Development of an integrated nutrient management system for export agriculture crops by increasing the nutrient uptake efficiency with the use of mycorrhizal inoculations

A thesis submitted for the Degree of Master of Philosophy

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This thesis is my original work and has not been submitted previously for a degree at this or any other university / institute. To the best of my knowledge it does not contain any material published or written by another person, except as acknowledged in the text.

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This thesis is dedicated to my parents and teachers, all of them expected me to fulfill this task one day.

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Abstract

In Sri Lanka export agricultural crops (EAC) such as black pepper, cinnamon, cocoa, clove, nutmeg, cardamom, beetle are becoming more important during the last two decades in comparison to traditional plantation crops such as tea, rubber and coconut. Cinnamon and black pepper are the most important EAC. Existing fertilizer recommendations for most of the EAC are based on inorganic N, P, K, and Mg mixtures and they are not appropriate to cater for nutrient management requirements of organic farming systems of EACs. On this context, application of mycorrizal inoculation techniques are becoming an important plant nutrient management tool, as arbuscular mycorrhizae induced growth improvements are widely reported in many perennial plants. Therefore, a set of experiments were carried out at the EAC Research station with the aim of developing an efficient AM inoculation protocol for pepper rooted cuttings and cinnamon seedlings as a component of integrated plant nutrient management .

As the first step, an experiment was carried out with the objectives of finding the appropriate host crop species and time of uprooting the host crop to obtain maximum possible number of spores in the inoculum of arbuscular mycorrhizae (AM) species *G. mosseae*. Three host crops namely maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and finger millets (*Eleucine coracana.* L) were studied using clay pots. Under local conditions, finger millet was found to be a good alternative as a host crop for mass propagation of AM. This crop would give an additional income as a grain harvest also at 12th week.

Experiment for black pepper (*Piper nigrum* Linn.), was carried out to select a suitable spore density of AM fungus *G. mosseae* for inoculation of black pepper rooted cuttings in the nursery stage using four mycorrhizal inoculum levels of *G. mosseae* namely 25 g (T1), 75 g (T2), 150 g (T3) and 300 g (T4) with a control (T5). Incorporation of the AM inoculum at the rate of 75 g containing approximately 795 (mean) spores with host crop roots and fungal structures in moist soil mixture as medium into one standard size polythene bag before planting a rooted cutting of black pepper was found to be suitable to obtain good quality planting materials for field planting at the 6th month of growth in the nursery. The first AM inoculation experiment for Cinnamon (*Cinnomomum verum* Presl Syn. *Cinnomomum zeylanicum* Blume) was conducted at nursery stage applying objective and treatments similar as black pepper pot trial. Inoculation of cinnamon seedlings with 75 g of above inoculum seems to be appropriate for enhancement of seedling growth of cinnamon.

A large size pot experiment was conducted to investigate the combine effect of Eppawala Rock Phospahate (ERP™) levels and AM (*G. mosseae*) on pepper rooted cuttings beyond the nursery stage. Three P fertility levels (1) F_1-5 μg P/ g soil (2) F_2-50 μg P/ g soil and (3) F_3-100 μg P/ g soil were tested with three mycorrhizal inoculum levels of *G. mosseae* (1) M_1-No Mycorrhizal inoculum, (2) M_2-75 g of Mycorrhizal inoculum and (3) M_3-150 g of Mycorrhizal inoculum in a large size pot experiment with black pepper (local selection MB12) rooted cuttings. Application of moderate rate of P fertilizer (50μg P/ g soil) likely maximized the beneficial effect of abuscular mycorrhiza (*Glomus mosseae*) symbiosis on pepper grown in Reddish Brown Latasolic soil at Matale area in Sri Lanka.

Obsevations warranted further research on rhizoshpere chemistry of pepper and cinnamon in both pot level and the field scale to draw firm recommendation on efficient utilization of applied fertilizer as well as native nutrients along with different AM types.

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Abbreviations

AM –Arbuscular Mycorrhizae

VAM- Vesicular Arbuscular Mycorrhizae

DEA-Department of Export Agriculture

EAC- Export Agricultural Crops

PSM- Phosphorus Solubilizing Microorganisms

PSF- Phosphorus Solubilizing Fungi

PSB- Phosphorus Solubilizing Bacteria

OM- Organic Matter

ERP- Eppawala Rock Phosphate

CEC-Cation Exchange Capacity

Chapter 01

General introduction

1.1 Introduction

In tropical agriculture as well as in forestry, there is growing emphasis for improvement of soil fertility through manipulation of soil biological processes rather than through heavy applications of inorganic fertilizers for economic and environmental reasons. Traditional soil management practices using organic residues, rotation and mixing of crops *etc.* are more appealing to the small scale farmers of the tropics for economic as well as socio-cultural reasons. On this context, application of mycorrizal inoculation techniques is becoming an important plant nutrient management tool, as mycorrhizae induced growth improvements are widely reported in many perennial plants (Liu *et al.*, 2004;Trovlove *et al.*, 2003). Mycorrhizae are highly evolved symbiotic associations formed between some soil fungi and plant roots. These associations are broadly categorized into two groups, based on morphological and anatomical characters of the mycorrhizal roots, as ectomycorrhizae and endomycorrhizae (Lakshmipathy, Bagyaraj and Balakrishna, 2007). Ectomycorrhizae are common among temperate forest tree species and can be cultured on laboratory media. Endomycorrhizae, include arbutoid, ericoid, orchid and arbuscular mycorrhizal (AM) forms (Lakshmipathy *et al;* 2007). Vesicular arbuscular mycorrhizae (VAM) synonym. AM are the most common sub group coming under endomycorrhizae. VAM fungi are associated with the majority of terrestrial plants (Quilambo, 2003). It has been estimated that over 80% of all vascular plants form arbuscular mycorrhizae (Hafeel and Gunatilleke, 1989). They occur in most ecosystems of the world and are found in many important crops species (Peterson *et al.*, 2004). AM play an essential role in plant growth, plant protection and soil quality.[1] The increased plant growth is attributed to enhanced uptake of diffusion limited nutrients, hormone production, biological nitrogen fixation, drought resistance and suppression of root pathogens (Bagyaraj, 2006). The increased capacity of plant roots for water and nutrients uptake from the soil when colonized by AM is the main mechanism proposed to explain the effect of AM on plant performance. This behavior is particularly evident with soil nutrients that are more immobile such as phosphorus (P), zinc (Zn) and copper (Cu). [1]

[1](http://www.plantmanagementnetwork/org/pub/cm/review/2004/amfungi). Dalpe and Monreal. AM Inoculum to Support Sustainable Cropping Systems. 12/08/2009

Hence AM fungi can be employed for the enhancement of effective utilization of nutrients in the native soils. AM fungi inoculated to crop plants colonize the plant root system and increase the growth and yield of crop plants including pepper (Durgapal, Pandey and Palni, 2002; Thanuja, 2002; Rao, 1993). A reduction in P application is recommended in order to stimulate and maintain the symbiosis effectively[1]. According to Xioutang (1994) *Glomus mosseae* is an AM species and that can form mycorrhizal associations with many plant species with significant effects on plant height, stem diameter and biomass, compared with uninoculated plants. Moreover, there is considerable potential for the development of an inoculum of *G. mosseae* to be used for inoculating tree crops.

In Sri Lanka export agricultural crops (EAC) are becoming more important during the last two decades in comparison to traditional plantation crops such as tea, rubber and coconut. Cinnamon is the most important crop in terms of export earnings coming under the purview of the Department of EAC. The area under cinnamon cultivation is estimated to be approximately 25,300 ha (Central Bank Report, 2003). Sri Lanka is the world's largest producer and exporter of cinnamon accounting for nearly two-third of the global output. A total quantity of 14015 t cinnamon quills was exported in 2003. Black pepper is the second important crop of EAC but the pepper export volume declined by 2% to 7740 t in 2003 (Central Bank Report, 2003). There are about 400,000 families engaged in the cultivation of EAC and majority of them are small holders (< 1 ha). Nevertheless, the increase of fertilizer prices and withdrawal of fertilizer subsidies from 2002 onwards, affected the production levels of two major EAC, black pepper and Cinnamon (two major EAC). Many small holders, mainly pepper growers resorted to organic farming in recent past mainly due to the high cost of inorganic fertilizers and pesticides and the increase price of organically cultivated produces.

Existing fertilizer recommendations for most of the EAC are based on inorganic N, P, K, and Mg mixtures formulated from the results of field experiments designed and carried out during the existence of fertilizer subsidy schemes. Currently there is no information available on nutrient management practices that can be used by organic farmers whose numbers are on the increase in Sri Lanka. The research division of the Department of EAC recently commenced research on developing novel integrated nutrient management practices focusing on green manure use as well as the effective utilization of nutrients in the native soils. Only the naturally occurring phosphate rock is recommended as a phosphorous source for the organic framing systems in Sri Lanka, and overseas. As Sri Lanka has a locally available phosphate rock (Eppawella phosphate rock- EPR), this rock is recommended as a P fertilizer source for organic farms and other perennial crops.

Most of the EAC species are perennial, and their roots are associated with mycorrhizea species (Palipane and Bandara, 1985). These mycorrhzea species can improve the plant-uptake efficiency of P and other nutrients from the soils. They can also solubilise the Eppawella phosphate rock (EPR) applied to the soil and increase the plant-availability of P from this rock. Recent overseas studies showed that ectomycorrhizal infection of *Pinus radiata* seedlings increased P uptake from Sechura phosphate rock by the trees through increased phospatase activities and root excretion of organic anions in rhizhosphere soils (Liu *et al.*, 2004; Liu *et al.*, 2005). Palipane (1999) demonstrated the beneficial effect of VAM in coffee seedling growth. In Sri Lanka, the Department of EAC received a pure inoculant of VAM- *Glomus mosseae* from Bangalore University. Initial pot experiments indicated that VAM inoculation enhanced the growth of black pepper rooted cuttings (Wimalarathne, 2005). Nevertheless, further pot experiments with varying VAM spore densities was proposed to improve the effectiveness of VAM inoculation.

It will be more valuable if we can increase the availability of plant-available nutrients such as P, N. Zn etc. through successful mycorrhizal inoculation of EAC plants at nursery stage. Ultimately, it would reduce the cost of fertilizer, save foreign exchange while providing conditions for efficient utilization of nutrients through biological methods leading to environmental sustainability of agricultural systems and improved soil quality. The findings of this research program would also reduce the poverty level of most of the EAC small holders by way of compensating the negative effect associated with the abolition of the fertiliser subsidy for this sector in recent the past.

Therefore, as an initial step towards this goal, the current investigations on mycorrhizal applications have been conducted with respect to pepper and cinnamon.

1.2 Aim

To develop an efficient AM inoculation protocol for pepper rooted cuttings and cinnamon seedlings as a component of integrated plant nutrient management. This AM inoculation protocol will act as a part of potting mixture preparation methodology in Pepper and Cinnamon nurseries. This research will be a guidance in future researches like "reducing the fertilizer quantity for Pepper and Cinnamon.

1.3 Objectives

1) Identify an appropriate host crop species and time of uprooting the host crop to get the maximum possible number of spores for inoculum preparation for species *Piper nigrum* Linn. (Pepper) and *Cinnomomum verum* Presl (Cinnamon) using *Glomus mosseae* AM species.

2) To develop and introduce a procedure to inoculate pepper rooted cuttings and cinnamon seedlings at nursery stage with available AM inoculum, *Glomus mosseae*, as a cultural method. Identify the effect of growth parameters (shoot dry weight, root dry weight, root length, leaf area, plant height etc.) on inoculating the differrent quantities of available inoculum.

3) To investigate the effect of mycorrhizal associations of pepper/cinnamon on increasing plant-availability of phosphorus from Eppawala Rock Phosphate (ERP). Identify the effect of growth parameters (shoot dry weight, root dry weight, root length, leaf area, plant height etc.), shoot tissue and root nutrient contents and soil chemical properties of rhizosphere soil and bulk soil (soil pH, Bray P, Olsen P, Water soluble P, Total N %, Mg, K) on inoculating the differrent quantities of available inoculum.

Chapter 2

Literature review

2.1 Export Agricultural crops in Sri Lanka

"Sri Lanka is a Spice Island" is renowned for the spices export from times immemorial. Cinnamon, pepper, cardamom, clove and nutmeg are the major spices which have export significance to Sri Lanka.[2] Except true cinnamon (*Cinnomomum verum* Presl Syn. *Cinnomomum zeylanicum* Blume), almost all the other EACs are cultivated in many other countries in large bulk form and as such Sri Lanka has the comparative advantage only in the Cinnamon market. For the other spices comparative advantage lies in niche markets. The Department of Export Agriculture (DEA) plays a major role in earning foreign exchange through export agricultural crops. In year 1972 at the establishment of the Department, the rate of foreign exchange through exporting export agricultural crops is 3%, but it has increased up to 9% in year 2008 (Department of Export Agriculture facts, 2010).The total exported amount of export agricultural crops in year 2008 was 34540.7 Metric tones and has earned 18196.9 million rupees (DEA, 2010). Black pepper is the most widely used spice all over the world. Of the total world trade in spices of about 270 million US$, 37% is pepper (Anon, 1996). In 2007 pepper production has increased by 5% (Central bank report, 2007). In Sri Lanka, pepper is cultivated over an area of 11600 ha mostly in the districts of Matale, Kandy, Kegalle, Kurunegala and Nuwara Eliya (Anon, 1996). According to Ekanayake (1990), price is not a dominant factor in demand for pepper in the U.S.A., Sri Lanka has to pay attention to other factors which influence the demand if Sri Lanka wants to expand its market for pepper in the U.S.A. The quality of the product, the consistent supply, and recognition of the specifications of the buyers, and establishing direct contact with processors and retail packers are some of the factors which can be helpful for market expansion (Ekanayake, 1990).

True Cinnamon, an another important spice in Sri Lanka commands about 60% of the world export market and exports about 7,000 tonnes of quills and chips per year (Anon, 1996). In 2007 Cinnamon production has increased by 5% (Central bank report, 2007). Cocoa, clove, nutmeg, cardamom, beetle, ginger, areca nut are some of other export agricultural crops grown in Sri Lanka under the guidance of DEA.

[2]. http://www.agri.ruh.ac.lk/cinnamon/Cin%20stat.htm. Cinnamon in Sri Lanka. 12/05/2010

2.1.1 Cinnamon in Sri Lanka

Genus *Cinnamomum* consists of about 250 species from South East Asia to Australia. There are six species known from Ceylon of which one, *C. verum* Presl (syn. *C. zeylanicum* Blume) is the widely cultivated (Dassanayake, Fosberg and Clayton, 1995). Cinnamon comes from Sri Lanka, and the tree also is grown commercially in India, Java, Sumatra, the West Indies, Brazil, Vietnam, Madagascar, Zanzibar, and Egypt.[3]

2.1.1.1 Growing conditions and varieties of cinnamon

Cinnamon is a hardy plant which can grow on any soil under a wide variety of tropical conditions, but to derive the characteristic flavour, careful cropping conditions must be observed (Anon, 2006). In Sri Lanka, it is cultivated under varying conditions ranging from semi dry to wet zone conditions and soils varying from the silver sands to loamy and lateritic gravelly soils (Anon, 1996). However, the quality of the bark is greatly influenced by the soil and ecological factors. The best quality bark is obtained from plants cultivated on sandy soils mixed with humus. Although a higher bark yield can be obtained when cinnamon is grown on other soils, the quality is inferior (coarser bark) to those grown on sandy soil. The most suitable temperature is between 20 °C-30 °C. Annual rain fall should be in the region of 1250 mm to 2500 mm and the most favourable elevation is 300 m to 350 m from the mean sea level. (Anon, 1996).

With the use of these genetic resources, production of cinnamon plants with elite characteristics can be developed. Potential technologies available for propagation are tissue culture and selection of vegetative propagated plants, which is widely practiced now. In addition to that controlled cross pollination can also be used in crop improvement.

It has been reported that there are eight different types (Cultivars) of cinnamon (*Cinnamomum zeylanicum*) with commercial value in Sri Lanka, based chiefly on pungency of bark and petiole, texture of bark and the structure of leaves (Anon, 1996).

[3]. New world encyclopedia (http://www.newworldencyclopedia.org/entry/Cinnamon.)/ 06/03/2010

According to Dassanayake *et al* (1995), there are eight different Cinnamon species in Sri Lanka, which are given below,

1) *Cinnamomum verum* Presl. (Kurundu)
2) *Cinnamomum dubium* Nees. (sewel kurundu)
3) *Cinnamomum ovalifolium* Wight.
4) *Cinnamomum litseaefolium* Thw. (Kudu kurundu)
5) *Cinnamomum citriodorum* Thw. (pengiri kurundu)
6) *Cinnamomum capparu-coronde* Blume. (Kappuru kurundu)
7) *Cinnamomum sinharajaense* Kostermans.
8) *Cinnamomum rivulorum* Kostermans.

(a) True cinnamon

Cinnamomum verum syn.*Cynnamomum zeylanicum* is one of the oldest and most significant cinnamon species grown in Sri Lanka.[2] and named as true cinnamon. Popularly labelled simply as cinnamon or as Ceylon cinnamon, *Cinnamomum verum* is a small evergreen tree which is 10-15 meters (32.8-49.2 feet) tall and is native to Sri Lanka and Southern India. The Department of Export Agriculture introduced two Cinnamon varieties namely Sri Wijaya and Sri Gamunu (DEA, 2010). The bark is widely used as a spice. Its flavor is due to an aromatic essential oil that makes up 0.5 to 1 percent of its composition. This oil is prepared by roughly pounding the bark, and then quickly distilling the whole. It is of a golden-yellow color, with the characteristic odour of cinnamon and a very hot aromatic taste. The pungent taste and scent come from cinnamic aldehyde or cinnamaldehyde and, by the absorption of oxygen as it ages, it darkens in color and develops resinous compounds.

Chemical components of the essential oil include ethyl cinnamate, eugenol, cinnamaldehyde, beta-caryophyllene, linalool, and methyl chavicol.

Cinnamon is harvested by growing the tree for two years and then coppicing it. The next year a dozen or so shoots will form from the base. These shoots are then stripped of their bark, which is left to dry. Only the thin (0.5 mm) inner bark is used; the outer woody portion is removed, leaving meter long cinnamon strips that curl into rolls ("quills") on drying; each dried quill comprises strips from numerous shoots packed together. These quills are then cut to 5-10 cm long pieces for sale.

Sri Lanka cinnamon is a very thin smooth bark, with a light-yellowish brown color, having a highly fragrant aroma.

2.1.1.2 Uses

Cinnamon bark is widely used as a spice. It is principally employed in cookery as a condiment and flavoring material, being largely used in the preparation of some kinds of desserts, chocolate, spicy candies, tea, hot cocoa, and liqueurs. Cinnamon is used as a spice or condiment for flavouring cakes and sweets, in curry powder and in incense, dentrifices and perfumes (Anon, 1996). In mexico, the largest importer of Sri Lanka cinnamon, the bark is boiled in water and the extract taken like tea as a beverage (Anon, 1996). Cinnamon bark is one of the few spices which can be consumed directly. Cinnamon is also used as an insect repellent.[3]

2.1.1.3 Economic analysis of cinnamon production

Cinnamon makes a considerable contribution towards the export income generated from Agricultural plant products in Sri Lanka.

Sri Lanka produces more than 90 percent genuine true cinnamon (*Cinnamomum Zeylanicum* Blume) in the world. Our exports account for 63 percent of all spice exports in the world .[1] Cheap low quality alternatives have begun to enter the global market leading to increased competition.[1]

The best quality cinnamon was produced in Negombo District. The major product of the cinnamon plant is quills. It accounts for 63% of the volume and 72% of exchange earning. Other bark products are quilling, featherings, chips, ground cinnamon, cinnamon powder, leaf oil and bark oil. According to Department of Export Agriculture reports (Herath and Weerasinghe, 2004), Over 90,000 ha of wet zone land is Under Export Agriculture Crops (EAC), accounting for 9% of the land under all perennial crops. There are over 250,000 small scale growers involved in cultivation and about 60,000 of them depend on EAC as their main family income.

The main producing area of cinnamon is the costal belt; Galle (9,929 ha), Matara (7,847 ha), Rathnapura (2622 ha), Kalutara (2457 ha) and Hambantota (1,735 ha) (Anon, 2005). According to the statistics of the DEA, existing extent of cinnamon plantation at the end of 2002 was 25,294 ha. As per the data of the Department of Export Agriculture for 2005, total extent of cinnamon was increased by 819 ha during last 5 years. Cinnamon at present is the dominant spice in Sri Lanka in terms of the foreign exchange earnings. The share lies on cinnamon in terms of the total export of Agricultural products in 2004 and 2005 were 0. 86%

and 0.96%, respectively (Central bank report, 2004 and 2005). In 2005 total foreign exchange earnings from cinnamon was above the earnings from rubber by 11.3 million dollars (Central Bank report, 2005). Cinnamon revenue growth in 2005 was 23.1% above the export earnings over 2004 figures (Table 2.1).

Table 2.1: Agricultural Exports in US $ millions in Sri Lanka (Central Bank report, 2005) (Share % - the percentage share of total agriculture exports)

Commodity	2004	Share %	2005	Share %
Total Agric. Export	1065.2	18.5	1153.8	18.2
Tea	738.9	12.8	810.2	12.8
Rubber	51.3	0.9	46.9	0.7
Coconut	113.1	2	113.3	1.8
Cinnamon	49.7	0.86	61.2	0.96

About 10,000 families are actively involved with cinnamon industry. Sri Lankan Government thus emphasizes on developing the cinnamon industry by providing investment assistance credits, extension and research inputs and assistance for cinnamon based value added products. (Admin Report DEA, 2003)

The potential production level of quills as per the estimates made by the DEA (2003), is about 1000 kg/ha, in the age of 7 year plantation, which can be maintained at this level till about 40 years with the adoption of good management practices. Gross income and expenditure analysis for a new establishment of a plantation is given in the Table 2.2

Table 2.2: Annual cost and returns for establishment and maintenance of a 1 ha. Cinnamon land in Sri Lanka.
(Administrative report, 2003)

component	1st year	2nd year	3rd year	4th year	5th year	6th year	7th year	8th year	Rest
Labour cost Rs.	36049	10370	17284	30617	41481	46420	51358	56296	56296
Material inputs Rs.	10150	7370	9250	9250	9250	9250	9250	9250	9250
Total cost Rs.	46199	17740	26703	40206	51577	58208	65684	74006	74006
Gross income Rs/ha.			60720	151440	214600	253300	296600	343500	343500
Net return Rs/ha			34017	11234	163023	195092	230916	269494	269494

2.1.2 Black Pepper in Sri Lanka

Black pepper (*Piper nigrum* L.) is the most widely used spice all over the world and it is popularly known as the "King of spices". It is native to Western Gharts in India, but is now cultivated in tropics of both hemispheres (Anon, 2003). Distribution and status of recorded Piper species in Sri Lanka are given in Table 2.3.

Black pepper belongs to the family *Piperaceae.* The plant is a perennial evergreen woody climber that grows up to 10 m or more in height (Anon, 2003). Pepper shows a dimorphic branching pattern. The orthotropic branches or vegetative climbing branches give the framework to the plant. The lateral plagiotropic branches are considered as fruiting branches. Leaves are simple and alternately arranged. The spike bears 50-105 minute flowers.

Black pepper is the second most important perennial spice crop grown in Sri Lanka and it is mainly concentrated to small-scale farms. At present the national average yield of pepper is about 250 kg per hectare, but the potential dry pepper yield is over 1000 kg per hectare for high yielding cultivars (Anon, 2003).

Distribution and status of recorded Piper species in Sri Lanka are listed in table 2.3

Table 2.3: Distribution and status of recorded Piper species in Sri Lanka.

Botanical name	Common name	Global distribution	Status
Piper betle L. Bulath (S) Vettilai (T)	Betel pepper (E)	East Africa, India, Malay peninsula, Philippines, Sri Lanka	In
P. chuvya (Miq.) C. DC.	Mala bulath (S)	Cultivated in India, Sri Lanka,	In
Seewiya wel (S) Java and Sumatra		Malay Island and native to	
P. hymenophyllum Miq.	-	Southern India and Sri Lanka	Na
P. longum L. Tippili (S) Tippili (T)	Long pepper (E)	Native to North East India, Cultivated through out India Malay peninsula, Philippines, Sri Lanka and Timor	In
P. nigrum L. Gammiris (S)	Black pepper (E)	Native to South India, Cultivated in Malay Peninsula,	Na
Milaku (T)		West India, South America, Sumatra, Borneo, Philippines Sri Lanka	
P. siriboa L. Rata karal (S)	Rata bulat wel (S)	Supposedly native to Sumatra Island	In
P. sylvestre Lam.	Wal gammiris (S)	South India, Sri Lanka and introduced to Mascarene Island	Na
P. trineuron Miq.	-	Sri Lanka	En
P. walkeri Miq.	-	Sri Lanka	En
P. zeylanicum Miq.	-	Sri Lanka	En

Source: Edirisinghe (2009)
Note: E= English name; S= Sinhala name; T= Tamil name; En= Endemic; In= Introduced and Na= Native.

2.1.2.1 Climate and soil conditions for Black pepper

It can be grown from sea level up to about 1200 m above sea level in areas receiving an annual rainfall of not less than 1750 mm (Anon, 1996). It is also essential that there is sufficient rainfall during the flowering season to ensure good pollination. It can tolerate a minimum temperature of 10 ^{0}C and a maximum 40 ^{0}C (Anon, 1996). Continuous strong wind is harmful. Sheltered valleys and leeward slopes are, therefore, the best situations for growing pepper in the mid and upcountry areas. In general, suitable lands found in wet and intermediate agro-ecological zone of low country and mid-country are ideal for black pepper

cultivation (Anon, 1996). However, the irregular occurrence of short dry spells having longer than 5 consecutive dry days become frequent in recent past for the traditional pepper growing areas. Such a short dry spell may accumulate up to 15 or even 25 consecutive dry days. These dry spells occur in the period out of the designated inter-monsoonal dry periods for the wetter part of the Island. This environmental phenomenon is the major cause for poor performance of field establishment following field planting of rooted pepper cuttings. Similarly, high variation in annual yield of bearing vines is also attributed to the variations in distribution of short dry spells rather than total rainfall (Sumanasena, 2007). Therefore, adoption of good agronomic practices would be highly beneficial for good post-nursery field establishment as well as subsequent improvement in productivity of pepper vines under the limitation of both ideal lands and irrigation facilities.

2.1.2.2 Pepper cultivars

Pepper plant can yield economically for a few decades and this feature makes it very necessary to use only genetically superior high yielding types (Anon, 1996). In order to identify superior lines from among the local vines, a selection programme has been launched with over 500 accessions and the selected materials are being evaluated at the Export Agriculture Research Station, Matale, Sri Lanka (Anon, 1996). Ten lines have been identified after a rigorous screening procedure for certain important characteristics such as high pungency, bold berries, continuous bearing habit and resistance to pests and diseases etc. (Anon, 1996). Thus at this stage, the Department of Export Agriculture is making mass propagation of these selected 10 local lines as one composite mixture named as PNMI (Anon, 1996). The two commercial varieties Panniyur-I of India and Kuching of Malayasia have been evaluated in Sri Lanka for the last 15 years (Anon, 1996). Experimental observations of the research division revealed that the cultivation of Panniyur-I along with the local selections will be economically beneficial (Anon, 1996).

2.1.2.3 Uses

Pepper has a variety of uses. From the ancient use in embalming and medicine it is now largely used by meat packers and in canning, pickling, baking, and confectionary and preparation of beverages (Anon, 1996). Black pepper constitutes an important component of culinary seasonings of universal use and an essential ingredient of numerous commercial food stuffs (Anon, 1996). The oil of pepper obtained by the steam distillation of crushed black pepper or as a by product in the manufacture of white pepper by steaming is a valuable

adjunct in the flavoring of sausages, canned meats, soups, table sauces and certain beverages and liquors. It is also used in perfumery (Anon, 1996).

2.2 Fertilizer use pattern in export agricultural crops

In Sri Lanka export agricultural crops (EAC) are becoming more important during the last two decades in comparison to traditional plantation crops such as tea, rubber and coconut. Sri Lanka is the world's largest producer and exporter of cinnamon accounting for nearly two-third of the global output and black pepper is the second The increase of fertilizer prices and withdrawal of fertilizer subsidies from 2002 onwards, affected the production levels of black pepper and cinnamon (two major EAC). Apart from this, many small holders, mainly pepper growers resorted to organic farming in recent past mainly due to the high cost of inorganic fertilizers and pesticides and the increased price of organically cultivated produces.

2.2.1 Fertilizer application for Cinnamon

Fertilizer application is important for commercial cultivation of cinnamon for higher productivity, as cinnamon is normally grown as a long-term monoculture. In the absence of fertilizer application, the supply of nutrients available in the soil will become exhausted leading to mineral deficiencies and drop of yield; dry matter yield as well as the oil content of the bark and leaves and poor stooling. The requirement of fertilizer is based on the results of field experiments, soil and plant tissue analysis or the symptoms of mineral deficiencies or toxicities. As such the optimum applications will vary from one region, plantation or field to another in acco.dance with local conditions. The Department of Export Agriculture of Sri Lanka recommends following fertilizer mixture and quantities, for cinnamon in Sri Lanka (Table 2.4).

Fertilizer mixture

Table 2.4: Recommended fertilizer mixture for Cinnamon (by DEA)

Composition	Ratio by Weight	Mineral content in the Mixture
Urea	2	N - 23%
Rock phosphate (28%) P_2O_5	1	P_2O_5 - 07%
Muriate of Potash (60%) K_2O	1	K_2O - 15%

Rate of Application

As Fertilizer requirement varies according to the age of plantation, the recommended rates of fertilizer for young plantations (Anon, 2005) are: 200 kg/ha/annum in the first year, 400 kg /ha/annum in second year and 600kg/ha/annum in third year.

Those quantities have to be applied as two splits at six months intervals. It is important to apply fertilizer when soil is under moist conditions or at the commencement of the rains. Fertilizer is to be applied at 50 cm radius around the plant or between rows. After application of fertilizer it is important to fork it into soil. In addition when there are symptoms of magnesium deficiency, the application of Dolomite at the rate of 500 kg/ha 2-3 months before applying the recommended fertilizer mixture would be advantageous. Maintaining soil pH around 4.5-5.5 facilitates better absorption of nutrients by cinnamon roots (Anon, 2005).

After 3 years when the cinnamon is mature, the dose of fertilizer should be doubled for every successive application thereafter. (Anon, 2005).

2.2.2 Fertilizer application recommendations for Pepper

Application of inorganic fertilizer would be helpful for obtaining good field establishment and higher yield sufficiently early. A vine in full bearing (i.e. 3rd year onwards) should receive an annual dose of 196 g N, 154 g P_2O_5, 196 g K_2O and 28 g of MgO per vine. During the first and second years of planting, the vines will receive 1/3 and 2/3 of the above dose respectively. The mixture should be applied in two splits at the beginning of each rainy season. Ring application at 15cm away from base is recommended (Anon, 2002).

According to a recent experiment, the inorganic fertilizer dosage can be reduced by 50%, if 10-15 kg fresh *Gliricidia* lopping (leaves and tender shoots) can be applied as mulch at a rate of four times per year per vine.

2.3 Need for improved nutrient management practices in organic farms

Existing fertilizer recommendations for most of the EAC are based on inorganic N, P, K, and Mg mixtures formulated from the results of field experiments designed and carried out during the existence of fertilizer subsidy schemes. Currently there is no information available on nutrient management practices that can be used by organic farmers whose numbers are on the increase in Sri Lanka. The research division of the Department of EAC recently commenced research on developing novel integrated nutrient management practices focusing on green manure use as well as the effective utilization of nutrients in the native soils. The consumption of fertilizer in Sri Lanka has been increasing steadily during the last 25 years. At the present time the imports of phosphate to Sri Lanka are; single super phosphate 80,000-120,000 metric tons and triple super phosphate 30,000-40,000 metric tons per year (Illeperuma, 1998). For a developing country like Sri Lanka which imports almost all its requirements of chemical fertilizers, a rapid increase in the world market price of fertilizer causes heavy drain on the foreign exchange reserves (Wijewardena, 1990). Developing plants that efficiently tap soil P reserves and low grade PR is therefore a priority for agricultural research (Trolove *et al*, 2003). The cost of P fertilizer will increase as the currently accessible deposits of high grade phosphate rock (PR) diminishes. Only the naturally occurring phosphate rock is recommended as a phosphorous source for the organic framing systems. As Sri Lanka has a locally available phosphate rock (Eppawella phosphate rock- EPR), this rock is recommended as a P fertiliser source for organic farms and other perennial crops.

2.4 Chemical and physical properties of Sri Lankan soil

Available P status of twenty acidic soils (pH 4.5-6.83) was determined by extracting with five different extractants, namely Olsen, Bray-1, Bray-2, Calcium lactate (CAL) and De-ionized water (Table 2.5) (Withana and Kumaragamage, 1995).

Table 2.5: Amount of P extracted by different methods and cumulative P uptake values for some acidic soils of Sri Lanka.

Soil	Olsen	Bray-1	Bray-2	CAL water	De-ion uptake	Cum. P
Minipe	2.90	0.68	1.29	0.54	0.07	2.09
Naula	1.15	0.34	0.55	0.19	0.06	1.88
Maha Illuppalama	1.35	0.14	0.22	0.12	0.10	1.81
Girandurukotte	2.49	0.88	1.31	0.61	0.10	2.15
Dodangolla	1.50	0.31	0.92	0.29	0.06	2.25
Nikaweratiya	4.40	1.99	6.04	1.96	0.47	2.85
Pallama	1.05	0.18	0.22	0.15	0.05	2.24
Pelawatta	2.40	0.39	0.46	0.33	0.07	2.13
Udadumbara	0.99	0.32	0.37	0.26	0.04	2.04
Matale	0.65	0.07	0.29	0.04	0.03	1.84
Hunnasgiriya	1.15	0.32	0.35	0.29	0.03	2.15
Nuwaraeliya	1.99	0.69	0.89	0.61	0.03	2.16
Lunuwila	0.95	0.20	0.45	0.12	0.08	2.06
Nanuoya	2.10	0.91	1.66	0.83	0.04	2.09
Kiribathkumbura	1.60	0.06	0.11	0.04	0.03	2.33
Awissawella	2.35	0.25	0.29	0.19	0.03	2.01
Mawathagama	0.75	0.07	0.22	0.06	0.03	2.18
Kegalle	1.25	0.53	0.55	0.39	0.03	2.02
Nawalapitiya	0.85	0.29	0.42	0.19	0.03	1.80
Rathnapura	1.71	0.32	0.37	0.26	0.03	2.07

Source: Withana and Kumaragamage (1995)

Suitability of these extractants in relation to soil chemical properties, mainly soil pH and P buffer capacity was studied considering the relationship between P quantity extracted and P uptake by an indicator plant under greenhouse conditions over 120 days (Table 2.6) (Withana and Kumaragamage, 1995).

Table 2.6: Soil physical and chemical properties of some selected locations in Sri Lanka

Location /soil type	pH	CEC (meq/100g soil)	OM (%)	Texture	Exchangeable Ca (meq/100g) soil)	MBC (µg P sorbed/ µg P solution)
Minipe	6.83	21.7	1.26	Sandy Loam	5.53	78.55
Naula	6.50	31.8	2.07	Loamy Sand	6.66	1854.60
Maha Illuppalama	6.71	24.2	2.21	Sandy Loam	5.80	240.50
Girandurukotte	6.12	18.1	1.64	Loamy Sand	1.46	34.19
Dodangolla	6.16	26.0	1.17	Sandy Loam	3.85	27.46
Nikaweratiya	6.13	13.7	1.47	Loamy Sand	3.40	22.35
Pallama	6.05	14.6	0.31	Sand	0.69	11.90
Pelawatta	5.77	16.9	1.09	Sandy Loam	4.41	185.08
Udadumbara	5.78	16.4	2.50	Sand	4.66	86.53
Matale	5.85	32.9	2.50	Sandy Loam	3.63	3060.60
Hunnasgiriya	5.80	25.0	3.16	Loamy Sand	0.91	233.80
NuwaraEliya	5.03	41.9	5.36	Loamy Sand	1.20	5716.70
Lunuwila	5.77	22:.7	0.85	Loamy Sand	0.80	5.30
Nanuoya	4.17	27.0	5.74	Loamy Sand	0.49	83.43
Kiribathkumbura	4.93	17.6	1.53	Sand	1.19	870.88
Awissawella	4.65	15.3	2.50	Sand	0.43	421.40
Mawathagama	4.83	19.3	1.52	Sandy Loam	1.00	208.57
Kegalle	4.73	22.3	3.69	Sandy Loam	0.34	2906.90
Nawalapitiya	4.75	17.7	2.36	Loamy Sand	0.56	2310.90
Rathnapura	4.50	21.3	2.55	Loamy Sand	0.21	433.60

CEC=Cation exchange capacity OM=Organic matter MBC=maximum buffer capacity

Source: Withana and Kumaragamage (1995)

Loganathan *et al* (1984) showed that soil supporting coconut in Sri Lanka were generally deficient in total as well as the active and available forms of P. Except some of the sandy soils (Negombo, Katunayake and perhaps Madampe) of the entisols the rest of the soils were severely deficient in P. Phosphorus deficiency in the soils was confirmed by the low levels of leaf P in the coconut palms (Loganathan *et al.,* 1984).

Application of P fertilizers is therefore essential to achieve high production of crops on soils in Sri Lanka. With the present trend of intercropping in coconut lands, deficiency of P would be aggravated unless adequate P fertilizers are added (Loganathan *et al*; 1984). As described by Zoysa and Loganathan (2001) in Phosphorus nutrition of Tea, Tea soils are generally strongly acidic (pH 4.0-5.5) and are rich in oxides and hydroxyoxides of ion and aluminium which are known to fix phosphorus. This led to the belief that high rates of phosphorus fertilizers need to be applied in order to obtain yield responses in tea. However, in practice rates as low as 5-15 kg phosphorus $ha^{-1}yr^{-1}$ were found to be sufficient to achieve maximum yield (Zoysa and Loganathan, 2001).

Jayawickrama *et al.*, (1991), have carried out an investigation on phosphate adsorption in six Sri Lankan soils in relation to their chemical and mineralogical properties (Table 2.7). In here soils were ranked according to their P adsorption maxima as follows: IBL < REG < RBE < RYL < RBL <RYP. In Sri Lanka pepper is cultivated over an area of 15,000 ha mostly in the districts of Matale, Kandy, Kegalle, Kurunegala and Nuwara-Elliya having soil type reddish brown latasolic (Anon,1996). Cinnamon is cultivated under varying soil conditions like silver sand of Kadirana, Ekala and Ja-Ela to loamy and lateritic gravelly soils of Kalutara, Galle and Matara districts (Anon, 1996).

Table 2.7: Some physical and chemical properties of the six Sri Lankan soils

Soil	Abbreviation	CLAY %	O.C. %	pH	CEC cmol/Kg	EX.Ca cmol/Kg
#Reddish Brown Earth *Rhodustalfs	RBE	18.9	0.61	5.4	10.80	76.0
#Reddish Brown Latasolic *Rhodudults	RBL	42.1	0.46	5.0	20.60	98.8
#Immature Brown Loam *Ustropepts	IBL	9.2	0.65	5.3	10.50	93.8
#Red Yellow Podsolic *Tropudults	RYP	66.9	0.19	6.2	34.30	80.8
#Red Yellow Latasol *Eutrustox	RYL	22.1	0.65	5.9	11.50	103.1
#Regosol *Quatzpsamments	REG	15.2	0.50	6.4	9.15	98.1

O.C-Organic Component, CEC- Cation Exchange Capacity, Ex. Ca.- Exchangeable Ca

Great soil group name
* 7th Approximation name

Source: Jayawickrama *et al* (1991)

2.5.1 Role of Phosphorus (P) in plants

Essentially all soils require regular inputs of fertilizer or manure P to raise their solution P concentration such that P can be transported to plant roots at rates sufficient to meet requirements for continuous crop production (Trolove *et al*, 2003).

Phosphorus is a nutrient required by all organisms for the basic processes of life. Phosphorus is a natural element found in rocks, soils and organic material. Phosphorus clings tightly to soil particles and is used by plants, so its concentrations in clean water are generally very low. However, phosphorus is used extensively in fertilizers and other chemicals, so it can be found in higher concentrations in areas of human activity. Many seemingly harmless activities added together can cause phosphorus overloads.[4]

Phosphorus exists in water in either a particulate phase or a dissolved phase. Particulate matter includes living and dead plankton, precipitates of phosphorus, phosphorus adsorbed to particulates, and amorphous phosphorus. The dissolved phase includes inorganic phosphorus and organic phosphorus. Phosphorus in natural waters is usually found in the form of phosphates (PO_4^{-3}). Phosphates can be in inorganic form (including orthophosphates and polyphosphates), or organic form (organically-bound phosphates). [4]

It is difficult to state in detail the functions of phosphorus in the economy of even the simplest plants. According to Brady (1974) only the more important functions will be considered here. Phosphorus makes its contribution through its favorable effect on the following:

i. Cell division and fat and albumin formation.
ii. Flowering and fruiting, including seed formation.
iii. Crop maturation, thus counteracting the effect of excess nitrogen applications.
iv. Root development, particularly of the lateral and fibrous rootlets
v. Strength of straw in cereal crops, thus helping to prevent lodging.
vi. Resistance to certain diseases.

