# ADDIS ABABA UNIVERSITY
# SCHOOL OF GRADUATE STUDIES

## Arbuscular Mycorrhizal Fungal diversity of Coffee and Shade trees of Bonga forest, South Western Ethiopia.

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## Abstract

To date, information regarding densities and identification of dominant coffee shade trees and explanation of their interaction with physical parameters in southwestern part of Ethiopia is extremely very scanty. Moreover, mycorrhizal fungi spore densities, colonization rate, diversity and interaction with physical and chemical parameters of soil are not evaluated in any of the coffee forest in the country and this work is to bridge such information gap. So, reconnaissance survey was conducted and the number of coffee and coffee shade tree species was counted with in a 20m x 20 m quadrat. Spores were separated from the bulk of the soil by the wet sieving and decanting method followed by sucrose centrifugation and counted per 100 g dry soil. Percent root colonization was determined clearing the root segments in 10 % KOH and stained with 0.05 % trypan blue.During the reconnaissance survey extremely large number of coffee seedlings and saplings were observed in the study area. *Olea capensis, Schefflera abyssinica*, *Phoenix reclinata* and *Prunus africana* were encountered as dominant shade tree species with percentage occurrences of 15, 12, 11 and 10 %, respectively. *Milletia ferruginea, Albizia gummifera, Cordia africana, Croton macrostachyus* and *Dracaena steudneri* comprise 40 % of the total number of plant species. Twenty four percent of the dominant shade trees were principally represented by leguminous trees such as *M. ferruginea* and *A. gummifera.* Arbuscular mycorrhizal fungal status was also investigated. Investigation of roots and rhizosphere soils of 14 shade tree species, *M. ferruginea, S. abyssinica, C. macrostachyus, Ficus vasta, A. gummifera, O. capensis, C. africana, Ehretia abyssinica, Pouteria adolfi-friederici, Pavetta oliveriana, Ficus sur, P. africana, P. reclinata and Polyscias fulva,* and the coffee tree underneath revealed arbuscular mycorrhizal colonization and different types of AM spores. The number and morphological type of spores were determined in 100g of rhizosphere soil. All species had a high level of AM colonization. *F. vasta, C. macrostachyus, S. abyssinica and O. capensis spp.* had the highest level of colonization. *E. abyssinica, P. africana and P. fulva* exhibited the lowest level of colonization. The coffee trees revealed generally maximum colonization than respected shade trees. The number of spores forming AM in all rhizosphere soils ranged between 578-1313 spores/ 100g of dry soil. Spores were grouped in four principal morphological types Glomus, Gigaspora, Scuttelospora and Acaulospora in descending order of relative abundance, respectively. Spore density and colonization percentage were found to be negatively correlated with O.C, total N, available P, EC and Zn. Population of spore types were also some how determined by soil physical and chemical

properties. Arbuscular Mycorrhizal fungi are commonly considered to be non specific with respect to their host and their ability to colonize particular plant species. Conversely, it is investigated that plant species differ in their ability to form and benefit from mycorrhizal relationship. This study didn't investigate the whole range of organisms in the rhizosphere which could give benefits to trees. So, it is difficult to draw conclusion that the well being of the shade trees and coffee plants in the natural forest is due to only AMF colonization. However, AMF could be considered to be a future tool in agriculture, i.e. fertilizer substitute and as biocontrol agent. So, it is recommended that: (a) studying the ecology, diversity and host range of AMF is of enormous importance before application. (b) their relation with nutrient dynamics and other soil characteristics should be evaluated before use. (c) these organisms should receive the proper management. (d) the impact of litter fall on AMF spore density and diversity should be evaluated in the future. Finally, this symbiosis command great interest as the subject of future research with relation to food security.

# 1. Introduction

Forest can be viewed as an important gene reserve and natural regeneration area for a tremendous number of economically important plant species and other forms of life. The remarkable roles played by the forest are well documented (Wolf, 1999; Daba, 2002).

Ethiopia's economy is predominantly dependent on agriculture, and this agriculture based economy is highly dependent on coffee production, as it contributes more than 67% to the total exchange earnings and over 6% to the gross national product (Wondimu, 1998). Despite its economic importance, however, small holder farmers dominate the production of coffee. It can be noted that around 25% of the Ethiopian population is engaged in coffee production, processing and marketing services, and drives its livelihood from coffee industry. In addition, coffee is of enormous cultural, social and economic importance to the nation. It grows in many parts of the country in natural coffee forests and managed agroforestry systems. The total estimated coffee area in the country is about 500,000 hectares of which one-tenth is considered to be forest coffee.

