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Creating Climate Resilient Crop Management by Enhancing Arbuscular Mycorrhizal Fungi Associations in Coffee

Mya Mya[1], Soe Naing Win[2] and Myat Mon Thin[3]

[1] *Professor (Rtd.), Yezin Agricultural University, Yezin; Teaching head, Myanmar Partner Organization (Germany), Mandalay, Myanmar*

[2] *Staff Officer, In-charge Inspector, Plant Quarantine Section, Department of Agriculture, Lweje Township, Kachin State, Myanmar*

[3] *Assistant lecturer, Department of Physics, University of Mandalay, Myanmar*

Abstract

Arbuscular mycorrhizae symbiotic association formed between the roots of most plant species and fungi is one of the approach in creating climate resilient agriculture. These symbiosis are characterized by bi-directional movement of nutrients where carbon flows to the fungus and inorganic nutrients move to the plant, thereby providing a critical linkage between the plant root and soil. Research data on arbuscular mycorrhizal association in coffee seedlings in Myanmar are summarized in this paper. Research has placed emphasis on a wider, multifunctional approach on development and effectiveness of arbuscular mycorrhizal fungi (AMF) in the host, host dependency, resistance to transplant shock, nutritional and ecological aspects including nutrient and metal uptake of AMF inoculated plants, and beneficial association with soil microorganism. Verification of the effectiveness of different AMF for promoting growth in the commercial nursery production of coffee seedlings indicated that growth response, nutrient and heavy metal uptake were significantly greater in soil inoculated with AMF when compared with those in soil without AMF. Study on association of AMF with soil microorganism indicated that combined growth-promoting effect of AMF, *G. manihot* and *T. harzianum*, T8 was found to occur in AMF inoculated plants when compared with those of non-inoculated coffee seedlings. Higher nutrient uptake of AMF inoculated coffee seedlings grown with leguminous cover crops show as building beneficial soil fungal communities. Over view of mycorrhizal symbiosis, based on nutritional and ecological aspects improve the understanding of responses to environmental and climatic perturbations for getting healthy plants and thriving mycorrhizal networks enhance crop resist to stressful conditions. Finally, suggestions of mycorrhizal technology application are drawn important in the AMF management of the climate resilient sustainable agriculture. Then, nutritional and ecological aspects indicate that mycorrhizl fungi is fundamental in re-designing a viable agricultural outcome for the future of food production.

Keywords: Arbuscular mycorrhizal fungi (AMF), symbiosis, coffee seedlings, plant growth, nutritional and ecological aspects, *Trichoderma harzianum,* T8, mycorrhizal technology, effective AMF, *Glomus manihot*, climate resilient sustainable agriculture

1. Introduction

In general, the symbionts trade nutrients, and the arbuscular mycorrhizal (AM) fungus obtains carbon from the plant while providing the plant with an additional supply of phosphorus (Smith 1997). Coffee (*Coffea arabica*) is economically important crop in coffee growing countries. One of the effective soil organisms was arbuscular mycorrhial fungus which has been found to improve growth and nutrient uptake of coffee. AM fungi are important as they improve plant-water interactions and thus increase residence to drought (Vaast *et al*., 1996). Recent studies have shown the diversity of AMF, elucidated growth effect mechanisms and demonstrated the importance of of AMF symbiosis to coffee in low fertility soils (Sieverding 1991). Mycorrhizal coffee seedlings grew much faster, exhibit improved nutrition and gave higher yields than those without mycorrhizas at the nursery stage. Coffee seedlings in the nursery intercropped with cover crops have shown to protect crops from reduced soil water availability in agroforestry system (Siqueira et al., 1998). The objectives of these studies were [1] to study the effective association of arbuscular mycorrhizal fungi in coffee seedlings, [2] to apply mycorrhizal technology in creating climate resilient mycorrhizal management in coffee, and [3] to share the knowledge to the coffee growers for quality and organic coffee production.

2. Materials and Methods

2.1.1 Test microbes

The test AMF, *G. manihot* (1), *G. etunicatum* were provided by Dr. Moaward, Göttingen University and Dr. H. von Alten, Hannover University, Germany, respectively. *G. manihot* (2) was collected from cassava (*Manihot esculenta* L) root, Yezin area, Myanmar (Plate 2.1). Morphology of three different AMF, *Glomus* spp. was studied based on characteristics of root colonization pattern and chlamydospores. Inocula were cultured on soghumn (*Sorghum bicola*) in pot containing sterilized soil. *Trichoderma harzianum*, T8 was provided by Dr. H. von Alten, Hannover University, Germany (Plate 2.2A and B).