[4] General Information on Phosphorus-

<http://bcn.boulder.co.us/basin/data/NEW/info/TP.html>/06/03/2010/Sheila

Phosphorus is a vital component of DNA, the genetic "memory unit" of all living beings. The structures of both DNA and RNA are linked together by phosphorusBonds.[4] Phosphorus is a vital component of ATP, the "energy unit" of plants. ATP forms during photosynthesis, has phosphorus in its structure, and processes from the beginning of seedling growth through to the formation of grain and maturity.[5] Some specific growth factors that have been associated with phosphorus are: stimulated root developments, increased stalk and stem strength, improved flower formation and seed
production, more uniform and earlier crop maturity, increased nitrogen N-fixing capacity of legumes, improvements in crop quality, and increased resistance to plant diseases.[5]

2.5.2 Phosphorus compounds in soils

Phosphorus compounds in soil can be placed into the following three classes:

i. Organic compound of the soil humus,

ii. Inorganic compounds in which the P is combined with Ca, Mg, Fe, Al and with clay minerals,

iii . Organic and inorganic P compounds associated with the cells of living matter. Microorganisms are involved in transformations of phosphorus between organic and mineral forms.[6]

[5] Efficient Fertilizer Use — Phosphorus: Dr. Bill Griffith <http://www.rainbowplantfood.com/agronomics/efu/phosphorus.pdf>/06/03/2010/

[6] Organic Phosphorus (http://karnet.up.wroc.pl/~weber/fosfor2.htm) 06/03/2010

Both inorganic and organic forms of phosphorus occur in soils and are important to plants as sources of this element (Brady, 1974). Thus there has been, and still is, ample justification for research directed at identifying plant species and plant mechanisms that improve the ability of plants to utilize sparingly-soluble forms of soil and fertilizer P (Trolove *et al*, 2003). The chemical properties of solid-phase soil P is diverse, including inorganic P strongly adsorbed or chemisorbed on amorphous hydrous oxides of Fe and Al, discrete more crystalline phases of P (which tend to be dominantly Fe and Al phosphates in more strongly weathered acid soils but include more Ca phosphates in less weathered weakly acidic and neutral to alkaline soils) and complex mixtures of organic P (Trolove *et al*, 2003). The organic P can also be present in an adsorbed form - some is present as discrete low-molecular-weight ions adsorbed on the hydrous oxide surfaces, however the majority of organic P is present as humic and fulvic organo-metallic P complexes (Trolove *et al*, 2003).

2.5.2.1 Organic Phosphorous compounds

Organic phosphorus is found in plant residues, manures, and microbial tissues. Soils low in organic matter may contain only 3% of their total phosphorus in the organic form, but high organic matter soils may contain 50% or more of their total phosphorus content in the organic form.[5] From 15 to 80% of the phosphorus in soils occurs in organic forms, the exact amount being dependent upon the nature of the soil and its composition. The higher percentages are typical of peats and uncultivated forest soils.

From the standpoint of plant nutrient, phosphorus is adsorbed by plants largely as the negatively charged primary and secondary orthophosphate ions ($H_2PO_4^-$ and HPO_4^{2-}) which are present in the soil solution. Small quantities of soluble organic P compounds are also present in water extracts of soil.[6]

There has been relatively less work done on the organic phosphorus compounds in soils, even though this fraction in some cases comprises more than one half of the total soil phosphorus. One of the reasons for lack of information on these compounds is because they apparently are exceedingly complex (Brady, 1974). The meager data available, however, indicate that the three main groups of organic phosphorus compounds found in plants are also present in soils (Brady, 1974). These are (a) phytin and phytin derivatives, (b) nucleic acids, and (c) phospholipids. There are likely other organic phosphorus compounds present in soils; some investigators doubt that those listed account for all the organic phosphorus (Brady, 1974).

2.5.2.2 Inorganic P compounds

Inorganic forms of soil phosphorus consist of apatite (the original source of all phosphorus), complexes of iron and aluminum phosphates, and phosphorus absorbed on clay particles. The solubility of these phosphorus compounds, as well as organic phosphorus is extremely low and only very small amounts of soil phosphorus are insolution at any one time. Most soils contain less than a pound per acre of soluble phosphorus, with some soils containing considerably less. Through adequate phosphorus fertilization and good crop/soil management, soil solution phosphorus can be replaced rapidly enough for optimum crop production.[5]

Most inorganic phosphorous compounds in soils fall into one of two groups: (a) those containing calcium, and (b) those containing iron and aluminium (Brady, 1974). Fluorapatite, the most insoluble and unavailable of the group, usually is an original mineral (Brady, 1974). It is found in even the more weathered soils, especially in their lower horizons. This fact is an indication of the extreme insolubility and consequent unavailability of the phosphorus contained therein (Brady, 1974). The simpler compounds of calcium, such as mono and dicalcium phosphate, are readily available for plant growth. Except on recently fertilized soils, however, these compounds are present in extremely small quantities since they easily revert to the more insoluble forms (Brady, 1974).
Much less is known of the exact constitution of the iron and aluminium phosphates contained in soils. The compounds involved are probably hydroxy phosphates such as dufrenite, wavellite, strengite, and variscite. These compounds are most stable in acid soils and are extremely insoluble. (Brady, 1974).
Phosphates react with certain iron or aluminium silicate minerals such as kaolinite. There is some uncertainty, however, as to the exact form in which this phosphorus is held in the soil. Most evidences indicate that it, too, is probably fixed as iron or aluminium phosphates (Brady, 1974).

Inorganic phosphate is phosphate that is not associated with organic material. Types of inorganic phosphate include orthophosphate and polyphosphates.[4]

Orthophosphate is sometimes referred to as "reactive phosphorus." Orthophosphate is the most stable kind of phosphate, and is the form used by plants. Orthophosphate is produced by natural processes and is found in sewage. Polyphosphates (also known as metaphosphates or condensed phosphates) are strong complexing agents for some metal ions. Polyphosphates are

used for treating boiler waters and in detergents. In water, polyphosphates are unstable and will eventually convert to orthophosphate.[4]

2.5.3 Soil Phosphorus analysis methods

The purpose of determining phosphorus in soils is to characterize the phosphorous in the soil system. Three different procedures which yield different indices would be relevant to this study are described below. In general, soil P determinations have two distinct phases.

First, the preparation of a solution containing the soil P or fraction thereof and second, the quantitative determination of the P in this solution. The molybdenum blue method is sensitive and suitable for extracts containing small amounts of P as well or for total P in soil. The method is based on the principal that in an acid molybdate solution containing orthophosphate ions, a phosphomolybdate complex formed that can be reduced by stannous chloride and other reducing agents to a molybdenum blue colour. The intensity of the blue colour varies with the P concentration but is affected also by other factors such as acidity, arsenates, silicates and substances which influence the oxidation-reduction condition of the system.

Phosphorus soluble in sodium bicarbonate (Olsen method)

Phosphorus is extracted from the soil with 0.5M $NaHCO_3$ at a nearly constant pH of 8.5. In calcareous, alkaline or neutral soils containing calcium phosphates, this extractants decreases the concentration of Ca in solution by causing precipitation of Ca as $CaCO_3$; as a result, the concentration of P in solution increases. In acid soils containing aluminum and iron phosphates such as variscite and strengite, P concentration in solution increases as the pH rises (Olsen and Dean, 1965).

Phosphorus soluble in Dilute Acid Fluoride (Bray method)

This method has been used widely as an index of available P in soils. The combination of HCl and NH_4F is designed to remove easily acid soluble forms of P, largely calcium phosphates and a portion of the aluminum and iron phosphates. The NH_4F dissolves aluminum and iron phosphates by its complex ion formation with these metal ions in acid solution. In general this method has been most successful on acid soils (Olsen and Dean, 1965).

Phosphorus soluble in Water

This method simply involves measuring the concentration of P in a water extract of the soil. With soils low in available P, absorption of P and growth of plants increase as concentration of P increases in the soil solution up to a limit. As an index of P availability, the objective of this method is to determine the P concentration level in the soil solution that limits the growth of plants (Olsen and Dean, 1965).

2.5.4 Natural rock phosphate from deposits

Mining of apatite deposits is the only economical source of phosphorus for phosphate fertilizers. Phosphorus is a vital resource for sustaining world agriculture. There are about 414 apatite deposits in some 40 countries in the world. The United States is the world's leading producer of phosphate fertilizers with about 30% of the total production, followed by China, Morocco and Russia. The other prominent phosphate production countries are Brazil, Israel, Kazakhastan, Senagal, South Africa, Togo and Tanzania (Lanka Phosphate Limited Estate Company facts, n.d.).

2.5.5 Eppawala Phosphate Deposit

The Eppawala carbonatite complex is the first recorded occurrence of carbonatite in Sri Lanka and was discovered in early 1971 during the course of systematic geological mapping of the Anuradhapura 1" topographical sheet (Jayawardane, 1976).

This apatite contains Calcium phosphate that can be used as a phosphate fertilizer. According to LPLEC news present reserve position is at 60 million metric tons containing about 33-40% of phosphate. This deposit is considered to be one of the richest and unique apatite deposits in the world. For the last 30 years we have consumed about 2% of the deposit. This deposit is a high-grade one because it is rich in phosphate crystals. Our deposit is an igneous type deposit. This deposit has come to the present position due to process of uplift occurrence though millions of years.

Eppawala deposit represents less than 1% of the world phosphate reserves. According to the studies the world's reserve position is about 10,000 million metric tons. The annual phosphate consumption in the world is about 200 million metric tons. As such the phosphate deposits all over the world will last for another 50 years and after such time phosphorus will be a one of the scarcest element in the world.

Eppawala apatite ore has two components that are separately identified by naked eye. They are greenish colour primary apatite crystals and brownish colour aluminous-ferruginous-siliceous secondary apatite. Due to inclusion of aluminium, iron and silica secondary apatite has less water solubility. Phosphate mining at Eppawala is carried out to market these two components separately. The commonly available variety is aluminous- ferruginous-siliceous phosphate is marketed as Eppawala Rock phosphate (ERP). The other component that is primary crystal is marketed as High Grade Eppawala Rock Phosphate (HERP). Both fertilizers are used for perennial crops. Application is not recommended for short-term crops such as paddy and vegetables due to low solubility in water. However, Eppawala Phosphate fertilizer is the only fertilizer extracted locally except Dolamite. More than 92% of the total fertilizer consumption of the country is done though imports. In this context the locally available Eppawala deposit has an important role to play for the betterment of the agriculture. At present 45,000 mt of Eppawala phosphate is marketed locally per year. Tea plantations dominate 60% of the use of Eppawala phosphate whereas rubber and coconut sectors share 30% of the use. The export cash crops and the fruit crops mainly share the balance. By using our own phosphate fertilizer, a substantial amount of foreign exchange, about Rs. 400 million annualy can be saved.

The solubility will depend also on the soil conditions and the research work carried out by various agricultural organizations have indicated that the ground apatite bearing ore from Eppawala is suitable for long term crops such as tea, rubber and coconut (Jayawardane, 1976, Rathnayake *et al*; 1994, Illeperuma, 1998, Lanka Phosphate Limited Estate Company facts, n.d.).
In Sri Lanka, imported phosphate fertilizers, such as Triple superphosphate (TSP) are being applied for the production of rice and other annual crops (Rathnayake, 1994).

Phosphate mineral is very insoluble and it cannot be directly applied for crops like paddy and vegetables. For this reason, Eppawala phosphate has to be treated with strong acids like sulfuric to produce more soluble phosphate fertilizers. The government has taken initiatives to produce Single Super Phosphate (SSP) by using Eppawala Phosphate. Single Super Phosphate is highly water soluble and can be used as an effective Phosphate fertilizer for short terms crops. (Lanka Phosphate Limited Estate Company facts, n.d.).

However, recent geological, geochemical and mineralogical studies have shown that ERP could be separated in to two components: coarse primary apatite crystals and a finer

aluminous- ferruginous-siliceous secondary phosphate matrix (Dahanayake, 1995). The primary apatite crystals have higher P_2O_5 contents, higher solubilities and lower R_2O_3 contents than those of the secondary matrix. The Eppawala carbonatite have a higher concentration of P relative to titanium in agreement with carbonatites from other occurrences (Jayawardena, 1976). Preliminary agronomical experiments have shown that primary apatite has the potential as a directly applied fertilizer to grow short term crops on certain acid soils (Dahanayake, 1995). Another preliminary laboratory study was conducted by Weeraratne (1983) on increasing phosphorus availability in Eppawala apatite.

The country spends about Rs. 1,200 million every year to import Triple Super Phosphate (TSP); which is the major phosphate fertilizer used for paddy and vegetables. By manufacturing Single Super Phosphate we will be able to save a substantial amount of foreign exchange vested on imports yearly. With the energy crises in the world, prices of phosphorus fertilizers are steadily increasing over the years, resulting an increase in the cost of cultivation adding burden to farmers and the government (Lanka Phosphate Limited Estate Company facts, n.d.).

In this context, incooperation of micorrizal inoculum for ERP into already using perennial crop sector may enhance the resource efficiency of the system in principle.

2.5.6 The phosphorus problem

Although the amount of total phosphorus in an average mineral soil compares favorably with that of nitrogen, it is much lower than potassium, calcium, or magnesium (Brady, 1974). Of even greater importance, however, is the fact that most of the phosphorus present in soils is currently unavailable to plants. Also, when soluble sources of this element are supplied to soils in the form of fertilizers, their phosphorus is often "fixed" or rendered insoluble or unavailable to plants, even under the most ideal field conditions (Brady, 1974). Large reserves of P have accumulated in soils of developed countries because additions of P fertilizer to sustain agricultural production have exceeded crop removal (Trolove *et al.*, 2003). By contrast, in many developing countries in the tropics and subtropics, soil P reserves are gravely low and large additions are required before maintenance levels begin to decline (Trolove *et al.*, 2003). The unavailability of applied Phosphorus fertilizer is a huge problem in Sri Lanka, too. This problem is affecting Tea, Rubber and Coconut plantations of Sri Lanka (Kalpage, 1973). The study conducted by Kendaragama *et al.*, (2003) indicated that practice of correct P supply is needed for sustaining available P status in soil and crop yield in Alfisols

in Sri Lanka although rice does not immediately respond for irregularities in phosphate fertilizer application.

Fertilizer practices in many areas exemplify the problem of phosphorus availability. As already emphasized, the tonnage of phosphorus-supplying materials used as fertilizers definitely exceeds all except the nitrogen carriers (Brady, 1974). The removal of phosphorus from soils by crops, however, is low compared to that of nitrogen and potassium, often being only one third or one fourth that of the latter elements. The necessity for high fertilizer dosage when relatively small quantities of phosphorus are being removed from soils indicates that much of the added phosphates become unavailable to growing plants, or, especially water soluble phosphates are lost as runoff polluting water bodies (Brady, 1974).

The overall phosphorus problem is threefold: (a) a small total amount present in soils, (b) the unavailability of such native phosphorus, and (c) a marked "fixation" of added soluble phosphates. Increasing the availability of native soil phosphorus and the retardation of fixation or reversion of added phosphates are, therefore, the problems of greatest importance (Brady, 1974).

Crops absorb phosphorus in the form of soluble orthophosphate ion (Ponmurugan and Gopi, 2006). The solubility of phosphate is inhibited by the presence of iron and aluminium in acidic soils and calcium in neutral and alkaline soils (Ponmurugan and Gopi, 2006). This leads to fixation of phosphorus, making it not available to crop plants.

2.5.7 Environmental problems with P fertilizers

Phosphorus cannot be readily washed out of the soil, but is bound to soil particles and moves together with them. Phosphorus can therefore be washed into surface waters together with the soil that is being eroded. Phosphorus is not considered to be dangerous, but it stimulates the growth of algae in slow moving water leading to eutrophication of the water body.[7]

It is important to remember that there are a number of sources of these pollutants including industrial waste, sewerage disposal, detergents and manures.

[7]Environmental problems with fertilizers
<http://www.agroservicesinternational.com/Environment/Problems.html> 06/03/2010

2.5.8 Factors that control the availability of inorganic soil phosphorus

The availability of inorganic phosphorus is largely determined by the following factors: (a) soil pH; (b) soluble iron, aluminium, and manganese content; (c) presence of iron, aluminium and manganese containing minerals; (d) available calcium and calcium minerals; (e) amount and decomposition of organic matter; and (f) activities of microorganisms. The first four factors are interrelated because their effects are largely dependent upon soil pH.

2.5.9 Phosphorus solubilizing Microorganisms (PSM)

Natural PR's have been recognized as a valuable alternative source for P fertilizer, especially for acid soils. The economic value of the rocks increases considerably along with the increasing costs of SP production (Goenadi *et al.*, 2000). Many studies have demonstrated ineffectiveness of finely ground Phosphate Rock use due to the low solubility of its P contents (Didek *et al.*, 2000). Microorganisms play an important role in effecting the availability of soil P to plant roots and increasing P mobilization in soil, though the development of effective microbial inoculants remains a major scientific challenge
(Alikhani *et al.*, 2006). These microorganisms includes rhizobia, bacteria, fungi and actinomycetes.

2.5.10 Application of Phosphorus solubilizing Microorganisms (PSM) in Agriculture

Microbial solubilization of hardly soluble mineral phosphates in soil is an important process in natural ecosystems and in agricultural soils. In addition to traditional methods of mineral phosphate fertilization, microbial P solubilization may increase the availability of phosphates in arable soils (Mikanova and Novakova, 2002).

The phosphate solubilization activity of rhizobia native to Iranian soils were investigated by Alikhani *et al.,* (2006) and it was found that many rhizobia isolated from Iranian were able to mobilize P from organic and inorganic sources (Alikhani *et al.*, 2006). A similar study was conducted by Molla and Chowdhury (1984) of Bangladesh. They examined the microbial mineralization of organic phosphate in soil by some selected phosphate dissolving microorganisms from non- rhizosphere and rhizosphere of plants. These isolates included bacteria, fungi and actinomycetes. It was also recorded that microbial solubilization have applications in saline soils as well (Rajankar *et al.*, 2007). In India, it was observed that the fungi viz; *Aspergillius* spp., *Penicillium* spp. and *Fusarium* spp. have the more solubilizing ability of inorganic insoluble phosphate than bacteria, viz; *Basilus subtilis*, and *Basilus megatherium* (Rajankar *et al.*, 2007).

Phosphate solubilizing fungi (PSF) are an important contributor in microbial P-mobilization and would be important possible way to increase available P for plant (El-Azouni, 2008). They have concluded that the amendment of soil with TCP (tri-calcium-phosphate) along with the application of P solubilizing fungi is suggesting as a sustainable way for increasing crop yield and also improving the physio-chemical properties of the soil (El-Azouni, 2008).

It is important to note that Babana and Antoun (2006) reported that by inoculating seeds with Tilemsi Phosphate rock (TPR) solubilizing microorganisms and AM fungi under field conditions in Mali it was possible to obtain wheat grain yields comparable to those produced by using the expensive diammonium phosphate (DAP) fertilizer (Babana and Antoun, 2006). In an another study, combined inoculation of Rhizobium and 'Phosphate solubilizing' *Pseudomonas striata* or *Bacillus polymyxa* with and without added chemical fertilizer on chickpea yield and nutrient content was studied under greenhouse conditions. While the single inoculation of *Rhizobium* increased the nodulation and nitrogenase activity, the 'phosphate-solubilizers' increased the available phosphorus content of the soil. Combined inoculation of *Rhizobium* and *Pseudomonas striata* or *Bacillus polymixa* increased the above parameters and also the dry matter content, the grain yield and nitrogen and phosphorus uptake significantly over the uninoculated control. The inoculation effects were more pronounced in the presence of added fertilizers. The possibilities of saving half the dose of N and replacing superphosphate with rockphosphate and inoculation with 'phosphate solubilizers' are discussed (Alagawadi and Gaur, 1988). There was a significant reduction of nitrogen fertilizer when applied with phosphate solubilizing fungi and bacteria combinely.

A super strain of *Bacillus firmus* (NCIM-2636) having high ability to solubilize insoluble inorganic phosphates were applied in acid soils of Nagaland, India. Rice (*Oryza sativa* L.) variety Jaya and IR-8 were grown in two successive years 1980 and 1981 after proper manuaring the soils received single super phosphate (S.S.P) and Mussoorie Rock Phosphate (R.P) separately at different doses.

[8]Curtis E. Swift, Mycorrhiza and soil phosphorus levels (http://mining.state.co.us/TechnicalBulletins/MycorrhizaAndSoilPhosphorusLevels.pdf.)/1/11/2008

Yield of crop in both the years increased significantly due to bacterial inoculation. From the study, the authors conclude to recommend the application of the efficient phosphate solubilizing and phytohormone producer together with supplementation of organic matter where necessary with moderate dose of R.P. (17.50 Kg P ha^{-1}) for increased crop yield and phosphatic fertilizer economy (Datta *et al.*, 1982).

2.6.1 Arbuscular mycorrhizae (AM) synonym Vesicular Arbuscular mycorrhizae (VAM)

Mycorrhizae are formed with the roots of most vascular plants, taking the form of ectomycorrhizae (characterised by dense mycelial sheaths around the roots and intercellular hyphal invasion of the root cortex), which are limited to mostly temperate forest trees, or endomycorrhizae (characterised by external hyphal networks in the soil and extensive growth of arbuscles (and commonly vesicles) within the root cortex cells of the host), which are formed by nearly all other plants (Trolove *et al.*, 2003).

The AM fungi expand their filaments in soil and within plant roots. This filamentous network promotes bi-directional nutrient movement where soil nutrients and water move to the plant and plant photosynthates flow to the fungal network.[1] The partners in this association are members of the Kingdom Fungi (Basidiomycetes, Ascomycetes and Zygomycetes) and most vascular plants (Brundrett *et al.*, 1996). The carbohydrates are translocated from their source location (usually leaves) to the root tissues of plants and then to the fungal partners. In return, the plant gains the use of the mycelium's very large surface area to absorb water and less soluble mineral nutrients such as P and Zn from the soil. The mechanisms of increased absorption are both physical and chemical. Mycorrhizal mycelia are much smaller in diameter than the smallest root, and can explore a greater volume of soil, providing a larger surface area for absorption. The cell membrane chemistry of fungi is different from that of plants. Mycorrhizae are especially beneficial for the plant partner in nutrient-poor soils.[1]

In fact almost all vascular plants (which excludes mosses, other fungi etc) benefit in nature from a mycorrhizal association of one kind or another. Although mycorrhizae are by no means essential to the well-being of any plant, their associations are of tremendous benefit in less than ideal circumstances. For example, a tree planted in fertile, moist yet well-drained soil with a good supply of readily available nutrients will already be growing at its maximum rate with maximum health, and so has little need of mycorrhiza. Indeed, as we will see later,

mycorrhizal fungi would probably not survive for long in such conditions anyway. On the other hand, trees planted in marginal conditions would probably not survive without a mycorrhiza and it is in these conditions that mycorrhyzae will thrive.[9]

As described by Dalpe and Monreal[1] and Xioutang (1994) many factors may influence the dependency (Plants differ greatly in their need for and response to mycorrhizal infection) on mycorrhiza, such as species of mycorrhiza, root morphology, plant growth rate, tillage system, fertilizer application, mycorrhizal fungi inoculum's potential and host crop dependency to mycorrhizal colonization.

Arbuscular mycorrhizal (AM) fungi are obligate symbionts and cannot be cultured on synthetic media (Lakshmipathy *et al*; 2007). Their penetration takes place through root hairs or epidermal cells grow intercellularly or intra-cellularly in the root cortex, ultimately developing short haustoria like structures called arbuscules within the cortical cells (Lakshmipathy, 2007). These arbuscules function as sites of nutrient exchanges between the fungus and host roots (Lakshmipathy, 2007). The arbuscles are tightly bunched hyphae which take carbohydrates from the cells, growing as they do so. Once they have completely filled the cells, they break down, releasing their nutrients to the host and the fungus proceeds to colonise another cell.[8]

Vesicles are formed in the cortical cells, which are thin walled structures of various sizes and shapes and function as storage organs (Lakshmipathy *et al.*, 2007).The presence of vesicles and arbuscles is the criteria for identifying AM fungus in the roots (Lakshmipathy *et al.*, 2007).

VAM reproduce from chlamydospores, which are long-lived, thick-walled spores, produced by the fungus near the surface of the host root, and are able to withstand the rigors of underground life until the roots of a potential host grow close by. Ectomycorrhizae can reproduce from spores or vegitatively from various types of clusters of hyphae or from resting hyphae. In all cases, germination is stimulated by near proximity of roots of a potential host plant, via their effect on the microflora in the rhizosphere.[9]

[9] Colin Lewis Bonsai, Mycorrhizae, Nature's minute miracle-workers <http://www.bonsaideals.com/descriptions/myco.html>/ 11.03.2010

Due to their obligate symbiotic status as described by Narayan *et al.*, (1993), AM fungi need to associate with plant for growth and proliferation. Consequently, the cultivation of AM fungal strains and the maintenance of reference collections require methodologies and infrastructures quite different from those used with other microbial collections and inoculum production. Interest in AM fungi propagation for agriculture is increasing due to their role in the promotion of plant health, in soil nutrition improvement, and soil aggregate stability (Narayan, 1993).

There are seven different types of mycorrhizal associations recognized on the basis of the extent of root penetration, production of external mantle or sheath and the inter and intra-cellular structures they form once inside the plant root (Sharma, 2003). Those types are discussed below.

1) Ectomycorrhiza

25 families of Basidiomycotina, 7 families of Ascomycotina and 1 genus of Zygomycotina belong to this type of mycorrhizal association. Their plant symbionts include both Gymnosperms and Angiosperms. They can be characterized by the presence of fungal sheath or mantle, septate hypha, no hyphal penetration of cells, and presence of Hartigs net between the cells.

2) Ectendomycorrhiza

The fungi belong to Basidiomycotina, which covers both Gymnosperm and Angiosperm plants. If fungal sheath is present it is usually very thin. They have septate hyphae which can penetrate the root cells and coil inside it. A fine network of fungal hyphae is present in the intercellular spaces as well.

3) Endomycorrhiza/Arbuscular mycorrhizal fungi (AM) synonym Vesicular Arbuscular mycorrhizae (VAM)

They only belong to the order Glomales of Zygomycotina. The plant symbiont vary from bryophytes to Angiosperms. There is no fungal sheath. The hyphae are aseptate and they enter the root cortical cells and form characteristic arbuscles and/ or vesicles.

4) Arbutoid mycorrhiza

They belong to the Basidiomycetes fungi and colonize the members of order Ericales. They form a fungal sheath, septate hyphae and Hartigs net in the inter-cellular spaces.

5) Monotropoid mycorrhizal

The fungi belong to Basidiomycotina and colonizes the members of the family Monotropaceae. They form a fungal sheath. Their septate hyphae penetrate only the epidermal cells and coils inside it. Hartigs net is present in the intercellular spaces.

6) Ericoid mycorrhizal

The fungal members of this group are found in the roots of the plants belonging to order Ericales. Rootlets are covered by a loosely woven mesh of dark brown septate hyphae from which branches penetrate the cortical cells forming intra-cellular coils invaginating and enclosed by the host's plasmalemma. Hartigs net is formed in the inter-cellular spaces.

7) Orchidoid mycorrhizal

This type of fungi belongs to Basidiomycotina and Mycelia Sterlia. They colonize only the members of the family Orchidaceae. Their septate hyphae enter the cells and develop into coils often called peltrons. This association is probably pseudomycorrhizal but plays and important role in the establishment of orchid seedlings (Sharma, 2003).

2.6.2 Benefits of Mycorrhizal Fungi

Research is continuing into the many benefits to plants of mycorrhizal associations and there are probably many yet to be discovered. Mycorrhizae increase plant P uptake by more thorough exploration of the soil volume than by roots alone, thereby making 'positionally unavailable' nutrients 'available'. The extensive hyphal growth of mycorrhizae increases the surface area for absorption and hyphal-transport of P to the host root, which effectively 'short-circuits' the distance for diffusion and stops the P from entering into sorption/desorption reactions with the hydrous Fe and Al oxide surfaces of soil particles. Thus P uptake to the plant is accelerated (Trolove *et al.*, 2003).

For the semi-arid tropics, where some African countries are located, there is a great possibility of using mycorrhizae as a biological tool for sustainable agriculture. Quilambo (2003) had shown the mycorrhizal benefits and recommends some management practices for the semi-arid Africa conditions.

Mehrvarz *et al.*, (2008) conducted an experiment to measure the effect of Phosphate Solubilizing Microorganisms and Phosphorus chemical fertilizer on yield and yield components of Barely (*Hordeum vulgare* L.). It was found that application of Mycorrhiza along with bacteria (*Pseudomonas petida*) significantly increase the chlorophyll content of Barely. Sole application of bacteria produced the maximum biological yield, while the application of the same bacteria along with mycorrhiza achieved the maximum one thousand seed weight.

A field study was conducted in Thailand for three fruit tree species namely durian, cashew, and longan with Arbuscular mycorrhizal species- *Glomus mosseae* and *Glomus manihotis* as biofertilizer for fruit tree production with the objective of measuring the effectiveness of growth. The results indicated enhanced growth of the three fruit types.[10]

A pot study results shows that inoculation of Buffel Grass (*Cenchrus ciliaris*) plants with mycorrhizal fungi *Gigaspora rosea*, *Glomus intraradices* and *Glomus etinicatum* is highly beneficial for the growth and biomass production in the absence or presence of P_2O_5 under sterile soil conditions (Khan *et al.*, 2007)

A pot experiment was conducted in Senegal to study the Effect of arbuscular mycorrhizal inoculation on the growth and the development of sesame (*Sesamum indicum* L.) by Boureima *et al.*, (2007) and the results indicate that inoculation with mycorrhizal fungi significantly increases leaf number and leaf area of *Sesamum*. Moreover, inoculation improved the root system by increasing volume and dry weight of roots.
Mycorrhizal fungi significantly change the host physiology. This results in altered root exudation and in turn a changed rhizosphere microflora. Colonization by AMF alters the physiology, morphology and nutritional status of the host plant, and host- soil biota and structure (Bagyaraj, 1992., Bagyaraj and Varma, 1995).
Biocontrol which preserves envioromental quality is an alternative approach to control root diseases (Bagyaraj and Chawla, 2009). Like most instances of biocontrol, mycorrhizae cannot offer complete immunity against any root diseases. They would only impart a degree of resistance against soil born pathogenic fungi, bacteria and nematodes.

Bagyaraj and Chawla (2009) have concluded that AM fungi has the potential to alleviate the severity of diseases caused by plant parasitic nematodes. Like most instances of biological control, AM fungi cannot offer complete immunity against the infestation by plant parasitic

nematodes. The diversity of interactions between VAM and plant pathogenic microorganisms shows that each pathogen- VAM fungus-plant combination is unique which is further influenced by the environment and hence generalizations regarding such interactions are difficult to make (Bagyaraj, 2006). The mechanism of suppression of root pathogens by VAM fungi is only partly understood. Current research using molecular techniques and immunological and histochemical analysis will probably provide more information about these mechanisms. However, the possibility of biologically controlling the root pathogens looks promising (Bagyaraj, 2006).

Over 160 known species of AMF colonize almost all crop plants. Work during the last two decades showed that AMF have potential benefits to agriculture. Yet this potential has so far not been realized, because research efforts are fragmented, data synthesis and modeling on the ecosystem level are lacking, and there is no information on the genetic potential of AMF to tolerate environmental or cultural conditions to modulate host plant and host soil responses (Bagyaraj and Varma, 1995).

The increasing soil degradation, imbalances in regional productivity and envioronmental concerns are some of the issues for developing future research and development strategies for evolving sustainable resource management. Mycorrhizal technology can play an important role in production of low cost quality seedlings for afforestation and reforestation programmes in forestry. (Bagyaraj *et al.*, 2002).

[10]Mycorrhizal fungi as biofertilizer for fruit tree production in Thailand (http://www.agnet.org/activities/sw/2006/paper-531302897.pdf)/01/02/2010

2.6.3 Mycorrhizal dependency of plants

The potential benefits resulting from the inoculation of plants with mycorrhizal fungi may be large or small depending on properties of the host plant, mycorrhizal fungi or soil where they are grown (Brundrett *et al.*, 1996). Agricultural practices such as fertilizer applications, crop rotations, tillage and liming affect field AM potential and root colonization levels. For example, high levels of P fertilization have been found to slow down or inhibit mycorrhizal efficiency in soy bean fields.[1] Plant species differ in their fertilization requirements, and consequently their dependency on AMF varies considerably from one crop to another (Bagyaraj and Varma, 1995).

Relative mycorrhizal dependency (RMD) has been as the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility (Bagyaraj, 2003). This was to know the extent of growth increase attributed to mycorrhizal condition (Bagyaraj, 2003). Plenchette *et al*; (1983) proposed another formula to calculate relative field mycorrhizal dependency (RFMD) of crop plants under field conditions. They compared plants in fumigated and non-fumigated soils. This measures the extent of growth increase due to native endophytes. The calculated values lie between 0 and 100%.

$$\text{RFMD} = \frac{\text{(Dry weight of mycorrhizal plant} - \text{Dry weight of non- mycorrhizal plant)}}{\text{Dry weight of non- mycorrhizal plant}} \times 100$$

This formula is of great practical importance for ascertaining the response of crop plants to native mycorrhiza in fields before applying field inoculation to crops.

Bagyaraj *et al.*, (1988) proposed a formula which enables calculation of the mycorrhizal inoculation effect (MIE) to assess the growth improvement brought about by inoculation with a mycorrhizal fungus in unsterile soil with indigenous vesicular arbuscular mycorrhizal fungi.

$$\text{MIE} = \frac{\text{Dry weight of inoculated plant} - \text{Dry weight of uninoculated plant}}{\text{Dry weight of inoculated plant}} \times 100$$

MIE is very useful for the assessment of the extent to which introduced fungi compete with native endophytes to bring about plant growth response (Bagyaraj *et al.*, 1988). This

information is of great value in practical agriculture, especially in developing countries, where farmers do not fumigate or sterilize either nursery or field sites.

2.6.4 The Effect of Mycorrhizae on Phosphorus and other nutritions uptake

The acquisition of phosphate through VAM associations involves transport of phosphate from the soil solution across the membrane of the fungal hyphae, movement of that phosphate along the huyphae to the arbuscule, unloading the phosphate from the fungal arbuscules at the arbuscule-cortical cell interface and uptake of that phosphate by the plant cortical cells (Smith, 2002)

When the amount of available phosphate in a soil is low, heavy infections of vesicular arbuscular mycorrhizas are developed (Woolhouse, 1975). If plants are grown in the presence of high concentrations of available phosphate, few if any arbuscules are formed (Woolhouse, 1975).

Bowen *et al.*, (1975) showed that vesicular arbuscular mycorrhizas can absorb several times more phosphate from solution than uninfected roots. The autoradiographic studies indicate that in mycorrhizas much of the increase in uptake is due to uptake and translocation by the fungal hyphae external to the root, rather than to a fungal stimulation of ion uptake by uninfected cells (Bowen *et al.*, 1975).

Findings of Pearson and Tinker (1975) confirm that hyphae of *Glomus mosseae* transport phosphorus from some distance (perhaps 1-2 cm) in to host root.

Granules, 0.1-0.2 μm in diameter are seen in the vacuoles of the fungus. These display cytochemical properties of polyphosphate granules (Cox *et al.*, 1975). Chemical analysis suggests the presence of higher levels of polyphosphate in mycorrhizal roots than non-mycorrhizal roots, or shoots (Cox *et al.*, 1975).

There is the possibility that hyphae have some special property of dissolving rock phosphate surfaces, beyond that caused by simple uptake of phosphate ions (Woolhouse, 1975). This might arises from changes in pH in or cation concentration in the very small volume of solution between the phosphate mineral surface and pressed hyphae (Woolhouse, 1975).

Khan (1975) studies the growth effects of VA mycorrhiza on crops maize and wheat in the field Pakistan. A significant feature arising from the study is the practical implication that mycorrhizal inoculation of crop seed may be beneficial (Khan, 1975). This technique could be of great practical value in increasing grain production in those areas of the world where phosphorus limits plant growth and phosphorus fertilization is not economical (Khan, 1975).

Janos (1975), measures the effects of vesicular arbuscular mycorrhizae on lowland tropical rainforest trees. As the observations of his experiment growth increases would be expected in the tropical soils used which are low in available phosphorus and generally bear plants which are heavily mycorrhizal. Such plants are likely to be extremely dependent on VA mycorrhizae for uptake of phosphorus and perhaps other minerals (Janos, 1975).

Effective Phosphorus management is important to optimize crop yield potential, reduce production costs and decrease the risk of environmental damage. Phosphorus uptake in many crops is improved by associations with AM fungi, particularly in low P soils. Use of effective P management practices, whether through efficient fertilizer use or encouragement of mycorrhizal associations can optimize the economics of crop production while avoiding negative effects of P on environmental quality (Grant *et al.*, 2004)

Bharadwaj and Sharma (2006) suggested a net saving of 25% of the recommended dose of inorganic Phosphorus i.e., triple super phosphate with AM fungi in alkaline soils for the proper growth of mulberry.

AM colonization of lettuce tended to decrease with the use of chemical inputs, such as pesticides and high amounts of P and N fertilizers. This was revealed by Miller and Jackson (1998) in the experiment of survey of vesicular-arbuscular mycorrhizae in lettuce production in relation to management and soil factors. Farahani *et al.*, (2008) found that arbuscular mycorrhiza is able to enhance the growth of coriander under water stress through enhancing P uptake.

Soil P levels have a greater influence on the development and functioning of mycorrhizal associations. The benefits are greatest in P-deficient soils and decrease as soil phosphate levels increase. Very high and very low phosphorus levels may reduce mycorrhizal infection/colonization. It is well established that infection by mycorrhizal fungi is significantly reduced at high soil phosphorus levels. Phosphate fertilization results in a delay in infection as well as a decrease in the percentage of infection of roots by mycorrhizal fungi. Also an increase in the level of soil phosphate results in a reduction in chlamydospore

production by the fungus. These spores are involved in root infection and spread of the fungus through the soil profile.[7]

The increased growth of plants inoculated with VA mycorrhizal fungi is not only attributed to improved phosphate uptake but also to better availability of other elements like Zn, Cu, K, Al, Mn ect. AM fungi affect the levels of plant hormones like cytokinins and gibberellin-like substances. (Bagyaraj, 2003). Xioutang (1994) reported that the concentrations of P, K, Ca and Mg in leaves and roots of VAM inoculated *Litichi chinensis* plants were increased relative to those in the uninoculated plants. But potassium uptake is less likely to be enhanced by mycorrhizas, since soluble K is usually maintained at a reasonably high concentration in the soil solution by a diffusion rate 10 to 20 times faster than that of P (Powell, 1975).

Studies to promote the activity of effective indigenous AM fungi, through judicious manipulation of agronomic practices, should be intensified. The possibility of reducing phosphate fertilizer input and of using cheaper fertilizers such as rock phosphate along with AM fungi should be investigated. It appears to be legitimate to regard the use of AM fungi as being an alternative strategy for a more rational and sustainable agriculture (Bagyaraj, 1992).

2.6.5 Present threats to Arbuscular Mycorrhizal Fungi

Human activities like application of P fertilizers, crop rotation and soil management may alter the population and diversity of arbuscular mycorrhizal fungi (Lakshmipathy *et al.*, 2007). Modern intensive farming practices are evidently a threat to AM fungi, as indicated by studies on these fungi (Lakshmipathy *et al.*, 2007). In recent days, AM fungal population and diversity are declining because of agricultural / land use intensification (Lakshmipathy *et al.*, 2007). Forest degradation by logging, burning etc has badly affected forest ecosystems as well as mycorrhizal associations within them and thereby causing severe damage to the environment (Weerawardane, 2006). Attempts to restore these lands have always been problematic probably due to low inoculum potential declines in the absence of host plants (Weerawardane, 2006).

2.6.6 Plant species involved with arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi have the widest host range and distribution of all the mycorrhizal associations (Lakshmipathy *et al*, 2007). It has been estimated that over 80% of all vascular plants form arbuscular mycorrhizas (Hafeel and Gunatilake, 1989). Arbuscular

mycorrhizae have been identified in a broad spectrum of plants, including some non – vascular plants, ferns and other seedless vascular plant groups within the gymnosperms including conifers such as. *Ginkgo biloba*, the cycads and the majority of angiosperm families (Peterson *et al.*, 2004). The few angiosperm families that do not have arbuscular mycorrhizas either form other categories of mycorrhizae or lack mycorrhizae. Among the latter families are the Brassicaceae (this family includes canola, mustard, cabbages, ect) and the Chenopodiaceae (this family includes garden and sugar beets, spinach and the large genus Chenopodium) although even here arbuscular mycorrhizal associations have been reported for a few species (Peterson *et al.*, 2004). A few aquatic plant families and the sedges may have low levels of colonization by arbuscular mycorrhizal fungi, however these can be overlooked if roots are collected at the wrong time of the year or if the sample size is too small. The relative abundance and the seasonality of arbuscular mycorrhizas in many plant species remains undetermined. Fossil records from the Devonian of the first vascular plants show that arbuscular mycorrhizal associations were present in underground rhizomaous structures confirming the presence of this mutualistic association as far back as 400 million year or more (Peterson *et al.*, 2004).

Arbuscular mycorrhizas have been studied intensively: they occur in most ecosystems of the world and are found in many important crop species (e.g. wheat, maize, rice, soybeans, forage crops, grapes, fruit trees, cotton) and horticultural species (e.g. roses, petunias, lilies, carnations) (Peterson *et al.*, 2004).

2.6.7 Fungal species involved with arbuscular mycorrhizal fungi

The fungi involved are ubiquitous soil-borne organisms (Phylum Glomeromycota: Class Glomeromycetes) belonging to four orders: Archaeosporales, Paraglomerales, Diversisporales and Glomerales. Eight genera of arbuscular mycorrhizal fungi have been recognized based mainly on morphological characteristics of asexual spores, although molecular methods and various biochemical parameters are now being used in systematic studies. These genera *Glomus, Paraglomus, Sclerocystis, Scutellospora, Gigaspora, Acaulospora, Archaeospora* and *Entrophospora* include approximately 150 species: however species delineation remains uncertain and continues to change as more isolates are examined and as the use of molecular techniques increases. A major challenge in the systematic of this group of fungi is the considerable variation within isolates of defined species and the lack of sexual reproductive structures (Peterson *et al.*, 2004). According to Lakshmipathy *et al* (2007) arbuscular mycorrhizal fungi come under the class Zygomycetes, under the order Glomales. The order

Glomales is divided into 2 sub-orders Glomineae and Gigasporineae. The sub order Glomineae has four families namely Glomaceae, Acaulosporaceae, Paraglomaceae and Archeosporaceae. Further, Glomaceae has two genera, viz. *Glomus* and *Sclerocystis,* Acaulosporaceae has two genera, viz. *Acaulospora* and *Entrophospora,* Archaeosporaceae has only one genus *Archaeospora* and Paraglomaceae has one genus *Paraglomus.* The suborder Gigasporineae has only one family Gigasporaceae. Gigasporaceae includes the genera *Gigaspora* and *Scutellospora* (Lakshmipathy *et al*, 2007).

All arbuscular mycorrhizal fungal species are obligate biotrophs, depending entirely on host plants for carbon compounds. This means that, unlike many ectomycorrhizal fungi, these fungi cannot be cultured in the absence of plants. As a result, researchers have used root organ cultures and genetically transformed root cultures to maintain arbuscylar mycorrhizal fungi and for use in experimental studies (Peterson *et al.*, 2004).