It has been estimated that more than 60% of the coffee plantations grow under naturally growing shade trees in evergreen forest areas of southwestern Ethiopia (Paulose and Zebene, 1994). The well-known and dominant shade trees reported from Ethiopia embody genera like *Albizia*, *Acacia*, *Bersama*, *Cordia*, *Croton*, *Dracaena, Entada, Erythrina, Ficus, Leucaena, Millettia,* and *Syzygium* (FAO, 1968; Demel and Tigeneh, 1991).

Recently, it has been reported that in a managed natural plantation forest at Yayu, greater than 40 percent of the important shade trees were legumes, *i.e.*, species of *Albizia*, *Acacia*, and *Millettia* (Taye, 2001). Likewise, most farmers traditionally grow coffee in agroforestry systems in eastern part of Ethiopia (Harerge) under shade trees of which 69% of them are leguminous and *Ficus* spp. (Demel and Tigeneh, 1991).

Coffee and shade trees are symbiotically associated with various microorganisms in their rhizosphere, the most important of which is the association of the roots with certain group of fungi known as mycorrhiza. Mycorrhizal fungi are the only one of the innumerable organisms living in soil that provides a direct link between plant root and soil matrix (Haselwandter, 1997).

It is the mutualistic symbiosis (non-pathogenic association) between soil-borne fungi with the roots of higher plants (Sieverding, 1991). Two types of mycorrhiza are known today: ecto- and endomycorrhizas. The ectomycorrhizas are characterized by an extracellular fungal growth in the root cortex. They are more common in temperate and boreal forest trees that number over 5000 species mainly within the Basidiomycetes (Sieverding, 1991). Some tropical trees such as pine and eucalyptus plants, however, have also been found to form ectomycorrhizal associations. The endomycorrhizas are characterized by inter-and intracellular fungal growth in root cortex, forming specific fungal structures, referred to as vesicles and arbuscles. This characteristic growth gives the endomycorrhiza the alternate name, vesicular arbuscular mycorrhiza.

The association helps the plants in water and nutrient uptake, especially phosphorus and nitrogen in many agroecosystesms (Sieverding, 1991), and not the least in the tropical forests (Janos, 1996). Jeffries *et al.*, (2002) have reported the diversity of (V) AM has significant ecological consequences because individual species or isolates vary in their potential to promote plant growth and adaptation to biotic and abiotic factors. Evidently, Vaast et al., (1998) and Bhattacharya and Bagyaraj (2002) have demonstrated the existence of significant variation in effectivity among the isolates of (V) AM fungi. Thus the composition and dynamics of populations of (V) AM have a marked impact on the structure and diversity of the associated plant communities, both in natural and agricultural ecosystems (Gange *et al.*, 1990). Currently, such observations have attracted the attention of international researchers and this and other features have made the mycorrhiza the hottest area of study.

Influence of mycorrhizal fungi on plant community composition and plant succession have been suggested due to the effect of mycorrhizal on the competitive ability of plants (Francis and Read, 1994; Van der Heijden *et al.*, 1998). The success of any ecosystem reforestation effort are, therefore, likely to depend on the establishment of mycorhhiza (Sieverding, 1991; Fracis and Read, 1994; Hasel Wandter, 1997). Mycorrhizal benefits are greatest and most obvious under low-input growing conditions of developing countries of the tropics with subsistence agriculture (Sieverding, 1991).

Many studies have revealed the occurrence of AM fungal propagules in coffee soils, enhanced growth and increase in P and Zn uptake of young coffee seedlings in nursery conditions (Lopes *et al.*, 1985), tolerance to nematodes and survival of coffee plants after field transplanting (Vaast and Zasoski, 1992; Vaast *et al.*, 1998). In general, the positive effects of mycorrhizal fungi on plant nutrition, health, and soil stability have a valuable agro-biotechnological importance for low-input agriculture in developing countries of the tropics (Douds *et al.*, 2000).

Several of the hitherto studies in Ethiopia have focused on indigenous trees with regard to their seed viability (Demel and Granstrom, 1997), regeneration ecology (Demel, 1997) and asexual propagation methods (Legesse, 1995; Kassa and Legesse, 1996; Desta and Legesse, 1998) in response to the frightening trends of deforestation and land degradation in the country.