2.1.2 Experimental design and statistical analysis

The experiments were conducted from 2000 to 2006 (Plate 2.3).. Treatments were arranged in a randomized complete block design, replicating 4 times. Coffee plants were harvested at 8 months after sowing and total dry weight g plant^{-1} were determined. Phosphorus, N, K, Zn, Fe and Mn uptake mg plant^{-1} (Myint *et al*., 2003) were analyzed by using Atomic absorption Spectrometer (AAS), Department of Agricultural Research, Yezin . Phosphorus, Zn, Fe, Ca and Mn uptake mg plant^{-1} (Myint *et al*., 2003, Mya *et al*., 2008) were analyzed by using EDX 700, Universities' Research Centre, Yangon. Data were statistically analysed by using standard analysis of variance by IRRISTAT. The least significant Difference (LSD = 0.05) test was used to compare the mean data of different treatments.

2.1.3 Relative mycorrhizal dependency (RMD) (Plenchette *et al*. 1983)[4] and mycorrhizal inoculation effect (MIE) reported by Bagyaraj et al. (1988)[5] were calculated.

3. Results and Discussion

3. 1. Development of arbuscular mycorrhizal fingi, *Glomus intraradices* in the root of sorghum, *Sorghum bicola* (Myint and Mya 1999)

Arbuscular mycorrhizal fungi are characterized by the formation of branched hostorial structures (arbuscules) within root cortical cells, and by terminal and interclary hyphal swellings (vesicles) which occur between the cells. Extrametrical hyphae grow from the root out into the rhizosphere soil (Plate 2). The chlamydospores produced by AMF are largely borne on the extrametrical hyphae beyond the root surface. Chlamydospores of different *Glomus* spp. were formed in the roots (plate 3, 4, and 5).

3.2 Occurrence of AMF in the natural soil (Mya *et al*., 2008)

Higher densities of AMF spores were observed under soil covering leguminous plants compared with non-leguminous ones. Distribution of AMF genera in coffee rhizospheres, with 3gerera occur more frequently under leguminous trees than under without leguminous soil (Table 3.1).

3.2.1 Relative mycorrhizal dependency (RMD)

As shown in Table 3.2, AMF inoculated coffee with significantly high plant growth was announced as significantly high relative mycorrhizal dependency (RMD) (Mya 2015).

3.3 Varietal response of arbuscular mycorrhizal association (Myint *et al*., 2002)

The results on varietal response and effects of *Glomus manihot* on plant growth and nutrient uptake of coffee varieties at 8 months after sowing indicated that coffee variety S 795 and Catimore show the significantly higher response to

AMF inoculation in comparison with those of different varieties (Table 3.3) (Myint *et al*., 2002, Myint *et al*., 2003).

3.4.1 Effectiveness of arbuscular mycorrhizal association (Myint *et al*., 2002, Myint *et al*., 2003)

The significant growth promoting effect of two different isolates of *G. manihot* may be attributed to its higher P uptake efficiency. Greater P accumulation mg $plant^{-1}$ in the coffee seedlings inoculated with *G. manihot* may be due to AM growth promotion in coffee plants being mainly attributed to the nutritional effects of the symbiosis. The results on growth and nutritional improvement of coffee (*coffea arabica L.*) by different arbuscular mycorrhizal fungi, *Glomus* spp. indicated that mycorrhizal inoculation effect (MIE) was found to be higher in AMF inoculated coffee with significantly high plant growth in comparison with non-inoculated plants (Myint *et al*., 2003) (Figure 3.4.1A). Mycorrhizal inoculation effect (MIE) was significantly higher in *G. manihot* (1) inoculated coffee seedlings. Supported finding was that plant mycorrhizal dependency and symbiotic efficiency (MIE) are directly influenced by soil P availability (Siqueira 1996), making the degree of availability of adequate soil P which is a very important factor in the establishment of efficient mycorrhizal associations.

Correlation between total dry weight and MIE, MIE and infection percent are significant, r^2 = 0.89 and r^2 = 0.99 in sterilized and non-sterilized soil, respectively (Figure 3.4.1B).

3.4.2 Nutritional and Ecological aspects of arbuscular mycorrhizal association (Myint *et al*., 2003)

Nitrogen, potassium, zinc and uptake of coffee seedlings inoculated with three AMF: *G. manihot* (1), *G. manihot* (2) and *G. etunicatum* were significantly increased in sterilized soil at 5months after sowing. In this study essential plant microelements such as zinc (Zn), iron (Fe) and manganese (Mn) were generally found in higher concentrations in mycorrhizal coffee plants which as a result showed better growth and higher P uptake than non-mycorrhizal plants (Figure 3.2 and 3.3). Buwalda *et al*., 1983 reported that higher concentrations of zinc (Zn), iron (Fe), manganese (Mn), and chlorine (Cl) were generally found in mycorrhizal coffee plants. Enhancements of P and Zn uptake due to AM colonization have also been well documented particularly in marginal soils. In this study it was observed that reduction of Zn deficiency symptoms occurred in AM colonized plants, on the other hand higher content of Zn concentrations in AMF coffee seedlings suggesting a correlation between AM colonization rates and Zn nutrition in coffee plants (Figure 3.4.2, Plate 3.2).