2.6.8 Isolation, propagation and storage of Vesicular Arbuscular Mycorrhizal fungi

As with most of the filamentous fungi, arbuscular mycorrhizal fungi propagation can occur either by spore differentiation and germination or by mycelium extension through soil and roots. The main obstacle in the production of efficient and reliable arbuscular mycorrhizal fungal inoculum lies in their symbiotic behaviour, the fungi obligatory requiring a host plant for growth (Jarstfer and Sylvia, 2001).

2.6.8.1 Isolation of VAM fungi

VAM fungi in a soil sample should be separated before the isolation step. Spore density data can be obtained by counting spores in a soil sample (Brundrett *et al.*, 1996). However, spore numbers may be poorly correlated with mycorrhizal fungus activity in soils. The standard procedure for separating Glomalean (VAM) fungus spores from soil sample is described in the material and method section. Pot culture isolation procedures often help to identify VAM fungi from soils collected in the field, when these do not contain spores of sufficient quality or quantity to allow fungi to be accurately identified (Brundrett *et al.*, 1996).

2.6.8.2 Inoculum propagation

The culture of arbuscular mycorrhizal fungi on plants in disinfected soil using spores, roots or infested soil as inocula has been the most frequently used technique for increasing propagule numbers (Jarstfer and Sylvia, 2001). As described by Brundrett *et al.* (1996). Glomalean (vesicular arbuscular mycorrhiza) fungi are usually propagated by growing them with a living host plant in soil pot cultures. These pot cultures, which consist of soil, spores, root pieces and

hyphal fragments, can be used as inoculum for experiments or to introduce fungi into soil. The choice of host plant used to propagate mycorrhizal fungi can have a large influence on fungal sporulation and mycorrhizal root formation and consequently on resulting inoculum levels (Brundrett *et al.*, 1996). However, the most important considerations for choosing host plants concern their tolerance to growing conditions in the glasshouse or growth chamber where they will be grown (temperature, light levels and drought stress) which must also be favourable to the mycorrhizal fungi. Grasses such as Sorghum species, corn (maize) and bahiagrass (*Paspalum* sp.) are often used as host plants because their extensive root systems result in more mycorrhiza formation (Brundrett *et al.*, 1996). Examples of plants that have been used successfully are: alfalfa, maize, onion, Sudan grass and wheat (Jarstfer and Sylvia, 2001). Generally the host selected should become well colonized (> 50% of the root length), produce root mass quickly, and be able to tolerate the high level light conditions required for the fungus to reproduce rapidly (Jarstfer and Sylvia, 2001). Hosts that can be propagated from seeds are preferable to cuttings since they are more easily disinfected. Most seeds may be disinfected with 10 % household bleach (0.525 % NaOCl) for 5 to 15 minutes followed by five washes of water.

Vesicular arbuscular mycorrhizal fungi can also be propagated by growing plants in aeroponic and hydroponic cultures and monoxenic cultures (Jarstfer and Sylvia, 2001). These other methods can be used to propagate existing isolates of mycorrhizal fungi and should provide greater control of inoculum quality, but are not practical for isolating new fungi from field soils.

2.6.8.3 Maintenance of pot cultures

Pot cultures can be grown in free draining pots or non draining buckets. It is easier to use water draining pots, but water dripping from them may cause cross contamination between isolates, so the use of non draining pots is recommended (Brundrett *et al.,* 1996). The water use of plants can be moderated by avoiding the use of excessive amounts of fertilizers, especially phosphorus.

2.6.8.4 Soils and nutrient supplements

For pot cultures, most researchers use a coarse textured (sandy) soil with moderate nutrient levels, supplemented with mineral nutrients (Brundrett *et al.*, 1996). All components of the culture system should be disinfested prior to initiation of a pot culture. The method of soil disinfestations is especially important, the objective is to kill existing AM fungi, pathogenic organisms, and weed seeds while preserving a portion of the nonpathogenic microbial community (Jarstfer and Sylvia, 2001). Pasteurized, steamed, or irradiated growing substrates

are required in order to avoid culture contamination which could affect the quality of the inoculum.[1]Sandy soils can be pasteurized by steaming for 1 hour at 90 ^{0}C on two consecutive days, or by other techniques such as solar heating (Brundrett *et al.*, 1996). Soil pH should be similar to that of the soil from which fungi were isolated (the pH of acidic soils can be raised by adding the appropriate amount of lime ($CaCO_3$) (Brundrett *et al.*, 1996).
Mineral nutrient supplements are usually provided, but can adversely influence fungal development if they are too high.[1] Inadequate mineral nutrient composition may affect fungal development. Optimum P levels vary with the host plant and cultivated fungal strains and an excess of available phosphorus can inhibit arbuscular mycorrhizal fungi propagation.[1] Nutrients can be provided by incorporating them into soils before the commencement of experiments or by periodic applications of a dilute hydroponics solution.[1] However, it will be difficult to regulate nutrient levels in free draining pots, since there will be uncontrolled losses due to leaching.

2.6.8.5 Storage of vesicular arbuscular mycorrhizal fungi

Large scale production of mycorrhizal inoculum requires inventory of product and the ability to provide clients with products of high and consistent quality. Although the detailed procedure for inoculum preservation is proprietary, methodologies for its preservation remain simple and inexpensive.[1] Fungal viability and mycorrhizal efficiency can be maintained for several months at room temperature (20 to 25 ^{0}C) especially when semi dry inocula are kept in plastic containers or packaging.[1] The major inconvenience of such a storage period is the occurrence of spore dormancy.[1]Long term storage (up to 1 to 2 years) may be conducted at 5 ^{0}C cold temperature storage. This method is efficient for both *in vivo* and *in vitro* propagated strains.[1] Narayan *et al.* (1993) has found that storage of vesicular arbuscular mycorrhizal fungi inoculants at 7 ^{0}C, compared with 25 ^{0}C, enhanced spore viability and this was evident from a reduction in vesicular arbuscular mycorrhizal fungi colonization and spore production in wheat (*Triticum aestivum* L.) and sorghum- sudangrass hybrid plants inoculated with G. *mosseae* spores stored at 25 ^{0}C. Pot cultures are usually stored by refrigerating damp inoculum at 4 ^{0}C or by air drying inoculum, but the responses of individual fungi to these treatments may vary (Brundrett *et al.*, 1996).

2.6.9 Specificity in VA mycorrhizas

Mosse (1975) confirms that non- indigenous endophytes (VAM) can be introduced in to both irradiated and unsterile soils, that they can become established in competition with the indigenous endophytes and can improve plant growth. This suggests that the practical

possibility of field inoculation should now be seriously considered (Mosse, 1975). It is improbable that the benefits of field inoculation will be as great as those of inoculation in irradiated soils in pots where optimum growth conditions are provided. But unless the introduced endophyte fails to get established or interacts in some particular way with the existing soil microflora, some improvement in the plant growth should result (Mosse, 1975).

2.6.10 Effectiveness of Mycorrhiza field studies

Most field studies have indicated an effect of fertilizers on the mycorrhizal population (Hayman, 1975). Although many locations (and pot experiments) show this effect to be a negative one, some locations indicate a positive effect (Hayman, 1975). There are many variables in the field; the original fertility of the soil before fertilizers are added and the nutrient content of the host plant are probably major factors to consider. Therefore to understand the field situation more fully and to facilitate comparisons between different locations, it is suggested that samples be mostly collected in late summer when the mycorrhizal population is highest, and that additional data be obtained, including N and P contents of the plant tissues and N, P, Organic matter contents and pH of the soil, measured according to universally accepted techniques (Hayman, 1975).

2.6.11 Arbuscular mycorrhizal inoculum production for commercial use

The most frequently use AMF species for commercial inoculum is typically *Glomus intraradices.*[1] This species is well adapted to both in vivo and in vitro propagation, can colonize a large variety of host plants, survive to long term storage and is geographically distributed all over the world. These characteristics make the *Glomus intraradices* species an excellent candidate for commercial inoculum. Several other AMF belonging mainly to *Glomus* species, but also to *Gigaspora, Scutellospora* and *Acaulospora* geneara are gradually used for commercial inoculum production.[1] Mycorrhiza workers rarely provide the rationale for selection of particular vesicular arbuscular mycorrhizal endophytes in their experiments. There are a few "favourite" spore types for field inoculation including *Glomus fasciculatum, G. mosseae, G.etunicatum, G.tenue* and *Gigaspora margarita.* These fungi were probably chosen because of their ready availability (Bagyaraj, 1992).

Generally, mycorrhizal fungi propagules, such as colonized roots, spores and hyphae are mixed with a growing substrate and the pots are seeded and incubated under controlled conditions.[1] Colonized roots (0.08 to 0.16 inches long) containing hyphae and / or vesicles and fungal spores may be used as starting fungal propagules for the production of mother

inoculum.[1] Research collections can provide such material from either in vivo or in-vitro propagated fungi.

The establishment of mycorrhizal cultures may proceed in different ways;

- Mother inoculum added directly at seedling in large trays or pots
- Mother inoculum incorporated at seedling transplantation of 4 to 6 week old plantlets;
- Mother inoculum added at transplantation of micro-propagated plantlets
- Colonized seedlings produced in greenhouses and transplanted to the field

2.6.12 Composition of Commercial inoculum

The inoculum sold on the market are provided as granular substrates made from mixed materials such as peat, compost, vermiculite, perlite, sand and / or expanded clay in which segments of colonized roots, spores and filamentous networks are distributed. Most of the time these roots, spores, and hyphal networks are not detectable because of their microscopic sizes. In terms of fungal content, the tendency is to introduce a mix of several AMF in commercial inoculum.[1]

2.6.13 Application of Mycorrhizae for commercial use

In developed countries, seedlings are usually raised in fumigated soil or potting mix in nursery beds. Inoculation of such substrate, with selected VAM fungi for the crop, is a easy and appropriate technology. Commercial use of VAM inoculation by this method, in the nursery production of citrus is done in USA (Bagyaraj, 2003). Inoculation of the nursery with efficient VAM fungi has been successful in the large scale production of many horticultural plants in France (Bagyaraj, 2003).

The potential of *Glomus epigaeus* for commercial use was evaluated with respect to *epigaeus* spore production, host growth stimulation and method of spore storage by Daniels and Menge (2006) in California, USA. It was reported that the spore producing capacity, efficiency on a wide range of host plants and ease of storage make *Glomus epigaeus* spore production host growth stimulation and method of spore storage (Daniels and Menge, 2006).

The commercial production of mycorrhiza innoculants is now a reality in the UK. In the USA there is a product called Transplant One-Step, from ROOTSinc 800 342-6173.[8]

In Sri Lanka a few research attempts were made to produce AM fungi and which are seems to be in a preliminary state of AM fungi commercial production. Jayaratne and Siriwardene

(2000), have introduced a AM inoculum production method for commercial use. They reported a simple and low cost method of AM inoculum production using an organic medium that can be used in commercial scale inoculum production using *Pueraria phaseoloides* as the host plant. Pre-inoculated (with *Acaulospora spinosa*) *Pueraria* seedlings were grown in large wooden boxes filled with a mixture of fine coir dust and finely ground rice husk (1:1) as the medium. These were grown for 3-4 months to multiply the AM fungus in the media before it was tested as a suitable inoculum (Jayaratne and Siriwardene, 2000).

2.6.14 Constrains for the commercialization of Arbuscular Mycorrhiza in Sri Lanka

As described by Perera (2007), three major obstacles to the commercialization of mycorrhizal fungi in Sri Lanka. 1) The lack of large scale field tests under typical agricultural conditions in a variety of locations, 2) Adequate cost benefit analysis to determine the economics of the utilization of mycorrhizal fungi, 3) A reluctance on the part of growers to switch from an energy dependent, heavy fertilizer system to a new, but cheaper, energy conservative system using mycorrhizal fungi (Perera, 2007).

2.6.15 Application of AM for perennial species including spice and beverage crops (EACs)

Thanuja, (2002) reported a results of a study of AM influence on induction of rooting and other root characteristics of pepper cultivar Panniur-1 under Indian conditions. Her investigation clearly showed the beneficial effects of inoculation with AM fungi which resulted in enhanced rooting and root growth in Black pepper cuttings.

In a number of initial studies, significant growth improvements were reported in black pepper rooted cuttings through inoculation of VAM fungi at Export Agriculture Research Station, Matale, Sri Lanka (Wimalaratne, 2005). Palipane and Bandara (1985) reported that Vesicular Arbuscular Mycorrhizae (VAM) *(Glomus species)* were found to be associated with vigorously growing unfertilized 10- year old coffee and cocoa plants in Sri Lanka. Similarly, coffee and cocoa seedlings inoculated with VAM had a significantly higher growth rate than that of uninoculated seedlings. Palipane (1999) demonstrated the beneficial effect of VAM in coffee seedling growth.

Soil fertility depletion of coconut lands is a major concern in coconut cultivation (Fernando and Tennakoon, 2006). Research on AMF associations in coconut is very sparse. As described by Fernando and Tennakoon (2006), it is supposed to develop an AMF based biofertilizer to

improve seedlings vigor of coconut from the indigenous AMF population presently available in coconut lands. Also studies revealed that AMF has also enhanced the uptake of Eppawala rock phosphate significantly than the respective controls (Fernando and Tennakoon, 2006).

Mycorrhiza plays an important role in natural forest ecosystems. Trees in moist tropical forests are predominantly VA mycorrhizal (Weerawardane, 2006). Mycorrhizal fungi plays a major role in forest succession, which is the natural process of restoration after disturbance, by helping facultative and obligatively mycorrhizal tree species to establish in the site gradually replacing pioneer species which occupy the open lands in early stages of disturbance. Forest plantation establishment also needs to pay attention on mycorrhizal inoculation to improve the establishment, early growth and productivity of plantations (Weerawardane, 2006).

It has reported that mycorrhizae inoculation, especially vasicular arbuscular mycorrhizae (VAM) are most common and important mycorrhizae in fruit crops (Rajapakse, 2006). Researches have been shown in that artificial inoculation of VAM improve growth and plant nutrition conditions of avocado, citrus, banana, grapevine, annona, mango and papaya especially in nursery seedlings and early growth stage of fruit crops (Rajapakse, 2006).
VAM can benefit soil-plant interactions in a number of ways including that of plantation crops like tea (*Camellia sinensis*) (Balasuriya, 2006). Different tea cultivars can support different degrees of VAM associations. Long term trials (>40 years) at the TRI, on fertilizer usage have shown their potential influence on the degree of VAM associations (Balasuriya, 2006).

Hafeel and Gunatilleke (1989), have showed that several species of *Gigaspora* and *Glomus* were common in their three study sites (undisturbed natural forest, site abandoned after shifting cultivation, nine year old *Pinus caribaea* plantation). This study showed that the modified sites contain inocula of at least some Endomycorrhizal species similar to those found in natural forest, thus providing a suitable soil environment for the successful growth of the broad-leaved species requiring endomycorrhizal associations at these modified sites. In an another study, Jayaratne and Siriwardana (2000) reported that the transplanted perennial crops such as rubber and tea can be inoculated with VAM at nursery stage.

It seems that there is a possibility in application of AM fungi for other export agricultural crops in Sri Lanka.

Chapter 03

General Material and Method

3.1 General

Some of the common experimental procedures that are relevant for a number of experiments are described in this section. Four experiments were carried out at the Export Agriculture Research Station (EARS), Department of Export Agriculture, Matale, Sri Lanka from January 2008 to January 2010. The altitude is 355 m above mean sea level and located at 7^0 27' N latitude and 80^0 34' E longitude and the area belongs to the agro-ecological zone of IM3a (Punyawardena, Bandara, Munasinghe and Nimal Jayarathna banda, 2002). Mainly Reddish Brown Latesoloic soils of Matale series (Typic Rhodudalfs, Clayey-USDA or Rhodic Dystric Nitisols-FAO) can be found in the research farm (Mapa, Somasiri and Nagaraja, 2005).

3.2 Mycorrhizal inoculum

The initial inoculum AM fungus *Glomus mosseae* available at the EARS, Department of Export Agriculture, Matale was used for each experiment. The inoculum consists of mycorrhizal spores and structures with sorghum (*Sorghum bicolor* L.) roots (host crop) and moist soil obtained by harvesting AM (*Glomus mosseae*) regeneration bed. Available inocula have been regenerated in sorghum beds.

3.3 Conversion of moist soil weight to equivalent dry weight

A sample of 25.0 g soil was weighted into a weighed moisture can with the lid. The soil sample was dried in an electric oven at the temperature of 105 ^{0}C without the lid for at least 24 hr until the achievement of a constant weight. Then it was removed from the oven, fitted with the lid, cooled in a desiccator at least for 30 minutes and weighed. The dry weight was calculated suing the equation given below.

Calculation

Initial weight of the soil = 25.0 g

Final weight of the soil = X

$$\text{Gravimetric moisture content per 100 g of soil } (\theta g) = \frac{(25\text{- X})\text{ g x 100 g}}{25.0\text{ g}}$$

$$\text{Moisture factor} = 1 + \theta g$$

$$\text{Estimated oven dry weight of the sample used for experiment} = \frac{\text{Mass of moist soil sample}}{\text{Moisture factor}}$$

3.4 Isolation of arbuscular mycorrhizae (AM) fungal spores

Arbuscular mycorrhizae (AM) fungi were collected from field and/or pot culture soils. Clamydospores or azygospores, soil-borne vesicles and mycelia or infected root pieces in soil form mixed inoculums, which cannot be quantified. Therefore, spores separated from soil are more pure and can be easily quantified. The spores of AM fungi are larger than those of most other fungi, ranging from 10-1000 μm in diameter. Most spores are between 100 and 200 μm in diameter and could be observed with a dissecting microscope (Jarstfer and Sylvia, 2001). There are numerous methods to recover VAM propagules from soil. The most basic and the most widely used method of these is the wet sieving and decanting, density gradient centrifugation method (Daniels and Skipper, 1982).

3.4.1 Wet sieving and decanting method to isolate and quantify spore density

The soil samples were weighed and determined their moisture content by oven drying (at 105 ^{0}C at least for 24 hours) a small sub sample in a pre-weighed container. This allowed spore numbers to be expressed relative to the dry soil weight. Coarse debris and rocks were removed from the samples by sieving with a 2 mm sieve. Dry soil samples had been thoroughly wetted for at least 30 minutes before sieving them. Soil was mixed in a substantial volume of water and sieved through a series of sieves (750 μm, 250 μm, 100 μm, 50 μm) after allowing heavy soil particles to settle for a few seconds. This washing and decanting process is repeated until the water was clear. Roots and coarse debris were collected on a coarse screen (750 μm) while spores were captured on one or more finer screens. It was ensured that soil aggregates were dispersed by vigorous washing with water and hand mixing to free spores from aggregates of clay or organic materials.

The first water centrifugation was done and it removes substantial amounts of floating organic debris from many soils. After this step the supernatant and floating debris were discarded. A small amount of finely powdered kaolin clay was added to centrifugation tubes with water

and sieving to help form stable pellet during centrifugation. It was ensured that the centrifuge is properly balanced before switching it on.

The next step involve re-suspending the pellet in 50% sucrose by vigorously shaking tightly stoppered tubes. The samples were then centrifuged for 1 minute at 2000 rpm to separate spores (and any remaining organic debris) from denser soil components. Immediately after centrifugation, spores in the sucrose supernatant were poured onto the finest sieve (50 μm) and carefully washed with water to remove the sucrose.

After rinsing the spores, they were washed onto a pre-wetted filter paper in a Buchner funnel before vacuum filtration. Filter papers could be marked with parallel lines to separate microscope fields for spore counting (Brundrett *et al.*, 1996).

3.5 Method for clearing and staining roots

Root samples (approximately 0.5 g) were placed in perforated plastic holders and stored in cold water until they were to be processed. A volume of 1.8 M KOH enough to cover the root samples was poured into a beaker Then the solution was heated up to 80 ^{0}C and placed the root samples in the heated KOH for a desired time, i.e. 15 minutes for tender roots and 30 min for other roots. Browning of the solution was taken as an indication of clearing of roots.

If samples are still pigmented after treating with 10% KOH, they were rinsed in a beaker wiih either 30% (wt/wt) H_2O_2 at 50 ^{0}C or 3% (wt/vol) NaOCl acidified with several drops of 5M HCl. Times of rinsing varied from several seconds to several minutes, depending on strength of the root pieces like Black pepper and Cinnamon. Roots were checked frequently to avoid destruction of the cortex and fungal tissue. Root pieces were examined through stereo microscope and determine the optimum rinsing time.

The roots were rinsed with copious amounts of water as soon as the samples were bleached white or became transparent. Then the samples were covered with tap water, stirred and drained. This step was repeated once. Enough trypan blue was dispensed in to a beaker to cover the samples and was heated up to 80 ^{0}C.

To prepare the solution added in the following in the given order to a flask while stiring: 800 ml of Glycerin, 800 ml of Lactic acid, 800 ml of distilled water and finally 1.2 g of trypan blue. Samples were placed in the stain for at least 30 min, cooled overnight and then drained the stain in to the large flask for reuse. The samples were rinsed with two changes of water to de-stain them (Jarstfer and Sylvia, 2001).

3.6 Assessment of colonization by VAM fungi

Mycorrhizal infection was determined according to Durgapal *et al.*, (2002) as percent roots infected with mycorrhizal fungi on a per plant basis. Microscopic observations were made to quantify infection which was calculated as follows.

$$\text{Percent infection} = \frac{\text{Number of positive roots}}{\text{Total number of roots observed}} \times 100$$

3.7 Measuring the leaf area and biomass

Leaves were separated from the stem of the sample plants and each leaf was passed through a leaf area meter of brand CI-202 of CID inc. The total count was taken for each sample.
All the above ground material of the plant including the leaves were then taken together and placed in a paper bag. These paper bags with the samples were tagged according to the respective treatment number and dried in an oven at 70 ^{0}C for 24 hours. Then dry weights were recorded as shoot biomass. Similarly, all the below ground roots were separately dried and recorded the dry weight of respective samples for each plant and finally tabulated on treatment basis.

3.8 Modified line intersect method for estimating root length

Simplified version of Newman's line intersection method as described by Tennant (1975) was adopted in this experiment for root length determination. As a pre-preparation, pots were washed out with a spout of water and roots were separated from the plant. For length estimates the grid in use was placed on the bottom of the dish, water containing the roots poured in and the roots positioned over the grid with a piece of sheet glass. When necessary, the root material was teased apart or cut into smaller pieces. Counts were made with the aid of a magnifying glass of the intercepts of the roots with the vertical and horizontal grid lines. Root length (R) was measured by counting the number of intercepts (N) of roots in a regular area (A) with randomly located and oriented lines of total length (H). In principle, the longer the root, the more intercepts it made with the randomly arranged lines. Best results were obtained when counts of one (01) were given to a root crossing a line, a root ending touching a line and a curved portion touching a line. Counts of two (02) were allocated to curved portions which lay on or along a line. A systematic traversing of vertical and horizontal lines

was found preferable to following single roots. All counts were accumulated on the hand tally counter.

Complete counts were converted to length measurements using the modified formula inclusive of the grid unit:

Root length (R) = 11/14 x Number of intercepts (N) x Grid unit

The 11/14 of the equation was combined with the grid unit to give a length conversion factor. These for the ½, 1, 2 and 3 cm grid squares, were 0.3928, 0.7857, 1.5714 and 2.3571 respectively. The modified formula therefore took the easily managed form:

Root length (R) = Number of intercepts (N) x Length conversion factor.

3.9.1 Soil sample collection and preparation

Soil samples were taken from pots and sampling points as described in Bigham (1996). When working with mycorrhizae, rhizosphere soil sampling is more relevant and therefore rhizhosphere soil sampling was collected as described by Wang and Zabowski (1998). The whole plant / vine was taken out of the soil with minimum injury to its roots, generally shaken the roots until the soil not tightly adhering to roots was removed and then collecting the soil closely adhering to the root system by putting the roots into a paper bag and vigorously shaking the roots (Wang and Zabowski, 1998). Soil outside the rooting area was collected as bulk soil. The 'bulk' soil (soil not influenced by roots) was collected from areas in pots/field where there were no roots (Liu *et al.*, 2004).

During soil sample collection, the sample details were entered in the relevant register. Lab number was entered in the serial order. The lab number was written on both tags and on the bag. The soil samples were brought to the drying room emptied from their containers and spread out to dry on flat trays made up of aluminum, or plastic. Large lumps of moist soil were broken by hand. When the soil was air drying, roots and other residues were manually removed. Then it was taken to the preparation room for further treatment. The air-dried soil samples were crushed gently by hand (using a porcelain pestle and mortar if necessary) and sieved through a 2 mm stainless steel sieve. Crushing was continued until the soil retained in the sieve contained no aggregates. Material larger than 2 mm were discarded. All determinations were carried out on the fraction having particle diameter less than 2 mm. The prepared samples were stored in cardboard or plastic containers with appropriate labels in the

soil store according to experiments. The ground samples were mixed well before a sample is weighed for analysis.

3.9.2 Analysis of soil chemical parameters

Soil chemical parameters include soil organic matter, Phosphorus soluble in Sodium Bicarbonate, Phosphorus soluble in Dilute Acid Fluoride, Phosphorus soluble in water, Soil Nitrogen, Soil K and Mg.

3.9.2.1 Determination of soil organic matter (Walkley-Black Method) (Broadbent, 1965)

Accurately 1 g of finely ground soil (passing through 0.5 mm sieve) was transferred into a 500 ml conical flask. 10 ml of 1/6 M Potassium dichromate was added followed by 20 ml of concentrated sulfuric acid. The flask was shaken for 1 min and left to stand on a sheet of asbestos for about 30 minute. About 200 ml of water, 10 ml of phosphoric acid and 1 ml diphenylamine indicator solution were added.

The solution was titrated by adding ferrous ammonium sulphate solution drop by drop until the colour flashes to green (This color change occur with little or no warning). Exactly 2 ml of 1/6 M $K_2Cr_2O_7$ was added to restore and excess of dichromate and completed the titration adding ferrous ammonium sulphate. A blank titration was conducted simultaneously, to calculate the molarity of ferrous ammonium sulphate solution.

Calculation:

$$2Cr_2O_7^{-2} + 3C + 16H^+ = 4Cr^{+3} + 3CO_2 + 8H_2O$$

According to the balanced equation;

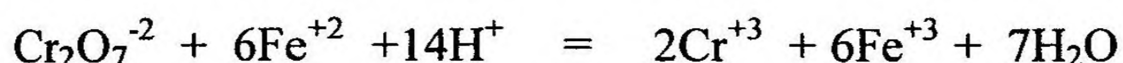
2 moles of $K_2Cr_2O_7$ will oxidize 3 moles of carbon

Volume of Potassium dichromate used = 10ml

Molarity of dichromate = 1/6 M

Burette reading (with soil) = V_1

Burette reading (blank) = V_2



$$Cr_2O_7^{-2} + 6Fe^{+2} + 14H^+ = 2Cr^{+3} + 6Fe^{+3} + 7H_2O$$

Moles of dichromate used = 1/6*10/1000

= X moles

Moles of Ferrous ammonium sulphate	= 1/6*10/1000*6
Molarity of Ferrous ammonium sulphate	= 1/6*10/1000*6/ V_2*1000
	= M_1
Moles of dichromate reacted with Ferrous	= M_1* V_1 *1/6
	= Y moles
Moles of dichromate reacted with C	= X-Y
	= Z moles

2 moles of dichromate will react with 3 moles of carbon;

Moles of Carbon reacted with dichromate	=3/2 * Z
Grams of Carbon reacted with dichromate	= 3/2 * Z * 12
Organic Carbon percentage (%)	= 3/2 * Z * 12 * 100/1
	= C% * 1.724

(1.724 Vam Bemmelens Factor)

3.9.2.2 Determination of Phosphorus soluble in Sodium Bicarbonate (Olsen's bicarbonate extraction) (Olsen and Dean, 1965)

This method may be used for soils having pH value more than 5.5.

Accurately 5 g of air-dried 2 mm sieved soil was transfered into a 100 cm^3 Erlenmeyer flask. One half tea spoon of Carbon black (Darco-G) and 50 cm^3 of 0.5 M $NaHCO_3$ were added. The flasks were shaken for 30 minutes using an orbital shaker and filtered through a Whatman No.42 filter paper. If the filtrate was coloured, some more carbon black were added. The solution was shaken and filtered. A suitable aliquat of the filtrate were pippetted in to a 50 cm^3 volumetric flask. 2 drops of P-nitro phenol indicator was added and 1M H_2SO_4 was added drop wise till the solution became colourless. The solution was made up to 30 cm^3 with pure water. 10 cm^3 of the molybdic acid reagent was added and made the volume up to the mark. The flask was left for 30 minute and measured the absorbance at 660 nm using a Spectrophotometer.

A reagent blank was also run and employed for zero absorption setting at the same wave length maximum.

To make the standard curve, 0, 1, 2, 3, 4, 5 and 6 cm^3 of standard 5 ppm phosphorous solution were pippetted each in to 50 cm^3 volumetric flask and $NaHCO_3$ extracting solution was added such that the total volume of each flask is 10 cm^3. The required steps to obtain absorption were followed as described earlier. Plotted the concentration of P in ppm against the absorption was followed. The concentrations of the standard solutions were 0, 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 ppm.

Calculation:

The concentration of P in the test solution is referred to the standard curve that corresponds to the measured absorption.

ppm in soil = X * 50/V * 50/5

= X * 500/V

Where X = ppm P in the test solution

V = Aliquot used from the extract cm^3

P.N.P. Indicator-0.2 g of 4-Nitrophenol dissolve in 100 ml de-ionized water

Reagent A

- Dissolve 12 g ammonium paramolibdate in 250 ml of De-ionized water
- Dissolve 0.2908 g Pottasium antimony tartrate in 100 ml of De-ionized water.
- Mix the above two reagent together and add 5 N H_2SO_4 1 L and mix
- Dilute up to 2 L.

Reagent B

- Dissolve 1.056 g ascorbic acid in 200 ml of reagent A

Standard Solutions

- Weight 0.4393 g KH_2PO_4 in to 1L volumetric flask
- Add 500 ml De-ionized water
- Shake till dissolve and volume rise up to 1L.(This is 100 ppm solution)

3.9.2.3 Determination of Phosphorus Soluble in Dilute Acid-Fluoride (Bray method)
(Olsen and Dean, 1965)

Accurately 1 g of soil was weighed into an extraction bottle or tube, and 7 ml of the extracting solution was added. The container was shaken for 1 minute, and filtered the contents through Whatman No.42 paper. If the filtrate is not clear, poured the solution back through the filter. To 2 ml of the filtrate, 5 ml of distilled water was added. 2 ml of the ammonium molybdate solution was added, and mixed the contents well. Added 1 ml of the $SnCl_2$ dilute solution and mix the solution again. After 5 or 6 minutes and before 20 minutes, measured the color photo metrically using 660 mμ incident light. A standard curve was prepared including the 2 ml of extracting solution in the range of 0.1 to 1 μg of P per ml. Plot the transmittances of the standards against the μg of P per ml on semilogarithmic graph paper. (Spectrophotometer used- Spectronic 21 D Milton roy)

Preparation of extracting solution:
Add 15 ml of 1.0 N ammonium fluoride (NH_4F) and 25 ml of 0.5 N hydrochloric acid (HCl) to 460 ml of distilled water. This gives a solution 0.03 N in NH_4F and 0.025 N in HCl. It will keep in glass > 1 year.

Calculated the concentration of extractable phosphorus as follows:

Ppm of P in soil = ppm of P in solution X 35
Preparation of the extracting solution:
Add 15 ml of 1.0 N NH_4F and 25 ml of 0.5 N HCl to 460 ml of distilled water. This gives a solution 0.03 N in NH_4F and 0.025 N in HCl. It will keep in glass more than 1 year.

3.9.2.4 Determination of Phosphorus Soluble in water
(Olsen and Dean, 1965)

Accurately 5 g of air dry soil was added to a flask suitable for continuous shaking and then 50 ml of water was added to the flask The contents of the flask were shaken continuously for 5 minutes. The mixture was centrifuged until the solution is free of soil mineral particles. This usually occurs in 15 minutes in a high speed centrifuge at a setting of 100 rpm's. Clear extract was obtained also by repeated filtration through Whatman No.42 filter paper. Return to the suspension the first portions coming through filter paper. The aliquots containing 2 to 20 μg

of P (15 ml aliquot maximum) were pipetted in to 125 ml separatory funnels with a 20 ml mark. 5 ml of the molybdate solution was added and distilled water added to the 20 ml mark. 10 ml of isobutyl alcohol was added and shaken the funnel for 2 minutes. After 5 minutes, the aqueous layer was discarded and washed the isobutyl alcohol mixture by shaking it for one minute with 10 ml of 1N H_2SO_4. The aqueous layer was discarded and added 15 ml of dilute $SnCl_2$ solution; Shaken the funnel for one minute and then discarded the aqueous layer. The blue isobutyl alcohol layer was transferred into a 25 ml volumetric flask and washed the funnel with ethyl alcohol. The solution was diluted to 25 ml with ethyl alcohol. Allowed the color to develop for 30 minutes. The color was stable at least 4 hours. The transmittance of the blue solutions was determined with 660 mμ or 720 mμ incident light. Set at 100% transmittance using a blank solution containing all the reagents.

A calibration curve was prepared by pipetting known concentrations of P in the range of 0 to 22 μg of P into 125 ml separatory funnels, and developed the colour as outlined in the procedure. The logarithem of the percent transmittance against the phosphorus concentration was plotted.

3.9.2.5 Soil nitrogen analysis

(Bremner, 1965)

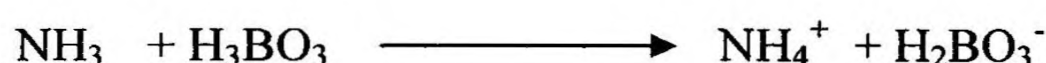
Principle:

Three steps are involved.

1) Digestion of the sample with concentrated sulphuric acid and catalytic mixture to convert organic nitrogen to ammonium

2) Distillation of NH_4^+ in digest with strong alkali and collection of NH_3 liberated in boric acid.

$NH_4^+ + OH^-$ $NH_3 + H_2O$

$NH_3 + H_3BO_3$  $NH_4^+ + H_2BO_3^-$

NO_3^- Nitrogen also gets converted to NH_3 with devadas alloy in alkaline medium.

3) Titration of borate ions of the ammonium borate formed against the standard acid

$$H_2BO_3^- + NH_4^+ + HCL \longrightarrow H_3BO_3 + NH_4Cl$$

Accurately weighed 0.5 g of soil was transfered into a digestion tube and about 1 g of Catalyst mixture (K_2SO_4 , $CuSO_4.3H_2O$ and Se powder) and 4 ml of concentrated sulfuric acid were added. The solution was heated on a digestion stand for about 08 hours or until the digest becomes colourless or pale straw in colour. After cooling, the whole digest was transferred in the flask / tube fully in to a macro kjeldhal flask using about 150 ml of distilled water. 10 ml of boric acid was placed in a receiving flask and two drops of mixed indicator was added. The receiving flask was placed beneath the condenser, So that the end of the delivery tube almost touches the surface of the boric acid solution. 25 ml of 10 N NaOH was poured into the micro Kjeldhal flask carefully along the wall. A small quantity of Devadas alloy and about 100 ml of distilled water were added into the distillation flask attached in to distillation apparatus.
The digest was distilled until the volume of the distillate in the receiving flask increase up to about 40 ml. The tip of the condenser was washed down and titrate the ammonium ions in the distillate against 0.01M H_2SO_4 until colour change from green to pink.

Calculation:

Moles of H_2SO_4 used to convert borate to boric acid could be found by the titration.

1 mol of H_2SO_4 = 1 mol of NH_4^+

Volume of 0.01 M H_2SO_4 required = X ml

X ml of 0.01 H_2SO_4 react with $= \dfrac{0.14 * X * 100}{5 * 100}$

Percentage of total Nitrogen in sample = X * 0.28

3.9.2.6 Soil K and Mg analysis

(Heald, 1965, Pratt, 1965)

The soil sample was air dried and sieved through 2 mm diameter sieve. 5 g of soil sample was weighed accurately and collected to a conical flask. 25 ml Ammonium Acetate was added. The container was closed with Parafilm and kept at shaker for 30 minutes then centrifuged 10 minutes at 2000 rpm. The upper part was filtered through No. 42 Whatman filter paper and collected to a plastic bottle. Amount of 2 ml was measured accurately to a beaker and 28 ml distilled water was added. The values were measured in ppm for K and Mg using Atomic Absorption Spectrophotometer (Spectronic 21 D Milton roy).

3.9.2.7 Soil pH Determination (1:2.5 H_2O)

Ten grams (10 g) of exactly weighed soil was transfered into a 50 ml beaker or a plastic container and 25 ml of distilled water was added with a 25 ml measuring cylinder or a 0-50 ml dispenser. It was stirred thoroughly with a glass rod and let to stand for 30 minutes. The pH meter was calibrated using the standard buffer solutions of pH 4.0 and 7.0. The sample suspension was stirred and immediately determined the pH to the nearest 0.01 pH unit. The electrodes were rinsed with distilled water and wiped with tissue paper before measuring the next sample.

(Tandan, 1999)

3.10 Chemical analyses of tissue samples (shoo/root)

Determination of total nitrogen: After oven drying (70^0C) and weighing, the plant samples were ground to less than 1 mm size particles. Shoot sample were composed of a mixture of leaves, petioles and stems. The ground samples were weighed accurately ($\pm$ 0.001 g) and placed in a Kjeldhal digestion tubes and digested with concentrated H_2SO_4 and after cooling, steam distillation was done under alkali medium (1.0 N NaOH). Liberated ammonia was collected in Boric acid indicator mixture and it was titrated with 0.1 N H_2SO_4 (Bigham, 1996).

3.10.1 Determination of P, K and Mg

The ground samples were weighed accurately and burned to ash in a muffle furnace at 450^0C. The tubes were cooled and then ahs samples were transferred into 50 ml volumetric flask

with 2.5 ml of con HCl acid through a filter paper. The extract was used for the determination of P, K and Mg.

Four ml of plant extract was pippeted into a beaker and 10 ml of ammonium metavandate reagent and distilled water was added to it. P concentrations of the samples were measured using spectrophotometer at 425 nm wave length. Potassium and Magnesium content of the extracts were measures using GBC903A Atomic Absorption Spectrophotometer.

3.11 Statistical analysis

Data were analysed by one way and two ways Analysis of Variance (ANOVA), with the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS Institute, 1990). To avoid problems arising from heterogeneity of error, all data were converted to natural logarithm values before statistical analysis when necessary. In such cases, actual means are presented in the tables or figures. After analysis, means were back-transformed to the original scale, and least significant differences for those means also back-transformed to give least significant ratios (LSR). The experimental designs involved Completely Randomized Design or Randomized Complete Block Design with factorial combinations as appropriate for each experiment.

Chapter 04

Effect of host crop on mass propagation of Vesicular Arbuscular Mycorrhizae (*Glomus mosseae*)

4.1 Introduction

Inoculation experiments have shown that different AMF species produce a wide range of growth responses in the host plant, from significantly positive to significantly negative. Often the concentration of soil P influences the effectiveness of inoculation (Gosling *et al.*, 2006). The Department of Export Agriculture (DEA) recently commenced a research programme to exploit the potential of VAM for productivity improvement of pepper and cinnamon. Therefore, it is necessary to mass propagate VAM spores with good characters (inocula) to inoculate Export Agricultural crop (EAC) plants to obtain above benefits. For that purpose, it is needed to select host plant species which can multiply the fungal mass and spores rapidly. The choice of host plant used to propagate VAM fungi can have a large influence on fungal sporulation and mycorrhizal root formation (Brundrett *et al.*, 1996). It seems that prior cropping with a strongly mycorrhizal plant eg. Maize can increase soil infectivity even further (Ocampo and Hayman, 1981). Association of arbuscular mycorrhizal (AM) with agricultural crops was assessed at four agro-ecological zones of Bangladesh during 1999-2000. Mainly cereals, pulses, oilseeds,vegetables and spices crops were selected for assessment. Among cereal crops; maize, wheat and sorghum scored higher root colonization and number of AM spores in their rhizosphere soils in all AEZs during both years (Khanam *et al.,* 2006). As an initial step, selection of a good host crop for mass propagation of available VAM (*Glomus mosseae*) spores is one of the primary objectives of the integrated nutrient management project for EACs. Therefore, as a part of the above project, investigations are necessary on the VAM spore regeneration ability and temporal distribution of VAM spore density in rhizosphere of different host crop species. The objectives of this research are to find out the appropriate host crop species and time of uprooting the host crop to get the maximum possible number of spores as inoculum (mycorrhizal spores and structures with host crop roots and moist soil).

4.2 Materials and methods

4.2.1 Experimental Design

Experimental lay out was completely randomize design (CRD) with 12 replicates.

4.2.2 Trial establishment

Three host crops, namely maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and finger millet (*Eleucine coracana.* L) were planted in clay pots (Plate 4.1). The clay pots having 24 cm in top diameter, 14 cm in bottom diameter and 18 cm in height with 0.0314 m^2 of surface area at the level of planting surface were used. Each pot was pre-soaked with water and then filled with 2 kg of sterile top soil (Reddish brown latasolic soil with pH 5.81) collected from Research Station, Department of Export Agriculture, Matale. Two hundred and fifty grams of inoculum consisted of mycorrhizal spores and structures (*Glomus mosseae*) with sorghum roots and moist soil were incorporated into the sterile soil in each pot. Thus initial spore density value became to 23 brown spores and 6 black spores per 50 gm of the potting media of each pot. Seeds of each host crop were surface sterilized with 70% (V/V) ethyl alcohol for 1 minute and washed 8 times with sterile distilled water (Brundrett *et al.*, 1996). Two seeds of either Maize or sorghum were planted per pot as per the treatment requirement and seedlings were thinned to one plant per pot after emergence (Groth and Martinson, 1983). In the case of finger millets, ten seeds were sowed per pot and seedlings were thinned to 6 plants per pot after emergence following the recommended seed rate (Anon, 2005). Pots were covered with blotting papers and kept in a shade house and watered daily.

The inoculum (mycorrhizal spores and structures with host crop roots and moist soils) which was used in this experiment was obtained following harvesting of exotic VAM (*Glomus mosseae*) regeneration bed, available at the Export agriculture research station, Matale (Plate 4.2).

Plate 4.1: Clay pots with different host crops, at two month after AM inoculation

Plate 4.2: Exotic AM (*Glomus mosseae*) regeneration bed at research station, DEA.