Although, recently, a few studies on the pattern of mycorrhizal symbiosis have been undertaken on acacia trees of arid and semi-arid ecosytems (Million, 2002; Shasho, 2002; Yonas, 2005), and infection rate of some trees of Afromontaine agroecosystem (Tesfaye *et al*, 2003a), there is a dearth of information on the interrelationship of mycorrhizal diversity, density, and nutrient dynamics of coffee and shade trees in the country.

To date, information regarding densities and identification of dominant coffee shade trees and explanation of their interaction with physical parameters in southwestern part of Ethiopia is extremely very scanty. Moreover, mycorrhizal fungi spore densities, colonization rate, diversity and interaction with physical and chemical parameters of soil are not evaluated in any of the coffee forest in the country and there is a strong need to bridge such information gap. Thus, this study was initiated with the following objectives:

a. Systematically identify the dominant coffee shade trees,

b. Determine the densities of dominant coffee shade trees in Bonga coffee forest.

c. Evaluate the spore density of AMF and to tentatively assign them to their respective fungal genera.

d. Investigate the relation ship among the spore density of AMF, AMF root colonization and soil physical and chemical parameters.

# 2. Literature review

## 2.1 Coffee production system and Importance of shade trees

Generally, four types of coffee production systems can be recognized in Ethiopia (Woldetsadik and Kebede, 2000). These include forest coffee, semi forest coffee, garden coffee and plantation coffee.

Forest coffee is a production system where coffee berries are simply picked from naturally growing coffee under shade trees. In this system, there is no management to improve coffee production. It is estimated to cover about 10% of the total coffee production in the country (Demel *et al*., 1998).

In the semi-forest coffee system, coffee berries are also collected from naturally grown coffee. However, inorder to improve the productivity of wild coffee, competing under story trees and shrubs are regularly removed from the system. This system accounts for about 35% of the total coffee production in the country.

In the garden coffee system, coffee is planted and managed in the area surrounding the farmer's home. It accounts for about 35% of the total coffee production in the country (Demel *et al*., 1998; Woldetsadik and Kebede, 2000).

Plantation coffee is grown on a large scale by private coffee farmers (investors) or the government. It is usually well managed and accounts for about 15% of the total coffee production (Demel *et al*., 1998; Woldetsadik and Kebede, 2000).

Trees which provide shade for coffee are used temporarily or permanently, depending up on the age of the coffee plants (Demel and Tigeneh, 1991). Temporary shade trees (Euphorbaiaceae and Fabaceae), usually fast growing species, are planted at the time of establishment of the coffee seedlings until the permanent ones grow well enough to provide shade. The well-known and dominant shade trees reported from Ethiopia are the genera like *Albizia*, *Acacia*, *Bersama*, *Cordia*, *Croton*, *Dracaena, Entada, Erythrina, Ficus, Leucaena, Millettia,* and *Syzygium* (FAO, 1968; Demel and Tigeneh, 1991).These trees are multi-purpose plants that provide forage, firewood, and medicine to increase alternative income sources to the population (CEC, 1999).

The main advantages of growing coffee under shade tree are: (a) reduction of light intensity and day time temperature in hot areas: this helps to control the cropping level and reduce overbearing dieback in areas where rapid growth might otherwise lead to these problems (Baggio *et al*., 1997; Beer *et al*., 1998; Muschler, 2001); (b) reduction of diurnal variations in areas where there is a marked difference between day and night temperatures that reduces incidence of distorted leaf growth as in the 'hot and cold' and 'crinkle leaf' syndromes; (c) protection from low night temperatures and from frost incidence; (d) protection from wind and hail; (e) protection of soil from the impact of falling rain and reduction of soil temperature and evaporation (Clarke and Macrae, 1988); (f) reduction in growth of weeds especially couch grass (*Digitaria scalarum*) (Baggio *et al*., 1997; Beer *et al*., 1998; Muschler, 2001). The weeds found in shaded coffee are less harmful and are easier to control; (g) addition of leaf litter to the soil acts as a mulch and eventually adds organic matter and minerals (Granhall, 1994); (h) addition of nitrogen to the soil by the root nodules of leguminous shade trees (Roskowski, 1982; Babbar and Zak, 1994; Snoeck *et al*, 2000). Some of the Ethiopian leguminous coffee shade trees have been demonstrated to nodulate by root nodule bacteria of the genera ***Rhizobium*** and ***Bradyrhizobium*** (Fassil, 1993; Fassil and Kleiner, 1997, 1998).