3.5. Interaction with soil microorganism (Myint *et al*., 2005)

From the enphasis on effect of *Trichoderma harzianum*, T8 and different *Glomus* spp. on total dry weight, P, Zn, Fe and Mn uptake mg plant^{-1} of 8 month-old coffee seedlings indicated that total dry weight of coffee inoculated with *T. harzianum* in combination with *G. manihot* (1) was significantly higher than that of plants inoculated with *T. harzianum* alone. In this study it was observed that induced plant growth occurred in AM colonized plants may be due to increasing the absorptive surface area of their host plant root systems providing an increased surface area of interaction with other soil microorganisms. This result was confirmed by Calvet *et al*., (1993) who observed enhanced growth of marigold (*Tagetes erecta*) after treatment with *T. aureoviride* in combination with the mycorrhizal fungus, *G. mosseae* (Figure 3.5).

3.6 Managing soil cover

The results on growth and nutritional improvement of coffee (*coffea arabica L.*) and cover crops: *Desmodium* spp. in the soil with pH 6.8 (Figure 3.6.1) and cover crops (*Arachis pentoi* in the soil with pH 5.54 (Figure 3.6.2) inoculated with arbuscular mycorrhizal fungi, *Glomus* spp. indicated that P uptake and N uptake were found to be higher in AMF inoculated coffee grown with cover crops in comparison with coffee without cover crops (Mya 2016). Increase in nutrient uptake and protecting the soil from water runoff losses and erosion of leguminous cover crops in coffee nursery is a fundamental strategy for enhancing resilience (Siequira 1996).

3.7 Transplant shock

Transplant shock at 7 month old coffee seedlings was reduced in mycorrhizal inoculated plants (Mya *et al*., 2008) (Table 3.7). Coffee seedlings inoculated with AMF resistance to drought and transplant shock may be due to improvement of plant-water interaction by AMF.

3.8 Suggestion on mycorrhizal technology in sustainable arbuscular mycorrhizal management

The source of arbuscular mycorrhizal fungi inoculum is the first point to be considered because commercial substrates found on the market are usually poor in purification. Inoculation of highly effective AMF is expected to greatly improve the nutritional state of seedlings and their establishment after transplantation (Mya 1998, Myint and Mya 1999). Verification of the effectiveness of different AMF for promoting growth in the commercial nursery production of coffee seedlings indicated that growth response, nutrient uptake and heavy metal uptake were significantly greater in soil inoculated with AMF when compared with those in soil without AMF (Myint and Mya 1999. Morphology and development of AMF were studied to maintain effective association important in incorporation this sustainable technology. Coffee was highly dependent on AMF symbiosis due to the significant mycorrhizal inoculation effect was observed in the plants inoculated with *G. manihot*(1) + RP in both sterilized soil and non-sterilized soil at 8 months after sowing when compared with those in non-treated control due to highly dependence of coffee on the AMF or nutrient uptake and plant growth. This finding was confirmed by the report on Mycorrhizal dependency at the different soil (Mya 2015). Pre colonization was also important for beneficial AMF symbiosis because when coffee seedlings were pre-colonized with AMF, plant development was favored, when compared to seedlings without AMF. The improvement of plant growth and nutrient uptake by two isolates of *G. manihot* may be due to their higher mycorrhizal inoculation effect (MIE) and well adaptation in the soil. Thus, P is undoubtedly the most important nutrient taken up by AMF, which is reflected in the large number of reports focused on the improved P status of AM plants; this situation is no different for coffee.

The selection process for formulating the effective AMF inoculants for a target plant and/or variety should consider the ecological adaptations of native AMF to the target environment. Generally, the soil for commercial coffee seedling production in Myanmar are not conductive to mycorrhizal formation .in coffee seedlings (Myint and Mya 1999, Myint *et al*., 2003)

According to the results, higher effectiveness of native AMF, *G.manihot* (2) from cassava was also effective for promoting growth in the commercial nursery production of coffee varieties. Available mycorrhiza inocula used in this research normally leads to an extremely high density of AMF propagules per 100 gram volume of soil, thereby enabling a rapid mycorrhizal establishment of coffee seedlings and making AMF inoculation a highly recommended practice at the nursery stage (Mya *et al*., 2003).

Thus, AMF inoculation may constitute a viable economic alternative for efficient seedling production, decreasing the use of fertilizers and pesticides, diminishing the time for field transplantation and producing more vigorous plants able to better withstand environmental stresses during the acclimatization period. The way to obtain the economic benefits of arbuscular mycorrhizas is to ascertain the link between their presence and function, and the impacts for effective. In order to estimate economic values of mycorrhizas at farm level, various factors affecting mycorrhizal influences on plants and soil need to be assessed, characterized and quantified. Therefore, AM fungi are gaining popularity as biofertilizers and biocontrol agents. The use of efficient mycorrhizal inoculum in coffee nurseries may be a promising technology for the production of healthy and vigorous coffee plantlets, thus increasing survival of environmental stress after field transplantation.