4.2.3 Data Collection

Spore density was studied in samples collected at 8, 10 and 12 weeks after planting. In order to reduce the variability, three samples were taken from each pot for analysis. Wet sieving technique (Brundrett *et al*., 1996) was adopted to find the spore density as described in material and method (Section 3.4.1) section. Number of spores per sample was counted under a stereo microscope, subsequently number of spores per pot was estimated. Data were statistically analysed using GLM procedure of SAS package. Duncan's Multiple Range Test (DMRT) was used to overcome the problem with the LSD test. Mycorrhizal infection of roots of these host crop species was observed through light microscope after staining with 0.1% tryphan blue (Jarstfer and Sylvia, 2001).

4.3 Results and discussion

Two types of spores, namely brown and black coloured were observed in analyzed samples (Table 4.1 and 4.2). Neither black spores nor brown spores were significantly ($p<0.05$) different (Appendices 4.1 and 4.2) in one way ANOVA. Treatment effects were not significant ($p<0.05$) for brown spore density (Appendices 4.2). It is important to note that brown spore density value of each crop species became almost equal at 12 weeks after planting (Table 4.1). However, the brown spore density value remained more or less similar through out the study period for finger-millet (Fig. 4.1).The pattern of black spore density (Table 4.2) variation with time is similar to that of brown spore density distribution showing fluctuations in Sorghum and Maize crops (Fig.4.2). Although it is not significant in contrast to the other two crop species, black spore density in finger millet is in slightly increasing order towards 12 weeks after planting .

Table 4.1: Mean brown spore density per 50 g soil with SEM values in different host crops at the different time intervals.

	mean brown spores/50 g soil at different time intervals		
Host crop	**Wk 08**	**Wk 10**	**Wk 12**
Sorghum	193±18.4[a]	96±7.5[a]	157±19.5[a]
Maize	58±4.2[b]	192±17.0[b]	132±9.4[b]
Finger millet	132±9.8[c]	111±10.1[c]	137±9.7[c]

n=3, $P < 0.05$
Same letters in column indicates significantly different data

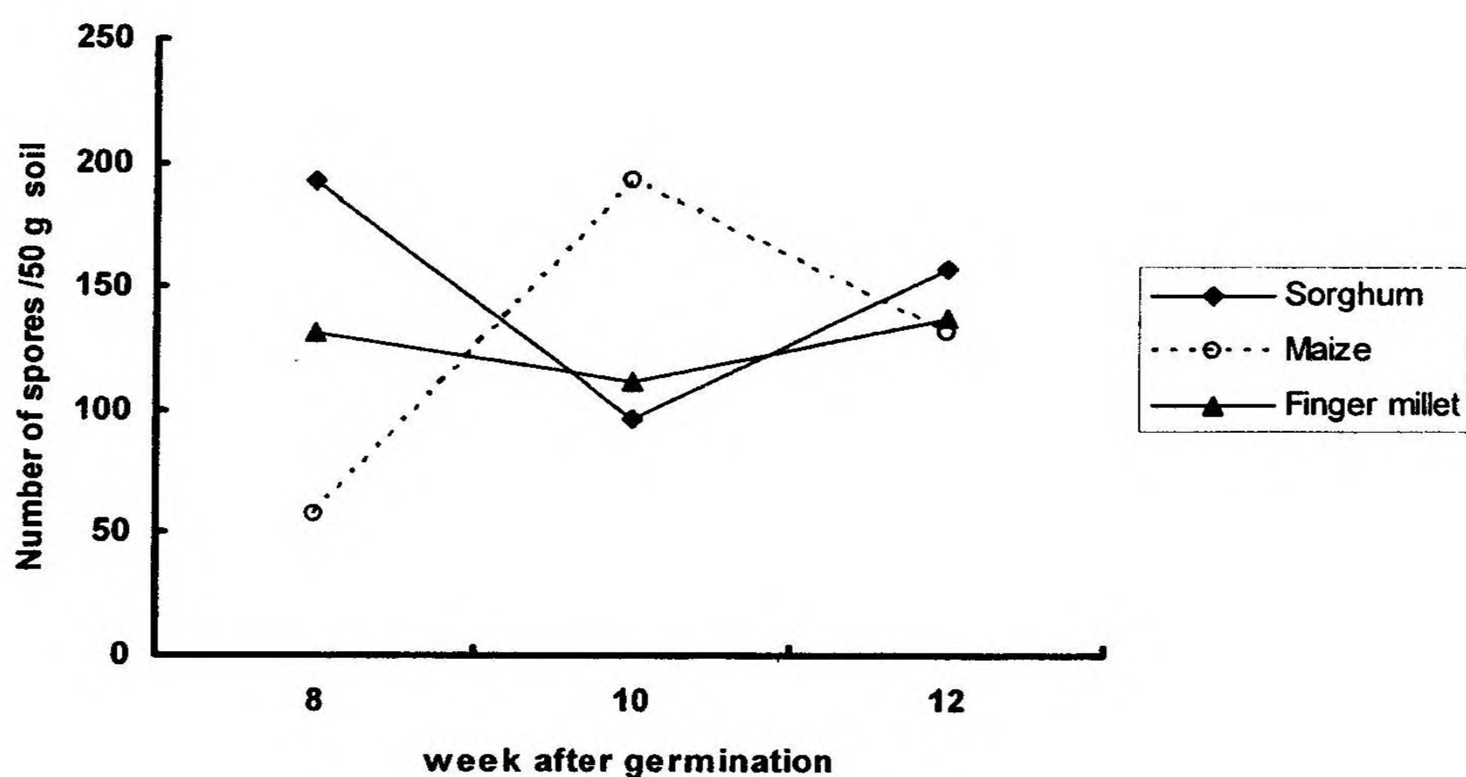


Figure 4.1: Variation of mean values of brown spore density with host crop and time

Table 4.2: Mean black spore density per 50 g soil in different host crops at the different time intervals

Host crop	mean black spores/50 g soil at different time intervals		
	Wk 08	Wk 10	Wk 12
Sorghum	214± 22.2[a]	98±6.9[a]	242±22.7[a]
Maize	40±7.9[b]	228±23.8[b]	119±7.8[b]
Finger millet	154±12.6[c]	202±18.7[c]	224±23.5[c]

n=3, P< 0.05
Same letters in column indicates significantly different data

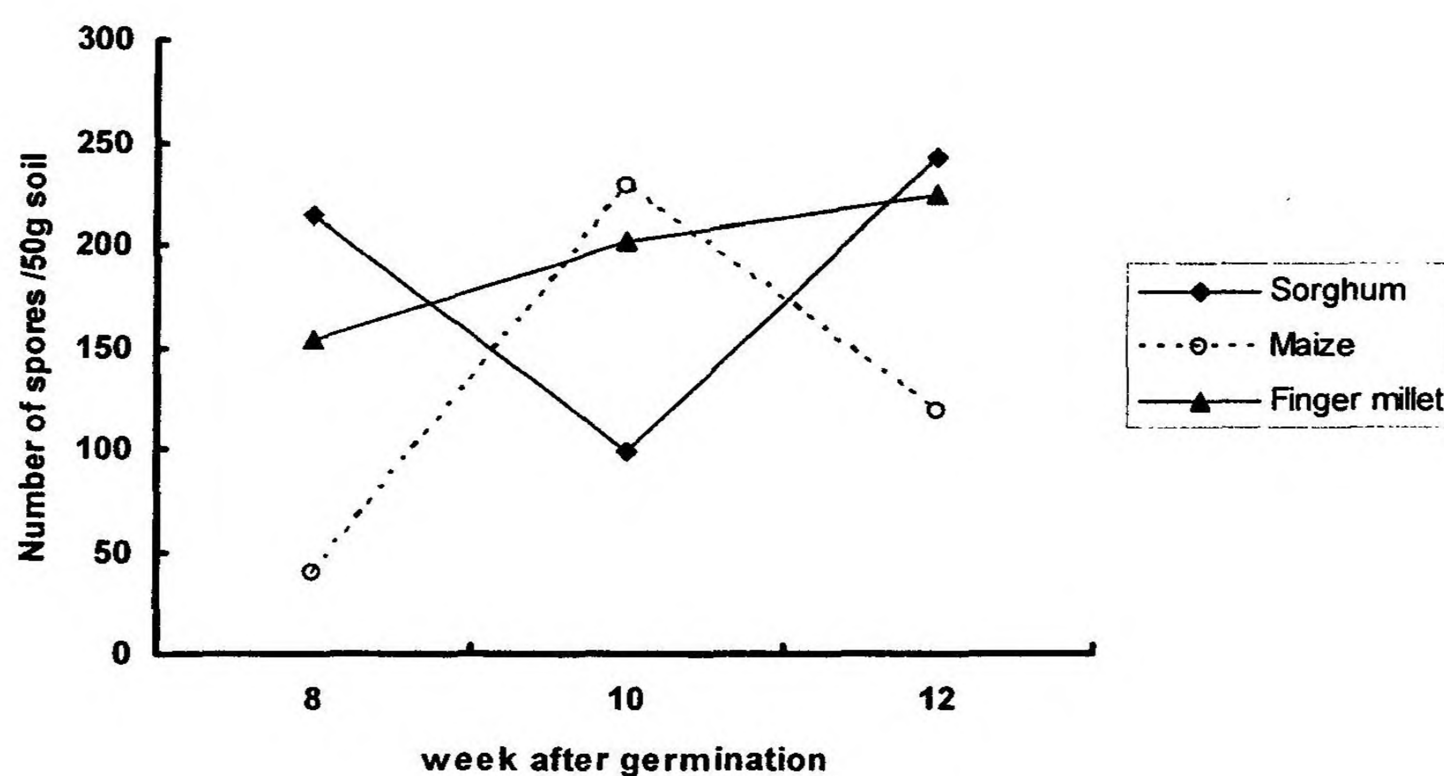


Figure 4.2: Variation of mean values of black spore density with host crop and time

The observations of this study are comparable with the spore density observations reported by Sharif and Moawad (2006) for certain other cereal crops like Barley, Rice, Chick pea, Wheat and Oat. According to the scale (0-20 spores/15 g soil-low, 20-60 spores/ 15 g soil- medium, >60 spores/ 15 g soil) given by Sharif and Moawad (2006), brown spore density values were within medium level for all the observations except for maize at 8 weeks in this experiment. Quite comparably, black spore density values were within high and medium levels except for maize at 8 weeks. According to the literature (Miranda, 1989, Narayan, 1993), sorghum or

maize can be used as suitable host crops for mass propagation of VAM species (Brundrett *et al.*, 1996). This study showed that finger millet could also be used as a good alternative host crop.

The observation of stained internal mycorrhizal structures in root pieces confirmed (Plate 4.3) the success of initial inoculation of each species in this study.

Plate 4.3: Stained Sorghum root (10×40) at 12 weeks after inoculation (the structures shown by an arrow are the initial stage of vesicle formation)

4.4 Conclusion

Brown spore density values of three host crops studied became almost equal towards harvest at 12 weeks. Delaying of uprooting host crop for inoculum collection up to harvest stage of a cereal crop can give an additional income. Therefore, under local conditions finger millet can be recommended as a good alternative host crop species for mass propagation of VAM.

Chapter 05

Determination of effective spore density of the Arbuscular Mycorrhiza (AM), *Glomus mosseae,* for inoculation of pepper (*Piper nigrum* Linn.) rooted cuttings

5.1 Introduction

As Black pepper is one of the major EAC crop in Sri Lanka. Application of mycorrizal inoculation techniques are becoming an important plant nutrient management tool, as arbuscular mycorrhizae induced growth improvements are widely reported in many perennial plants. This was attempts by several tropical countries and findings were as follows. Incorporation of vesicular arbuscular mycorrhiza (VAM) fungus *Glomus fasciculatum* in the rooting medium of sand enhanced rooting of black pepper (*Piper nigrum*) cuttings (Anandaraj and Sarma, 1994). Rooting enhancement could be used as a criterian to identify efficient strains of VAM for black pepper. An another experiment conducted by Kandiannan, *et al.*, (2000) to test the effect of Azospirillum, Phosphobacteria and vesicular arbuscular mycorrhiza on growth and nutrient content of black pepper cuttings indicated that growth parameters were on par with control, when these three biofertilizers were applied individually, but their combination enhanced growth significantly over control. Rooting enhancement could be used as a criterion to identify efficient strains of VAM for black pepper. The investigation carried by Thanuja, *et al.*, (2002) clearly showed the beneficial effects of inoculation with AM fungi which resulted in enhanced rooting and root growth in black pepper cuttings.

Glomus mosseae form large asexual chlamydospores at a hyphal tip, usually one per tip which highly infective to genera of herbaceous and woody plants in a wide range of conditions. It has a high reproduction ability mediated through the production of spores. Spores are borne singly in soil and also formed in the root cortex or in sporocarps (James and Schenck, 1984). According to Xioutang (1994) *G. mosseae* can form Mycorrhizal associations with many plant species with significant effects. Moreover, there is considerable potential for the development of an inoculum of *G. mosseae* for use with tree crops. Therefore, as an initial step towards this goal, the present experiment was carried out with the objective to select a suitable spore density of the AM fungus *Glomus mosseae* for the inoculation of pepper rooted cuttings at the nursery stage.

5.2 Material and Methods

This experiment was carried out at the nursery of the Research Station, Department of Export Agriculture (DEA), Matale, from March to October, 2008.

5.2.1 Experimental treatments

Four mycorrhizal inoculum levels of *Glomus mosseae* 25 g (T1), 75 g (T2), 150 g (T3) and 300 g (T4) were tested with a control (T5) after quantification of initial spore density value of each inoculum using wet sieving and decanting technique with sucrose centrifugation (Brundrett *et al.*, 1996). Inoculum levels and corresponding quantifications are shown in Table 5.1.

Table 5.1: Inoculum levels and corresponding quantitative values of parameters assigned for each treatment

Treatment code	Amount of inoculum (g)	Quantity of potting mixture (g/pot)	Mean number of spores/ pot	Mean AM Spore Density (No. of spores/g of potting mixture)
T1	25	875	265 ± 74	0.3
T2	75	825	795 ± 146	0.88
T3	150	750	1590 ± 329	1.77
T4	300	600	3180 ± 591	3.53
T5	0	900	0	0

5.2.2 Experimental Design

The experiment was laid out in a randomized complete block design with 5 blocks. Each block consisted of 5 pots having one black pepper (cultivar GK 49) rooted cutting in each pot.

5.2.3 Trial establishment

The sterilized (autoclaved at 121 ^{0}C for 15 minutes) standard potting mixture consisting of equal parts of top soil, cow dung, coir dust and sand was used (Anon, 2003). The initial nutrient composition such as total Nitrogen, available Phosphorus, exchangeable Potassium, Magnesiun and the pH of the standard potting media were measured. The quantity of corresponding inoculum [mycorrhizal spores and structures with sorghum (*Sorghum bicolor* L.) roots and moist soil obtained by harvesting AM (*Glomus mosseae*) regeneration bed] was assigned to each treatment (Table 5.1) and well mixed with the standard potting mixture required to fill each polythene bag. Number of replicates for each inoculum treatment was 20 polythene bags and this procedure was repeated for 100 polythene bags following the treatment combinations (Table 5.1).

A two nodal cutting of black pepper cultivar GK 49 (local) was planted in each black polythene bag (gauge 150) of 21 cm×13 cm (8" x 5") and these pots were kept in a humid chamber on 14th March 2008 according to the recommended practices (Anon, 2003). The spore density in the inoculum was quantified through wet sieving and decanting technique (Brundrett *et al.*, 1996) described in material and method section (section 3:4:1).

In order to find the equivalent dry weight of moist soil sample, moisture factor was adopted using a sample for determination of dry basis moisture content as mention in the general material and methods. (Section 3.3)

5.2.4 General management

The black pepper cuttings planted in pots as above were kept in an air tight humid chamber at the nursery site. The humid chamber was opened at 3rd week after planting. The cuttings were gradually hardened by opening the polythene cover for few hours every day for about two weeks. The polythene cover was totally removed at 5th week after planting. General management practices such as watering, weeding and control of pest and diseases were done manually throughout the growing period.

5.2.5 Data collection

Data were collected at 8th week, 16th week and 24th week after planting.

5.2.5.1 General observations

Casualties of cuttings were counted at the time of complete opening of the humid chamber. Prior to the commencement of uprooting of the rooted cuttings following measurements were taken for each plant at 8th week, 16th week and 24th week after planting.

a) Number of leaves

   The number of new leaves emerged in each plant was recorded

b) The diameter of initial stem

   The diameter of the initial stem of the cuttings was measured by using a Vernier Caliper. The point at which the stem emerges from soil surface was measured.

c) The height of the new shoot

   The height of the newly emerged shoot (from the base of the new shoot to the tip of the terminal shoot) of each plant was measured.

5.2.5.2 Destructive sampling and observations

The following destructive measurements were taken at 8th week, 16th week and 24th week after planting by uprooting a set (5 pots from one treatment level) of samples from each replicate. The roots of the uprooted plants were thoroughly washed with tap water. Then roots were carefully separated using a surgical scissor and following measurements were taken on per plant basis.

a) Leaf area

   All the leaves of each uprooted plant were separated from the stem and leaf area was measured using an electronic area meter as described in the catalogue (CI-202 Area Meter).

b) Root length

   The washed roots were wiped off with a tissue paper and each root sample was spread randomly on a 1 cm grid transparent sheet. The number of intersections was counted across longitudinal as well as vertical direction as described in general M & M. (Section 3.8). Total number of intersections was used to obtain the total root length of each sample following Newman's method as described by Tennant (1975).

   Root length = Number of intercepts * length conversion factor

According to the Tennant (1975), the length conversion factor for the 1cm grid squares is 0.7857.

c) Shoot dry weight

Each shoot sample was transferred to a paper bag and dried for constant weight in an electronic oven at 70 ^{0}C. The final weight was measured using an analytical balance.

d) Root dry weight

After measuring the root length each root sample was transferred to a paper bag and oven dried at 70 ^{0}C for a constant weight. The final weight was measured using an analytical balance.

e) Root staining and percentage mycorrhizal colonization

In order to ascertain the success of inoculation, pepper root samples were stained using the procedure described by Jarstfer and Silvia (2001), (Refer Chapter 03 M & M-Section). Mycorrhizal infection was determined as percent roots infected with mycorrhizal fungi on a per plant basis (Durgapal *et al.*, 2002). The colonization was assessed depending on the presence or absence of hyphae, vesicles and arbuscules in each segment of root and was expressed as percentage of colonization according to the method described by Kapoor and Paroda (2005).

f) Final VAM spore population

Rhizosphere soils under each inoculum level were also assessed for VAM spore population through wet sieving and decanting method (Brundrett *et al.*, 1996) which is described in the Chapter General Materials and Methods (section 3.4.1). The spores were counted using a stereomicroscope. Data were statistically analyzed using general linear model (GLM) procedure of SAS package.

5.3 Results and Discussion

Initial chemical properties of the potting media were: 0.19% total nitrogen, 2.2 mg kg^{-1} available phosphorous, 1109 mg kg^{-1} exchangeable potassium, and 576 mg kg^{-1} magnesium and with a pH of 5.55 (1:2.5 water suspension). Nevertheless, chemical properties of the potting media were not tested after the experiment, as it is beyond the objectives of this experiment. Simple counting of live pepper cuttings at the opening of the humid chamber at 21 days after planting revealed that the overall success percentage was very high with 75% of survival irrespective of treatment effects.

According to the microscopic view of stained pepper root segments, darkly stained AM hyphae were observed (30% at T2 and T3 levels and 20% at T1 and T4 level) in the roots at the 2nd month after inoculation of rooted cuttings at all the inoculum levels except in T5 (control). Ten root pieces were tested from one sample in staining purpose. Neither vesicles nor arbuscules were seen in the control. This confirms the successful inoculation of *G. mosseae* in this experiment. Furthermore, extra-radical, intra-cellular and inter-cellular hyphae were observed at the 6th month after inoculation (Plate 5.1a and 5.1b). Different structures such as globose and sub-globose vesicles with hyphal connections were observed in the root samples of treated cuttings. These structures were recognized with reference to the illustrations of Brundrett *et al.* (1996) as well as Brown and King, (1982). Preddy *et al.*, (2003) also reported of observing similar mycorrhizal structures in root sample of different genotypes of turmeric. The heaviest infection of mycorrhizal fungi was observed in roots of T3 cuttings having the maximum root colonization, along with mycelium, vesicles, and spores at the 6th month after inoculation. (Table 5.2) It indicates that the time taken to produce vesicles and arbuscules is quite longer in black pepper roots than the time taken for the same process in *Sorghum bicolor* which was only two months (Kumari *et al.*, 2008). This is comparable with studies of Brundrett *et al.* (1996).

Table 5.2: Mycorrhizal colonization of root samples and spore density of rhizosphere soil at 6th month after inoculation

Inoculum level	Root colonization % (Mycorrhizal structures)	Mean no. of Vesicles	Mean no. of spores/50 g of potting medium
T1	90	0	239 ± 34
T2	100	10 ± 4	305 ± 39
T3	100	94 ± 12	769 ± 55
T4	100	07 ± 2	524 ± 47
T5 (control)	0	0	0

n=5

Replacement of one third of the nutrient rich potting medium with inoculum (top soil base material) in T4 may be one of the reasons for relatively low infection of AM in T4 compared to T3 cuttings. Apart from that, the introduction of a high number of infective propagules at T4 could have resulted in relatively low infection density in pepper rhizosphere due to competitive effect.

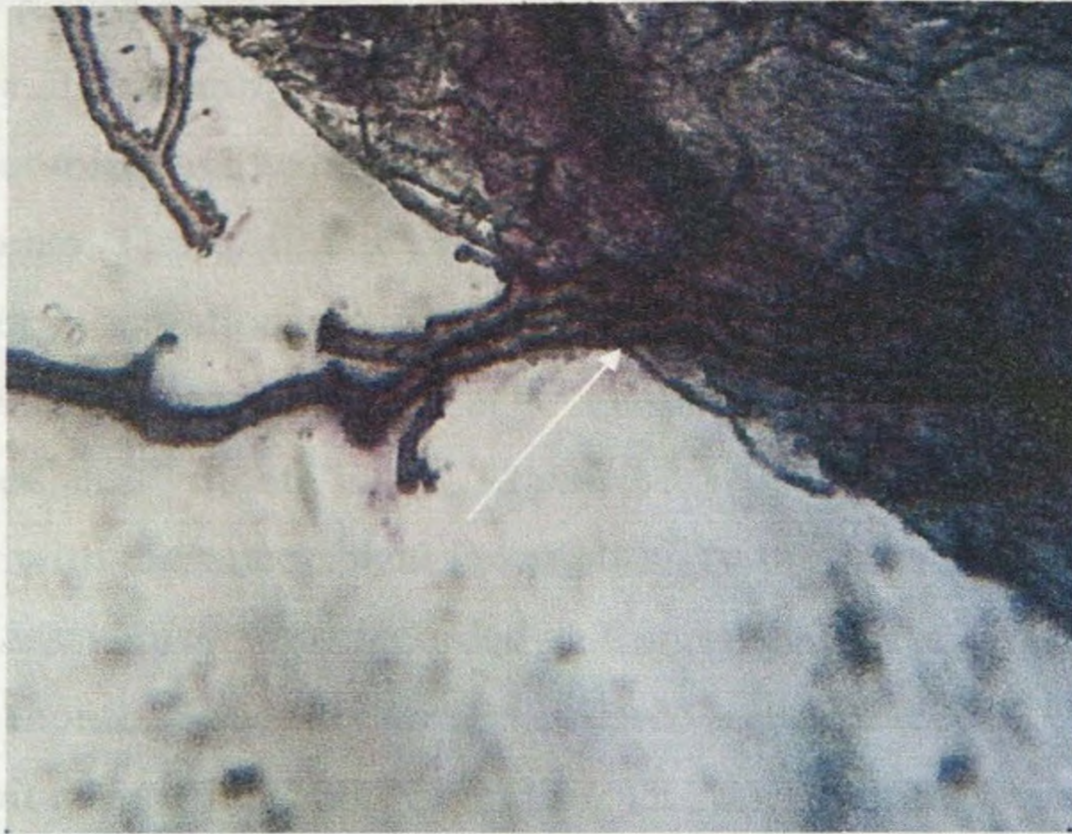


Plate 5.1a: Stained black pepper root epidermis segment (10×40) at 2nd month after inoculation showing fungal hyphae (indicated by an arrow) entry point to the root.

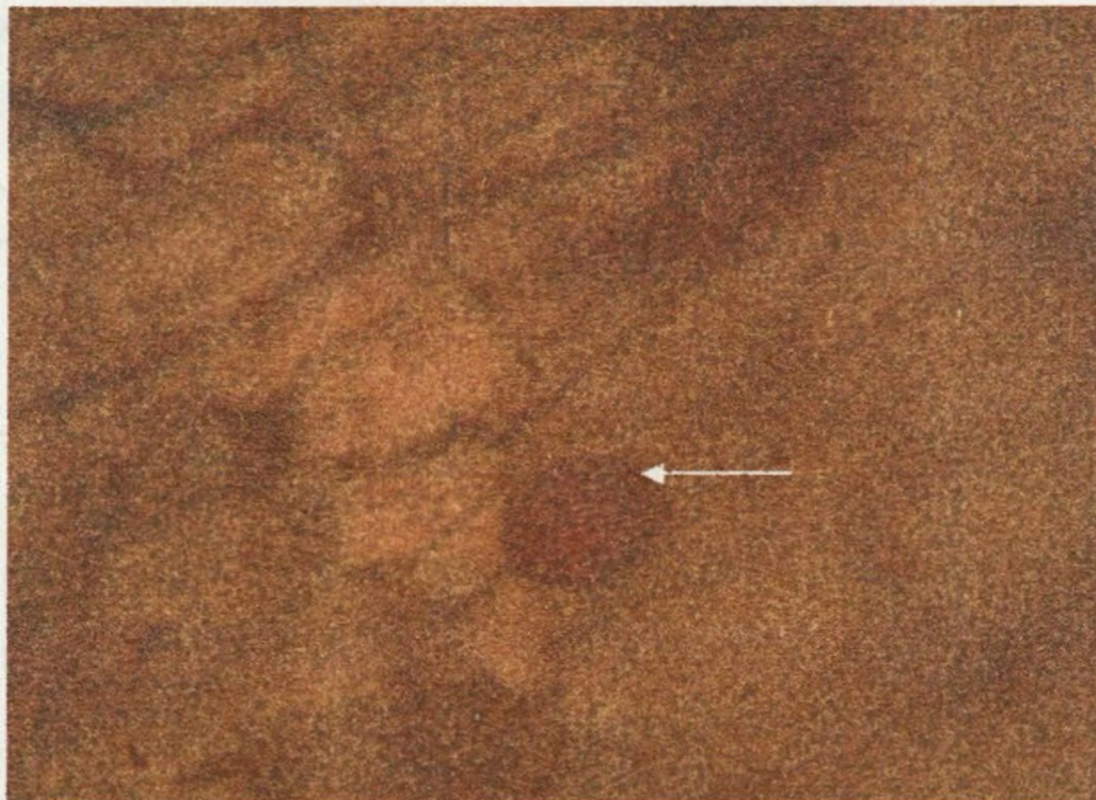


Plate 5.1b: Stained black pepper root cortex segment (10 x 40) at 4th month after inoculation showing vesicle formation (indicated by an arrow) within root tissues

5.3.1 Shoot dry weight/ biomass

A one way ANOVA was employed to determine the effect of time and treatment on shoot dry weight individually. The combined effect was determined by performing a two way ANOVA. As expected, shoot biomass value of each treatment increased with time irrespective of inoculation treatments. The treatment effect was not significant ($p<0.05$) for shoot dry weight until 4th month after inoculation. Although it was not significant, the highest shoot dry weight was recorded in T4 at 2nd month and T1 at 4th month. Interestingly, a non- significant low dry weight was recorded at 2nd month in T1, T2 and T3, when compared with the control showing growth retardation with the micorrhizal inoculation. According to the Table 5.3a, significantly higher shoot biomass values were recorded at 6 month after inoculation in comparison with the 2 month and 4 month. Shoot dry weight became significant ($p<0.05$) at 06th month having maximum shoot biomass (5.077±0.871 g) for T2 (75 g inoculum) cuttings (Fig.5.1). This suggests the possibility of existing temporary growth retardation due to VAM infection at the initial stage and it seems to be gradually disappeared towards 6th month in this experiment. According to Linderman and Hendrix (1982), VAM fungi initially act as parasites, absorbing organic nutrients from its host plant until it develops effective nutrient and water absorbing external mycelium. Indication of initial competition due to VAM infection in pepper rooted cuttings at higher inoculation levels have also been reported by Wimalaratne (2005). Xioutang (1994) found that inoculation of VAM fungi increased the shoot dry weight and root dry weight of *Mangifera indica* plants.

Table 5.3a: Effect of treatment on mean shoot dry weight (g) of pepper rooted cuttings at different constant time periods (one way ANOVA)

	Mean shoot dry weight (g) Time (month)		
Treatment	**2**	**4**	**6**
T1	1.38±0.044	2.412±0.393	3.162±0.299^{c}
T2	1.200±0.086	2.175±0.110	5.077±0.871^{abc}
T3	1.35±0.110	2.345±0.384	3.772±0.308^{e}
T4	2.94±0.079	1.300±0.533	3.593±0.192^{b}
T5	2.88±0.392	1.866±0.450	3.283±0.674^{a}
Pr (at 0.05)	0.623	0.213	0.048
LSD value	–	–	1.3769

n=5, α =0.05

Note: Means with the same letters (in column) are significantly different

Table 5.3b: Effect of time on mean shoot dry weight (g) of pepper rooted cuttings at different treatment levels (One way ANOVA)

	Mean shoot dry weight (g) Treatment				
Time (month)	**T1**	**T2**	**T3**	**T4**	**T5**
2	1.095±0.044[ab]	0.974±0.086[a]	1.355±0.110[ab]	0.861±0.079[a]	1.849±0.392[a]
4	2.412±0.393[bc]	2.174±0.110[b]	2.345±0.384[bc]	1.3±0.533[b]	1.866±0.450[b]
6	3.162±0.299[ac]	5.077±0.871[ab]	3.772±0.308[ac]	3.593±0.192[ab]	3.283±0.674[ab]
Pr (at 0.05)	0.0066	0.0453	0.0062	0.0116	0.0078
LSD value	0.7094	1.8425	0.9584	0.8957	0.625

n=5,α=0.05

Means with the same letters are significantly different

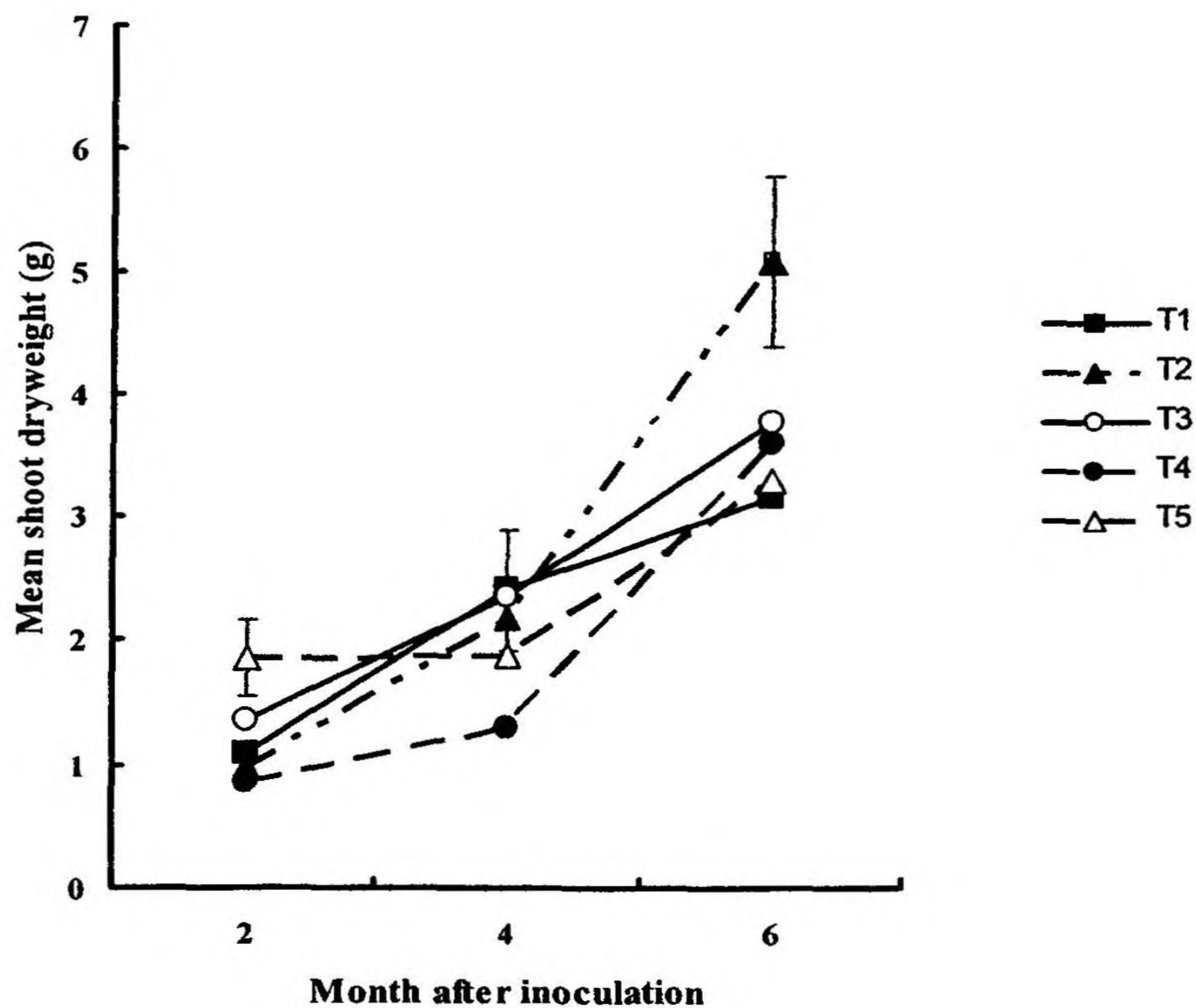


Figure 5.1: Effect of VAM inoculation on mean shoot dry weight of black pepper with time after inoculation, vertical bar indicates LSD at 0.05 probability

A highly significant effect of time was evident on mean shoot dry weight for all treatments. In T1, T2, T3 a steady significant increase was observed whereas for T4, T5 a significant increase was seen at 6th month (Table 5.3b). The combine effect of time and treatment was also significant, having the Pr value 0.0187.

5.3.2 Root length

Similar to the shoot dry weight increase, root length also increased with time irrespective of inoculation treatments. In contrast to the shoot biomass, treatment effect became significant for root length at 4th month only. During the period from 4th month to 6th month, a tremendous root elongation was observed while the order of magnitude with reference to treatments also slightly changed showing almost similar root length values for each of the inoculated treatments. All the inoculated rooted cuttings showed higher root length values than the un-inoculated plants at 4th month having the minimum root length for non inoculated (T5) plants (Fig. 5.2). Nevertheless, the differences in root length values among T2, T3 and T4 were not significant. The narrow differences in root as well as shoot growth response to different inoculation levels may be attributed to high nutrient status of standard potting mixture in this experiment. The effect of time was highly significant in all the treatments. Although for all the treatments showed no significant effect among 2nd and 4th month, by the end of the 6th month all the treatments exhibited a highly significant increase in all the treatments including the control (T5) (Table 5.4b). The highest root length was recorded in T2 treatment at month 6 with a mean of 3250.25(±680.2) cm. The lowest recorded value was observed in T4 treatment at 2nd month with a mean of 61.28±10.6 cm (Table 5.4a). In general mycorrhiza induced growth improvements in higher plants are prominent under low nutrient conditions. The root length improvement in this study indicates the effectiveness of VAM inoculation on initial growth of pepper rooted cuttings at nursery stage. Thanuja (2002) also reported beneficial effects of inoculation with VAM fungi which resulted in enhanced rooting and root growth in black pepper cuttings.

The improvement in rooting of pepper cuttings would be beneficial in acquisition of drought resistance and to reduce transplant shock at the post nursery field establishment stage of pepper cultivation. Apart from those, improvement in nutrient absorption capacity as well as enhancement of plant vigor can also be expected with root growth improvement in pepper rooted cuttings.

Table 5.4a: Effect of treatment on mean root length (cm) of pepper rooted cuttings at different constant time periods with ±SEM values.

	Mean root length (cm)		
	Time (month)		
Treatment	**2**	**4**	**6**
T1	107.71±37.4^{a}	496.51±152.0^{a}	2845.3±623.6^{a}
T2	152.03±22.5^{b}	482.26±117.1^{b}	3250.25±680.2^{b}
T3	163.89±41.1^{c}	304.69±47.1^{c}	2383.35±120.8^{c}
T4	61.28±10.6^{d}	590.32±64.97^{cd}	2855.55±408.2^{d}
T5	136.318±20.3^{e}	119.42±2.99^{abd}	2883.45±400.1^{e}
Pr (at 0.05)	0.311	0.0448	0.9394
LSD value	–	274.553	–

n=5, α=0.05
Note: Means with the same letters in a column are significantly different

Table 5.4b: Effect of time on mean root length (cm) of pepper rooted cuttings at different treatment levels (with ±SEM values.)

	Treatment				
Time (month)	**T1**	**T2**	**T3**	**T4**	**T5**
2	107.7±37.4^{a}	152±22.5^{a}	163.9±41.1^{a}	61.3±10.6^{a}	136.3±20.3^{a}
4	496.5±152.0a^{b}	482.3±117.1^{b}	304.7±47.1^{b}	590.3±64.97^{b}	119.4±2.99^{b}
6	2845.3±623^{ab}	3250.3±680^{ab}	2383.4±120^{ab}	2855.6±408^{ab}	2883.5±400^{ab}
Pr (at 0.05)	0.0376	0.0175	0.0001	0.0271	0.0271
LSD value	1410.22	1400.18	257.88	1025.1	1224.45

n=5, α=0.05
Means with the same letters in a column are significantly different

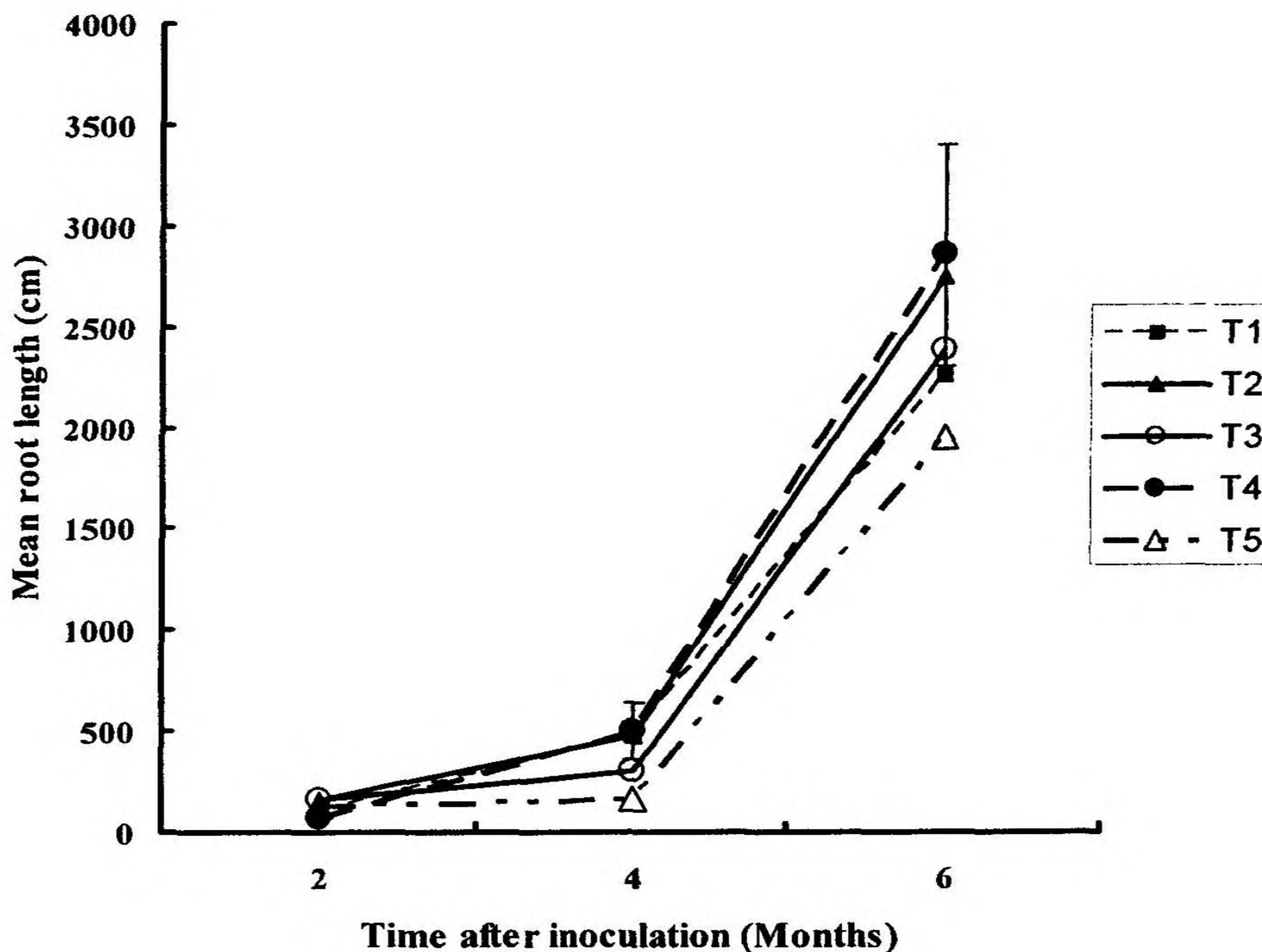


Figure 5.2: Effect of VAM inoculation on mean root length of black pepper with time after inoculation, vertical bar indicates LSD at 0.05 probability.

5.3.3 Other growth parameters

Mean values of other growth parameters taken at 2nd, 4th and 6th month after inoculation are shown in table 5.6- 5.9. Significant changes were not observed for certain growth parameters namely root biomass, leaf area and number of leaves among the treatments. Similarly some of the other selected growth parameters also did not show any trend with respect to treatments showing mean values of 0.39 cm, 0.54 cm and 39.2 cm for new shoot diameter, old shoot diameter and height of new shoot, respectively at 6th month after inoculation. This indicates that the later mentioned growth parameters may not be practical to record as sensitive indicators for a nursery stage study of pepper rooted cuttings.Usually farmers used to field plant pepper rooted cuttings collected during 4th month to 6th month and this depends on various factors such as onset of rainfall in the growing season, availability of well developed rooted cuttings in the nurseries and availability of labour and other resources. Overall observations including plant growth parameters and staining practices of this experiment indicated that adoption of mycorrhizal inoculations seems to be a beneficial practice for pepper nursery management.