On the other hand growing coffee under shade trees has disadvantages under different conditions: (a) under dry conditions, the shade trees compete with the coffee for soil moisture; (b) shade trees require regular pruning and thinning to prevent the shade becoming excessive; which can cause damage to the coffee; (c) if the shade is dense, the coffee stems become etiolated and weak and are liable to break if the normal (uncapped) multiple stem pruning system is followed. For this reason single stem or capped multiple stem pruning is often preferred where the coffee is grown under shade; (d) the cropping potential of coffee under shade is limited due to reduced flowering, and the yield response to nitrogen fertilizer is also limited. For this reason intensively managed coffee is grown with out shade, where as coffee grown on a low input/ low out put system is usually shaded (Clarke and Macrae, 1988).

## 2.2 Taxonomic description of AMF

The original taxonomy of the arbuscular mycorrhizal fungi was based on the morphology of the large soil-borne spores which were found near colonized plant host's roots (Simon *et al*., 1993). Distinguishing AMF spore characteristics used in classification include wall morphologies, size, shape, colour, hyphal attachment and reaction to staining compounds (Wright, 2005). With the advent of molecular techniques the classification of AMF has under gone major revision. An analysis of almost full-length zygomycotan sequences exposed a clear separation of the AMF from the other included fungal groups.

The arbuscular mycorrhizal fungi are now placed in the new division Glomeromycota (Schüßler *et al.,* 2001). The fungi of the Glomeromycota have coenocytic to sparsely septate mycelium. They reproduce asexually though blastic development of the hyphal tip and form symbiotic relation ships with photoautotrophs (Schüßler *et al*., 2001).

The arbuscular mycorrhizal fungi (AMF) belong to taxonomic division Glomeromycota order Glomerales and in sub order Glomineae with in the families Paraglomaceae (genus *Paraglomus*), Glomaceae (genus *Glomus*), Archaeosporaceae ( genus *Archaeospora*) , Acaulosporaceae (genera *Acaulospora* and *Entrophospora* ) and in sub order Gigasporinae with in the family Gigasporaceae (genera *Gigaspora* and *Scutellospora*) (Schüßler *et al*., 2001).

## 2.3 AMF and Soil fertility

Three main components are involved in AMF association: 1) the soil, 2) the fungus and 3) the plant. The fungal component involves the fungal structure within the cell of the root and the extraradical mycelium in the soil. The extraradical mycelium in the soil may be quite extensive under some conditions, but does not form any vegetative structures (Smith and Read, 1997). Its primary function is the absorption of resources from the soil.

AMF have been shown to improve productivity in soils of low fertility and are particularly important for increasing the uptake of slowly diffusing ions such as $PO_4{}^{3-}$ (Jacobsen *et al*., 1992), immobile nutrients such as P, Zn and Cu (Liu *et al*., 2002) and other nutrients such as Cadmium (Guo *et al.,* 1996). Under drought conditions the uptake of highly mobile nutrients such as $NO^{3-}$ can also be enhanced by mycorrhizal associations (Ázcón *et al*., 1996). In legume plants the importance of AMF symbiosis has been attributed to high P requirements on the nodulation and $N_2$ fixation process which requires enhanced P uptake (Guo *et al.,* 1996). Improved P nutrition has been shown to increase in infertile and P-fixing soils of the tropics (Dodd, 2000). Mycorrhizal fungi can also improve absorption of N from $NH_4^+$ -N mineral fertilizers, transporting it to the host plant (Johanssen *et al.,* 1993). Its transport and absorption can also increase biomass production in soils with low potassium, Calcium and Magnesium (Liu *et al.,* 2002).

### 2.3.1 AMF and Soil structure

Fungal hyphae, especially those of AM fungi, grow into the soil matrix to create the skeletal structure that holds primary soil particles together via physical entanglement. They create conditions conducive to formation of microaggregates, and they chemically enmesh and stabilize microaggregates and smaller macroaggregates into macroaggregate structures (Miller and Jastrow, 2000). AM fungi produce copious amounts of the glycoprotein glomalin (Rillig, 2004). Although soil aggregation is a complex hierarchical process, concentration of glomalin is tightly correlated with aggregate stability across many soils (Wright and Upadhyaya 1998; Rillig, 2004).