4. Conclusion

Results of the study reported here indicated that the AMF isolates used have dramatic effects on plant responses of coffee seedlings grown in different pH soil for uptake of mineral nutrients. Synergistic effect of AMF and *Trichoderma* observed in this study were essential for early development of coffee seedlings in the nursery plots for getting quality coffee seedlings resistant to transplant shock. The relevance of the symbioses in relation to ecological aspect is now of significant interest since agricultural lands are subject to changes in modern environmental conditions that threatened agricultural production and quality. Contributing improved crop yields and soil health are giving way to new theories with a broader functional basis, using more ecologically relevant species .

In this study we propose cultivar-AMF interaction on working, evaluating the contribution to coffee growth as well as the contribution of potential cultivar-symbiont selectivity on AMF populations in coffee nursery. Recommendations will focus on cultivar selection, adjusting nutrient and Zn and Fe, Mn uptake, and intercropping with soil cover leguminous plants to build beneficial soil fungal communities. These best practices will also help Coffee producers from perils of drought, as healthy plants and thriving mycorrhizal networks enhance crop tolerance to stressful conditions.

Over view of mycorrhizal symbiosis, based on nutritional and ecological aspects will improve the understanding of responses to environmental and climatic perturbations. This knowledge is an important prerequisite for future, sustainable management of terrestrial ecosystems.

5. References

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Table 3.1 Occurrence of indigenous arbuscular mycorrhizal fungi in coffee research farm (Mya *et al*., 2008,)

	Genus	Soil with leguminous soil cover (*Desmodium* spp.) (pH 6.8)	Soil without leguminous soil cover (*Desmodium* spp.) (pH 7.4)
		No. of spores in 100 gm of soil	
1	*Glomus* spp.	80	60
2	*Acaulospora* spp.	50	20
3	*Gigaspora* spp		20

Mean data of 20 plants from 20 places

Table 3.2 Dependency of Coffee on different *Glomus* species (Mya 2015)

No	AMF	RMD^X	^YDependency	References
	Glomus ectunicatum S-139	XX	Obligate	Myint *et al*., 2003
2	*Glomus intraradices* (S-49)	XX	Obligate	Myint *et al*., 2005
4	*Glomus intraradices*(S-510)	XX	Obligate	Myint *et al*., 2005
5	*Glomus manihot* (Germany)	XXX	Obligate	Myint *et al*., 2003
6	*Glomus manihot* (Myanmar)	XXX	Obligate	Myint *et al*., 2003

^XRMD Relative Mycorrhizal Dependency, ^YOBL = Obligate mycotrophic plant (Severding 1998, Myint and Mya 1999), Data are significantly different at LSD values ($P \leq 0.05$)

Table 3.3 Responses in total dry weight and Fe uptake of coffee varieties to arbuscular mycorrhizal fungi (*Glomus manihot*)at different soil types (Mya *et al*., 2008)

Varieties	Total dry weight g plant $^{-1}$ at 8 months after sowin	
	red soil	black soil
Catimor	3.45 aB	2.83 aB
S- 795	3.60 a A	2.65 abB
Caturra (R)	3.20 ab A	2.38 bc B
Caturra (Y)	3.20 ab A	2.25 bc B
SanRamon	3.00 b A	2.20 c B
Mean	3.29	2.46
	LSD (0.05)(a) = 4.44	**LSD (0.05) (b) = 6.29**
	CV%(a) = 9. 01	CV%(b) = 9.70
Varieties	Fe uptake mg plant $^{-1}$ at 8 months after sowing	
	red soil	black soil
Catimor	27.69 aA	**24.80 c**
S- 795	14.32 dA	24.80 cB
Caturra (R)	25.03 ab A	37.48 bB
Caturra (Y)	21.01 c A	25.79 c B
SanRamon	22.35 bc A	42.21 a B
Mean	22.08	31.01
	LSD (0.05)(a) = 2.44	**LSD (0.05) (b) = 3**
	CV%(a) = 7.11	CV%(b) = 9.50