Table 5.5a: Effect of treatment on mean root dry weight (g) of pepper rooted cuttings at different constant time periods (not significant)

	Mean root dry weight (g) Time (month)		
Treatment	**2**	**4**	**6**
T1	0.556±0.046	0.563±0.054	0.454±0.071
T2	0.506±0.070	0.568±0.106	0.65±0.183
T3	0.533±0.042	0.659±0.123	0.498±0.083
T4	0.387±0.079	0.522±0.127	0.542±0.087
T5	0.464±0.086	0.413±0.076	0.476±0.073
Pr (at 0.05)	0.4569	0.5271	0.6683
LSD value	–	–	–

n=5, α=0.05

Table 5.5b: Effect of time on mean root dry weight (g) of pepper rooted cuttings at different treatment levels (not significant)

	Treatment				
Time (month)	**T1**	**T2**	**T3**	**T4**	**T5**
2	0.556±0.046	0.506±0.070	0.5328±0.042	0.3874±0.079	0.4638±0.086
4	0.5635±0.054	0.5683±0.106	0.6594±0.123	0.5217±0.127	0.4135±0.076
6	0.454±0.071	0.65±0.183	0.498±0.083	0.5425±0.087	0.4767±0.073
Pr (at 0.05)	0.6274	0.6729	0.2465	0.6556	0.8966
LSD value	–	–	–	–	–

n=5, α=0.05

Table 5.6a: Effect of treatment on mean no. of leaves of pepper rooted cuttings at different constant time periods (not significant)

	Mean no. of leaves Time (month)		
Treatment	**2**	**4**	**6**
T1	1.93±0.04	6.7±0.31	6.8±0.30
T2	2.65±0.02	7.17±0.40	10±0.61
T3	2.18±0.11	5.46±0.25	8.4±0.47
T4	1.63±0.20	4.6±0.19	7.5±0.42
T5	2.45±0.21	6.2±0.30	8±0.50
Pr (at 0.05)	0.0585	0.3242	0.4366
LSD value	–	–	–

n=5, α=0.05

Table 5.6b: Effect of time on mean no. of leaves of pepper rooted cuttings at different treatment levels

	Treatment				
Time (month)	**T1**	**T2**	**T3**	**T4**	**T5**
2	1.93±0.04[ab]	2.65±0.02[ab]	2.18±0.11[ab]	1.63±0.20[a]	2.45±0.21[a]
4	6.7±0.31[b]	7.17±0.40[bc]	5.46±0.25[bc]	4.6±0.19[b]	6.2±0.30[b]
6	6.8±[a]	10±0.61[ac]	8.4±0.47[ac]	7.5±0.42[c]	8±0.50[c]
Pr (at 0.05)	0.0225	0.0054	0.0001	0.06	0.1126
LSD value	2.397	2.7272	0.668	-	-

n=5, α=0.05

Note: Means with the same letters are significantly different

The treatment effect on the mean number of leaves at different constant time periods was found to be non- significant. In contrast to this, the effect of time on the treatments T1, T2, T3 were highly significant. A general trend of significant increase was seen in all those treatments. T4 and T5 failed to show a significant time effect although a trend of an increase with time was observed. The highest no. of leaves was recorded in T2 treatment at month 6 with a mean of 10±0.61. The lowest recorded value was observed in T4 treatment at 2nd month with a mean of 1.63±0.20 (Table 5.6a).

Table 5.7a: Effect of treatment on mean shoot height of pepper rooted cuttings at different constant time periods

Treatment	Mean height of new shoot (cm) Time (month) 2	4	6
T1	11.13 ± 1.742^{b}	27.22±1.49	41.4±3.51
T2	13.83 ± 0.830^{a}	25.46±1.54	34.2±3.34
T3	11.21 ± 1.40^{c}	21.48±2.36	40.74±2.76
T4	8.54 ± 1.72^{a}	20.77±3.65	31.62±2.24
T5	12.03 ± 1.48^{d}	26.34±5.14	48.33±7.17
Pr (at 0.05)	0.0251	0.4762	0.0773
LSD value	4.6801	–	-

n=5, α=0.05

Note: Means with the same letters are significantly different

Only at 2nd month a significant treatment effect was recorded. At 2nd month the highest mean shoot height was observed in T2 with a mean of 13.83±0.830 whereas at month 6 the highest was recorded with the control having a mean of 48.33±7.17. A similar increase was seen on T1 and T3.

Table 5.7b: Effect of time on mean shoot height of pepper rooted cuttings at different treatment levels

Time (month)	Treatment T1	T2	T3	T4	T5
2	11.13 ± 1.742^{abc}	13.83 ± 0.83^{abc}	11.21 ± 1.40^{abc}	8.54 ± 1.72^{abc}	12.03 ± 1.48^{abc}
4	27.22 ± 1.49^{bac}	25.46 ± 1.54^{bac}	21.48 ± 2.36^{bac}	20.77 ± 3.65^{bac}	26.34 ± 5.14^{bac}
6	41.4 ± 3.51^{cab}	34.2 ± 3.34^{cab}	40.74 ± 2.76^{cab}	31.62 ± 2.24^{cab}	48.33 ± 7.17^{cab}
Pr (at 0.05)	0.0066	0.0453	0.0062	0.0116	0.0078
LSD value	0.7094	1.8425	0.9584	0.8957	0.625

n=5, α=0.05

Note: Means with the same letters are significantly different

Table 5.8a: Effect of treatment on mean leaf area (cm2) of pepper rooted cuttings at different constant time periods

	Mean leaf area(cm^2) Time (month)		
Treatment	**2**	**4**	**6**
T1	20.51±5.903	271.5±43.15	247.8±64.03
T2	13.12±2.770	252.53±44.26	359.6±67.45
T3	31.95±10.36	258.21±48.70	301.2±26.31
T4	15.59±3.64	259.9±27.17	248.25±40.99
T5	13.95±3.86	158.76±31.74	238.66±69.94
Pr (at 0.05)	0.3166	0.6439	0.4375
LSD value	–	–	–

n=5, α=0.05

Table 5.8b: Effect of time on mean leaf area (cm2) of pepper rooted cuttings at treatment levels

	Treatment				
Time (month)	**T1**	**T2**	**T3**	**T4**	**T5**
2	20.51±5.903^{ab}	13.13±2.770	31.95±10.36^{ab}	15.59±3.64^{ab}	13.95±3.86
4	271.51±43.15^{b}	252.53±44.26	258.21±48.70^{b}	259.9±27.17^{b}	158.77±31.74
6	247.8±64.03^{a}	359.6±67.45	301.2±26.31^{a}	248.2±40.99^{a}	238.67±69.94
Pr (at 0.05)	0.0318	0.0836	0.0044	0.0164	0.0963
LSD value	135.54	-	100.33	94.53	-

n=5,α=0.05

Note: Means with the same letters are significantly different

5.4 Conclusion

Inoculation of pepper rooted cuttings with inoculum containing mycorrhizal (*G. mosseae)* spores and fungal structures with sorghum (*Sorghum bicolor* L.) roots and soil lead to a successful inoculation. Incorporation of 75 g of inoculum (795 mean number of spores) at the preparation of nursery pots was found to be adequate for a potting mixture of approximately 900 g which is sufficient to fill one standard polythene bag of dimensions 21 cm x 13 cm that enhance root length and shoot dry weight.

5.5 Future work

Further experimentation to reduce the dependency of the high value components such as cow dung and coir dust in standard potting mixture along with mycorrhizal inoculum as a supplementary potting media component is proposed.

Chapter 06

Effective spore density of Vesicular Arbuscular Mycorrhizae (*Glomus mosseae*) for Cinnamon (*Cinnomomum verum* Presl Syn. *Cinnomomum zeylanicum* Blume) seedlings

6.1 Introduction

Vesicular arbuscular mycorrhizal (VAM) fungi are geographically ubiquitous, and occur over a broad ecological range. They are commonly found in association with agricultural crops, most shrubs, most tropical tree species and some temperate tree species (Bagyaraj, 2006). As such, effective utilization of VAM in agricultural crops would reduce the cost of fertilizer, save foreign exchange while providing conditions for efficient utilization of nutrients through biological methods leading to environment sustainability of agricultural systems and improved soil quality. Thus, agricultural practices including use of excessive doses of fertilizers and biocides, tillage, and monocultures of non-mycorrhizal crops are detrimental for the establishment of mycorrhizal association. These agronomic practices are often imposed on intensively cultivated spice lands in Sri Lanka. Chemical fertilizers, organic manure and liquid fertilizers with high micro-nutrient availability and fungicides are frequently applied through out the growing season. Although these inputs affect AM symbiont, accumulation of toxic substances and inherent low P availability of these soils may promote infection.

Cinnamon is the most important spice crop in Sri Lanka and it is the highest nutrient demanding export agricultural crop. Apart from that organic farming is also becoming popular among spice growers at present. It will be more valuable if we can increase the nutrient availability in soil such as P, N and Zn etc. through successful mycorrhizal inoculation of cinnamon seedlings at nursery stage. Therefore, exploitation of beneficial effect of mycorrhizal association for cinnamon seedlings is also timely. Therefore, a preliminary study was conducted to investigate the occurrence AM fungi and suitable spore density of *Glomus mosseae* of initially potted cinnamon seedlings. The present experiment was designed as a part of the research programme of DEA/NSF, to investigate the suitable spore density for inoculation of cinnamon seedlings at nursery stage.

6.2 Materials and methods

This experiment was carried out at the nursery of the Research Station, Department of Export Agriculture (DEA), Matale.

6.2.1 Experimental treatments

Four mycorrhizal inoculum levels of *G. mosseae;* 25 g (T1), 75 g (T2), 150 g (T3), 300 g (T4) were tested with the control (T5). Inoculum consisting of mycorrhizal spores and structures with sorghum (*Sorghum bicolor* L.) roots and moist soil were obtained by harvesting VAM (*Glomus mosseae*) regeneration bed. The initial mean spore density value of inoculum was counted and found to be 154 spores per 50 g of inoculum).

6.2.2 Experimental design

The experimental design was randomized complete block with 5 blocks having 3 replicates (pots) per each block.

6.2.3 Trial establishment

Three-month-old, three standard cinnamon seedlings were planted in pre-prepared clay pots. The clay pots having 11 cm height, 14 cm inner top diameter and 7 cm of inner bottom diameter were filled with 1:1 sterilized top soil and river sand. Above four inoculum levels that were assigned to each treatment were incooperated to respective pots.

Plate 6.1: Three months old cinnamon seedlings after inoculation with VAM

6.2.4 General management

The cinnamon seedlings planted in pots as shown in Plate 6.1 were kept in a shade house at the nursery site. The general management practices such as watering, weeding and control of pest and diseases were done manually throughout the growing period.

6.2.5 Data collection

Data were collected at 2nd and 4th month after inoculation

Prior to the commencement of uprooting of a set of seedlings from each replicate, following measurements were taken from each plant at 2nd and 4th month after inoculation.

6.2.5.1 General observations

a) Number of leaves

The number of new leaves emerged in each plant was recorded

b) The diameter of initial stem

The diameter of the initial stem of the seedlings was measured by using a Vernier Caliper.

c) The height of the new shoot

The height of the newly emerged seedlings (from the base of the new shoot to the tip of the terminal shoot) of each plant was measured.

6.2.5.2 Destructive sampling and observations

The following destructive measurements were taken at each 2nd and 4th month after trial establishment by uprooting 05 seedlings per treatment. The roots of the uprooted plants were thoroughly washed with tap water. Then roots were carefully separated using a pair of surgical scissors and following measurements were taken on per plant basis.

a) Leaf area

The leaves of the each uprooted seedlings were separated from the stem and leaf area was measured using an electronic area meter as described in the catalogue (CI-202 Area Meter).

b) Root length

The washed roots were blotted dry with a tissue paper and each root sample was spread randomly on a 1 cm grid transparent sheet. The number of intersections was counted across longitudinal as well as vertical direction as described by Tennant (1975). Total number of intersections was used to obtain the total root length of each sample following Newman's method as described by general material and method chapter (section 3.8).

c) Shoot dry weight

Each shoot sample was transferred to a paper bag and oven dried at 70 ^{0}C for 24 hours and weighed using an analytical balance.

d) Root dry weight

After measuring the root length root samples were transferred to a paper bag and oven dried at 70 ^{0}C for 24 hours and weighed using an analytical balance.

e) Root staining and percentage mycorrhizal colonization

In order to ascertain the success of inoculation, pepper root samples were stained using the following procedure as described by Jarstfer and Silvia (2001). An attempt was made to work out the percentage mycorrhizal colonization in roots of successfully inoculated rooted cuttings using stained root samples with an arbitrary scale as proposed in the modified technique described by Philips and Hayman (1970). Mycorrhizal infection was determined as percent roots infected with mycorrhizal fungi on a per plant basis (Durgapal *et al.*, 2002).The colonization was assessed depending on the presence or absence of hyphae, vesicles and arbuscules in each segment of root and was expressed as percentage of colonization according to the method described by Kapoor and Paroda (2005).

(F) Final VAM spore population

Rhizosphere soils under each inoculum level were also assessed for VAM spore population through wet sieving and decanting method (Brundrett *et al.*, 1996). The spores were counted using a stereomicroscope. Data were statistically analysed using GLM procedure of SAS package.

6.3 Results and Discussion

According to the microscopic view of stained cinnamon root segments, darkly stained VAM *G. mosseae* hyphae were observed at 4th month after inoculation of seedlings at all the inoculum levels. Nevertheless, certain stained mycelia were seen in the roots of non-inoculated seedlings and this may be due to the presence of naturally occurring VAM types in the enviorenment. Apart from that mycorrhizal spores were also observed in the rhizosphere soil samples collected from inoculated plants at 4th month after inoculation. Generally it is difficult to stain spice roots due to abundance of phenolic compounds and therefore, achievements of substantial staining in these cinnamon root samples seem to be satisfactory. This confirmed the success of VAM inoculation of cinnamon seedlings.

6.3.1 Root biomass

One way ANOVA was employed to determine the effect of treatment and time on root dry weight (Appendix: 6.5 and 6.6). The combined effect was determined by performing a two way ANOVA.

Table 6.1: Mean root dry weight (g) of cinnamon seedlings during study period (treatment comparison)

	Mean root dry weight (g) Time (month)	
Treatment	02	04
T1	0.154 ± 0.034^{c}	0.160 ± 0.025^{a}
T2	0.261 ± 0.012^{abc}	0.288 ± 0.054^{a}
T3	0.224 ± 0.008^{d}	0.272 ± 0.035^{b}
T4	0.185 ± 0.012^{b}	0.219 ± 0.012^{c}
T5	0.151 ± 0.029^{a}	0.226 ± 0.047^{d}
Pr (at 0.05)	0.0217	0.0338
LSD value	0.072	0.125

n=5, $\alpha = 0.05$

Note: means with the same letters are significantly different

In fact there were irregularities in performances of certain growth parameters with respect to the inoculation treatments during the study period. Certain parameters were highly responded for VAM inoculation. For example treatment effect was significant for root dry weight of

inoculated plants at both 2nd and 4th month after inoculation. Plants of T2 showed the maximum mean root dry weight (Fig. 6.1) of 261 mg at 02nd month after inoculation and it is significantly higher than non- inoculated control plants (151 mg) as well as T1 and T4. Relative positions of the treatments for root dry weights were almost same at the 4th month after inoculation showing the overall maximum weight (288 mg) for T2 plants (Fig. 6.2). The time comparison of root dry weight at constant treatment levels is also significant (Pr=0.0310, LSD=0.0417) having 0.1954 g and 0.2416 g mean root dry weight values for 2nd and 4th month, respectively.

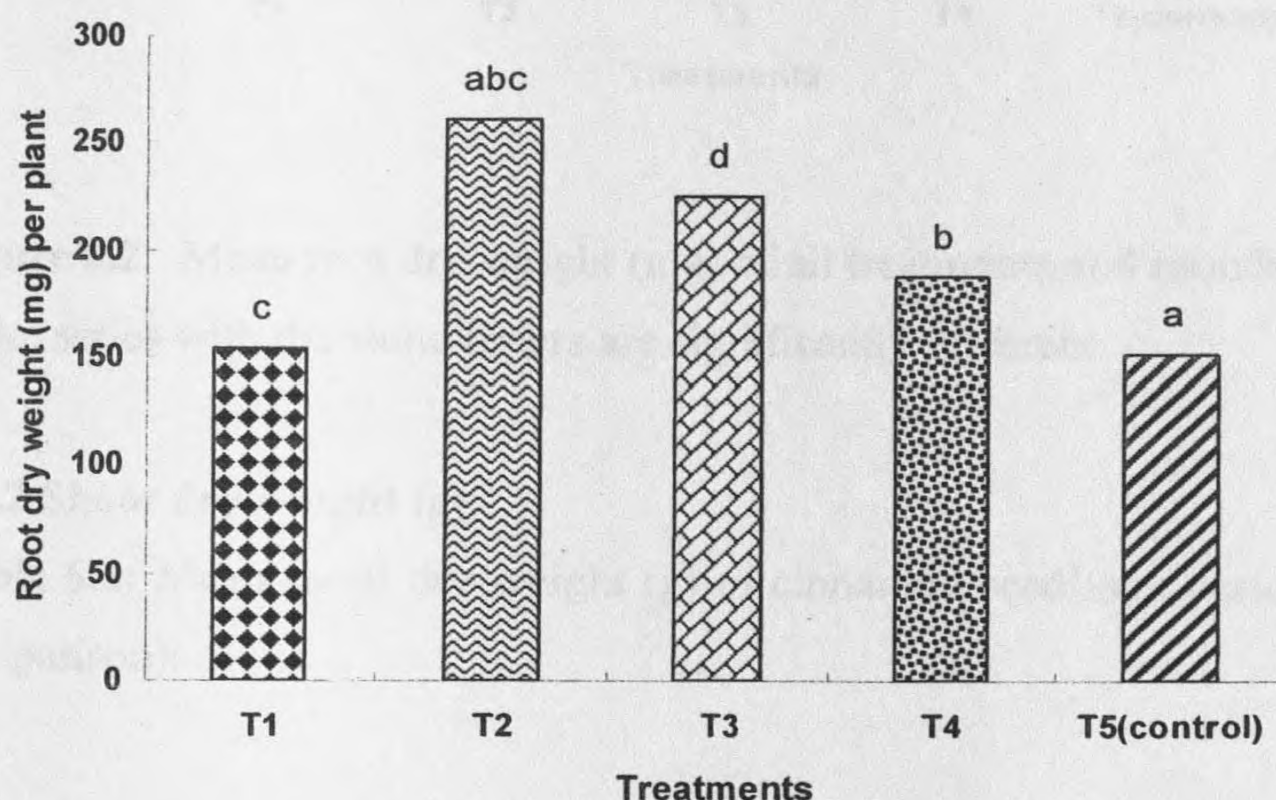


Figure 6.1: Mean root dry weight (mg) of cinnamon seedlings at 2 month after inoculation
Note: series with the same letters are significantly different

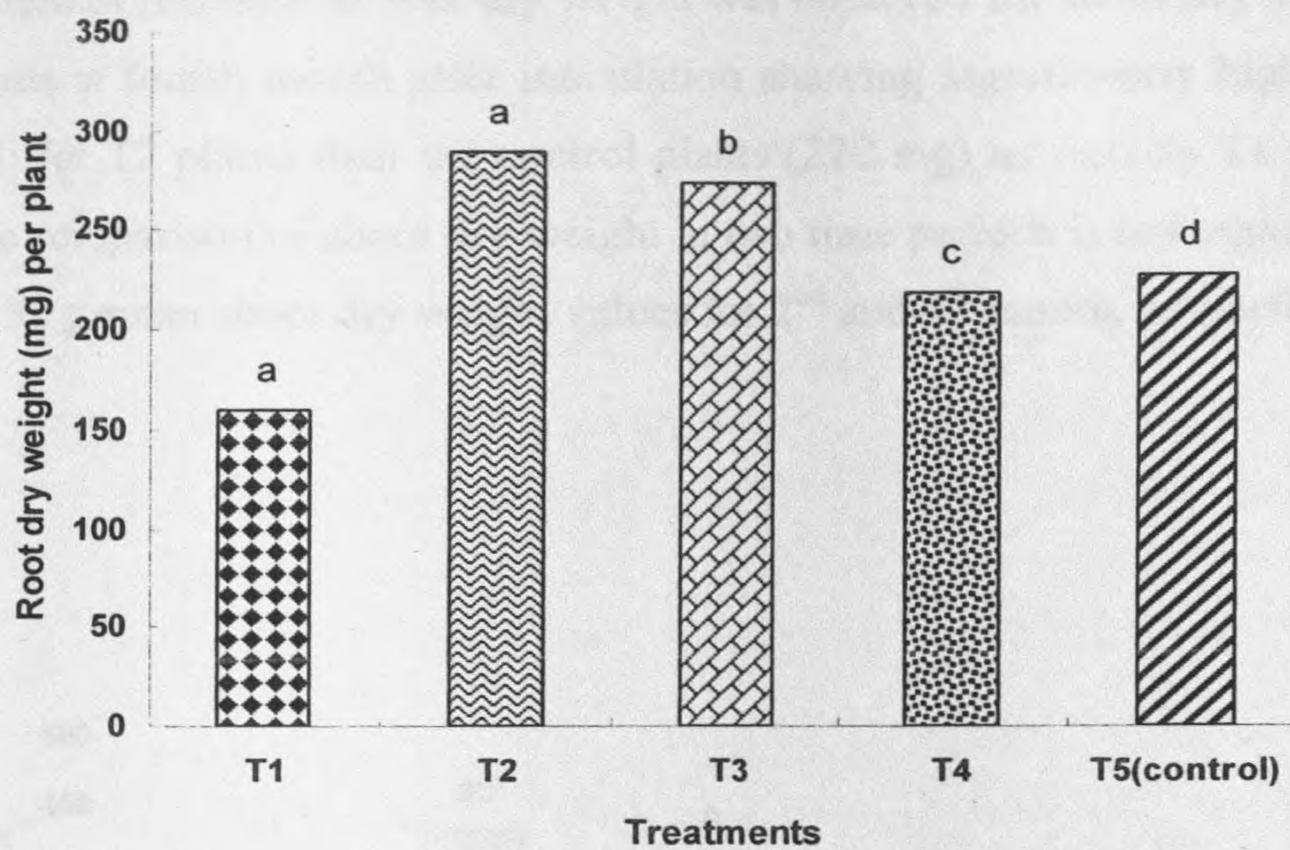


Figure 6.2: Mean root dry weight (mg) of all treatments at 4 month after inoculation

Note: series with the same letters are significantly different

6.3.2 Shoot dry weight (g)

Table 6.2: Mean shoot dry weight (g) of cinnamon seedlings during study period (treatment comparison).

Treatment	Shoot dry weight(g) Time (month) 02	04
T1	0.340±0.076[a]	0.278±0.047[b]
T2	0.345±0.038[b]	0.430±0.051[ab]
T3	0.390±0.029[c]	0.411±0.059[c]
T4	0.271±0.038[d]	0.306±0.027[d]
T5	0.326±0.062[e]	0.27±0.034[a]
Pr(at 0.05)	0.663	0.043
LSD value	–	0.1482

n=5, α=0.05

means with the same letters are significantly different

The shoot dry weight at 02nd month were not showing a significant difference at Pr (0.05), but a rather high shoot dry weight was recorded by treatment 3 (T3), 0.390±0.029 mg. A similar pattern of response as root dry weight was observed for shoot dry weight figures of cinnamon plants at fourth month after inoculation showing significantly higher shoot dry weight (430 mg) for T2 plants than the control plants (270 mg) as well as T1 plants (278 mg) (Fig.6.3). The comparison of shoot dry weight in two time periods is not significant having 0.335 g and 0.351 g mean shoot dry weight values for 2nd and 4th month, respectively.

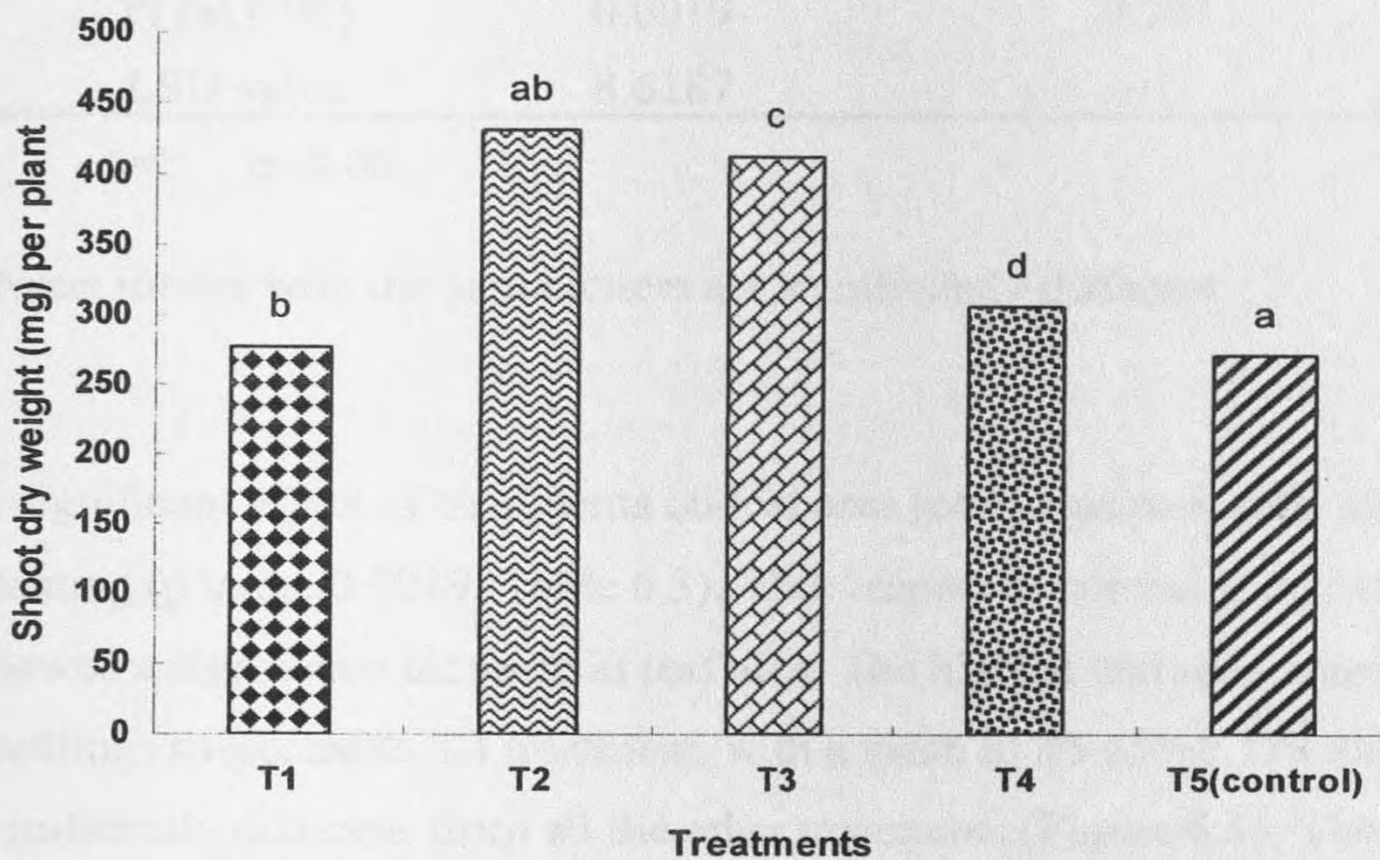


Figure 6.3: Shoot dry weight (mg) of all treatments at 4 month after inoculation

Note: series with the same letters are significantly different

6.3.3 Leaf area

Table 6.3: Mean leaf area (cm^2) of Cinnamon seedlings during study period (treatment comparison)

	Mean leaf area (cm^2) Time (month)	
Treatment	02	04
T1	24.10 ± 3.482^{a}	25.44 ± 3.611^{a}
T2	25.13 ± 3.458^{b}	31.54 ± 3.259^{b}
T3	25.65 ± 1.118^{c}	29.66 ± 4.646^{c}
T4	22.20 ± 3.469^{d}	24.06 ± 2.388^{d}
T5	7.01 ± 0.728^{abcd}	24.23 ± 3.051^{e}
Pr (at 0.05)	0.0019	0.291
LSD value	8.6287	–

n=5, α=0.05

Note: means with the same letters are significantly different

A significant effect of treatments on leaf area (cm^2) was seen only at 2nd months after planting (p value 0.0019, Table 6.3). With respect to control treatment T1, T2, T3, and T4 showed a significant increase in leaf area. The highest leaf area was recorded by cinnamon seedlings subjected to T3 treatment, with a mean of 25.65 ±1.118 although the effect is not significantly different from all the other treatments.(Figure 6.4). The time comparison of leaf area at constant treatment level is also significant (Pr= 0.0044, LSD=4.341) having 20.699 cm^2 and 27.190 cm^2 mean leaf area values for 2nd and 4th month, respectively.

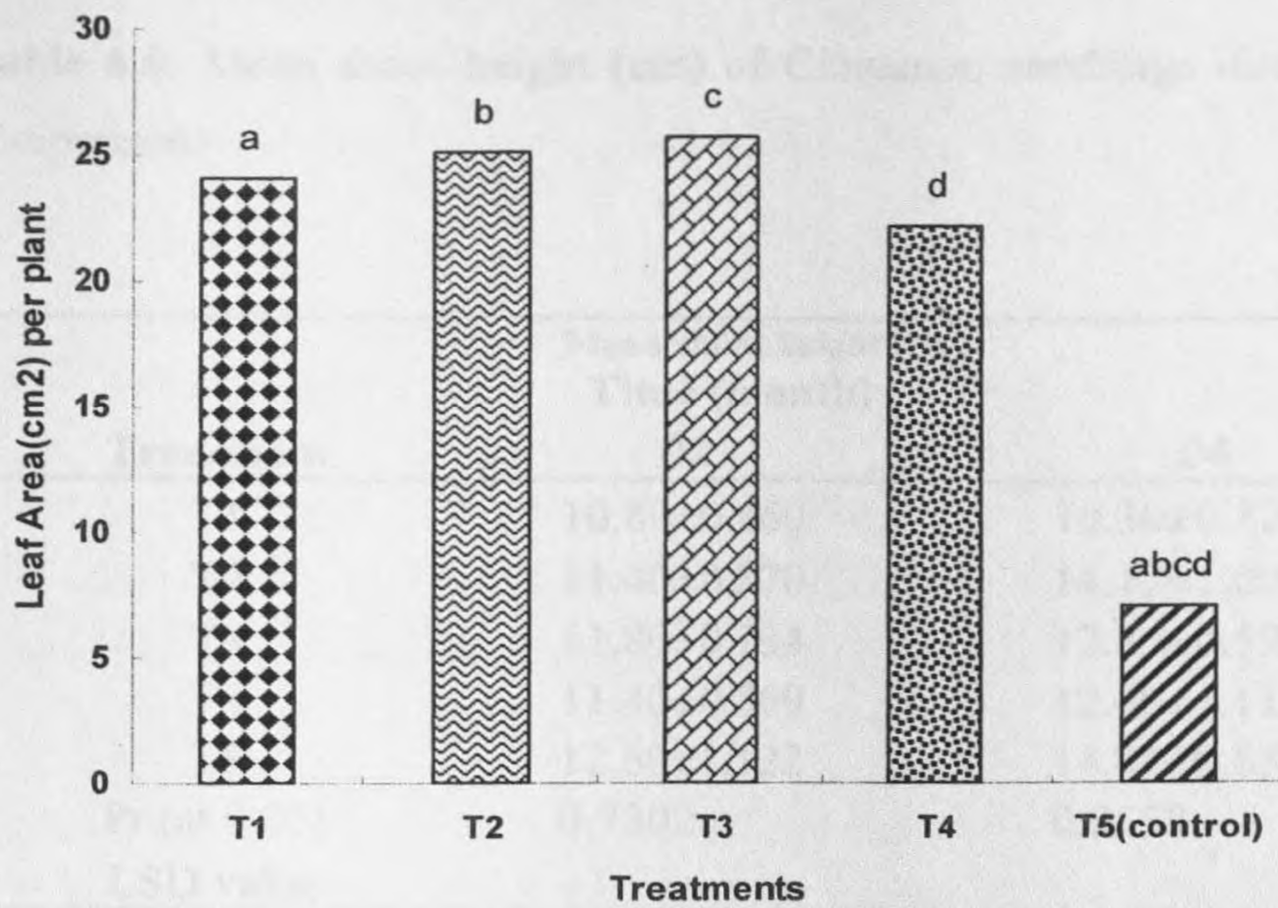


Figure 6.4: Mean leaf area (cm^2) of cinnamon seedlings at 2 month after inoculation

Note: series with the same letters are significantly different.

6.3.4 Other growth parameters

Other growth parameters namely mean root length, mean shoot height, mean stem diameter were not significantly different

Table 6.4: Mean root length (cm) of cinnamon seedlings during study period (treatment comparison)

Treatment	Mean root length(cm) **Time (month)** 02	04
T1	255.66±119.52	151.58±22.13
T2	233.29±17.50	265.25±36.47
T3	215.27±13.47	242.25±43.53
T4	186.05±20.46	215.69±20.70
T5	120.09±33.73	154.01±26.96
Pr (at 0.05)	0.5161	0.1628
LSD value	-	-

n=5, α=0.05

Table 6.5: Mean shoot height (cm) of Cinnamon seedlings during study period (treatment comparison)

	Mean shoot height (cm) **Time (month)**	
Treatment	02	04
T1	10.80±0.860	10.30±0.826
T2	11.40±0.870	14.12±1.005
T3	11.80±0.734	12.85±0.595
T4	11.40±0.509	12.40±1.117
T5	12.60±1.122	14.81±0.654
Pr (at 0.05)	0.7302	0.0558
LSD value	-	-

n=5, α=0.05

Table 6.6 : Mean stem diameter (cm) of Cinnamon seedlings during study period (treatment comparison)

	Mean stem diameter (cm) **Time (month)**	
Treatment	02	04
T1	0.23±0.023	0.214±0.013
T2	0.222±0.01	0.271±0.016
T3	0.456±0.236	0.261±0.012
T4	0.228±0.007	0.236±0.011
T5	0.22±0.011	0.259±0.129
Pr (at 0.05)	0.465	0.1326
LSD value	-	-

n=5, α=0.05

The growth parameters stem diameter, plant height and root length did not showed a significant different effect at 2nd month as well as 4th month after inoculation. The time effect on stem diameter and root length is also not showed a significant difference among 2nd and 4th months. These parameters may be considered as not successful in growth measurements in cinnamon seedlings.

The growth parameters like root biomass, shoot biomass and leaf area can used as better measurements in cinnamon seedlings. It can be considered that mycorrhizae activities are directly involves with selected growth parameters like root biomass, shoot biomass and leaf area.

6.4 Conclusion

Staining procedure for mycorrhizal structures of cinnamon roots found to be satisfactory and it confirmed the success of VAM inoculation of cinnamon seedlings. Only three growth parameters namely shoot biomass, root biomass and leaf area can be used as better measurements in Cinnamon seedlings and other measurements are seems to be not appropriate. Overall observations indicated that inoculation of cinnamon seedlings with 75 g of inoculum consists of mycorrhizal spores and structures with sorghum (*Sorghum bicolor* L.) roots and moist soil seems to be appropriate for enhancement of seedling growth.

Chapter 07

Investigating the effect of mycorrhizal associations of pepper on increasing the plant availability of phosphorus from Eppawala Rock Phosphate (ERP^{TR})

7.1 Introduction

In Sri Lanka black pepper is grown mainly on Reddish Brown Latasolic (RBL)- *Rhodudalfs*-soil at Matale area and RBL soil has naturally low plant available soil P status (less than 5 ppm of Olsen P) (Mapa *et al.*, 2005). Not only in Sri Lanka, in many developing countries of the tropics and subtropics, soil P reserves are gravely low and large additions are required before maintenance levels begin to decline. In addition, the cost of P fertilizer will increase as the currently accessible deposits of high grade phosphate rock (PR) diminish (Trolove *et al.*, 2003). In this context, research into plant mechanisms that enhance P uptake, including effect of root geometry, mycorrhizal associations, and root-induced changes in the soil are timely. As Sri Lanka has a locally available phosphate rock (Eppawella phosphate rock- EPR), this rock is recommended as a P fertilizer (Eppawala Rock Phospahate-ERP™) source for organic farms and other perennial crops including pepper. Nevertheless, reactive rock phosphate brands including ERP™ are recognized to be slow in dissolution and release of P for plants. Successfully inoculated mycorrhzea species can improve the plant-uptake efficiency of P and other native nutrients from the soils (Bagyaraj, 2003, Xioutang, 1994). It is important to note that not only in some perennial EAC species (Palipane and Bandara,1985) but also in natural rain forest tree species (Hafeel and Gunatilleke, 1989), Vesicular Arbuscular Mycorrhizae (AM) *(Glomus species)* have been reported in Sri Lanka. On this context, exploitation of beneficial effects of mycorrhizal association for utilization efficiency of ERP™ fertilizer for pepper vines is also timely. Therefore, as the second step a large size pot experiment was conducted to investigate the combined effect of Eppawala Rock Phosphate (ERP™) levels and AM mycorrhizae (*Glomus mosseae*) on pepper rooted cuttings beyond the nursery stage.

7.1.2 Fertilizer use in pepper cultivation

Present standard fertilizer recommendation for pepper (Anon, 2002) is given in the tables 7.1a and 7.1b).

Table 7.1a. Present standard fertilizer recommendation for pepper

Fertilizer mixture	Weight in parts	Percentage of nutrient in the mixture
Urea (46%)	4	14% N
Rock Phosphate (28% P_2O_5)	5	11% P_2O_5
Muriate of potash (60% K_2O)	3	14% K_2O
Keserite (24% MgO)	1	2% MgO

Fertilizer quantity required per one plant per year is 1400 g consisting of 196 g N/ Plant (392 kg /ha), 154 g P_2O_5 /Plant (308 kg /ha), 196 g K_2O /Plant (392 kg /ha and 28 g MgO /Plant (56 kg / ha). This annual requirement is broken down in to two major doses which are applied at the commencement of respective monsoon periods as given in the Table 7.1b.

Table 7.1b. Annual dose of chemical fertilizer mixture for pepper.

Year after field planting	At the beginning of 1st monsoon (g)	At the beginning of 2nd monsoon (g)
1st year	250	250
2nd Year	500	500
3rd year and there after	700	700

Due to the high cost of chemical fertilizers and the environmental issue pertaining to chemical fertilizer use, an alternative fertilizer mixture has been developed by the DEA.

According to the alternative fertilizer mixture, the above standard fertilizer mixture has been reduced by 50% along with application of 10-15 kg *Gliricidia* leaves and tender material (4

times of 2.5 kg or 3 times of 3.75 kg pruning materials of shade trees) per vine per year (Anon, 2002) (Table 7.1c).

Table 7.1c. Modified standard fertilizer recommendation with *Gliricidia* lopping for pepper

Year after field planting	At the beginning of 1st monsoon	At the beginning of 2nd monsoon
1st year	125	125
2nd Year	250	250
3rd year and there after	350	350

7.2 Materials and methods

This experiment was carried out at the nursery of the research station, Department of Export Agriculture (DEA), Matale. The nursery site environment is similar to post nursery field establishment stage environment. Several reasons like same soil type, same fertilizing, same weeding and same weather conditions were applied for our nursery pot trial.

7.2.1 Experimental treatments and design

Three Eppawala rock phosphate levels: (1) F_1-5 µg P/ g soil (2) F_2-50 µg P/ g soil and (3) F_3-100 µg P/ g soil were tested with three mycorrhizal inoculum levels of *G. mosseae* : (1) M_1-No mycorrhizal inoculum, (2) M_2-75 g of mycorrhizal inoculum and (3) M_3-150 g of mycorrhizal inoculum. Finely ground Eppawella phosphate rock- (ERP™) (150-250 µm of particle size) was used as the test reactive phosphate rock (RPR) fertilizer. Inoculum consisted of mycorrhizal spores and structures with sorghum (*Sorghum bicolor* L.) roots and moist soil that were obtained by harvesting AM (*Glomus mosseae*) regeneration bed at the EAC research station, Matale. The experimental design was a 3x3 factorial with 18 replicates giving a total of 162 experimental pots. Rooted cuttings of local pepper selection MB12 was initially planted in standard polythene bags.

7.2.2 Rationale of selecting P fertilizer testing levels

For the testing, the P fertilizer requirement was calculated based on the recommendations made by Anon (2002).

According to the above recommendation

First year requirement = 250 g mixture/ plant/ 12 month

Amount from rock phosphate = 250 x 5/13

= 96.15 g/ 12 month

P_2O_5 (@ 28%) equivalent = 26.92 g / 12 month

P Equivalent (P=31, O=16) $= \frac{62}{142} \times 26.92$

=11.75 g/ vine/ 12 month

= 11.75×10^6 µg

Standard planting pit size (for loam soil) = 45 x 45 x 45 (Anon, 2002)

Approximate soil bulk density = 1.2 g/ cm^3

Soil mass per planting pit = 109.35 kg

P quantity be added as ERP to a planting pit for 12 month on the basis as P µg/g soil

$$= \frac{11.75 \times 10^6}{109.35 \times 10^3}$$

= 107.45 µg/ g soil

ERP at standard recommendation for 1st year = 214.90 µg/ g soil (without *gliricidia* lopping application)

At modified recommendation with *Gliricidia* lopping, equivalent ERP^{TR} =107.45µg / g soil / year

The nutritional composition of *Gliricidia* reduced the calculated total nutrient requirement up to half. This is the reason for half fertilization requirement mentioned in above calculation.

Assuming some improvement of nutrient absorption in the presence of mycorrhizal association, the three fertilizer testing levels; F1-5 μg P/ g soil (low), F2-50 μg P/ g soil (medium) and F3-100 μg P/ g soil (high) were selected to be tested in this experiment.