AM symbiosis has been linked to changes in soil structure in both pot and field experiments (Rillig, 2004). Soil structure refers to pore space as well as to aggregates, and the number and dimension of the pore spaces between soil particles are important in functional considerations of soil structure, especially from the standpoint of soil water relations (Hamblin, 1985).

### 2.3.2 AMF and Plant water Relations

Many reports summarize the comparative water relations of AM and non AM plants (Augé, 2001). However, the influence of AM symbiosis on the water relations and moisture retention properties of soils remains largely unstudied. Fungi may be the most effective soil organisms in stabilizing soil structure and AM fungi often comprise the largest portion of the soil microbial biomass (Foster, 1994).

The moisture characteristic of a soil depends on the size and distribution of its pores, or void space (Hamblin, 1985). Because AM fungi affect soil structure, it seems logical to suspect that AM colonization of a soil might affect its moisture retention properties and, in turn, the behaviors of plants growing in the soil, particularly when soil is relatively dry.

Mycorrhizal plants often show physiological responses linked to increased drought resistance: e.g., the ability to maintain stomatal opening and hence carbon gain to lower soil water potential (Ψ) and leaf Ψ or water content (θ) (Duan *et al.* 1996). In addition to promoting stomatal opening during soil drying, AM fungi can also affect stomatal response when soil Ψ is lowered osmotically (Augé *et al.*, 1992). This suggests that AM root systems either scavenge water of low activity more effectively [a finding also implicated by Bethlenfalvay *et al.*, (1988)] or contribute to so-called nonhydraulic root-to-shoot communication (Davies *et al.,* 1994) differently than nonAM root systems.

Recent comparisons among six AM species revealed significant contributions by extraradical hyphae to mycorrhizal root water uptake (Marulanda *et al.*, 2003). There is support for the idea that AM plants may have access to water that is unavailable to nonAM plants. Better growth of AM plants in drying soils may be related to improved exploitation of bound water, with mycorrhizae providing access to soil water below the permanent wilting Ψ of nonmycorrhizal plants (Franson *et al.*, 1991). Duan *et al.,* (1996) concluded that AM fungi probably increased the capability of cowpea root systems to scavenge water in drier soil, resulting in less strain to roots and foliage and hence higher transpiration, gs and shoot Ψ, and lower concentrations of abscisic acid in xylem sap, at low soil θ. This possibility is supported by a study with alfalfa (Goicoechea *et al.*, 1997), in which AM roots had lower abscisic acid concentrations than non AM roots at similar low soil θ.

## 2.4 AMF – Plant pathogen Interaction

### 2.4.1 Role of AMF in Biocontrol of Plant diseases

It has been estimated that even in industrialized countries, billions of dollars are spent for disease management through conventional methods such as agrochemicals and breeding for resistant varieties (Qinn, 1999).

Currently, popautlions of rhisosphere microorganisms of plants have demonstrated an antagonism to ward phytopathogenic organisms. Natural farming or eco-friendly farming has emerged as the only answer to bring sustainability to plant productivity without environmental hazard. Potential alternatives that curtail chemical fertilizers and pesticides are available in nature (Sharma *et al.,* 1997).

Qinn, (1999) suggested that now is the right time to look at the natural systems that lie below our feet the soil microflora especially mycorrhizae. Apparently, such biological and natural inputs include bio-pesticides and bio-fertilizers (Dubey *et al*., 1997; Sharma *et al.,* 1997). Interestingly, Mycorrhizal fungi are the best candidate for both advantages. Thus, this makes the mycorrhizal fungi the most popular and significant groups of soil microorganisms. Therefore, currently, mycorrhizal fungi function as effective suppressors of pathogenic soil borne fungi (Vigo *et al*., 2000) and plant parasitic mematodes (Pandey *et al*., 1999).

Backer and Scher, (1987) ascribed the most desirable attributes of bio control agents. These are: (1) active in an environment conducive for the pathogen. (2) compatible with other agents. (3) survive agricultural introduction. (4) colonize the appropriate substrate or plant part. (5) efficiently suppress the pathogen.