^XMeans of four replications with five plants each

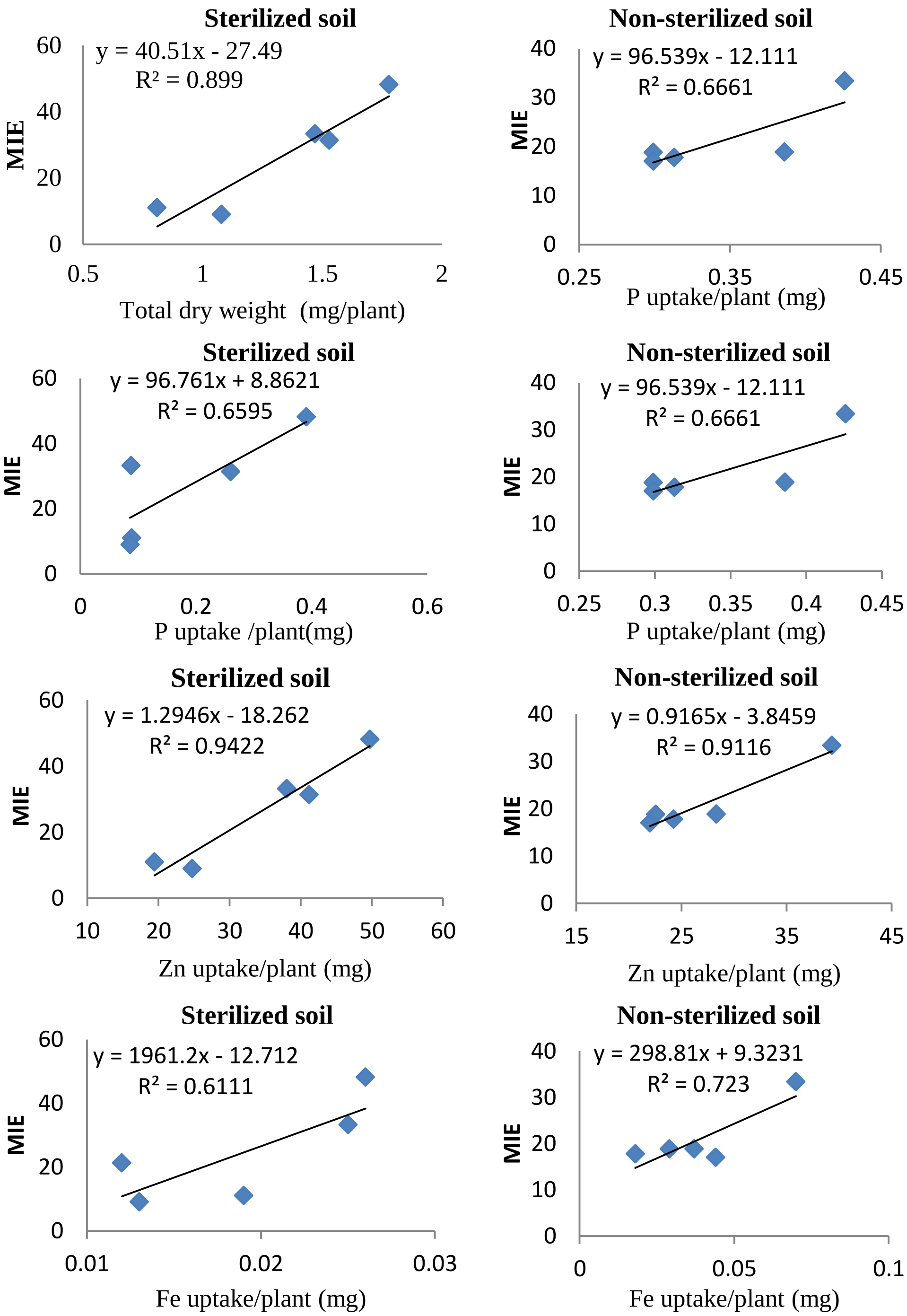


Figure 3.4.1B Correlation between total dry weight, P, Zn, Fe uptake /plant (mg) and mycorrhizal inoculation effect (MIE) at 5 month old coffee seedlings (Myint, *et al*., 2003)

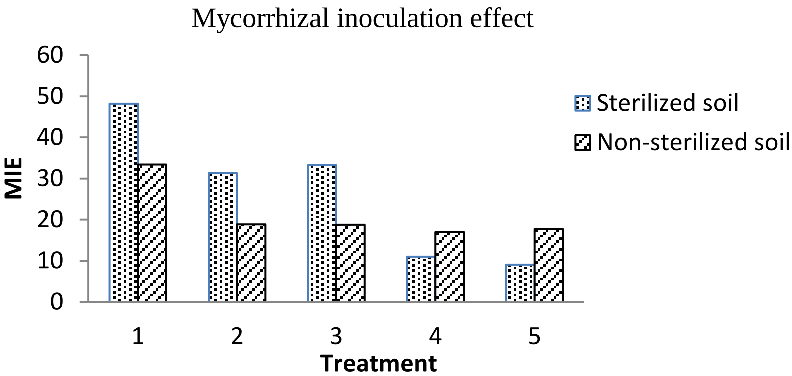


Figure 3.4.1A Mycorrhizal inoculation effect (MIE) of different Glomus spp. 1: *Glomus manihot*(1)+RP, 2:*Glomus manihot*(2)+RP, 3:*Glomus etunicatum*+RP , 4: Rock phosphate (RP), and 5: Control. Data are significantly different at LSD values (P ≤ 0.05) (Myint, *et al*., 2003)