The required amounts of ERP™ were calculated considering that P_2O_5 ratio as 28% of ERP™. The soil mass equivalent of 21 cm x 13 cm size of standard black polythene bag was 800 g and therefore, the three levels of ERP™ were 0.0326 g, 0.327 g and 0.654 g for low medium and high fertilizer testing levels, respectively. When transferring 21 cm x 13 cm size (standard) pot into 25 cm x 30 cm pots (bigger pot), three levels of ERP™ were readjusted according to mass basis of the new bigger pot.

7.2.3 Experimental pot preparation and trial establishment

Pots were prepared using nursery level standard black polythene bags (21 cm x 13 cm size, gauge 150). Mycorrhizal inoculum and ERP™ fertilizer were mixed with different quantities of sterilized (autoclaved at 121 ^{0}C for 15 minutes) potting mixture, and were incorporated into each polythene bag to make the final quantity of 800 g as per the treatment requirements. Inoculum levels, P fertilizer levels and corresponding quantities are shown in Table 7.2.
When mixing and filling the pots according to the treatments, following steps were followed. Firstly, the potting mixture was spread evenly on a clean plastic tray; secondly, a required quantity of ERP™ was spread on the potting mixture, then mixed them together thoroughly by hands and finally the required quantity of mycorrhizal inoculum was incorporated and well mixed. In this manner, 162 experimental pots were prepared according to each treatment combination. Immediately after preparation of experimental pots, they were thoroughly watered and one rooted cutting of local pepper selection (MB12) was planted in each polythene bag. Then these pots were stacked and covered with 250 gauge polythene sheets to form a humid chamber type propagator and sealed to avoid air circulation (Plate 7.1). The propagator was opened at 3 weeks after planting. Then experimental pepper rooted cuttings in the standard polythene bags were allowed to grow for 3 months under the net house at nursery condition. Then these pots were transferred to bigger polythene bags to expose for an extended period of 8 months under the simulated condition of post nursery field establishment stage. The transferring procedure without disturbing the identification of each tag was adopted as described in the next sub section.

Table 7.2 : ERP™ fertilizer levels, inoculum levels and corresponding quantitative values assigned on per pot basis for each treatment at nursery stage

Treatment code	Inoculum level (g)	P level (g)	ERP level (g)	Quantity of potting mixture (g)
F1M1	No inoculum	0.004	0.0327	800
F1M2	75	0.004	0.0327	725
F1M3	150	0.004	0.0327	650
F2M1	No inoculum	0.04	0.327	800
F2M2	75	0.04	0.327	725
F2M3	150	0.04	0.327	650
F3M1	No inoculum	0.08	0.655	800
F3M2	75	0.08	0.655	725
F3M3	150	0.08	0.655	650

Plate 7.1: Pepper rooted cuttings within the air tight propagator at first stage (nursery stage)

7.2.4 Transferring rooted cuttings from standard pots to bigger pots

Well established pepper rooted cuttings along with respective pot contents (potting mixture, P fertilizer and well grown *Glomus mosseae* structures) in standard polythene bags (21 cm x 13 cm size) were neatly transferred to bigger polythene bags (25 cm x 30 cm size) at 03 months growth after propagator opening. The bigger polythene bags (25 cm x 30 cm size) were filled with secondary potting media (equal part of top soil and river sand mixture). The total weight of the bag become 5 kg and P fertilizer levels were adjusted on mass basis to maintain the initial treatment levels at the bigger pot stage also. Corresponding quantities are given in Table 7.3. Respective quantities of secondary potting media, ERP™ fertilizer and initial pot contents (with AM + Phosphorous + primary potting media) along with 3 months old rooted cuttings was repotted into the large bags to make the final volume of each bag approximately 5 L. When mixing and filling the bigger pots with corresponding secondary potting media component and respective ERP™ fertilizer quantity, the steps given in the initial pot filling stage was followed exactly to get a homogeneous mixture. The initial tagging was maintained as it is and then well grown rooted cuttings in smaller bags (25 cm x 30 cm) with the corresponding tags were transferred without causing any injury. Immediately after preparation of experimental pots, they were watered adequately.

Table 7.3: ERP™ fertilizer levels, AM inoculum and corresponding quantitative values assigned for each treatment at bigger pot stage per each pot.

Treatment code	P level (g)	ERP™ level (g)	Standard potting mixture (g) + AM inoculum (g) distribution at nursery stage	Quantity of secondary potting media per pot (g)
F1M1	0.021	0.172	800 +0 = 800	4200
F1M2	0.021	0.172	725 + 75 = 800	4200
F1M3	0.021	0.172	650 + 150 = 800	4200
F2M1	0.21	1.718	800 + 0 = 800	4200
F2M2	0.21	1.718	725 + 75 = 800	4200
F2M3	0.21	1.718	650 +150 = 800	4200
F3M1	0.42	3.435	800 +0 = 800	4200
F3M2	0.42	3.435	725 +75 =800	4200
F3M3	0.42	3.435	650 +150 =800	4200

Plate 7.2: pepper rooted cuttings in 25 cm x 30 cm size polythene bags

7.2.5 General management

Back pepper rooted cuttings in bigger (25m x 30 cm) polythene bags were kept at the nursery site under the normal environmental conditions of shade trees. General agronomic management practices such as watering, weeding and control of pest and diseases were done manually throughout the growing period as per the standard recommendations (Anon, 2002).

7.2.6 Data collection

Casualties of cuttings were counted at the time of complete opening of the humid chamber. All the pepper vines on bigger bags were harvested at 08 month after repotting into the bigger bags and data were collected on per vine basis. Prior to the commencement of uprooting of the pepper vines following measurements were taken on per vine basis.

7.2.6.1 Non destructive measurements

a) Number of leaves

The number of new leaves emerged in each vine was recorded

b) The height of the new shoot

The height of the newly emerged shoot (from the base of the new shoot to the tip of the terminal shoot) of each vine was measured.

7.2.6.2 Destructive sampling and observations

a) Leaf area

The leaves of the each uprooted vine were separated from the stem and leaf area was measured using an electronic area meter as described in the catalogue (CI-202 Area Meter).

b) Shoot dry weight

Each shoot sample (stem and leaves of each vine) was transferred to a paper bag and oven dried at 70 ^{0}C for a constant weight (for 24 hours) and weighed using an analytical balance. Following the recording of the dry weight of each sample they were milled to < 1mm particle size.

c) Rhizhosphere soil sampling

At the harvest, the rhizhosphere soil was collected according to the method of Wang and Zabowski (1998). The whole seedling was removed from the pot with minimum injury to its roots, by shaking the roots until the soil not tightly adhering to the roots was removed and then collecting the soil closely adhering to the root system by vigorously shaking the roots. The 'bulk' soil (soil not influenced by roots) was collected from areas in pots where there were no roots.

d) Root measurements

Following the extraction of rhizosphere soil of each potted pepper vine, whole root content was thoroughly washed with tap water. Then roots were carefully separated using a surgical scissor and following measurements were taken on per plant basis.

e) Root dry weight

Each root sample was transferred to a paper bag and oven dried at 70 ^{0}C for constant weight and weighed using an analytical balance. Following the recording of the dry weight of each sample they were milled to < 1 mm particle size.

f) Root staining and percentage mycorrhizal colonization

In order to ascertain the success of inoculation, representative pepper root samples were taken and stained using the procedure described by Jarstfer and Silvia (2001). The procedure is given in detail in general methodology section.

7.2.6.3 Chemical analyses of soil and plant samples

Plant tissue nutrient content and selected soil chemical properties were determined using representative samples as described in general methodology section.

7.2.7 Statistical analyses

Data were analysed by one way and two ways Analysis of Variance (ANOVA), with the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS Institute, 1990). To avoid problems arising from heterogeneity of error, all data were converted to natural logarithm values before statistical analysis. In such cases, actual means are presented in the tables or figures. After analysis, means were back-transformed to the original scale, and least significant differences for those means also back-transformed to give least significant ratios (LSR). The experimental design was Randomized Complete Block Design with factorial combinations.

7.3 Results and discussion

7.3.1 General

Mean values of some selected chemical properties of the potting media are shown in Table 7.4. As expected chemical parameters of the initial potting mixture (PM) were higher than the same properties of the secondary potting mixture (TS) except for total nitrogen. Nitrogen constituent of the well decomposed cattle manure must have stabilized the equilibrium N level (low) during storage of the cattle manure at the nursery site. The mean spore density of the 75 g inoculum is 617 black spores and 184 brown spores after wet sieving and decanting technique as described in general material and method chapter.

Table 7.4: Mean initial soil chemical properties of planting media

Soil chemical property	PM (±SEM)	TS (±SEM)
Soil pH	6.95 ± 0.046	6.27 ± 0.035
Bray P (ppm)	64.6 ± 3.376	15.17 ± 1.969
Olson P (ppm)	-	3.8 ± 0.77
Water Soluble P (ppm)	2.7 ± 0.215	2.4 ± 0.208
Total Nitrogen (%)	0.57 ± 0.022	0.59 ± 0.029
Mg (ppm)	999 ± 7.572	335 ± 26.308
K (ppm)	946 ± 10.268	397 ± 25.640

PM- Potting Media used as rooting media (21 cm x 13 cm polythene bag- initial stage)
TS- Secondary stage potting media (30 cm x 25 cm polythene bag)

Simple counting of live cuttings at the opening of the humid chamber at 21 days after planting revealed that the overall success percentage was satisfactory with 52% of survival irrespective of treatment effects. The mean spore density values were 11, 297 and 674 per 50 g soil for M1, M2 and M3 respectively. Presence of air contaminated spores in non inoculated samples may be the reason for above results. According to the microscopic view of stained pepper root segments, darkly stained AM hyphae were observed in the roots of sample rooted

cuttings at all the inoculum levels except in M1 (control) at the 4th month after inoculation. Neither vesicles nor arbuscules were seen in the control. This confirms the successful inoculation of *G. mosseae* in this experiment. Furthermore, extra-radical, intra- cellular and inter-cellular hyphae were observed at the 8th month after repotting at all the inoculation levels. The heaviest infection of mycorrhizal fungi was observed in roots of M2 and M3 cuttings having the maximum root colonization, along with mycelium, vesicles, and spores at the 8th month after repotting.

7.3.2 Effect of treatments on plant growth parameters

The plant growth parameters are summarized in Table 7.5. ANOVA tables related to data analysis were summarized and are given in the Appendix No.7.9-7.12.

7.3.2.1 Effect of treatment on plant height (vine length)

None of the treatment effects were significant ($P<0.05$) for plant height at the time of final sampling. However, mean plant height for P fertility levels of F1, F2 and F3 were 110 cm, 120.9 cm and 131.8 cm, respectively with an increasing trend. Similarly, mean plant height for inoculation treatments of M1, M2 and M3 were 110 (±20.802) cm, 120.9 (±19.964) cm and 131.8 (±19.162) cm respectively with an increasing trend. Overall maximum height of 166.7 cm was observed for treatment combination of F3M3.

7.3.2.2 Effect of treatments on leaf area (cm^2)

The main effect of P fertilizer was significant ($P<0.05$) for fresh mean total leaf area having 1417.5 (±208.2) cm^2 , 994.1 (±178.3) cm^2 and 2017.0 (±295.2) cm^2 mean leaf area values for P fertility levels of F1 (5 µg P/ g soil), F2 (50 µg P/ g soil), and F3 (100µg P/ g soil) applications respectively at the time of destructive sampling. Nevertheless, it is important to note that the increasing order was not proportional to P increment levels showing significantly smaller leaf area at F2 level. The total leaf area for inoculation treatments of M1, M2 and M3 were 1137 (±206.754) cm^2, 1446 (±303.299) cm^2 and 1650 (±283.742) cm^2 respectively and there is an increasing trend.

A significant interaction was not seen between P fertility level and AM inoculations levels at the time of observation, i.e. 8 month after repotting. It is important to note that the lowest total leaf area was observed at no AM inoculation (M1) with 50 µg P/ g soil (F2) level and it is significantly smaller than the leaf area of each F1M3, F3M2 and F3M3 treatment combinations (Figure 7.1).This irregularity in response pattern may be attributed to the

Table 7.5: Mean plant growth parameters of pepper rooted cuttings with SEM (standard error of the mean) at 08 month afte...

Plant growth parameter	P fertilizer level (µg/g soil)	AM Inoculum level		
		M1-0 g	M2-75 g	M3-150 g
Leaf area (cm^2)	5 µg/g soil (F1)	1265.7 ± 199.8	1068.7± 411.1	1918.2 ± 299.0
	50 µg/g soil (F2)	741.9 ± 213.8	1208.6 ± 286.8	1031.9± 327.8
	100 µg/g soil (F3)	1935.4 ± 0.00	2061.7 ± 211.9	1999.5 ± 224.4
Plant height (cm)	5 µg/g soil (F1)	116.0 ± 10.69	116.0 ± 14.10	137.3 ± 13.3
	50 µg/g soil (F2)	103.0 ± 14.97	122.2 ± 12.31	91.3 ± 27.76
	100 µg/g soil (F3)	111.0 ± 44.99	124.6 ± 30.52	166.7 ± 16.44
Shoot dry weight (g)	5 µg/g soil (F1)	18.26 ± 3.28	17.51 ± 6.26	26.22 ± 3.03
	50 µg/g soil (F2)	10.92 ± 3.69	17.75 ± 5.12	14.63 ± 4.54
	100 µg/g soil (F3)	20.75 ± 13.67	26.07 ± 3.15	26.28 ± 3.10
Root dry weight (g)	5 µg/g soil (F1)	1.79 ± 0.20	1.94 ± 0.52	2.73 ± 0.50
	50 µg/g soil (F2)	3.30 ± 1.36	1.55 ± 0.28	1.65± 0.80
	100 µg/g soil (F3)	2.70 ± 1.20	2.55 ± 0.77	2.38 ± 0.44

Table 7.6 Mean nutrient contents in shoot and root material of pepper rooted cuttings with SEM (standard error of the mean) at 08 month after repotting

		Root			Shoot		
		AM Inoculum level			AM Inoculum level		
Nutrient	P fertilizer level (µg/g soil)	M1-0 g	M2-75 g	M3-150 g	M1-0 g	M2-75 g	M3-150 g
K content	5 µg/g soil (F1)	1.57 ± 0.04	1.48 ± 0.04	1.76 ± 0.17	2.12 ± 0.06	1.99 ± 0.09	2.07 ± 0.13
	50 µg/g soil (F2)	1.04 ± 0.34	1.74 ± 0.08	1.29 ± 0.31	2.37 ± 0.10	2.30 ± 0.10	2.44 ± 0.16
	100 µg/g soil (F3)	1.54 ± 0.17	1.39 ± 0.39	1.94 ± 0.09	2.22 ± 0.22	2.35 ± 0.08	2.16 ± 0.23
Mg content	5 µg/g soil (F1)	0.40 ± 0.01	0.43 ± 0.02	0.40 ± 0.04	0.32± 0.01	0.27 ± 0.02	0.27 ± 0.03
	50 µg/g soil (F2)	0.28 ± 0.05	0.44 ± 0.02	0.38 ± 0.11	0.30 ± 0.01	0.32 ± 0.03	0.32 ± 0.03
	100 µg/g soil (F3)	0.36 ± 0.02	0.44± 0.02	0.51 ± 0.04	0.35 ± 0.02	0.35 ± 0.02	0.24 ± 0.04
N content	5 µg/g soil (F1)	2.55 ± 0.18	2.69 ± 0.161	2.8 ± 0.097	1.20 ± 0.020	1.92 ± 0.106	1.99 ± 0.020
	50 µg/g soil (F2)	1.99 ± 0.62	2.63 ± 0.133	2.6 ± 0.284	2.106 ± 0.163	2.06 ± 0.101	2.13 ± 0.080
	100 µg/g soil (F3)	2.15 ± 0.04	2.82 ± 0.062	2.8 ± 0.094	1.855 ± 0.175	2.04 ± 0.170	1.86 ± 0.120
P content	5 µg/g soil (F1)	0.18 ± 0.03	0.15 ± 0.02	0.17± 0.01	0.15 ± 0.01	0.16 ± 0.013	0.19 ± 0.01
	50 µg/g soil (F2)	0.19 ± 0.02	0.19 ± 0.03	0.19 ± 0.02	0.21 ± 0.02	0.17 ± 0.005	0.18 ± 0.01
	100 µg/g soil (F3)	0.22 ± 0.40	0.17 ± 0.01	0.213 ± 0.02	0.17 ± 0.03	0.19 ± 0.010	0.16 ± 0.02

Table 7.7: Means of soil chemical parameters in rhizosphere soil and bulk soil with SEM (standard error of the mean) at 08 month after repotting

		Rhizosphere soil				Bulk soil			
		AM Inoculum level				AM Inoculum level			
Soil chemical parameter	P fertilizer level (µg/g soil)	M1-0 g	M2-75 g	M3-150 g	LSD value	M1-0 g	M2-75 g	M3-150 g	LSD value
Soil pH	5 µg/g (F1)	6.2±0.06	6.2±0.05	6.8±0.05		6.1±0.06	6.0±0.02	6.0±0.04	
	50 µg/g (F2)	6.1±0.04	6.2±0.06	6.4±0.05	0.082	6.1±0.07	6.1±0.060	6.2±0.120	0.121
	100 µg/g (F3)	6.4±0.04	6.3±0.01	6.3±0.02		6.4±0.06	6.1±0.056	6.2±0.065	
Soil Bray P (ppm)	5 µg/g (F1)	10.5±0.44	11.0±0.64	10.6±0.57		8.2±0.80	7.5±0.18	6.9±0.38	
	50 µg/g (F2)	10.8±0.49	10.9±0.43	12.6±0.08	1.499	7.9±1.26	8.9±0.48	10.1±2.29	1.843
	100 µg/g (F3)	14.4±2.11	13.7±0.29	13.4±1.43		5.6±0.69	7.3±0.67	6.6±0.17	
Olsen P (ppm)	5 µg/g (F1)	4.7±0.15	5.3±1.18	3.1±0.15		2.7±0.15	3.4±0.12	2.0±0.47	
	50 µg/g (F2)	6.0±0.05	5.2±0.55	7.7±1.30	1.586	2.5±0.47	2.3±0.24	4.2±1.61	1.025
	100 µg/g (F3)	5.2±0.65	7.0±1.42	5.4±0.86		3.2±0.10	4.5±0.6.1	2.9±0.23	
Water soluble P (ppm)	5 µg/g (F1)	3.2±1.27	0.3±0.09	1.9±0.78		2.3±0.89	1.7±0.84	4.3±0.62	
	50 µg/g (F2)	2.3±2.07	6.5±1.99	5.9±0.90	2.283	3.4±0.77	5.7±0.82	6.0±2.32	2.345
	100 µg/g (F3)	1.5±2.54	0.9±0.87	2.7±0.90		6.2±0.75	6.3±1.57	4.8±1.61	

variability induced by AM on P response pattern. As there are many environmental and biological factors on intensity and response of AM on plant mechanism (Trovlove, *et al.* 2003) interaction effect could be further complicated by not reflecting a consistent response in perennial species within a short duration after inoculation. Apart from that high variability in replicates could have masked the interaction effect between P fertilizer levels and AM inoculation on leaf area development.

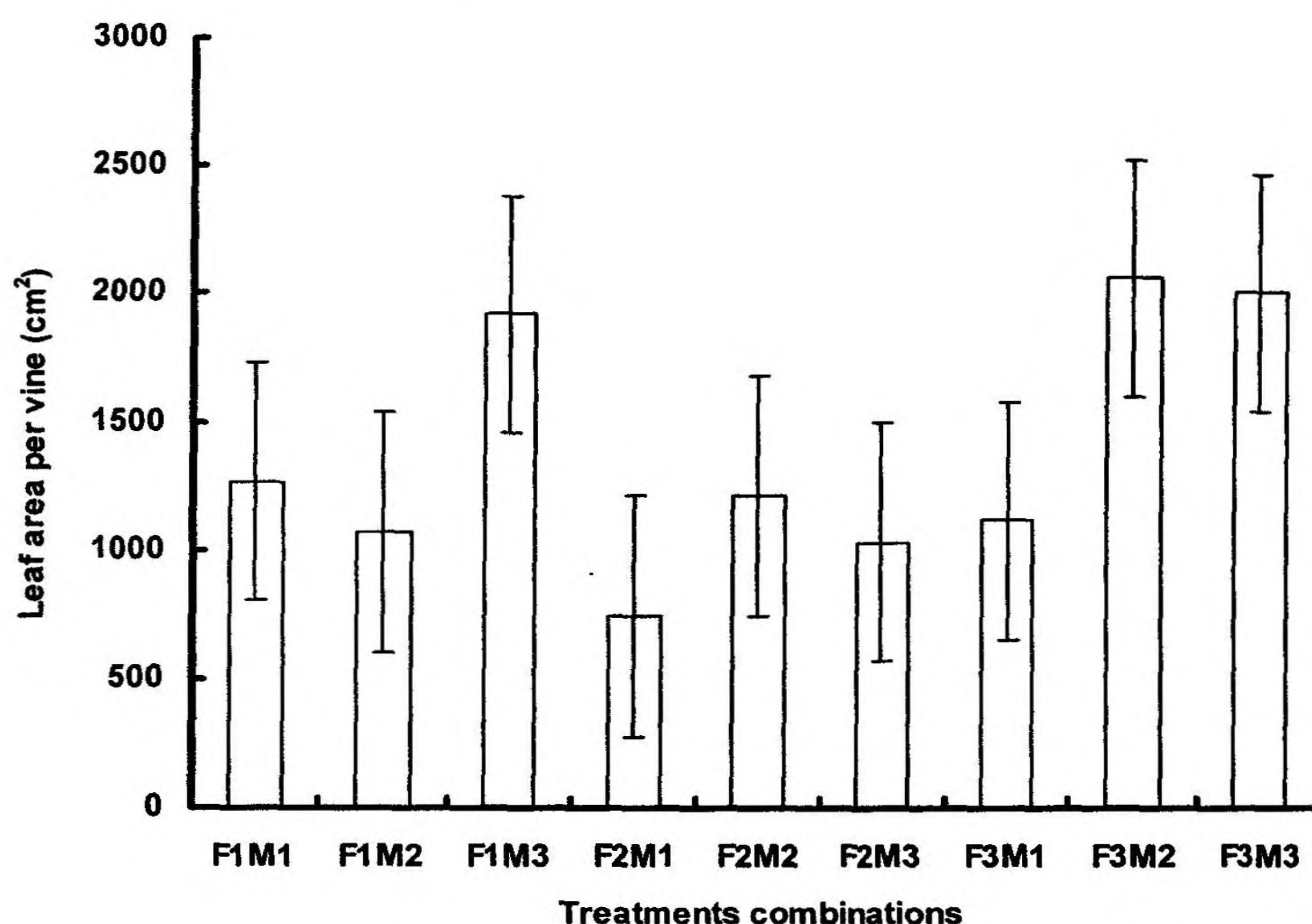


Figure 7.1. Effect of treatment combination on mean leaf area per vine (Error bar indicates LSD at P<0.05)

7.3.2.3 Effect of treatments on shoot dry weight

The main effect of P fertilizer was significant (P<0.05) for shoot dry weight having 20.66 (±4.190) g, 14.43(±4.450) g and 24.82 (±6.613) g shoot dry weights for 5 μg P/ g soil (F1), 50 μg P/ g soil (F2) and 100 μg P/ g (F3) fertilizer application levels respectively and the shoot dry weight of F2 level was significantly smaller than that of F3 level.

The mean shoot dry weights for inoculation treatments of M1, M2 andM3 were 16.13 (±6.882) g, 20.44 (±4.843) g and 22.38 (±3.528) g, respectively and there is an increasing

trend. Pattern of variation in shoot dry weight values are appeared to be similar for the variation patterns of leaf area too in this experiment.

7.3.2.4 Effect of treatments on root dry weight of Black pepper

None of the treatment effects were significant for root dry weight values. However, mean root dry weight for P fertilizer treatments of F1, F2 and F3 were 2.157 (±0.408) g, 2.167 (±0.813) g and 2.522 (±0.729) g, respectively with an increasing trend. The mean root dry weight for inoculation treatments of M1, M2 and M3 were 2.585 (±0.847) g, 2.015 (±0.521) g and 2.254 (±0.582) g, respectively. In contrast to the response pattern of above ground growth parameters i.e. leaf area and shoot dry weight, the maximum total root dry weight of 3.3 g was observed at F2M1 (P fertilizer level 50µg P/ g soil without mycorrhizae) treatment combination. This is in agreement with the general rule that in mycorrhizal plants, shoot growth is more enhanced than root growth (Marschner, 1996). However, this is contrary to the observation of black pepper root growth enhancement for AM inoculation at nursery stage where only AM inoculation densities were tested (Mala *et al.*, 2009). It is likely that this inconsistency may be attributed to the additional variability resulted from incorporation of P fertility levels in this experiment.

7.3.3 Effect on shoot tissue and root nutrient contents

The powdered shoot and root materials were subjected to nutrient analysis to ascertain the amount of nutrients absorbed by the plant. The summarized observations are given in Table 7.6.

7.3.3.1 Effect on shoot tissue nutrient content

Mean values are shown in the Table 7.6. Mean shoot tissue K (%) contents for P fertilizer treatments F1, F2 and F3 were 2.06 (±0.089), 2.37 (±0.119) and 2.25 (±0.161) %, respectively with a significantly lower tissue K content in F1 fertilizer level. The effect of inoculation treatment was not significant having 2.24 (±0.112), 2.21 (±0.087) and 2.23 (±0.170) % of K for samples of M1, M2 and M3 respectively. The minimum tissue K content was observed for treatment combination of F1M2. This is likely to be an initial plant physiological response of pepper rooted cuttings for AM inoculation at the lowest added P level.

Neither P fertility level nor AM inoculation treatment was significantly changed the shoot tissue Mg content. The smallest tissue Mg content of 0.27% was observed for each F1M2 and F1M3 and this pattern was seen for tissue K content too.

None of the treatment effects were significant for shoot tissue N content at the time of sampling.

Even though significant main effects were not seen for shoot tissue P content, a trend of interaction ($P<0.1$) was seen as a clear increase of tissue P content with the increase of AM inoculation density at the lowest P fertility level F1 (5 μg P/ g/ soil) (Table 7.6). This is an indication of improvement in P absorption by plants at the presence of AM colonization in pepper rooted cuttings.

7.3.3.2 Effect on root tissue nutrient Content:

Mean values with standard error are shown in Table 7.6. Neither P fertility level nor AM inoculation treatments significantly changed the root tissue K content, However, there is an increasing trend of tissue K content with increase of AM inoculation showing 1.36 (±0.171), 1.54 (±0.117) and 1.66 (±0.111) % of root tissue K content for M1 (no AM), M2 and M3 levels respectively.

The mean root tissue Magnesium (Mg) content was not significantly changed due to main effect of P fertility levels. Nevertheless, significantly ($P<0.05$) lower root tissue Mg content of 0.343 (±0.002)% was observed at no AM inoculation (M1) compared to 0.436(±0.004) and 0.428(±0.004)% of Mg for AM2 and AM3 respectively. Moreover, a systematic increase in mean root tissue Mg content was observed having 0.361 (±0.024), 0.437 (±) and 0.505 (±0.018) % of Mg for M1, M2 and M3, respectively at the highest P fertility level (F3), although the effect was not significant. This is an indication of improvement in Mg absorption with inoculation of AM for pepper rooted cuttings.

Even though, root tissue N content was not significantly changed due to main effect of P fertility levels, a significantly lower tissue N content of 2.23 (±0.277) % was observed at no AM inoculation (M1) compared to 2.72 (±0.118) and 2.73 (±0.158) % of N for M2 and M3 respectively. These observations confirmed the likely improvement of N absorption also due to AM inoculation of pepper rooted cuttings.

None of the treatment effects were significant for root tissue P content values. However, root tissue P content for P fertilizer treatments of F1, F2 and F3 were 0.17 (±0.145), 0.19 (±0.344) and 0.20 (±0.065) % g respectively with an increasing trend.

7.3.4 Soil chemical properties of rhizosphere soil and bulk soil

The chemical properties were summarized and given in Table 7.7 with LSD values. ANOVA tables related to data analysis are summarized in the Appendix No.7.1-7.8.

7.3.4.1 Effect of treatments on soil pH (1:2.5 H_2O) of rhizosphere soil and bulk soil

There was a significant ($P<0.05$) difference among rhizosphere soil samples and bulk soil samples for soil pH (1:2.5 H_2O) having 6.24 and 6.11 pH values for rhizosphere and bulk soil samples respectively. For rhizosphere soil samples, the main effect of P fertilizer levels was significant ($P<0.05$) having 6.17 (±0.052), 6.23 (±0.052) and 6.32 (±0.020) pH values for F1 (5µg P/ g soil), F2 (250µg P/ g soil), and F3 (100µg P/ g soil) applications respectively. For bulk soil samples also, the main effect of fertilizer level was significant ($P<0.05$) having 5.99 (±0.039), 6.13 (±0.082) and 6.20 (±0.057) pH values for F1 (5µg P/ g soil), F2 (50µg P/ g soil) and F3 (100µg P/ g soil) P applications respectively. There is an increasing order of soil pH with the increase of the application of Eppawala Rock Phosphate in this experiment (Figure 7.2a). The same pattern was obvious with different AM inoculum levels for Rhizosphere soil (Figure 7.2b).

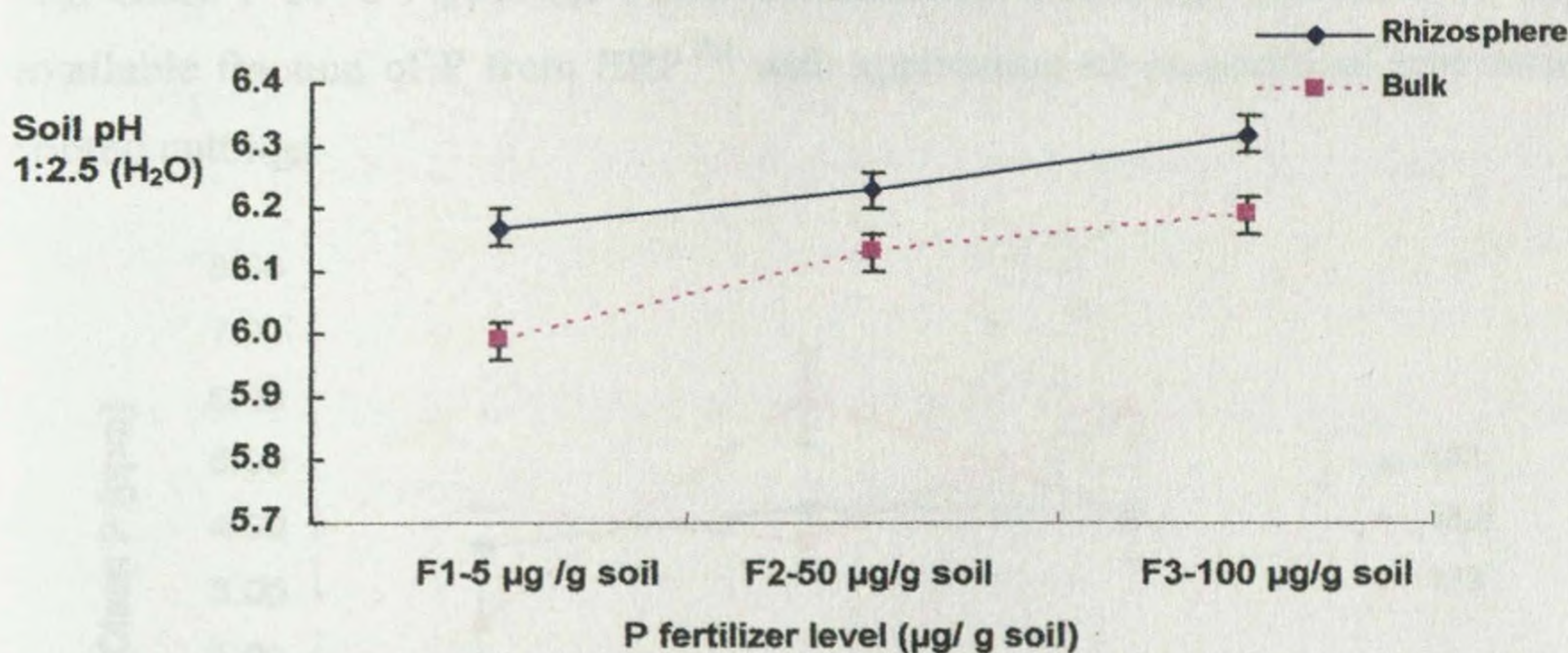


Fig. 7. 2a. Effect of P fertility level on soil pH of rhizosphere and bulk soil (Error bar indicates LSD at $P<0.05$)

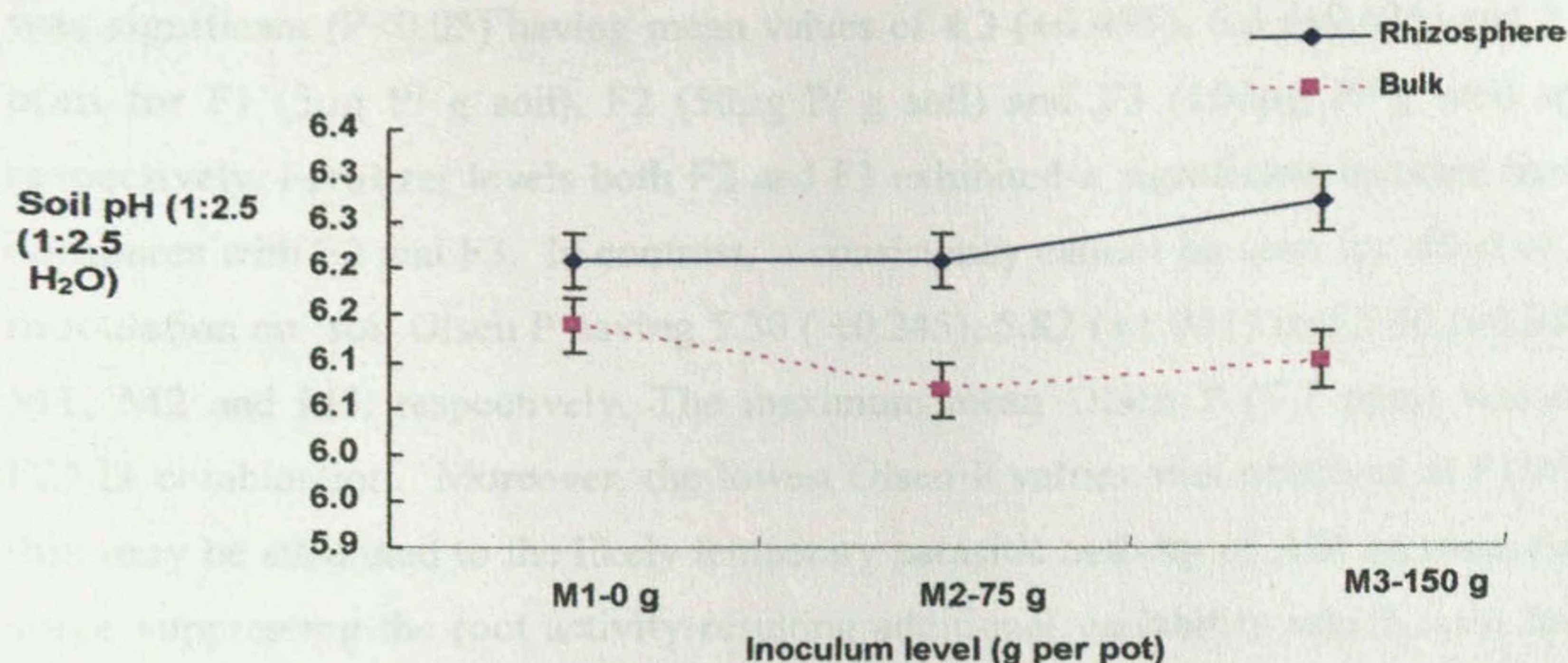


Fig. 7.2b. Effect of AM inoculum levels on pH values of rhizosphere and bulk soil (Error bar indicates LSD at P<0.05)

7.3.4.2 Olsen P content of rhizosphere soil and bulk soil

In general, mean value of soil Olsen P was 4.3 ppm and the rhizosphere soil Olsen P value (5.5 ppm) was significantly ($P<0.05$) greater than that of bulk soil (3.1 ppm) (Table 7.7). It is important to note that the interaction between P fertilizer levels and AM inoculation were highly significant ($P<0.001$) for soil Olsen P for the model of both rizhosphere samples as well as bulk soil samples together (Figure 7.3) and this interaction reflected as the maximum soil Olsen P of 5.7 ppm for F2M3 combination. This is an indication of improvement of available fraction of P from ERP^{TM} with application of mycorrhizal inoculation for pepper rooted cuttings.

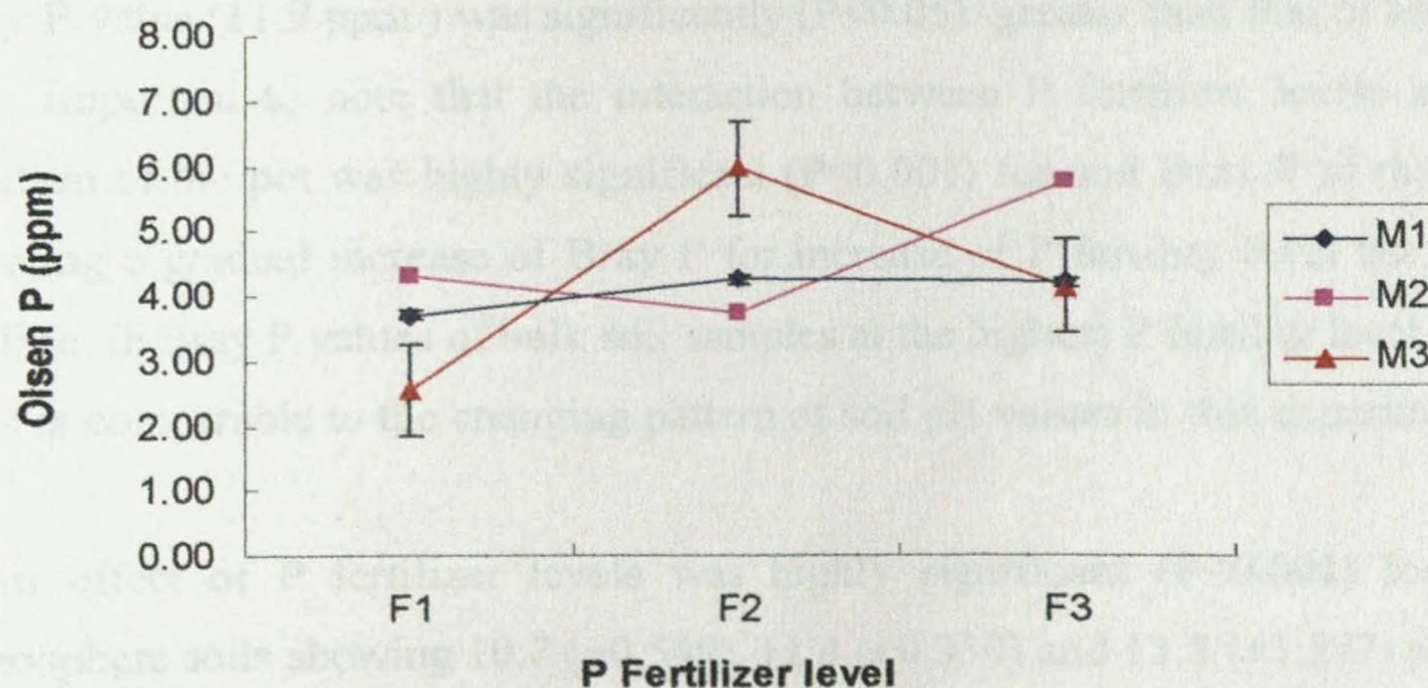


Fig 7.3: Interaction effect of P fertility levels and AM inoculum levels on soil Olsen P for both rizhosphere and bulk soil samples (Error bar indicates LSD at P<0.05)

When considering the rhizosphere soil samples alone, only the effect of P fertilizer levels was significant (P<0.05) having mean values of 4.3 (±0.495), 6.3 (±0.636) and 5.9 (±0.938) ppm for F1 (5μg P/ g soil), F2 (50μg P/ g soil) and F3 (100μg P/ g soil) applications, respectively. Fertilizer levels both F2 and F3 exhibited a significant increase from F1 when compares with F2 and F3. In contrast, a consistency cannot be seen for effect of mycorrizal inoculation on soil Olsen P having 5.30 (±0.245), 5.82 (±1.051) and 5.40 (±0.938) ppm for M1, M2 and M3, respectively. The maximum mean Olsen P (7.7 ppm) was observed at F2M3 combination. Moreover, the lowest Olsen P values was observed at F1M3 (3.1) and this may be attributed to the likely temporary parasitic activity of AM on roots during initial stage suppressing the root activity resulting additional variability which must have masked the treatment effects at the lowest P fertility level. This concept is supported by Marschner (1996) quoting that formation and maintenance of mycorrhizae requires considerable amounts of photosynthates and mycorrihae strongly compete with roots for photosynthates. Only the interaction between P fertilizer level and AM inoculum level was significant (P<0.05) for Olsen P values of bulk soil samples at the time of sampling in this experiment. Substantially high soil Olsen P values of 4.2 and 4.5 ppm were observed for bulk soil samples of F2M3 and F3M2 respectively. As Wang and Zabowski (1998) pointed out, the effect of P fertilization levels could be strong enough to mask the differences in the concentrations of fertilized nutrients between rhizosphere and bulk soil samples.

7.3.4.3 Bray-1 P content of rhizosphere soil and bulk soil

The Observed Bray P values were higher than Olsen P values irrespective of treatment combinations. Overall mean value of soil Bray P was 9.8 ppm and the mean rhizosphere soil Bray P value (11.9 ppm) was significantly (P<0.05) greater than that of bulk soil (7.8 ppm). It is important to note that the interaction between P fertilizer levels and soil sampling location of the pot was highly significant (P<0.001) for soil Bray P of rhizosphere samples showing a gradual increase of Bray P for increase of P fertility level but there was a clear decline in Bray P values of bulk soil samples at the highest P fertility level (F3) (Figure 7.4). This is comparable to the changing pattern of soil pH values in this experiment.

Main effect of P fertilizer levels was highly significant (P<0.001) for soil Bray P of rhizosphere soils showing 10.7 (±0.549), 11.4 (±0.330) and 13.8 (±1.277) ppm Bray P values for F1 (5μg P/ g soil), F2 (50μg P/ g soil) and F3 (100μg P/ g soil) levels respectively. The Bray P value (10.7) of F1 was significantly lower than that of each F2 and F3. A systematic

increase in soil Bray P was observed having 11.6 (±1.013), 11.9 (±0.450) and 12 (±0.693) ppm for M1, M2 and M3 respectively.

