recorded 0.48%  $\pm$ 0.019 in the non-

mycorrhizal seedlings. Non-mycorrhizal control plants produced highest nitrogen content in shoots (0.48%  $\pm$ 0.012) compared to the AM inoculated plants ranged 0.43%  $\pm$ 0.039 to 0.38%  $\pm$ 0.019 at higher level of P (Figure 4). In medium and higher level of P, there was no significant increase in plant Nitrogen.

Plant phosphorus and Plant Nitrogen content was found higher in the experiment containing low level of superphosphate, where half of recommended dose of superphosphate was used. Same results was reported by George (2000) and suggested that on soils deficient in P, mycorrhizal colonization supports plant development by supplying the plant with additional P and sometimes with N, K or Zn. Tanwar *et al.*, (2013) also supported that Mycorrhizal inoculation alone did not significantly influence the concentration of plant phosphorus and total nitrogen (N). AM fungi and P fertilizer together resulted in significant increase in the concentration of both

phosphorus and nitrogen compared to their respective control but higher P application doesn't support the increment in plant nutrient level. These results are in accordance with the findings of Rakshit and Bhadoria (2009) that highest phosphorus uptake occurs in mycorrhizal maize plants with added low P than non-mycorrhizal as well as plants without added P. Increase in P and N in plantlets at low level superphosphate might be due to the ability of the mycorrhizal fungi to extrametrical hyphae in the roots and ultimately help for absorption of water, available phosphorus and other nutrients for plant growth. Findings of Gosling *et al.*, (2005) and Tiamtanong *et al.*, (2015) supported our results. Although we found low P and N concentration in shoot tissues of higher P level. This may be due to less mycorrhizal infection at higher soil P level which showed similar result with Perner *et al.*, (2007). In our experiments, plantlets inoculated with few mycorrhizal fungi showed less plant phosphorus than the control plants. This might be the fact that phosphorus and nitrogen uptake in mycorrhizal plants through Mycorrhizal pathway can reduce Direct pathway of nutrient acquisition resulting reduced mineral nutrients in mycorrhizal plantlets as compared to the control plants containing only direct pathway of nutrient uptake (Nouri *et al.*, 2014).

In our experiment, the correlation coefficient of plant biomass with phosphatase ( $p > 0.001$ ,  $p > 0.005$ ,  $p > 0.005$ ) and phosphorus ( $p > 0.05$ ,  $p > 0.005$ ,  $p > 0.05$ ) was found significantly positive with respect to the levels of phosphorus concentration. But at higher concentration of P nitrogen content, biomass was found non significant in relation to nitrogen content of the plantlets. Biomass was found non significant in relation to the infection at all the levels of P in *Piper mullesua* plantlets.

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