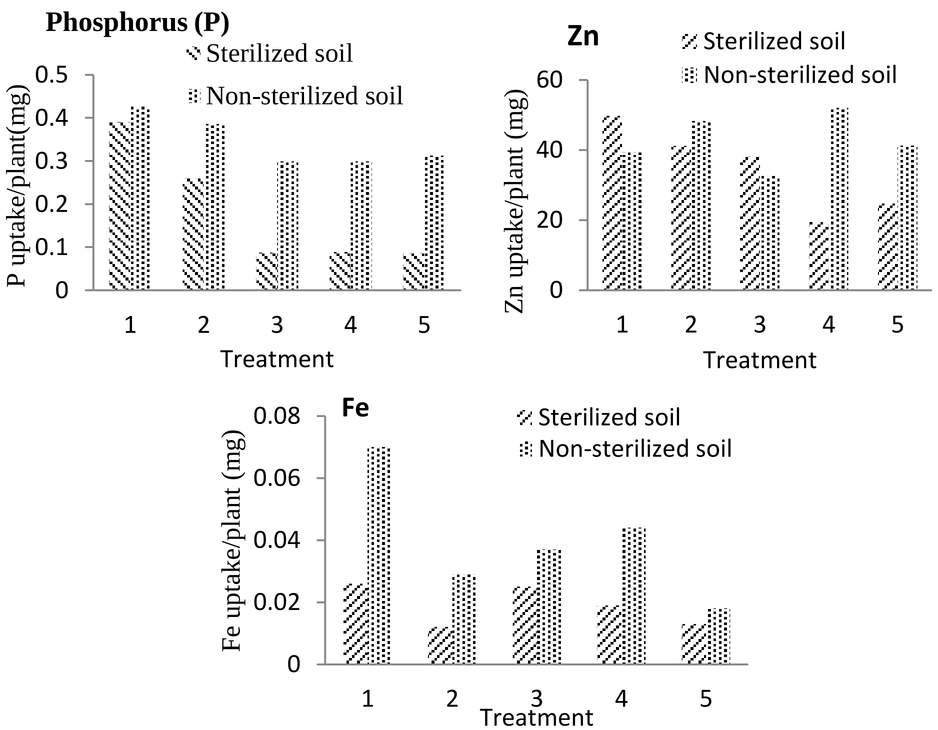


Figure 3.4.2 Effect of different *Glomus* species on Total dry weight plant[1] (mg), infection% and mycorrhizal inoculation effect, P, Zn and Fe uptake plant[1] (mg) of 5 month old coffee seedlings, 1: *Glomus manihot* (1)+RP, 2: *Glomus manihot*(2)+RP, 3: *Glomus etunicatum*+RP , 4: Rock phosphate (RP), and 5: Control. Data are significantly different at LSD values (P ≤ 0.05) (Myint, *et al*., 2003)