For bulk soil samples, main effect of P fertilizer level was significant ($P<0.05$) showing 7.6 (±0.454), 9.0 (±1.342) and 6.6 (±0.512) ppm of Bray P values for F1 (5µg P/ g soil), F2 (50µg P/ g soil) and F3 (100µg P/ g soil) levels, respectively. In contrast to Olsen P values, the interaction between P fertilizer levels and mycorrhizal inoculum levels was not significant for Bray P measurement. This may be attributed to the nature of Bray test which dissolves unreacted phosphate rock applied to acid soils and thus, for soils that have received rock phosphate applications recently, the Bray test could overestimate the P availability until all of the rock phosphate is fully dissolved (Bigham, 1996).

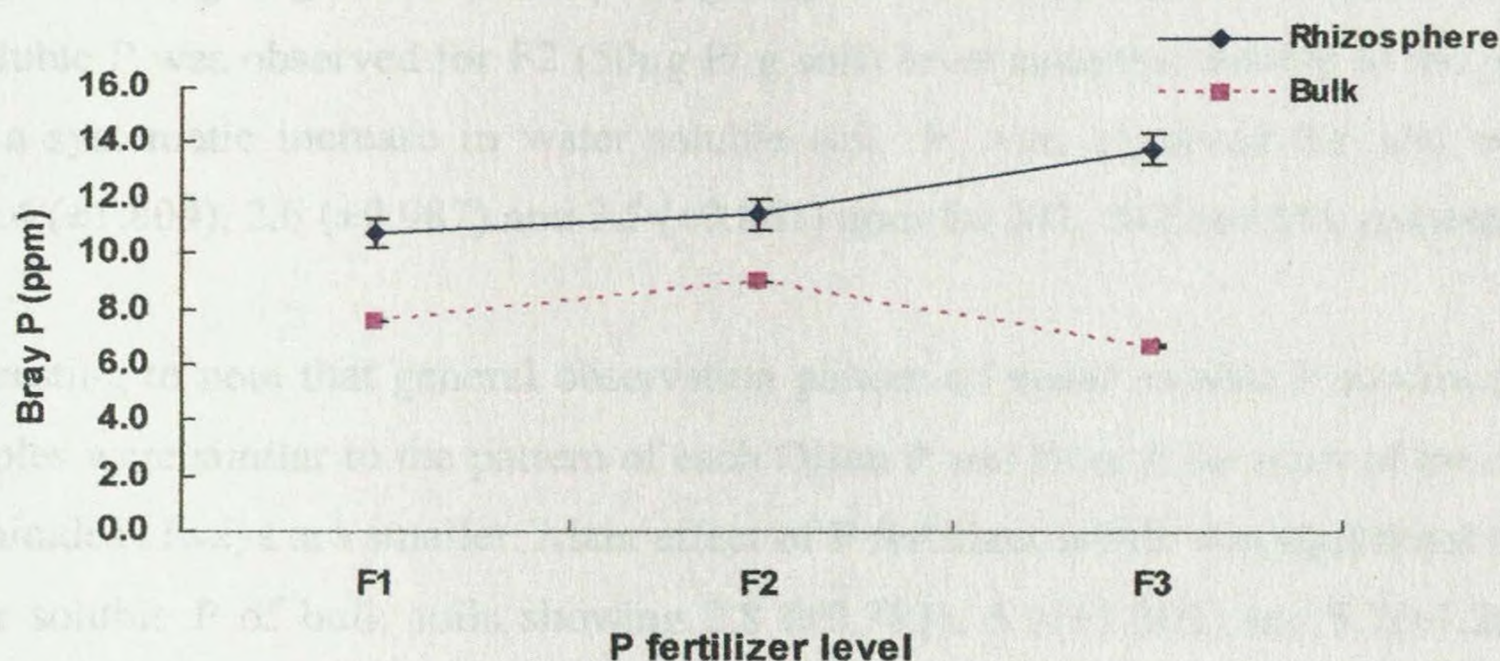


Fig. 7.4.Effect of P fertility level on Bray P of rhizosphere and bulk soil (Error bar indicates LSD at $P<0.05$)

7.3.4.4 Water soluble P of rhizosphere soil and bulk soil

As expected the observed water soluble P values were lower than each Olsen P values as well as Bray P values of corresponding treatment combinations. Overall mean value of water soluble soil P was 3.7 ppm and the water soluble P value of rhizosphere soil samples (2.9 ppm) was significantly ($P<0.05$) lower than that of bulk soil (4.5 ppm) and this is opposite from the trend observed for other two P fractionation methods in this study. Moreover, the interaction between P fertilizer levels and soil sampling location of the pot was significant ($P<0.05$) for soil water soluble P fraction of soils showing a gradual increase of water soluble P of bulk soil samples with the increase of P fertility level but there was a sharp decline in water soluble P value of rhizosphere soil samples at P fertility level of F3 (Figure 7.5).

For rhizosphere soil samples, main effect of P fertilizer levels was significant ($P<0.05$) having 1.8 (±0.715), 4.9 (±1.654) and 1.7 (±1.283) ppm water soluble P values for F1 (5μg P/ g soil), F2 (50μg P/ g soil) and F3 (100μg P/ g soil) levels, respectively where the highest water soluble P was observed for F2 (50μg P/ g soil) level samples. Similar to the pattern of Bray P, a systematic increase in water soluble soil P was observed for AM treatments having 2.4 (±1.804), 2.6 (±0.987) and 3.5 (±0.861) ppm for M1, M2 and M3, respectively.

It is interesting to note that general observation pattern of water soluble P content of bulk soil samples were similar to the pattern of each Olsen P and Bray P for most of the cases but the magnitudes always are smaller. Main effect of P fertilizer levels was significant ($P<0.05$) for water soluble P of bulk soils showing 2.8 (±0.783), 5.1(±1.303) and 5.7(±1.263) ppm water soluble P values for F1 (5μg P/ g soil), F2 (50μg P/ g soil) and F3 (100μg P/ g soil) levels respectively. The water soluble P value (2.8) of F1 was significantly ($P<0.05$) smaller than that of each F2 and F3. Similar to the pattern of Bray P for rhizosphere soil, a systematic increase in water soluble P of bulk soil samples was observed having 3.7 (±0.756), 4.6 (±1.077) and 5.0 (±1.516) ppm for inoculum treatments M1, M2 and M3, respectively.

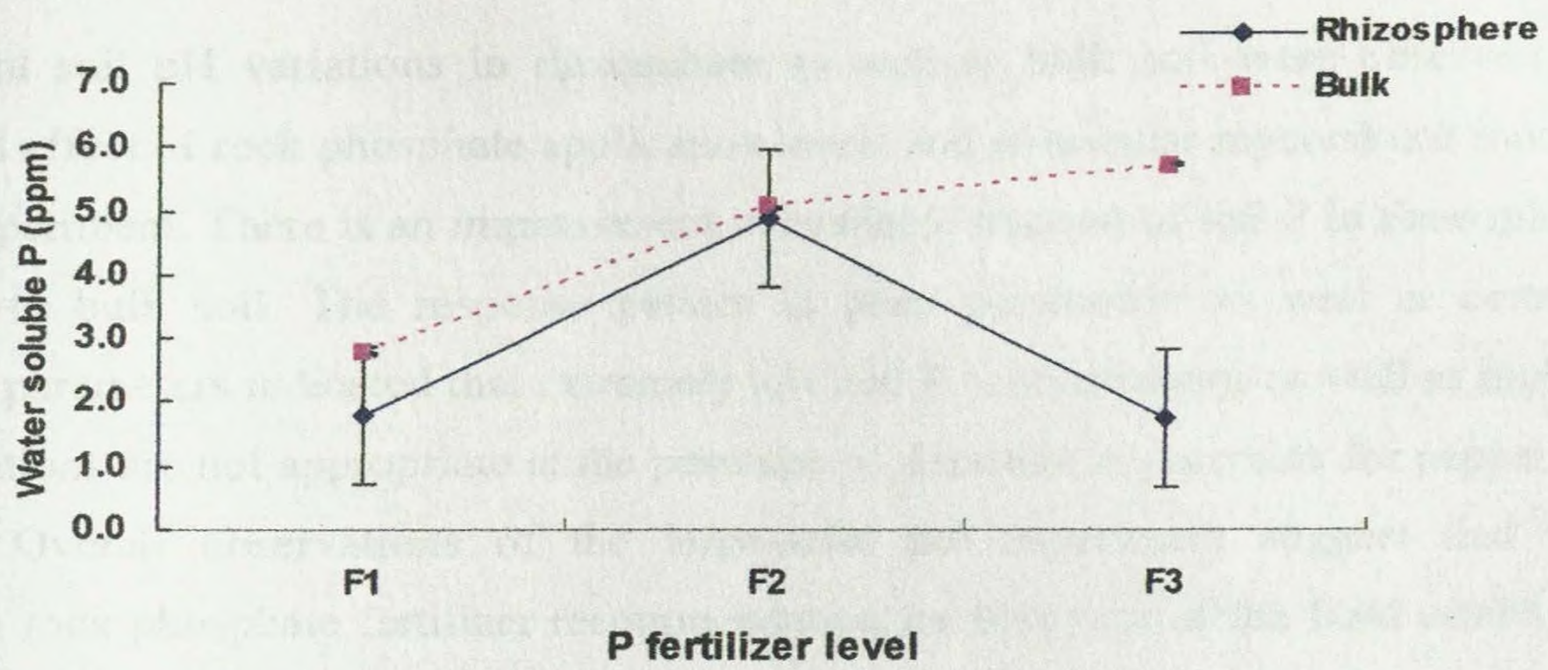


Fig. 7.5 Effect of P fertility levels on water soluble P of rhizosphere and bulk soil (Eror bar indicates LSD at $P<0.05$)

7.4 Conclusion

Significant soil pH variations in rhizosphere as well as bulk soil were observed due to combined effect of rock phosphate application levels and arbuscular mycorrhizal inoculation in this experiment. There is an improvement in available fraction of soil P in rhizosphere soil compare to bulk soil. The response pattern in plant parameters as well as certain soil chemical parameters indicated that extremely low soil P concentrations as well as high soil P concentrations are not appropriate at the presence of abuscular mycorrhiza for pepper rooted cuttings. Overall observations of the large-scale pot experiment suggest that current Eppawala rock phosphate fertilizer recommendation for first year of the field establishment appeared to be too high.

This means that the application of moderate rate of P fertilizer (50μg P/ g soil) may maximize the beneficial effect of abuscular mycorrhiza (*Glomus mosseae*) symbiosis on pepper grown in Reddish Brown Latasolic soil at Matale area in Sri Lanka.

Chapter 08
General Discussion

Results of the first pot experiment on appropriate host crop species and time of uprooting are presented in Chapter 4. The host crop to obtain maximum possible number of spores in the inoculum of arbuscular mycorrhizae (AM) species *G. mosseae* was studied. Two types of spores, namely brown and black in colour, were observed and observations of darkly stained internal mycorrhizal structures in root pieces confirmed the success of initial inoculation of each host crop species namely maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and finger millets (*Eleucine coracana.* L). The observations are in comparable with observations of Brundrett, *et al.* (1996) as they reported wide range of spore colours are associate with *Glomus* spores, comparatively dark colour spores can be old, dead spores are typical of those found in field soils. As described by Brundrett, *et al.* (1996), (1) presence of relatively straight hyphae ramifying along the root cortex, often producing 'H' branches which result in simultaneous growth in two directions (2) relatively dark staining of these hyphae and (3) presence of oval vesicles which usually form between root cortex cells in many cases, which persist in roots often developing into thickened and /or multilayered walls are several characteristics that can be used to identify genus *Glomus.* The observations of stained hyphal structures in host crop root samples of this experiment were also similar to those of Brundrett, *et al.* (1996), irrespective of host crop species. The characteristic mean brown spore density value recorded was 142 (± 98 SD) per 50 g of potting media at 12th week after inoculation of each host crop species. Brown spore density values of each species became almost equal towards harvest at 12th week and spore density of finger millet remains above 100 spores per 50 g during three uprooting times i.e. 8, 10 and 12 weeks after planting. Ocampo and Hayman (1981) reported that maize became heavily mycorrhizal much faster from the endophytes native to Long Hoos soil (mainly *Glomus macrocarpus* var. *geosporus*) than barley or lettuce in this soil whereas with the E3 endophyte in Woburn soil in U.K. It is important to note that there is a paucity of literature evidence on arbuscular mycorrhizal association with finger millet. Nevertheless, the results of the current experiment indicated that finger millet as a good alternative host crop for mass propagation of AM under local conditions. Apart from that finger millet can give an additional income as a grain harvest also at 12th week. Therefore, finger millet was also included as a suitable alternative host crop species in the protocol (Annex 09) prepared for mass propagation of AM fungus *G. mosseae* at local nursery level.

The results of the experiment with black pepper on suitable spore density of AM fungus *G. mosseae* are presented in Chapter 5. Observations of darkly stained AM hyphae at the 2nd month after inoculation in all the inoculated treatments confirmed the success of inoculation for pepper rooted cuttings at the nursery stage. The heaviest infection of mycorrhizal fungi was observed in roots with 150 g of inoculum per standard pot (T3) showing root colonization, along with mycelium (100%), vesicles (94%), and spores (769/ 50 g) at the 6th month after inoculation. Significantly higher ($P<0.05$) shoot biomass (5.07 g) and root length (2740 cm) were observed at the 6th month in the rooted cuttings inoculated with 75 g of inoculum per pot (T2) than in the unionoculated control (3.28 g and 1952 cm of roots). The observations of the current experimentis comparable with general literature on successful inoculation and growth improvement of black pepper rooted cuttings with different AM species as inoculation. For example, Anandaraj and Sarma (1994) showed that, incorporation of vesicular arbuscular mycorrhizae (AM) fungus *Glomus fasciculatum* in the rooting medium of sand enhanced rooting of black pepper (*Piper nigrum*) cuttings and suggested that rooting enhancement could be used as a criterion to identify efficient strains of AM for black pepper. According Thanuja *et al.* (2002), among three AM fungi tested with pepper cultivar Panniyur-1, the maximum percentage rooting was observed in cuttings inoculated with *Acaulospora laevis* (69.96%). Orthotropic cuttings showed a higher rooting percentage on inoculation with AM. Mycorrhizal inoculation also improved other root characteristics. Among the different AM fungi tried, *Gigaspora margarita* was efficient in producing the maximum number of primary roots and dry weight of roots, whereas the longest primary root was observed with the inoculation of *Glomus fasciculatum*. The percentage root colonization and spore count were found to be higher in cuttings inoculated with *A. laevis*. In an another study conducted in India by Kandiannan *et al.* (2000) reported that growth and nutrient content of black pepper (*Piper nigrum* L.) cuttings were influenced by inoculation with biofertilizers. Moreover, inoculation of AM with combination of other biofertilizer/s enhanced plant height, leaf area, biomass and dry matter production and nutrient content significantly over uninoculated control (Kandiannan *et al.*, 2000).

In general our observations of the current study are appeared to be sufficient to draw the conclusion;incorporation of the AM inoculum at the rate of 75 g containing approximately 795 (mean) spores with host crop roots and fungal structures in moist soil mixture as medium into one standard size polythene bag before planting a rooted cutting of black pepper was

found to be suitable to obtain good quality planting materials for field planting at the 6th month of growth in the nursery.

The results of the experiment with true cinnamon on suitable spore density of AM fungus *G. mosseae* are presented in Chapter 6. According to the microscopic view of stained cinnamon root segments, darkly stained VAM, *G. mosseae* hyphae were observed at 4th month after inoculation of seedlings at all the inoculum levels and it confirmed the success of inoculation for cinnamon seedlings. It is important to note that general staining procedure with tryphan blue was not acceptably successful with cinnamon root samples and this may be due to the abundance of phenolic compounds and many other characteristic metabolic by-products in cinnamon roots. Nevertheless, it was able to successfully stain and clear the cinnamon roots for AM structures subsequent to the staining of more than hundred of root pieces were used for the staining process development. It can be concluded that the staining method described by Jarstfer and Silvia (2001) has been perfected for staining of cinnamon root pieces in the absence of literature on either staining or presence of AM with cinnamon (*Cinnomomum verum* Presl) in other countries. Plants inoculated with 75 g of inoculum (*G. mosseae*) showed the maximum root dry weight of 261 mg at the 2nd month after inoculation which is significantly higher ($P< 0.05$) than non-inoculated control plants (151 cm). Therefore, conclusion, inoculation of cinnamon seedlings with 75 g of above inoculum seems to be appropriate for enhancement of seedling growth of cinnamon.

The results of the experiment on the combined effect of finely divided (150-250 µm of particle size) Eppawala Rock Phospahate (ERP™) levels and AM (*G. mosseae*) on pepper rooted cuttings beyond the nursery stage are given in Chapter 7. The main effects of P fertility level as well as AM inoculum were significant for certain plant growth parameters e.g. leaf area and shoot dry weight. The smallest leaf area was seen at F2M1 combination and it was significantly smaller than that of few treatment combinations containing AM inoculum. In general, shoot dry weight also followed the same pattern as leaf area. In contrast to the shoot growth parameters, the maximum root dry weight was recorded at F2M1 combination and the possible reasons have already been discussed and this is comparable with the theory of root growth inducement under stress situation created by either limited food or mineral nutrients (Sumanasena, 2003).

Quite comparably, minimum tissue K content was observed at F1M2 combination while a clear increase of tissue P content was seen with the increase of AM inoculum level at the lowest P fertility level (F1). On the other hand, a significant tissue Mg content improvement

was observed with increase of inoculation density indicating the effectiveness of AM inoculation for pepper rooted cuttings in this experiment.

The higher soil pH in rhizhosphere soil samples in this experiment is in agreement with Marschner (1996). Moreover, Marschner (1996) stated that the rhizosphere pH may differ from that of the bulk soil by up to two units, depending on plant and soil factors. In plants, grown in, and adapted to, acid soils, the pH of apical root zones is often considerably higher than basal zones and the bulk soil. Such a pH increase might not only favour uptake rate of cations such as Mg^{2+} but also play and important role in detoxifying monomeric Al in the rhizosphere. According to Wang and Zabowski (1998) the difference in the solution pH between rhizosphere and bulk soil of Douglas-fir was probably due to unequal NH_4^+ and NO_3^- uptake causing differential root exudation of H^+ and OH^- ions. Furthermore, with a higher concentration of NH_4^+ relative to NO_3^- in the rhizosphere soil solution, the solution pH of the rhizosphere was lower than that of bulk soil, but with a lower concentration of NH_4^+ relative to NO_3^-, the solution pH of the rhizosphere was higher than that of the bulk soil. More uptake of cations relative to anions will result in the release of H^+ while more uptake of anions relative to cations will cause the release of OH^- from roots to maintain electro-neutrality inside the plant. In the literature, the question of whether AM hyphae are able to solublize soil P that is other wise unavailable to the plant remains a matter of debate (Trolove *et al.*, 2003). In another literature, Li *et al.* (1991) attributed some of the hyphosphere P depletion to acid production by the hyphal network probably caused by NH_4^+ uptake. The reasons for the variations in results may be due to soil type, species of mychorrizal fungi and host plants and plant age. As plant physiology of a perennial spice crop like pepper (*Piper nigrum* L.) can be characteristically different from other tree species giving essential oils as metoboilc byproducts, cation and anion exchange mechanism in the rhizosphere could also be different from most of the other plant species. This fact also a possible reason for the pH difference between rhizosphere soil and bulk soil in this experiment and above all, soil pH is an important parameter to characterize the chemical availability of mineral nutrients in soils. Certainly the reasons for these differences in soil pH in this study warrant further investigation on rhizosphere chemistry of black pepper.

According to observation pattern of the soil chemical properties, there is a significant ($P<0.05$) difference between rhizosphere soil and bulk soil for certain parameters. Always a significantly ($P<0.05$) higher pH, Olsen P and Bray P values were recorded with rhizosphere soil but significantly ($P<0.05$) higher water soluble P values were recorded with bulk soil for

few instances and this is acceptable as certain labile organic P components are counted only with Olsen P and Bray P procedure but not with water soluble extraction procedure. The main effects of P fertility levels were significant ($P<0.05$) for each Olsen P, Bray P and water soluble P. Overall, higher soil pH and Olsen P for bulk soil were recorded at F2 level. There is an indication for improvement of Bray P (in bulk soil), Olsen P (in rhizosphere soil and bulk soil), water soluble P (in rhizosphere soil) and root dry weight with moderate level of (50 µg P/ g soil) Eppawala rock phosphate application level at the presence of AM inoculation in pepper. A similar response pattern was reported for different rock phosphate levels and ectomycorrhizal infection level of *Pinus radiata* seedlings in an Allophanic soil (Liu *et al*, 2004). Nevertheless, irregularities in observation pattern for different fraction of P in this experiment are likely to be reflected the fact that phosphates release from mycorrhizae is likely to play a significant role in the acquisition of Pi(inorganic P) from labile organic forms of P(Po) (Plassard and Dell, 2010). Factors that contribute to this include the extent of extraradical hyphal penetration of soil and the physiology and biochemistry of the fungal/soil and fungal /plant interfaces. As labile forms of Po can constitute the major fraction of the total P in some tropical soils, a greater understanding of the forms of Po available to the phosphates is warranted.

Chapter 09
Summery and future research

9.1 Summery

As the initial step, an experiment was carried out with the objectives of finding the appropriate host crop species and time of uprooting the host crop to obtain maximum possible number of spores in the inoculum of arbuscular mycorrhizae (AM) species *G. mosseae*. Two types of spores namely brown and black coloured were observed and observations of stained internal mycorrhizal structures in root pieces confirmed the success of initial inoculation of each hot crop species. Mean brown spore density value became 142 (± 98 SD) per 50 g of potting media at 12th week after inoculation of each species of sorghum, maize and finger millet. Brown spore density values of each species became almost equal towards harvest at 12th week and spore density of finger millet remains above 100 spores per 50 g during three uprooting times i.e. 8, 10 and 12 weeks after planting. Under local conditions, finger millet was found to be a good alternative as a host crop for mass propagation of AM. This crop would give an additional income as a grain harvest also at 12th week. A protocol has been prepared for mass propagation of existing *G. mosseae* on the basis of experimental results and the protocol is in Annex 9. However, Sorghum (*Sorghum bicolor* L.) has been used for the mass propagation of AM for research purpose considering the facts that availability of supportive literature.

As introduction of mycorrhizal associations is a complementary alternative to improve fertilizer absorption efficiency of black pepper (*Piper nigrum* Linn.). The first inoculation experiment was carried out to select a suitable spore density of AM fungus *Glomus mosseae* for inoculation of black pepper rooted cuttings in the nursery using four mycorrhizal inoculum levels of *G. mosseae* with control. Observations of darkly stained AM hyphae at the 2nd month after inoculation in all the inoculated treatments confirmed the success of inoculation. The heaviest infection of mycorrhizal fungi was observed in roots with 150 g of inoculum per standard pot showing root colonization, along with mycelium (100%), vesicles (94%), and spores (769/ 50 g) at the 6th month after inoculation. Significantly higher ($P<0.05$) shoot biomass (5.07 g) and root length (2740 cm) were observed at the 6th month in the rooted cuttings inoculated with 75 g of inoculum per pot than in the unionoculated control (3.28 g and 1952 cm of roots). In conclusion, incorporation of the AM inoculum at

the rate of 75 g containing approximately 795 (mean) spores with host crop roots and fungal structures in moist soil mixture as medium into one standard size polythene bag before planting a rooted cutting of black pepper was found to be suitable to obtain good quality planting materials for field planting at the 6th month of growth in the nursery.

True Cinnamon is the most important spice crop of Sri Lanka while it is the highest nutrient demanding export agricultural crop species. Apart from that organic farming is also becoming popular among spice growers at present. On this context, with the objective of harnessing the beneficial effect of mycorrhizal association for cinnamon seedlings, this experiment was conducted to investigate the suitable spore density of AM (*Glomus mosseae*) for inoculation of cinnamon seedlings at nursery stage. Four mycorrhizal inoculum levels of *G. mosseae* 25 g, 75 g, 150 g, 300 g were tested with the unionoculated control. Inoculum consists of AM -*Glomus mosseae* spores and structures with sorghum (*Sorghum bicolor* L.) roots in moist soil having mean spore density of 154 spores/50 g (3 spores/ g). Plants inoculated with 75 g of inoculum showed the maximum root dry weight of 261 mg at the 2nd month after inoculation and it is significantly higher ($P< 0.05$) than non- inoculated control plants (151 cm). Similarly shoot dry weight was also significantly higher for plants inoculated with 75 g of inoculum (431 mg). It was concluded that inoculation of cinnamon seedlings with 75 g of above inoculum seems to be appropriate for enhancement of seedling growth of cinnamon.

Finely divided Eppawala Rock Phospahate (ERP™) is recommended as a P fertilizer for perennial crop species including pepper and cinnamon in Sri Lanka. Many sources of reactive rock phosphate brands including ERP™ are recognized to be slow in dissolution and release of P for plants and this is aggravated by high P fixing nature of tropical acid soils. On the other hand, successfully inoculated mycorrhzea species can improve the plant-uptake efficiency of P and other native nutrients from the soils. Considering these facts, three Eppawala rock phosphate levels (1) F_1-5 µg P/ g soil (2) F_2-50 µg P/ g soil and (3) F_3- 100 µg P/ g soil were tested with three mycorrhizal inoculum levels of *G. mosseae* (1) M_1-No Mycorrhizal inoculum, (2) M_2-75 g of Mycorrhizal inoculum and (3) M_3-150 g of Mycorrhizal inoculum in a large size pot experiment with pepper (local selection MB12) rooted cuttings beyond the nursery stage.

Significant soil pH variations in rhizosphere as well as bulk soil were observed due to combined effect of rock phosphate application levels and abuscular mycorrhizal inoculation in this experiment.

The response pattern in plant parameters as well as certain soil chemical parameters indicated that extremely low soil P concentrations as well as high soil P concentrations are not appropriate at the presence of abuscular mycorrhiza for pepper rooted cuttings. Significant interactions between P fertilizer levels and AM inoculation for soil Olsen P for both rizhosphere samples as well as bulk soil samples and the interactions reflected as the maximum soil Olsen P of 5.7 ppm for F2M3 combination. This is an indication of improvement of available fraction of P from ERP™ with application of mycorrhizal inoculation for pepper rooted cuttings. This means that the application of moderate rate of P fertilizer (F2-50μg P/ g soil) may maximize the beneficial effect of abuscular mycorrhiza (*Glomus mosseae*) symbiosis on pepper grown in Reddish Brown Latasolic soil at Matale area in Sri Lanka.

Overall observations of the large-scale pot experiment suggest that current Eppawala rock phosphate fertilizer recommendation for first year of the field establishment appeared to be too high.

9.2 Future research

Further experimentation on potting media with different mycorrizal species appeared to be worthwhile to reduce the dependency of the high value components such as cow dung and coir dust in standard potting mixture along with mycorrhizal inoculum as a supplementary potting media component is proposed.

Success of initial inoculation of cinnamon seedlings indicated that further experimentation on combination effect of different P fertility levels and AM inoculation is warranted.

The soil pH is an important parameter to characterize the chemical availability of mineral nutrients in soils. Certainly the reasons for these differences in soil pH in large pot trial warrant further investigation on rhizosphere chemistry of black pepper. Moreover, observations suggest that further research on rhizoshpere chemistry of pepper is warranted for improvement of plant nutrient management. These experiments should be conducted not only in the pot level but also at the field scale to draw firm recommendations on efficient utilization of applied fertilizer as well as native nutrients in the export agricultural cropping systems.

References

Administrative report, (2003) Department of Export Agriculture,

Alagawadi, A.R.and Gaur, A.C. (1988). Associative effect of *Rhizobium* and phosphate-solubilizing bacteria on the yield and nutrient uptake of chickpea. *Plant and soil, 105*, pp.241-246.

Alikhani H. A., Saleh-Rastin N., Antoun H., (2006). Phosphate solubilization activity of rhizobia native to Iranian soils, *Plant and soil 287*:pp.35-41

Anandaraj, M. and Sarma, Y. R. (1994). Effect of Vesicular arbuscular mycorrhizae on rooting of Black pepper (*Piper nigrum* L.). *Journal of spices & aromatic crops. 3 (1):* pp.39-42.

Anon (1996). Technical bulletin on Pepper cultivation. Technical bulletin No: 4. Department of Export Agriculture, 1095, Peradeniya.

Anon (2002). Technical bulletin on Pepper cultivation. Technical bulletin No: 4. Department of Export Agriculture, 1095, Peradeniya.

Anon (2003). Technical bulleting on Pepper cultivation. Technical Bulleting No: 4. Department of Export Agriculture, 1095, Peradeniya.

Anon (2005). Technical leaflet on cultivation of finger millet (Kurakkan) in Sinhala. Field crop Research and Development Institute, Department of Agriculture, Maha Illuppallama

Anon (2006). Technical bulleting on Pepper cultivation. Technical Bulleting No: 4. Department of Export Agriculture, 1095, Peradeniya.

Babana, A.H. and Antoun, H. (2006). Effect of Tilemsi phosphate rock solubilizing microorganisms on phosphorus uptake and yield of field-grown wheat (Triticum aestivum L.) in Mali. *Plant and soil, 287*:51-58.

Balasuriya, A. (2006). Potential role of Vesicular Arbuscular Mycorrhiza in Sri Lankan tea gardens. *Abstracts of papers,* A seminar on Potential of Mycorrhizal Associations for perennial crop management organized by Department of Export Agriculture and Section B of the Sri Lanka Association for the Advancement of Science, Sri Lanka.

Bagyaraj, D.J. (1992) Vesicular Arbuscular Mycorrhiza; Application in Agriculture. *In* J.R. Norris, D.J. Read and A.K. Varma (Ed), *Methods in Microbiology*: Academic Press, London. 24: pp359-373.

Bagyaraj, D.J. (2003). Microbial biotechnology for improved crop productivity. *In* S.S. Marwaha and J.K. Arora (Ed), *Biotechnological Strategies in Agro-processing.* Asiatech Pub. Inc., New Delhi. pp80-84.

Bagyaraj, D.J. (2006). VA Mycorrhizal Fungi and Their Role in Plant Disease Control. In B. Ramanujam and R.J. Rabindra (Ed), *Current status of Biological Control of Plant Diseases Using Antagonistic Organisms in India.* PDBC Pub. Bangalore. pp125-134.

Bagyaraj, D.J. and Chawla, G. (2009). Current Status and Relevance of Arbuscular Mycorrhizal Fungi in Nematode Management. In N. Nagesh, R.J. Rabindra and D.S.Bhumannavur (Ed), Proceedings of the Brain Storming Session on status, Prospects and Road Map for Enhancing the Uptake of Antagonistics Organisms in Nematode Management in India. Jwalamuki Pub. Bangalore.(In press)

Bagyaraj, D.J., Lakshmipathy, R. and Balakrishna, A.N. (1988). Agricultural Intensification in Tropics: Its Influence on AM Fungi. In T. Satyanarayana and B.N. Johri (Ed), *Microbial Diversity- Current Prospective and Potential Applications.* I.K. International Pvt. Ltd., New Delhi. pp345-360.

Bagyaraj, D.J., Mehrotra, V.S. and Suresh, C.K. (2002). Vesicular Arbuscular Mycorrhizal Biofertilizer for Tropical Forest Plants. *In* S. Kannaiyan (Ed), *Biotechnology of Biofertilisers*. Narosa Pub. House, New Delhi. pp299-311.

Bagyaraj, D.J. and Varma, A. (1995). Interaction between Arbuscular Mycorrhizal fungi and plants: Their Importance in Sustainable Agriculture in Arid and Semi-arid Tropics. *In* J.G. Jones (Ed), *Advances in Microbial Ecology*. Plenum Press, New York. pp119-142.

Bharadwaj, A. and Sharma, S. (2006). Reducing Phosphorus Requirement Using AM Fungi in Mulberry Grown under Alkaline Conditions. *Journal of Agronomy*, 5(3): pp471-477.

Bigham, J.M. (1996). Method of Soil Analysis (Part 3) Chemical methods, SSSA, Book Series 5, Madison, Wisconsin, USA.

Boureima, S., Diouf, M., Diop, T.A., Diatta, M., Leye, E.M., Ndiaye, F. (2007). Effect of arbuscular mycorrhizal inoculation on the growth and the development of sesame (Sesamum indicum L.). *African Journal of Agricultural Research*. 3(3): pp234-238.

Bowen, G.D., Bevege, D.I., Mosse, B. (1975). Phosphate physiology of vesicular arbuscular mycorrhizas. In F.E. Sanders, B.Mosse and P.B.Tinker (Ed.), *Endomycorrhizas* Academic press. London, New York, San Francisco. pp.241-260.

Brady, N.C. (1974). The nature and properties of soils, 8th Edition, Macmillan Publishing Co. , Inc. Ney York.. pp639.

Bremner, J.M. (1965). Total Nitrogen. In C.A.Black (Ed.). *Method of Soil Anlysis-part 2, Chemical and Microbiological properties*, American society of Agronomy, Inc. Madison, Wisconsin, USA. 9: pp 1149-1176.

Broadbent, F.E. (1965). Organic Matter. In C.A.Black (Ed.). *Method of Soil Anlysis-part 2, Chemical and Microbiological properties*, American society of Agronomy, Inc. Madison, Wisconsin, USA. 9: pp 1397-1400.

Brown, M.F. and King, E.J. (1982). Morphology and histology of Vesicular-Arbuscular Mycorrhizae. Anatomy and cytology. *In:* Schenck N.C.(Ed). Principles and methods of mycorrhizal research. The American phytopathological society. St.Paul, Minn. U.S.A. Pp. 69-71.

Brundrett, M. N., Bougher, B., Dell, T., Groove and Malajczuk, N. (1996). Working with Mycorrhizas in Forestry and Agriculture. Canberra: Australian Centre for International Agricultural Research; GPO box 1571.pp 141-183.

Central Bank Report, (2003). Annual report of Central Bank, Sri Lanka. P 85.

Central Bank Report, 2004, Annual report of central bank, Sri Lanka. P 81

Central Bank Report, 2005, Annual report of central bank, Sri Lanka. P 79

Central Bank Report, 2007, Annual report of central bank, Sri Lanka. P 82

Cox, G., Sanders, F.E., Tinker, P.B., Wild, J.A. (1975). Ultra structural evidence relating to host endophyte transfer in a vesicular arbuscular mycorrhiza. In F.E. Sanders, B.Mosse and P.B.Tinker (Ed.), *Endomycorrhizas* Academic press. London, New York, San Francisco. pp.297-312.

Dahanayake, K. (1995). Towards better utilization of Eppawala Phosphate. In K.Dahanayake (Ed), *Handbook on Geology and Mineral Resourses of Sri Lanka* GEOSAS-II, Colombo, Sri Lanka. pp69-73.

Daniels, B.A.,and Skipper,H.D.(1982).Method for the recovery and quantitative estimation of propagules from soil. In N.C.Schenck (Ed). *principles and methods of mycorrhizal research.*The American phytopathological society.St.Paul,Minn.pp 48-50.

Daniels, B.A. and Menge, J.A. (2006). Evaluation of the commercial potential of the Vasicular- arbuscular Mycorrhizal Fungus, *Glomus epigaeus. New phytologist, 87*(2): pp345-354.

Dassanayake, M.D., Fosberg, F.R. and Clayton, W.D. (Eds.). (1995). A Revised Handbook to the FLORA OF CEYLON, Volume IX, Amerind Publishing Co. Pvt. Ltd., New Delhi.

Datta, M., Banik, S., Gupta, R.K. (1982). Studies on the efficacy of a phytohormone producing phosphate solubilizing *Bacillus firmus* in augmenting paddy yield in acid soils of Nagaland. *Plant and soil*, *69*, pp365-373.

Department of Export Agriculture facts, (2003). 1095. Galaha road, Peradeniya.

Department of Export Agriculture facts, (2010). 1095. Galaha road, Peradeniya.

Didiek, K.R., Siswanto, M., Yudho, H.G. (2000). The status of soil phosphate fractions and the ability of fungi to dissolve hardly soluble phosphates. *Applied soil ecology, 29, pp73-83.*

Durgapal, A., Pandey, A. and Palni, L.S. (2002). The use of Rhizosphere soil for improved establishment of conifers at nursery stage for application in plantation programmes. *The Journal of Sustainable Forestry 15*(3):pp. 57-73

Edirisinghe, R.W. (2009). Observations on *Piper Hymenophyllum* Miq. : A rare wild Piper species in Sri Lanka. *Ceylon Journal of bioscience*. 38 (1) pp23-26.

Ekanayake, M.B.A. (1990). Demand for Pepper in the USA and prospects for Sri Lanka's Pepper exports. M.Sc. thesis, The Ohio State University.

El-Azouni, I.M. (2008). Effect of Phosphate Solubilizing Fungi on Growth and Nutrient Uptake of Soybean (*Glycine max* L.) Plants. *Journal of Applied Sciences Research*, *4*(6), pp592-598.

Farahani, H. A., Lebaschi, M.H. and Hamidi, A. (2008). Effects of Arbuscular Mycorrhizal Fungi, Phosphorus and Water Stress on Quantity and Quality Characteristics of Coriander. *Advances in Natural and Applied Sciences*, 2(2):pp55-59.

Fernando, W.C. and Tennakoon, N.A. (2006).Mycorrhizal associations in coconut. *Abstracts of papers,* A seminar on Potential of Mycorrhizal Associations for perennial crop management organized by Department of Export Agriculture and Section B of the Sri Lanka Association for the Advancement of Science, Sri Lanka.

Goenadi, D.H., Siswanto and Sugiarto, Y., (2000). Bioactivation of Poorly Soluble Phosphate Rocks with a Phosphorus-Solubilizing Fungus. *Soil Science Society American Journal. 64:* pp927-932.

Gosling. P, Hodge, A., Goodlass, G., Bending, G.D.(2006). Arbuscular mycorrhizal fungi and organic farming, *Science Direct.* Agriculture, Ecosystems and Environment *113* : 17–35

Grant, C., Bittman, S., Montreal, M., Plenchette, C. and Morel, C. (2004). Soil and fertilizer Phosphorus: Effects on plant P supply and mycorrhizal development. *Canadian journal of plant science. 85*: 3-14.

Groth, D.,E. and Martinson, C.A. (1983) Increased Endomycorrhizal infection of Maize and Soy beans after soil treatment with Metalaxyl. *Plant disease 67*:pp1377-1378.

Hafeel, K.M., Gunatilleke, I.A.U.N. (1989), Spatial and Temporal Distribution of Endomycorrhizal Spores in the Primary and Modified Forest Sites at Sinharaja, Dept. of Botany, Faculty of Science, University of Peradeniya

Hayman, D.S. (1975). The occurrence of Mycorrhiza in crops as affected by soil fertility. In F.E. Sanders, B.Mosse and P.B.Tinker (Ed.), *Endomycorrhizas* Academic press. London, New York, San Francisco. pp.495-506.

Heald, W.R. (1965). Calcium and Magnesium. In C.A.Black (Ed.). *Method of Soil Anlysis-part 2, Chemical and Microbiological properties*, American society of Agronomy, Inc. Madison, Wisconsin, USA. 9: pp 999-1009.

Herath. M. and Weerasinghe, A. (2004). Economic analysis of cinnamon production in Sri Lanka. Administrative report. Pp.16-32.

Illeperuma, O.A. (1998). Eppawala apatite deposit- A national asset of immense economic importance. *Chemistry in Sri Lanka. 15* (2): pp24-26.

James, M.T. and Schenck, N.C. (1984). Taxonomy of the fungi forming endomycorrhizae-A Vesicular Arbuscular Mycorrhizal fungi (Endogonales). *In:* Schenck N.C.(Ed). Principles and methods of mycorrhizal research. The American phytopathological society. St.Paul, Minn. U.S.A. pp 1-10.

Janos, D.P. (1975). Effects of vesicular arbuscular mycorrhizae on lowland tropical rainforest trees. In F.E. Sanders, B.Mosse and P.B.Tinker (Ed.), *Endomycorrhizas* Academic press. London, New York, San Francisco. pp.437-446.

Jarstfer, A.G. and Sylvia, D.M. (2001). Isolation, culture and detection of arbuscular mycorrhizal fungi. *In:* Hurst C.J. *et al.* (Ed). Manual of environmental Microbiology. American society of Microbiology. Washington D.C. PP.535-542.

Jayaratne A.H.R. , Siriwardene D. (2000). Arbuscular mycorrhizal inoculum production for commercial use, *Tropical Agricultural Research and Extension 3* (2): 2000

Jayawardane, D.E. de S. (1976). The Eppawala carbonatite complex in North- West Sri Lanka (Ceylon). Geological Survey Department publication, Colombo, Sri Lanka, *Econ. Bull.3*: pp38-39.

Jayawickrama, S.P., Thenabadu, M.W., Kumaragamage. (1991). An Investigation on Phosphate Adsorption in Six Sri Lankan Soils in Relation to Their Chemical and Mineralogical Properties. In Deepthi C. Bandara (Ed). *Tropical Agricultural Research.* 3: pp 135-143.

Kalpage, F.S.C.P. (1973). Phosphate Problems in Relation to the Soils of Sri Lanka, Recent Advances and Review. In K.A. De Alwis (Ed), Soil Science Society of Sri Lanka (Ceylon), Proceeding of the fourth annual Sessions. pp5-9.

Kandiannan, K., Sivaraman, K., Anandaraj, M. and Krishnamurthy, K.S. (2000). Growth and nutrient content of Black pepper (*Piper nigrum* L.) cuttings as influenced by

inoculation with biofertilizers. *Journal of spices and Aromatic crops. 9(2):* pp145-147.

Kapoor, K.K. and Paroda, S. (2005). Experimental Soil Microbiology, Postgraduate lecture notes, Department of Microbiology, CCS, Haryana Agricultural University, Hisar 125004, India. pp 45-46.

Kendaragama, K.M.A., Senevirathne Banda, K.M., Bandara, P.T. (2003). Influence of rice crop on soil phosphorus availability in relation to phosphate fertilizer application. *In* H.B. Kotagama, G.A.W. Wijesekara, D.S.A. Wijesundara (Ed.). ASDA- Annals of the Sri Lanka Department of Agriculture. 5: 129-139.