### 2.4.2 Interactions with Soil borne fungal root pathogens

All available evidences indicate that mycorrhizal fungi interact with various types of root-borne pathogens (Chaube and Singh, 1991). Accordingly, there is a distinct possibility that resistance may be at hand to invasion by different types of soil-borne root pathogens. Therefore, AMF are currently studied as agents of bio-protection (Mark and Cassells, 1996) to a neutral action (Reddy *et al*., 1989 cited in Trotta *et al*., 1996) and occasional increasing disease severity (Chaube and Singh, 1991).

Investigation of effects of AMF associations on health of *Limm usitatissimum* infected by fungal pathogens indicated that AMF plants revealed enhanced resistance against the wilt necrotrophic pathogen (*Fusarium oxysporum* f.sp lini; Dugassa *et al.,* 1996). Although AMF plants were also highly susceptible to the shoot pathogen, *Oidium lini*, they suffered less than non-AMF plants in terms of shoot fresh weight, $CO_2$ assimilation and content of sucrose in shoot apex. This indicates that AMF not only stimulates resistance mechanism but also induces tolerance against pathogens (Dugassa *et al*., 1996).

The specific mechanisms by which AMF fungi exert their deleterious effects on soil borne root pathogens is to be elucidated (Maloy, 1993; Trotta *et al*., 1996). However, there are several valid assumptions to assert their interaction. First, AMF fungi improve the mineral nutrition of host plants, and more precisely P uptake (Douds *et al*, 2000). Extensive network of external mycelium could supply essential nutrients other than P and prevent water shortage by compensating for the loss of active roots rotted by the noxious pathogens (Trotta *et al*. 1996). Second, increase in growth promoting phytohormones (Dugassa *et al*., 1996), phenolic compounds (Morandi, 1996; Dubey *et al.,* 1997) and enzyme activity in the roots of mycorrhizal plants may occur (Dumas-

Gaudot *et al.,* 1996; Trotta *et al.*, 1996). Third, competition between AMF and pathogenic fungi for colonization sites (Trotta *et al.*, 1996). Fourth, the stimulation of predator populations (Andrade *et al.,* 1998). Fifth, AMF fungi may produce sugars, amino acids or other compounds that stimulate beneficial organisms that inhibit plant pathogens (Maloy, 1993).

A review by Tari and Anderson, (1988) revealed that the production of iron-chelating substances and antibiotics require adequate nutrition for beneficial rhizospere microorganisms such as pseudomonads. It has been repeatedly reported that PGPR interact synergistically with mycorrhizae in enhancing plant growth (Chanway and Holl, 1991). The majority of these bacteria are in the fluorescent Pseduomonads group which abundantly inhabit in the rhizosphere (Caruthers *et al.*, 1995). It is evident that AMF preferentially stimulated fluorescent pseudomonads and caused their numbers to increase (Andrade *et al.*, 1998). Additionally, Barea *et al* (1998) recognized that antifungal compounds exuded by ***Pseudomonas spp.*** did not interfere with AMF formation or functioning. Presumably however, root pathogen suppression of AMF fungi are strongly linked with these essentially important microbes.

In general, mechanisms such as production of antibiotics, siderophores, hydrogen cyanide, and plant hormone like substances and vitamins (O'sullivan and O'gara, 1992; Derylo and Skorupska, 1993) are involved in bio-control and plant growth promotion. Furthermore, the authors suggested that intensive competition for resource with deleterious rhizosphere organisms is directly linked to ability to successfully colonize a root surface (O'sullivan and O'gara, 1992). Schenck (1987) further suggested that morphological features play a great role in influencing disease causing soil microbes. These are (1) greater morphogenic response in cell walls to the pathogens on myconhizal roots, (2) increase in wound barrier formation and (3) increase in lignifications of the cell wall.

Nevertheless, a few studies revealed that AMF fungi also make the roots susceptible to root rot pathogens, thereby increase severity of disease rather than reducing it (Chaube and Singh, 1991). Larsen and Bodker, (2001) found that in severely infected root cortical tissue *Glomus mosseae* had reduced energy reserves and biomass and did not protect the plant from the biotrophic pathogen, *Aphanomyces euteiches*. In wheat, high levels of colonization by AMF did not protect crop roots from damage by root pathogens (Ryan *et al.,* 2002).

## 2.5 Ecological restoration versus AMF

Inoculation with native AM fungi increased plant uptake of phosphorus improving plant growth and health. The results support the use of AM fungi as biological tool in the restoration of self-sustaining ecosystems (Jeffries *et al*., 2002).