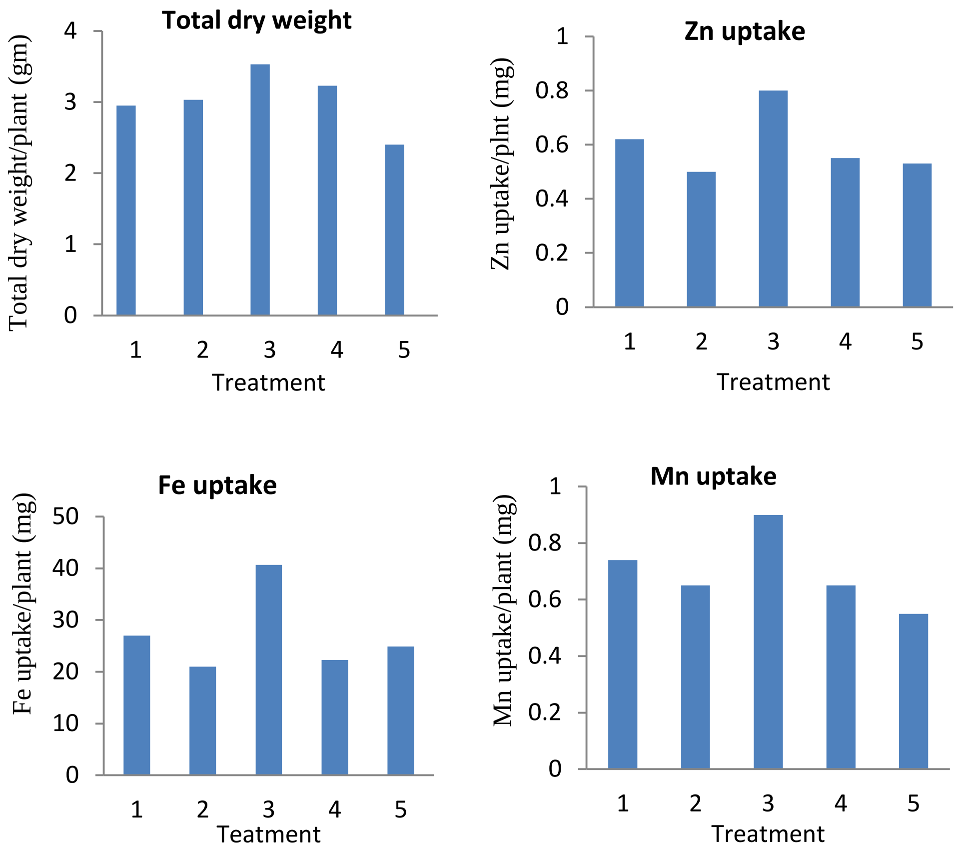


Figure 3.5 Effect of AMF associated with *T. harzianum*, T8 on total dry weight and P uptake of coffee (*Coffea Arabica* L.) 1: *T. harzianum*, *T*8, 2: T8+*G. etunicatum* , 3: T8+*G. manihot* (1) , 4: T8+*G. manihot* (2), 5: Control. Data are significantly different at LSD values (P ≤ 0.05) (Myint *et al*., 2005)

Table 3.7 Plant growth and transplant shock of coffee seedlings inoculated with Different AMF (Mya *et al*., 2008)

Treatment	Total dry weight (gm)	Resistance to transplant shock (%) after transplanting	
		3 month	7 month
Glomus manihot (1)	3.6	95	85
Glomus manihot (2)	3.25	94	81
Glomus etunicatum	3.13	92	85
Acaulospora spp.	2.95	71	61
Non-mycorrhizal	1.85	70	60

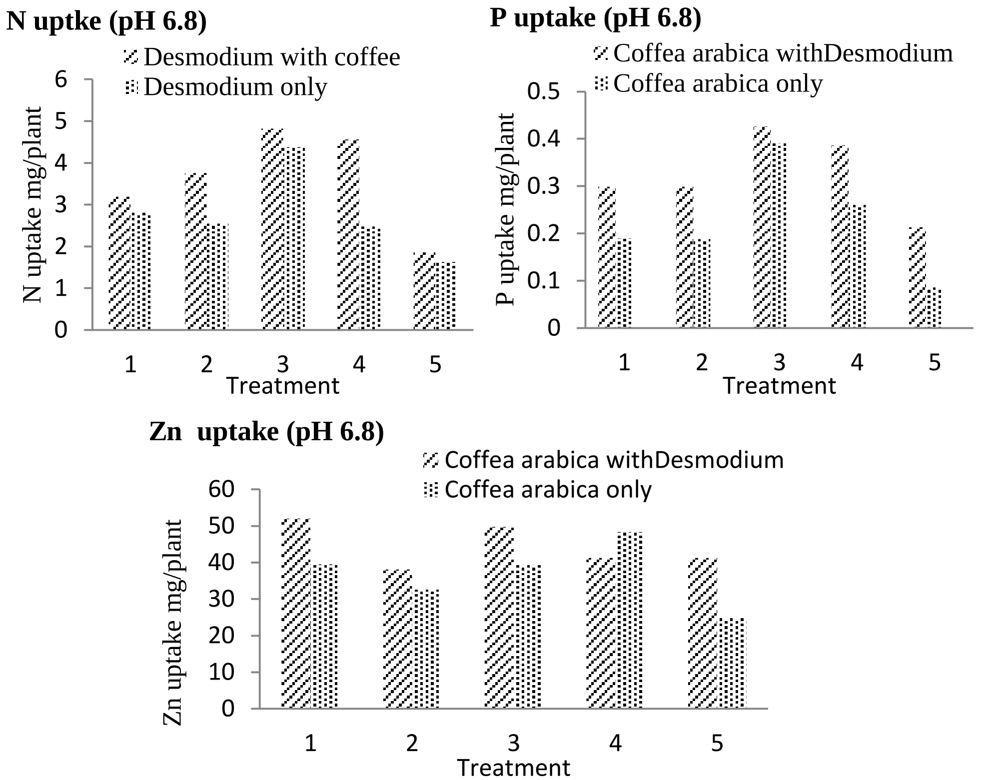


Figure 3.6.1 Nitrogen, Phosphorus and zinc uptake per plant (mg) of coffee seedlings and cover crop *Desmodium* spp. grown in coffee nursery soil inoculated with four AMF isolates : 1: *G. intraradices* , 2: *G.etunicatum*, 3: *G. manihot* (1), 4: *G. manihot* (2),5: Non- AMF. Data are significantly different at LSD values ($P \leq 0.05$) (Mya 2015, Mya 2016)