Khan, A.G. (1975). Growth effects of VA mycorrhiza on crops in the field. In F.E. Sanders, B.Mosse and P.B.Tinker (Ed.), *Endomycorrhizas* Academic press. London, New York, San Francisco. pp.419-437.

Khan, I.A., Ahmad, S., Mirza, S.N., Moazzam, N., Athar, M. and Shabbir, S.M. (2007). Growth Response of Buffel Grass (*Cenchrus ciliaris*) to Phosphorus and Mycorrhizal Inoculation. *Agriculturae Conspectus Scientificus.* 72 (2): 129-132

Khanam D., Mridha, M.A.U. and Solaiman, A.R.M. (2006). Comparative study of arbuscular mycorrhizal association with different agricultural crops among four aezs of Bangladesh *J. Agric. Res., 44(2).* Pp147-161.

Kumari, I.S., Mala, W.J., Jayasinghe, A., Sumanasena, H.A. (2008). Effect of host crop on mass propagation of vesicular Arbuscular mycorrhizae (*Glomus mosseae*). Proceedings of section B, Annual Sessions of Sri Lanka Association for Advancement of Science. *64(1):* pp38.

Lakshmipathy, R., Bagyaraj, D.J., Balakrishna, A.N. (2007). Can agricultural practices and land use patterns affect arbuscular mycorrhizal fungal population and diversity?. In B.N. Ganguli and S.K. Deshmukh(Ed), Fungi: *Multifaceted Microbes.* Anamaya Pub., New Delhi. pp304-315.

Lanka Phosphate limited estate company facts. (n.d.). Thalawa road, Eppawala. Sri Lanka

Linderman, R.G. and Hendrix, J.W. (1982). Evaluation of plant response to colonization by vesicular-arbuscular mycorrhizal fungi. A. Host variables. *In:* Schenck N.C.(Ed). Principles and methods of mycorrhizal research. The American phytopathological society. St.Paul, Minn. U.S.A. pp 69-71.

Liu, Q., P. Loganathan, M. J. Hedley and L.J.Grace (2005). Effect of mycorrhizal inoculation on rhizosphere properties of *Pinus radiata* seedlings. 3rd Australian and New Zealand Soils Conference. SUPER SOIL 2004. Sydney Published in the web.

Liu, Q., Loganathan, P., Hedley, M.J. and Skinner, M.F. (2004). The mobilization and fate of soil and rock phosphate in the rhizosphere of ectomycorrhizal Pinus radiate seedlings in an Allophanic soil. *Plant and Soil*. 264: pp.219-229.

Loganathan, P., Dayaratne, P.M.N., Shanmuganathan, R.T. (1984). Evaluation of the phosphorus status of some coconut growing soils of Sri Lanka. In D.T. Wettasinghe (Ed.). COCOS- Journal of the coconut research institute of Sri Lanka. 2: pp29-43.

Mala W. J., Kumari, I. S., Sumanasena, H. A., Nanayakkara, C.M. (2009), Effective spore density of *Glomus mosseae,* Arbuscular Mycorrhiza (AM), for inoculation of pepper (*Piper nigrum* Linn.) rooted cuttings, Tropical Agriculturist. 21(2). Pp189-197.

Mapa, R.B., Dassanayake,A.R., Nayakekorale (2005): Soils of the Intermediate Zone of Sri Lanka, Special Publication No-4, Soil Science Society of Sri Lanka, SRICANSOL center, P.O Box 10, Peradeniya, Sri Lanka. pp 243.

Marschner,H. (1996): Mineral nutrient acquisition in nonmycorrhizal and Mycorrhizal plants. Phyton (Horn, Austria) Special Issue:Bioindication..) 36 (3):pp61-68.

Mehrvarz, S., Chaichi, M. R. and Alikhani, H.A. (2008). Effects of Phosphate Solubilizing Microorganisms and Phosphorus Chemical Fertilizer on Yield and Yield Components of Barely (*Hordeum vulgare* L.). *American-Eurasian J. Agriculture and Environmental Science.*, 3(6): pp822-828.

Mikanova, O., Novakova, J. (2002). Evaluation of the P- solubilizing activity of soil microorganisms and its sensitivity to soluble phosphate, *ROSTLINNA V'YROBA, 48* (9): pp397-400

Miller, R.L. and Jackson, L.E. (1998). Survey of vesicular-arbuscular mycorrhizae in lettuce production in relation to management and soil factors. *Journal of Agricultural Science*, Cambridge, 130: pp173-182.

Miranda, J.C.C.D., Harris, P.J., Wild, A. (1989). Effects of soil and plant phosphorus concentrations on vesicular- arbuscular mycorrhiza in sorghum plants. New phytologist. Vol.112. (3).pp.405-410.

Molla, M.A.Z., Chowdhury, A.A. (1984). Microbial mineralization of organic phosphate in soil. *Plant and soil, 78*, pp393-399.

Mosse, B. (1975). Specificity in VA mycorrhizas. *In* F.E. Sanders, B.Mosse and P.B.Tinker (Ed.), *Endomycorrhizas* Academic press. London, New York, San Francisco. pp.469-481.

Narayan, C. T. and James, J.G. (1993). Propagation and storage of vesicular- arbuscular mycorrhizal fungi isolated from Saskatchewan agricultural soils. Canadian journal of Botany. 71: 1328-1335.

Olsen, S.R., Dean, L.A. (1965). Phosphorus. In C.A.Black (Ed.). *Method of Soil Anlysis-part 2, Chemical and Microbiological properties*, American society of Agronomy, Inc. Madison, Wisconsin, USA. 9: pp 1035-1049.

Ocampo, J. A. and Hayman, D. S. (1981). Influence of Plant Interactions on Vesicular-Arbuscular Mycorrhizal Infections. II.Crop Rotations and Residual Effects of Non-Host Plants. *New Phytologist*, Vol. *87*, No. 2. , pp. 333-343.

Palipane, J.B. (1999). Influence of Vesicular Abuscular Mycorrhizal fungi on the growth of Coffee plants. Proceedings of the National Conference on Mycorrhiza, 5-7, March

1999. Institute of microbiology and Biotechnology, Barakatulla University, Bhopal and Mycorrhiza Network Asia, Tata Research Institute, New Delhi. P 11.

Palipane, J.B. and Bandara, J.M.R.S. (1985). Influence of Vesicular Abuscular Mycorrhizal fungi on the growth of Coffee and Cocoa seedlings. In H.P.M. Gunasena (Ed), *Sri Lanka Journal of Agricultural Science*. 22(1):pp73-80.

Perera, M.P. (2007). Feasibility of using mycorrhizae to improve agriculture in Sri Lanka. B.Sc. thesis, University of Colombo, Sri Lanka.

Peterson, R.L., Massicotte, B., Melville, L.H. (2004), Mycorrhizas- Anatomy and cell biology, NRC Research press, Ottawa. Pp. 57-77.

Philips, J.M. and Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular Arbuscular Mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55:pp.158-161.

Plassard,C. and and B.Dell, (2010): Phosphorus nutrition of Mycorrhizal trees. Tree Physiology, 30(9): pp.1129-1139.

Plenchette, C., Fortin, J.A. and Furlan, V. (1983). *Plant soil* 70, 199-209.

Ponmurugan, P. and Gopi, C. (2006). In vitro production of growth regulators and phosphatase activity by phosphate solubilizing bacteria. African Journal of Biotechnology. 5(4). pp348-350.

Powell, C. L. (1975). Potassium uptake by endotrophic mycorrhizas. In F.E. Sanders, B.Mosse and P.B.Tinker (Ed.), *Endomycorrhizas* Academic press. London, New York, San Francisco. pp.461-468.

Pratt, P.F. (1965). Potassium. In C.A.Black (Ed.). *Method of Soil Anlysis-part 2, Chemical and Microbiological properties*, American society of Agronomy, Inc. Madison, Wisconsin, USA. 9: pp 1022-1030.

Preddy, M.N., Charitha Devi, M. and Sridevi, N.V. (2003). Evaluation of turmeric cultivars for VAM colonization, Indian Pathology. 56(4): 456-466.

Punyawardena, B.V.R., T.M.J.Bandara, M.A.K.Munasinghe and Nimal Jayarathna banda (2003): Agro-ecological regions of Sri Lanka (revised), Unpublished map, Department of Agriculture, Peradeniya, Sri Lanka

Quilambo, O. A., (2003), African journal of Biotechnology, Vol.2 (12), pp539-546.

Rajankar, P.N., Tambekar, D.H., Wate, S.R. (2007). Study of Phosphate Solubilization Efficiencies of Fungi and Bacteria Isolated From Saline belt of Purna river basin. *Research journal of Agriculture and Biological Sciences,* 3 (6), pp.701-703.

Rajapakse, R.G.A.S. (2006). Mycorrhizal associations in fruit crops. *Abstracts of papers,* A seminar on Potential of Mycorrhizal Associations for perennial crop management organized by Department of Export Agriculture and Section B of the Sri Lanka Association for the Advancement of Science, Sri Lanka,

Rao, N.S.S. (1993). Biofertilizers in Agriculture and Forestry. Science publishers. Inc. Post Office Box 699, Enfield, New Hampshire 03748, united state of America.

Rathnayake, M.P.K., Kumaragamage, D., Dahanayake, K. (1994). Comparison of Selectively Mined Apatite Crystals and Commercially Available Rock Phosphate from Eppawala, as P Fertilizers for Flooded Rice. In A.R. Ariyaratne (Ed), *Tropical Agricultural Research. 6*: pp.223-225.

SAS Institute, 1990: SAS/STAT User's guide, Version 6, Cary, NC: SAS Institute.

Sharif, M. and Moawad, A. M. (2006) Arbuscular Mycorrhizal Incidence and Infectivity of crops in North West Frontier Province of Pakistan. World Journal of Agricultural sciences 2(2):pp.123-132.

Sharma, A.K.(2003). Biofertizers for sustainable Agriculture.Jodhpur:Agrobios.pp 202-232.

Smith, F.W. (2002) The Phosphate uptake mechanism. *Plant and Soil.* 245: pp.105-114.

Sumanasena, H.A. (2003). A study of the combined effects of irrigation frequency and phosphorus fertility on summer pasture production. Ph.D. thesis, Massey University, Palmerston North, New Zealand.

Sumanasena, H.A. (2007). Effects of Short Dry Spells on Productivity of some Perennial Spice and Beverage Crop Species. Presidential Addresses, Section B, Sri Lanka Association for the Advancement of Science, 120/10, Vidya Mandiraya, Wijerama mawatha, Colombo 07, Proceedings of the 63rdAnnual Sessions , 63(II): pp.16-31

Tandan, H. L. S. (Eds.). (1999). Methods of analysis of soils, plants, water and fertilizers. Fertilizer development and consultation organization. New Delhi. Pp. 144-150.

Tennant, D. (1975). A test of a modified line intersects method of estimating root length. Journal of Ecology, *63*:pp.995-1001.

Thanuja T. V., Ramakrishna V. H. and Sreenivasa, M. N. (2002). Induction of rooting and root growth in Black pepper cuttings (Piper nigrum L.) with the inoculation of Arbuscular Mycorrhizae. *Journal of Science and Horticulture. 92* (3-4): pp.339-346.

Tinker, P. B. (1975). Relative effect of Vesicular Arbuscular Mycorrhizal fungi on the growth and yield of green house soya beans. In F.E. Sanders, B.Mosse and P.B.Tinker (Ed.), *Endomycorrhizas* Academic press. London, New York, San Francisco. pp.297-312.

Trolove, S.N, Hedley, .J, Kirk, G.J.D, Bolan, N.S and Loganathan, P. (2003). Progress in selected areas of Rhizosphere research on P acquisition. Aust. J. Soil Res. 42, pp.471-499

Wang, X. and Zabowski, D. (1998). Nutrient composition of Douglas-fir rhizosphere and bulk soil solutions. *Plant and Soil*, 200: pp.13-20.

Weeraratne, C.S. (1983). Preliminary laboratory studies on increasing phosphorus availability in Eppawala apatite. *Journal of the national science council of Sri Lanka. 11* (1): pp.57-63.

Weerawardane, N.D.R., (2006) .Importance of mycorrhiza in forestry. *Abstracts of papers,* A seminar on Potential of Mycorrhizal Associations for perennial crop management organized by Department of Export Agriculture and Section B of the Sri Lanka Association for the Advancement of Science, Sri Lanka,

Wijewardena, J.D.H., Amarasiri, S.L. (1990). Comparison of phosphate sources on growth of vegetables on an acid soil. In S. Nagarajah (Ed.). *Tropical Agriculturist.* 146: pp57-61.

Wimalaratne, H.G.M.C. (2005). Effect of Vesicular-Arbuscular Mycorrhiza (VAM) on shoot and root development of black pepper (*Piper nigrum Linn*) rooted cuttings. B.Sc. project report, Department of Crop science, Faculty of Agriculture, University of Peradeniya, Sri Lanka.

Withana, A.K. and Kumaragamage, D.K. (1995). Evaluation of Phosphorus Extraction Methods for Assessing Phosphorus Availability of Some Sri Lankan Soils. In E.R. Kalyani perera (Ed.). Tropical Agricultural Research. *Vol. 7*. pp.143-151.

Woolhouse, H.W. (1975). Membrane structure and transport problems considered in relation to phosphorus and carbohydrate movements and the regulation of endotrophic mycorrhizal association. In F.E. Sanders, B.Mosse and P.B.Tinker (Ed.), *Endomycorrhizas* Academic press. London, New York, San Francisco. pp.209-239.

Xioutang, L. (1994). Inoculation of forest and fruit trees with Vesicular Arbuscular Mycorrhizal fungi in Guangxi province, China.. *In:* Brundrett, M.,Dell, B. ,Malajuzuk, N. ,Mingqin, G.(Ed). Mycorrhizas for plantation forestry in Asia. Austrailian Centre for International Agricultural Research GPO Box 1571, Canbera. PP.114-118

Zoysa, A.K.N., Loganathan, P. (2001). Phosphorus Nutrition of Tea: A review. In W.W.D.Modder (Ed.). Twentieth century Tea Research in Sri Lanka. The Tea research institute of Sri Lanka, Talawakelle. Sri Lanka.

Appendices

ANOVA tables

(01). Effect of host crop on mass propagation of Vesicular Arbuscular Mycorrhizae (*Glomus mosseae*)

Appendix 4.1: Total black spores of different treatments

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	10	168040.1616	16804.0162	0.93	0.5351
Error	14	252952.4784	18068.0342		
Corrected total	24	420992.64			

R-Square	C.V.	MSE	Mean
0.399152	80.91584	134.4174	166.12

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	2	23993.89194	11996.94597	0.66	0.5303
WK	2	21855.50305	10927.75153	0.6	0.5598
R	2	53196.8549	26598.42745	1.47	0.2629
TRT * WK	4	67401.71349	16850.42837	0.93	0.4732

Appendix 4.2: Total brown spores of different treatments

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	10	52634.18824	5263.41882	0.73	0.6882
Error	14	101113.8118	7222.41513		
Corrected total	24	153748			

R-Square	C.V.	MSE	Mean
0.342341	64.0911	84.98479	132.6

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	2	1270.26231	635.13115	0.09	0.9163
WK	2	1575.04009	787.52004	0.11	0.8975
R	2	11110.8549	5555.42745	0.77	0.482
TRT * WK	4	33560.78756	8390.19689	1.16	0.3691

(4) Effective spore density of *Glomus mosseae,* Arbuscular Mycorrhiza (AM), for inoculation of pepper (*Piper nigrum* Linn.) rooted cuttings

Appendix 5.1: Leaf area of pepper rooted cuttings at 2 months after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	8	13790147345	172.393418	0.78	0.6234
Error	15	3295.695855	219.713057		
Corrected total	23	4674.8432			

R-Square	C.V.	MSE	Mean
0.295015	76.92123	14.82272	19.27

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	1136.970865	284.242716	1.29	0.3166
Block	4	207.426405	51.856601	0.24	0.9137

Appendix 5.1 Leaf area of pepper rooted cuttings at 4 months after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	8	59347.81471	7418.47684	0.89	0.5531
Error	12	100146.1928	8345.51607		
Corrected total	20	159494.0075			

R-Square	C.V.	MSE	Mean
0.372101	37.95489	91.3538	240.6905

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	21372.13588	5343.03397	0.64	0.6439
Block	4	25358.90259	6339.72565	0.76	0.5711

Appendix 5.2: Leaf area of pepper rooted cuttings at 6 months after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	8	116925.3436	14615.6679	1.14	0.4024
Error	13	167321.9292	12870.9176		
Corrected total	21	284247.2727			

R-Square	C.V.	MSE	Mean
0.411351	39.92165	113.4501	284.1818

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	52013.27083	13003.31771	1.01	0.4375
Block	4	69040.2875	17260.07188	1.34	0.3069

Appendix 5.3: Root dry weight of pepper rooted cuttings at 2 months after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	8	0.16397596	0.020497	0.91	0.5366
Error	15	0.33943604	0.02262907		
Corrected total	23	0.503412			

R-Square	C.V.	MSE	Mean
0.325729	30.79419	0.15043	0.4885

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	0.08702031	0.02175508	0.96	0.4569
Block	4	0.07600076	0.01900019	0.84	0.5212

Appendix 5.4: Root dry weight of pepper rooted cuttings at 4 months after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	8	0.34104819	0.04263102	1.06	0.4537
Error	11	0.44395236	0.04035931		
Corrected total	19	0.78500055			

R-Square	C.V.	MSE	Mean
0.434456	36.38436	0.200896	0.55215

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	0.13584489	0.03396122	0.84	0.5271
Block	4	0.20230026	0.05057506	1.25	0.3451

Appendix 5.5: Root dry weight of pepper rooted cuttings at 6 months after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	8	0.51914345	0.06489293	1.32	0.3164
Error	13	0.64064292	0.04928022		
Corrected total	21	1.15978636			

R-Square	C.V.	MSE	Mean
0.44762	42.06557	0.221991	0.527727

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	0.11854375	0.02963594	0.6	0.6683
Block	4	0.40409875	0.10102469	2.05	0.1463

Appendix 5.6: Root length of pepper rooted cuttings at 2 months after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	8	39859.78955	4982.47369	1.02	0.4654
Error	13	63286.66679	4868.20514		
Corrected total	21	103146.4563			

R-Square	C.V.	MSE	Mean
0.386439	55.68488	69.77252	125.2989

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	25872.26913	6468.06728	1.33	0.311
Block	4	11127.32755	2781.83189	0.57	0.6881

Appendix 5.7: Root length of pepper rooted cuttings at 4 months after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	8	683737.8324	85467.2291	2.16	0.1114
Error	12	475841.8598	39653.4883		
Corrected total	20				

R-Square	C.V.	MSE	Mean
0.589643	51.18807	199.1318	389.02

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	364360.1318	91090.033	2.3	0.1189
Block	4	146197.8005	36549.4501	0.92	0.483

Appendix 5.8: Root length of pepper rooted cuttings at 6 months after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	8	4521668.96	565208.62	0.34	0.9357
Error	13	21782676.04	1675590.465		
Corrected total	21	26304345			

R-Square	C.V.	MSE	Mean
0.171898	45.22645	1294.446	2862.144

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	1272656.522	318164.13	0.19	0.9394
Block	4	2848146.776	712036.694	0.42	0.788

Appendix 5.9: Shoot dry weight of pepper rooted cuttings at 2 months after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	8	3.42098662	0.42762333	2.03	0.1125
Error	15	3.15555034	0.21037002		
Corrected total	23	6.57653696			

R-Square	C.V.	MSE	Mean
0.52081	37.07976	0.458661	1.236958

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	2.86548006	0.71637002	3.41	0.0359
Block	4	0.41430466	0.10357617	0.49	0.7415

Appendix 5.10: Shoot dry weight of pepper rooted cuttings at 4 months after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	8	7.29359253	0.91169907	1.88	0.1643
Error	11	5.34002002	0.48545637		
Corrected total	19	12.63361255			

R-Square	C.V.	MSE	Mean
0.577316	33.62922	0.696747	2.07185

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	3.35771563	0.83942891	1.73	0.2135
Block	4	4.45807553	1.11451888	2.3	0.1243

Appendix 5.11: Shoot dry weight of pepper rooted cuttings at 6 months after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	8	13.16607922	1.6457599	1.6	0.2231
Error	12	12.33997792	1.02833149		
Corrected total	20	25.50605714			

R-Square	C.V.	MSE	Mean
0.516194	26.88814	1.014067	3.771429

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	9.76024374	2.44006094	2.37	0.0485
Block	4	3.64299874	0.91074969	0.89	0.5015

(03). Effective spore density of Vesicular Arbuscular Mycorrhizae (*Glomus mosseae*) for Cinnamon (*Cinnomomum verum* Presl Syn. *Cinnomomum zeylanicum* Blume) seedlings

Appendix 6.1: Leaf area (cm2) 2 month after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	8	1242.711668	155.338958	3.82	0.0139
Error	14	569.035793	40.645414		
Corrected total	22	1811.747461			

R-Square	C.V.	MSE	Mean
0.685919	30.8009	6.375376	20.6987

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	1219.585502	304.896375	7.5	0.0019
Block	4	20.618967	5.154742	0.13	0.9702

Appendix 6.2: Leaf area (cm2) 4 month after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	11	961.6126243	87.4193295	1.06	0.4353
Error	19	1560.480576	82.1305566		
Corrected total	30				

R-Square	C.V.	MSE	Mean
0.381276	33.3306	9.062591	27.19

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	440.7470109	110.1867527	1.34	0.291
Block	7	595.5266475	85.0752364	1.04	0.4394

Appendix 6.3: Shoot biomass (g) 2 month after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	8	0.06628328	0.00828541	0.55	0.802
Error	16	0.24144056	0.01509003		
Corrected total	24	0.30772384			

R-Square	C.V.	MSE	Mean
0.215399	36.6779	0.122842	0.33492

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	0.03660424	0.00915106	0.61	0.6637
Block	4	0.02967904	0.00741976	0.49	0.7419

Appendix 6.4: Shoot biomass (g) 4 month after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	11	0.20419987	0.01856362	1.48	0.22
Error	19	0.23901407	0.01257969		
Corrected total	30				

R-Square	C.V.	MSE	Mean
0.460725	31.9777	0.112159	0.350742

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	0.1399507	0.03498767	2.78	0.0565
Block	7	0.05142566	0.00734652	0.58	0.7606

Appendix 6.5: Root biomass (g) 2 month after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	8	0.04780528	0.00597566	2.1	0.0989
Error	16	0.04561496	0.00285094		
Corrected total	24	0.09342024			

R-Square	C.V.	MSE	Mean
0.511723	27.3144	0.53394	0.19548

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	0.04429784	0.01107446	3.88	0.0217
Block	4	0.00350744	0.00087686	0.31	0.8687

Appendix 6.6: Root biomass (g) 4 month after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	11	0.10326444	0.00938768	1.06	0.4434
Error	18	0.16008076	0.00889338		
Corrected total	29				

R-Square	C.V.	MSE	Mean
0.392126	39.0334	0.094305	0.2416

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	0.04331209	0.01082802	1.22	0.0338
Block	7	0.04179277	0.0059704	0.67	0.6938

Appendix 6.7: Root length (cm) 2 month after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	8	121761.3964	15220.1746	0.94	0.5137
Error	16	259847.3468	16240.4592		
Corrected total	24	381608.7432			

R-Square	C.V.	MSE	Mean
0.319074	63.0648	127.4381	202.0748

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	54993.30414	13748.32604	0.85	0.5161
Block	4	66768.09226	16692.02307	1.03	0.423

Appendix 6.8: Root length (cm) 4 month after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	11	92911.79699	8446.527	0.96	0.5111
Error	18	158184.1266	8788.00703		
Corrected total	29	251095.9236			

R-Square	C.V.	MSE	Mean
0.370025	43.7769	93.74437	214.1413

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	65121.83468	16280.45867	1.85	0.1628
Block	7	28826.93182	4118.13312	0.47	0.8446

Appendix 6.9: Plant height (cm) 2 month after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	8	10.8	1.35	0.31	0.9501
Error	16	69.2	4.325		
Corrected total	24	80			

R-Square	C.V.	MSE	Mean
0.135	17.9281	2.079663	11.6

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	8.8	2.2	0.51	0.7302
Block	4	2	0.5	0.12	0.9751

Appendix 6.10: Plant height (cm) 4 month after inoculation

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	11	75.50587364	6.86417033	1.13	0.3918
Error	19	115.3465006	6.0086845		
Corrected total	30	190.8523742			

R-Square	C.V.	MSE	Mean
0.395624	18.8447	2.463913	13.07484

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	4	65.26817611	16.31704403	2.69	0.0626
Block	7	7.68185944	1.09740846	0.18	0.9862

(02). Investigate the Effect of Mycorrhizal associations of pepper on increasing plant availability of phosphorus from ERP (Eppawala Rock Phosphate).

Appendix 7.1: Soil bray P of rhizosphere soil 08 month after repotting
F- Fertilizer levels
M- AM inoculum levels
R- No. of replicates

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	10	52.69974609	5.26997461	2.46	0.0559
Error	15	32.07598776	2.13839918		
Corrected total	25	84.77573385			

R-Square	C.V.	Bray MSE	Bray P Mean
0.621637	12.2906	1.462327	11.89792

Source	DF	Type III SS	Mean Square	F Value	Pr > F
F	2	44.74212069	22.37106034	10.46	0.0014
M	2	0.59371808	0.29685904	0.14	0.8715
R	2	2.24735958	1.12367979	0.53	0.6018
F*M	4	7.54747373	1.88686843	0.88	0.4978

Appendix 7.2: Soil bray P of bulk soil 08 month after repotting
F- Fertilizer levels
M- AM inoculum levels
R- No. of replicates

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	10	40.40794824	4.04079482	1.25	0.3366
Error	15	48.43909792	3.22927319		
Corrected total	25	88.84704615			

R-Square	C.V.	Bray MSE	Bray P Mean
0.454804	23.1735	1.797018	7.754615

Source	DF	Type III SS	Mean Square	F Value	Pr > F
F	2	24.74225579	12.37112789	3.83	0.0453
M	2	2.38110764	1.19055382	0.37	0.6977
R	2	3.19821875	1.59910937	0.5	0.6191
F*M	2	11.46563097	2.86640774	0.89	0.495

Appendix 7.3: Soil olsen P of rhizospher soil 08 month after repotting
F- Fertilizer levels
M- AM inoculum levels
R- No. of replicates

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	10	44.47232502	4.4472325	1.86	0.1349
Error	15	35.91119544	2.3940797		
Corrected total	25	80.38352046			

R-Square	C.V.	Olsen MSE	Olsen P Mean
0.55325	28.0688	1.547281	5.512462

Source	DF	Type III SS	Mean Square	F Value	Pr > F
F	2	19.1497955	9.57489775	4	0.0405
M	2	1.24428833	0.62214417	0.26	0.7746
R	2	2.18650189	1.09325094	0.46	0.6419
F*M	4	21.37627565	5.34406891	2.23	0.1144

Appendix 7.4: Soil olsen P of bulk soil 08 month after repotting
F- Fertilizer levels
M- AM inoculum levels
R- No. of replicates

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	10	17.20434167	1.72043417	1.72	0.1654
Error	15	14.98685833	0.99912389		
Corrected total	25	32.1912			

R-Square	C.V.	Olsen MSE	Olsen P Mean
0.534442	32.4533	0.999562	3.08

Source	DF	Type III SS	Mean Square	F Value	Pr > F
F	2	2.75482315	1.37741157	1.38	0.2821
M	2	1.51429722	0.75714861	0.76	0.4858
R	2	0.257675	0.1288375	0.13	0.88
F*M	4	12.22899056	3.05724764	3.06	0.0498

Appendix 7.5: Soil water soluble P of rhizospher soil 08 month after repotting
F- Fertilizer levels
M- AM inoculum levels
R- No. of replicates

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	10	130.5249338	13.05249338	2.63	0.0442
Error	15	74.3661778	4.9577452		
Corrected total	25	204.8911115			

R-Square	C.V.	MSE	Mean
0.637045	80.4609	2.226599	2.767308

Source	DF	Type III SS	Mean Square	F Value	Pr > F
F	2	64.6495037	32.32475185	6.52	0.0092
M	2	10.32992593	5.16496296	1.04	0.377
R	2	15.69880556	7.84940278	1.58	0.2378
F*M	4	41.44873778	10.36218444	2.09	0.1329

Appendix 7.6: Soil water soluble P of bulk soil 08 month after repotting
F- Fertilizer levels
M- AM inoculum levels
R- No. of replicates

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	10	72.58332949	7.25833295	1.39	0.2743
Error	15	78.45156667	5.23010444		
Corrected total	25	151.0348962			

R-Square	C.V.	MSE	Mean
0.480573	51.1576	2.286942	4.470385

Source	DF	Type III SS	Mean Square	F Value	Pr > F
F	2	40.25763889	20.12881944	3.85	0.0448
M	2	5.7224537	2.86122685	0.55	0.5898
R	2	4.19523333	2.09761667	0.4	0.6766
F*M	4	18.94217389	4.73554347	0.91	0.4855

Appendix 7.7: pH of rhizosphre soil 08 month after repotting
F- Fertilizer levels
M- AM inoculum levels
R- No. of replicates

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	10	0.23271362	0.02327136	3.61	
Error	15	0.09679792	0.00645319		
Corrected total	25	0.32951154			

R-Square	C.V.	MSE	Mean
0.706238	1.28792	0.080332	6.237308

Source	DF	Type III SS	Mean Square	F Value	Pr > F
F	2	0.1048669	0.05243345	8.13	0.0041
M	2	0.01843912	0.00921956	1.43	0.2704
R	2	0.01011875	0.00505937	0.78	0.4744
F*M	4	0.00440653	0.02860163	4.43	0.0146

Appendix 7.8: pH of bulk soil 08 month after repotting
F- Fertilizer levels
M- AM inoculum levels
R- No. of replicates

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	10	0.34860636	0.03486064	2.49	0.0541
Error	15	0.21020903	0.1401394		
Corrected total	25	0.55881538			

R-Square	C.V.	MSE	Mean
0.623831	1.93871	0.11838	6.106154

Source	DF	Type III SS	Mean Square	F Value	Pr > F
F	2	0.21185764	0.10592882	7.56	0.0054
M	2	0.03524097	0.01762049	1.26	0.3127
R	2	0.01379097	0.00689549	0.49	0.6209
F*M	4	0.11886431	0.02971608	2.12	0.1287

Appendix 7.9: Leaf area (cm^2) 08 month after repotting
F- Fertilizer levels
M- AM inoculum levels
R- No. of replicates

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	10	6205323.617	620532.362	2.69	0.0443
Error	14	3224930.063	230352.147		
Corrected total	24	9430253.68			

R-Square	C.V.	MSE	Mean
0.658023	33.4939	479.9502	1432.947

Source	DF	Type III SS	Mean Square	F Value	Pr > F
F	2	3429697.692	1714848.846	7.44	0.0063
M	2	374894.502	187447.251	0.81	0.4631
R	2	549157.993	274578.996	1.19	0.3326
F*M	4	1015677.797	253919.449	1.1	0.3938

Appendix 7.10: Plant height (cm) 08 month after repotting
F- Fertilizer levels
M- AM inoculum levels
R- No. of replicates

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	10	17784.72673	1778.47267	1.86	0.1347
Error	15	14354.34106	956.95607		
Corrected total	25	32139.06779			

R-Square	C.V.	MSE	Mean
0.553368	25.5055	30.93471	121.2865

Source	DF	Type III SS	Mean Square	F Value	Pr > F
F	2	3541.773524	1770.886762	1.85	0.1913
M	2	1903.795422	951.897711	0.99	0.393
R	2	6718.903941	3359.45197	3.51	0.0562
F*M	4	5042.258566	1260.564641	1.32	0.3085

Appendix 7.11: Root dry weight (g) 08 month after repotting
F- Fertilizer levels
M- AM inoculum levels
R- No. of replicates

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	10	11.12167821	1.11216782	0.71	0.7035
Error	15	23.49423333	1.5662822		
Corrected total	25	34.61591154			

R-Square	C.V.	MSE	Mean
0.321288	55.0674	1.251512	2.272692

Source	DF	Type III SS	Mean Square	F Value	Pr > F
F	2	0.57248333	0.28624167	0.18	0.8348
M	2	1.15698704	0.57849352	0.37	0.6973
R	2	2.94583333	1.47291667	0.94	0.4123
F*M	4	6.04610278	1.51152569	0.97	0.4551

Appendix 7.12: Shoot dry weight (g) 08 month after repotting
F- Fertilizer levels
M- AM inoculum levels
R- No. of replicates

Source	DF	Sums of squares	Mean Square	F value	Pr > F
Model	10	951.8139	95.18139	1.23	0.348
Error	15	1161.902179	77.4601453		
Corrected total	25	2113.716079			

R-Square	C.V.	MSE	Mean
0.450304	44.4822	8.801145	19.78577

Source	DF	Type III SS	Mean Square	F Value	Pr > F
F	2	441.8113426	220.9056713	2.85	0.0862
M	2	125.0191905	62.5095953	0.81	0.4647
R	2	230.6651617	115.3325808	1.49	0.2571
F*M	4	114.2958894	28.5739723	0.37	0.8271

Mean separation tables

(1) Effective spore density of *Glomus mosseae,* Arbuscular Mycorrhiza (AM), for inoculation of pepper (*Piper nigrum* Linn.) rooted cuttings

Mean differences-Shoot biomass at 6 month of different treatments

	T1	T2	T3	T4	T5
T5	0.121^{ns}	1.794^{s}	0.488^{ns}	0.309^{ns}	*
T4	0.431^{ns}	1.485^{s}	0.179^{ns}	*	
T3	0.61^{ns}	1.305^{ns}	*		
T2	1.916^{s}	*			
T1	*				

Names denoted by **ns** means not significant, s means significant

Mean differences-Root length at 6 month of different treatments

	T1	T2	T3	T4	T5
T5	377.1^{s}	362.9^{s}	185.3^{ns}	470.9^{s}	*
T4	93.8^{ns}	108^{ns}	285.6^{s}	*	
T3	191.8^{ns}	177.6^{ns}	*		
T2	14.2^{ns}	*			
T1	*				

Names denoted by **ns** means not significant, s means significant

Mean differences- height of new shoot at 2 month of different treatments

	T1	T2	T3	T4	T5
T5	0.9^{ns}	2.59^{ns}	0.08^{ns}	2.7^{ns}	*
T4	1.8^{ns}	5.29^{s}	2.62^{ns}	*	
T3	0.82^{ns}	2.67^{ns}	*		
T2	3.49^{ns}	*			
T1	*				

Names denoted by **ns** means not significant, s means significant

Mean differences- height of new shoot at 6 month of different treatments

	T1	T2	T3	T4	T5
T5	6.93^{ns}	9.78^{s}	0.66^{ns}	7.2^{s}	*
T4	14.13^{ns}	2.58^{ns}	6.54^{ns}	*	
T3	7.59^{ns}	9.12^{ns}	*		
T2	16.71^{ns}	*			
T1	*				

Names denoted by **ns** means not significant, s means significant

Mean differences- Leaf area of T1 during study period

M1- 02month after inoculation
M2- 04 month after inoculation
M3- 06 month after inoculation

	M1	M2	M3
M3	227.29^{s}	23.71^{ns}	*
M2	251.0^{s}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Leaf area of T2 during study period

	M1	M2	M3
M3	346.5^{s}	107.0^{ns}	*
M2	239.4^{s}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Leaf area of T3 during study period

	M1	M2	M3
M3	269.3^{s}	42.9^{ns}	*
M2	226.3^{s}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Leaf area of T4 during study period

	M1	M2	M3
M3	232.7^{s}	11.4^{ns}	*
M2	244.3^{s}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Leaf area of T5 during study period

	M1	M2	M3
M3	224.7^{s}	79.9^{ns}	*
M2	144.8^{s}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Root length of T1 during study period

	M1	M2	M3
M3	2737.6^{s}	2348.8^{s}	*
M2	388.8^{ns}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Root length of T2 during study period

	M1	M2	M3
M3	3098.3^{s}	2768^{s}	*
M2	330.3^{ns}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Root length of T3 during study period

	M1	M2	M3
M3	2219.5^{s}	2078.7^{s}	*
M2	140.8^{ns}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Root length of T4 during study period

	M1	M2	M3
M3	2794.3^{s}	2265.3^{s}	*
M2	529^{ns}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Root length of T5 during study period

	M1	M2	M3
M3	2747.2^{s}	2764.1^{s}	*
M2	16.9^{ns}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Shoot biomass of T1 during study period

	M1	M2	M3
M3	2.066^{s}	0.75^{s}	*
M2	1.316^{s}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Shoot biomass of T2 during study period

	M1	M2	M3
M3	4.103^{s}	2.903^{s}	*
M2	1.2^{ns}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Shoot biomass of T3 during study period

	M1	M2	M3
M3	2.418^{s}	1.427^{s}	*
M2	0.991^{s}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Shoot biomass of T4 during study period

	M1	M2	M3
M3	2.731^{s}	2.292^{s}	*
M2	0.438^{ns}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Shoot biomass of T5 during study period

	M1	M2	M3
M3	1.435^{s}	1.417^{s}	*
M2	0.017^{ns}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Height of new shoot in T1 during study period

	M1	M2	M3
M3	30.274^{s}	14.176^{s}	*
M2	16.098^{s}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Height of new shoot in T2 during study period

	M1	M2	M3
M3	20.37^s	8.734^s	*
M2	11.64^s	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Height of new shoot in T3 during study period

	M1	M2	M3
M3	29.52^s	19.25^s	*
M2	10.27^s	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Height of new shoot in T4 during study period

	M1	M2	M3
M3	23.08^s	10.85^s	*
M2	12.23^s	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Height of new shoot in T5 during study period

	M1	M2	M3
M3	36.29^s	21.99^s	*
M2	14.30^s	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- No. of leaves in T1 during study period

	M1	M2	M3
M3	4.86^s	0.098^{ns}	*
M2	4.768^s	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- No. of leaves in T2 during study period

	M1	M2	M3
M3	7.35^s	2.83^s	*
M2	4.52^s	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- No. of leaves in T3 during study period

	M1	M2	M3
M3	6.21^{s}	2.93^{s}	*
M2	3.28^{s}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- No. of leaves in T4 during study period

	M1	M2	M3
M3	5.86^{s}	2.9^{ns}	*
M2	2.968^{ns}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

Mean differences- No. of leaves in T5 during study period

	M1	M2	M3
M3	5.55^{s}	1.8^{ns}	*
M2	3.75^{s}	*	
M1	*		

Names denoted by **ns** means not significant, **s** means significant

(2). Effective spore density of Vesicular Arbuscular Mycorrhizae (*Glomus mosseae*) for Cinnamon (*Cinnomomum verum* Presl Syn. *Cinnomomum zeylanicum* Blume) seedlings

Mean differences-Shoot biomass at 4 month of different treatments

	T1	T2	T3	T4	T5
T5	0.008^{ns}	0.169^{s}	0.142^{ns}	0.036^{ns}	*
T4	0.027^{ns}	0.125^{ns}	0.106^{ns}	*	
T3	0.134^{ns}	0.019^{ns}	*		
T2	0.157^{s}	*			
T1	*				

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Root biomass at 2 month of different *treatments*

	T1	T2	T3	T4	T5
T5	0.003^{ns}	0.109^{s}	0.074^{s}	0.036^{ns}	*
T4	0.032^{ns}	0.074^{s}	0.038^{ns}	*	
T3	0.07^{ns}	0.036^{ns}	*		
T2	0.106^{s}	*			
T1	*				

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Root biomass at 4 month of different treatments

	T1	T2	T3	T4	T5
T5	0.067^{ns}	0.062^{ns}	0.046^{ns}	0.008^{ns}	*
T4	0.059^{ns}	0.069^{ns}	0.054^{ns}	*	
T3	0.113^{ns}	0.016^{ns}	*		
T2	0.129^{s}	*			
T1	*				

Names denoted by **ns** means not significant, **s** means significant

Mean differences- Leaf area at 2 month of different treatments

	T1	T2	T3	T4	T5
T5	17.08^{s}	18.12^{s}	18.64^{s}	15.19^{s}	*
T4	1.9^{ns}	2.93^{ns}	3.45^{ns}	*	
T3	1.55^{ns}	0.52^{ns}	*		
T2	1.03^{ns}	*			
T1	*				

Names denoted by **ns** means not significant, **s** means significant

Appendix 09

Mass propagation protocol for initial VAM (*Glomus mosseae*) stock - Central Research Station Matale - DEA

- Initial stocks are being maintained in host crop cultivation of either Finger millet (*Elucine coracana*) or Sorghum (*Sorghum bicolor*) seedlings.
- Polythene bags of 30x25 cm (12"x10") are being used and inoculum extraction can be made at 10 – 11 week after germination of host crop (75 days foreign literature)
- At the time of inoculum extraction above ground portion of the host crop should be cut off at the ground level.
- Then all pots would be dumped on to a polythene sheet at one pot at a time and all root-soil mixture containing fungal hyphae and VAM spore (inoculum) should be mixed together to form a one bulk mixture.
- Then, at least 10 samples should be collected using a hand spade and these samples can be pooled and three composite samples (50 g) should be analyzed for spore density if facilities are available

1. Continuation of inoculum regeneration: Use 1:1 mixture of top soil collected from A horizon or Ap horizon of the nursery site and bulk inoculum collected from previous cycle. If the surface soil is clay type then incorporation of a sufficient quantity of river sand is advisable. Sowing of the seeds of either finger millet or Sorghum can be done immediately after watering.

2. Use as an inoculum for black pepper (*Piper nigrum.* L): Incorporation of above inoculum at the rate of 75g (moist) for one standard polythene bag (21x13 cm) at the time of nursery preparation is adequate. But mean number of spores should be approximately 300 spores in the inoculum. If the spore density is different appropriate adjustment may be required for the quantity of inoculum that should be added to the potting mixture. For example 1:1 (about 400 g moist soil containing inoculum and 500g standard potting mixture per pot)

- Five kg of inoculum can be generated from one polythene bag of 30x25 cm (12"x10") size within a 10 wks time irrespective of the season of the year. However, nurseryman/farmer should maintain seed grain during the main two seasons.
- Seed requirement per one polythene bag is 3 g of Finger millet seeds.
- Regeneration can be continued if a requirement arises from commercial nursery. In that case attention of a skilled nurseryman is required for regeneration work as well as neat maintenance of the above inoculum. In general, two full labour days is sufficient for extraction and refilling of above 40 regeneration bags as described above with top soil mixture and sowing seeds with subsequent wetting of the pots.

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