Disturbance of native plant communities in desertification threatened areas is often followed by degradation of physical and biological soil properties, soil structure, nutrient availability and organic matter. When restoring disturbed land it is essential to not only replace the above ground vegetation but also the biological and physical soil properties (Jeffries *et al*., 2002).

A relatively new approach to store and protect against desertification is to inoculate the soil with arbuscular mycorrhizal fungi with the reintroduction of the vegetation. A long term study done by Jeffries *et al.,* (2002) demonstrated a significantly greater long term improvement in soils quality parameter was attained when the soil was inoculated with a mixture of indigenous arbuscular mycorrhizal fungi species compared to the non inoculated soil and soil inoculated with a single exotic species of AM fungi. The benefits observed were an increased plant growth and soil nitrogen content, higher soil organic matter content and soil aggregation.

By stimulating the development of beneficial microorganisms in the rhizosphere (Pennington, 1986), the use of AMF-infected plants could reduce the amount of fertilizer needed for the establishment of vegetation and could also increase the rate at which the desired vegetation becomes established by stimulating the development of beneficial microorganisms in the rhizosphere (Pennington, 1986).

Degraded soils are common targets of revegetation efforts in the tropics, but they often exhibit low densities of AMF fungi (Michelsen and Rosendahl, 1990). This may limit the degree of mycorrhizal colonization in transplanted seedlings and consequently hamper their seedling establishment and growth in those areas. Soil inoculation with *G. mosseae* has significantly enhanced plant growth and biomass production in limestone mine spoils (Rao, 2002).

The use of arbuscular mycorrhizal fungi in ecological restoration projects has been shown to enable their host plant establishment on degraded soil and improve soil quality and health (Jeffries *et al*., 2002).

## 2.6 Host specificity of AMF

AM fungi were considered to have low specificities in association with plant host species, but these conclusions were based almost exclusively on experiments in which individual isolates of species are grown separately, with out considering competitive interactions. When fungi are examined as a community, it was found that AM fungal growth rates are highly host specific.

In an experiment in which AM fungi were trapped on different plant hosts, isolates of different fungal species sporulated differentially, with the relative dominance of fungal species being reversed, depending on the plant species with which they were associated (Bever *et al*., 1996). For example, *Acaulospora colossica* was dominant in association with *Allium vineale*, field garlic, but this fungus was a minor component of the community associated with *Plantago lanceolata*. Alternatively, *Scutellospora calospora* sporulated profusely with *Plantago*, but was a minor component in association with *Allium*.

It was found that the distribution of fungi in the field to be similarly host specific (Bever *et al*. 1996, Schultz 1996). This pattern of host specificity in AMF-host association has been observed in many other systems, including tall grass prairie (Johnson *et al*., 1992), sand dunes (Koske 1981), California grasslands (Nelson and Allen 1993), chalk grasslands (Sanders and Fitter, 1992), and agricultural fields (Douds and Millner,1999). This specificity of fungal response could contribute to the maintenance of diversity within the AM fungal community.

## 2.7 Seasonality of AMF

It has also been found that AM fungi differ in their seasonality, with some fungi sporulating in late spring and others sporulating at the end of summer (Schultz *et al.,* 1999). As the spores represent the dormant state of the fungus, the physiologically active state is most likely the mirror image of the seasonal spore counts. Therefore, *Gigaspora gigantea*, which sporulates most abundantly in the fall and appears to overwinter as spores, is likely to be physiologically active during the warm season.

Similar patterns have been seen for *Gi. gigantea* in a sand dune on the coast of Rhode Island (Lee and Koske,1994). Alternatively, *Ac. colossica*,which sporulates most profusely at the beginning of summer and oversummers as spores, is physiologically active with the cool season plant community (e.g.,*Allium vineale*). Thus, such a kind of study helps in proper management of different genera of mycorrhiza.

## 2.8 Plant Diversity versus AMF

Van der Heidjen et al., (1998) have provided evidence that diversity of AMF determines plant community structure through the response of individual plant species to this diversity. AMF diversity is the major factor in the maintenance of plant biodiversity and ecosystem stability and function. Several studies show that AMF alters plant community structure by affecting the relative abundance of plant species and plant-species diversity (Gange *et al*., 1990; Sanders and Koide, 1994).