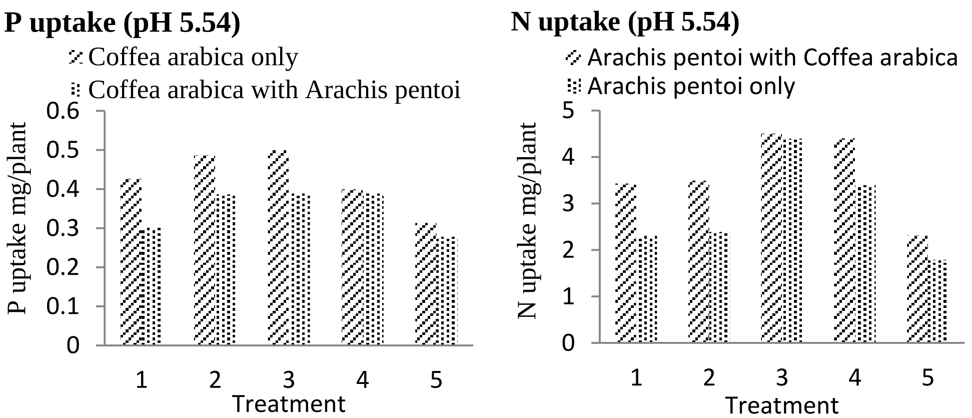
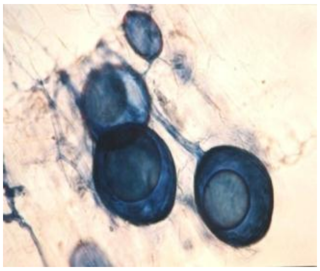
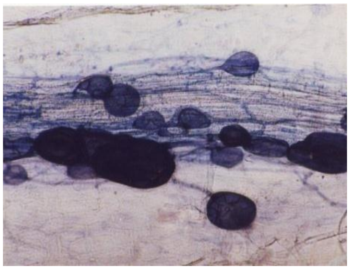


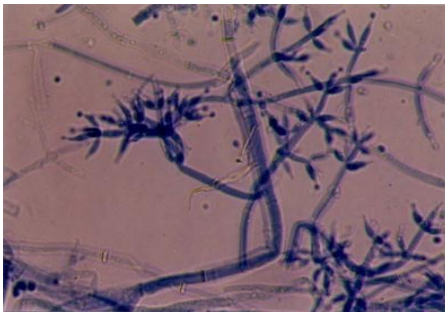
Figure 3.6.2 Nitrogen and Phosphorus uptake per plant (mg) of coffee seedlings and cover crop *Arachis pentoi* grown in coffee nursery soil inoculated with four AMF isolates : 1: *G. intraradices* , 2: *G.etunicatum*, 3: *G. manihot* (1), 4: *G. manihot* (2),5: Non- AMF. (Mya 2015, Mya 2016)





Glomus etunicatum *Glomus manihot* (1) *Glomus manihot* (2)

Plate 2.1 Source of AMF (Myint and Mya 1999)

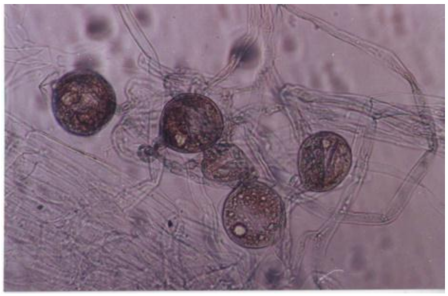


Morphology of *Trichoderma harzianum*, T8 (X400)

Colony of Trichoderma harzianum, T8 (X400)

Plate 2.2A Morphology and colony of *Trichoderma harzianum*, T8

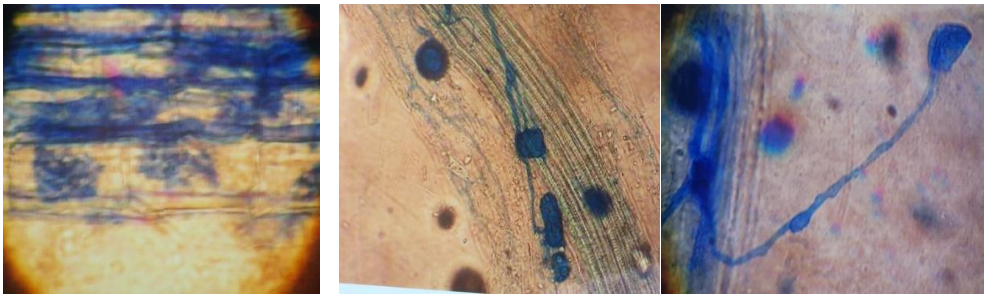
Sand and soil based inoculum



Spore based inoculum with *G. etunicatum*

Plate 2.2 B Inoculum starter and host

Plate 2.3. Experimental plots of effects of arbuscular mycorrhizal fungi on plant growth and nutrient uptake of Coffee (*Coffea arabica L.)* seedlings (Myint *et al*., 2003) and influence of soil microorganisms, *Trichoderma harzianum*, T8 and arbuscular mycorrhizal fungi, *Glomus* spp. on plant growth and nutrient uptake of coffee (*Coffea arabica*) seedling (Myint *et al*., 2005).



Arbuscules Vesicle and spore External mycelium

Plate 3.1 Development of arbuscular mycorrhizal fingi, *Glomus intraradices*

Plate 3.2 Zinc and Fe deficiency symptoms in AMF non inoculated and recovery symptoms in AMF inoculated coffee seedlings (Myint *et al*., 2003