Interplant transport of assimilates from the dominant canopy species via a common mycorrhizal network to subordinate plant species, has been suggested as a mecahnism by which AMF affect the floristic diversity of plant communities (Gange *et al*., 1990). Another mechanism by which AMF may affect plant community structure is the differential growth response of plant species to colonization by AMF, the so-called “mycorrhizal dependence” (Mitiku and Manjunath, 1991).

The species composition and diversity of AMF communities has the potential to determine plant population and plant community structure. The fact that plant species vary in the degree of response to AMF species has important implications for growth of individual plant species. In turn, this will affect a plant’s ability to coexist with other plant species in a community (Van der Heijden *et al.,* 1998). On the other hand, established mycorrhizal plants may serve as important sources of inoculum for initially nonmycorrhizal, conspecifics, which may affect regeneration and could contribute to patchy distributions of species within the community (Koide and Dickie, 2002).

## 2.9 AMF management and Perspectives

Manipulation of agricultural systems to favour AMF colonization must occur only if there is clear evidence that AMF make a positive contribution to yield or are vital for maintenance of ecosystem health and sustainability (Ryan *et al*., 2002).

The main areas, in which the benefits of introducing inoculant AMF into a plant growth system will accrue, are those that lack indigenous inoculum of AMF. These include sterilised soils or post *in vitro* plant micro-propagation, buried, extremely fertilised, degraded areas (Dodd, 2000) or rooting of pepper cuttings (Thanuja *et al.*, 2002). It is widely accepted that plants with highly branched root system (Gramineae) are less mycotrophic (less dependent on the fungi for normal growth) than those with coarser roots (e.g. cassava, onion). Root branching determines plant dependence on the symbiosis. Similarly soils under low-input management system show higher AMF fungus spore populations than soils under conventional management (Douds *et al*, 1993, 1995).

Salicylic acid contents in the plant reduced mycorrhization, suggesting that enhanced salicylic acid levels in plants delay AMF root colonisation. Although salicylic acid affects AMF root colonization, it has no effect on the potential of plants to be colonized by AMF (Medina *et al*., 2003).

Survival of AMF in soil may be affected by the presence or absence of crops and crop rotation (Troeh *et al*., 2003). Fallow fields had less spores than cultivation of corn followed by soybean. In cowpeas, inoculation and amendment with organic manure resulted in increased growth and yield (Muthukumar and Udaiyan, 2002).

The use of native Mycorrhizal fungi as a potential source of AMF inoculum was considered as a preferential strategy for ensuring the successful re-establishment of native shrub species in semi-arid degraded soil (Caravaca *et al*., 2003). Bell *et al*., (2003) found that the susceptibility of acacia seedlings to colonization by AMF appeared to be seasonal. Colonization increased with increasing daytime temperatures and day length. Despite the beneficial effects of AMF, their activity may be greatly limited by soil fumigation, non-responsive plant varieties, or rotations based primarily on non-mycorrhizal crops or crops of low AMF dependency.

Despite a high number of publications on AMF covering a variety of topics, the issues which most concern African countries such as drought and increased crop growth and yield are the least addressed. Under the conditions of many African countries, where no effective isolates are available and occurrence and distribution of AMF are still unknown, pot trials are an important step for correct management of indigenous AMF.

These trials must be designed to determine basic characteristics, such as host-fungus specificity interaction and competitiveness between AMF and, effectiveness of the AMF under possible environmental stresses, drought stress, salinity, N and P deficiency conditions. Cheap and mass production of AMF inoculum will represent a significant step in its use in African agriculture.

# 3. Materials and Methods

## 3.1 Description of the Study area

This study was carried out in Bonga forest coffee which is situated 15 Km. away from Bonga town (SNNPRS) and 445 Km. from the capital, Addis Ababa (07019’.376’’N and $036^0$14’.947’’E) Fig.3a&b). Recently, this forest has been identified and demarcated as forest coffee and forest genetic resources conservations (MOA, 1998). It has been considered as one of the national forest priority areas of forests in southern nations and nationalities people’s regional state (SNNPRS). Its altitude ranges from 1750-2000masl. The yearly average annual rainfall of this forest is over 1600mm. The mean annual temperatures range from 15-25$^o$c with the mean daily temperature minima range from 9.7 to 16.3 $^o$c and the corresponding maxima from 20 to 30.4 $^o$c (MOA, 1998).

## 3.2 Sampling

### 3.2.1 Vegetation sampling

This investigation was conducted at Bonga coffee forest in southwestern part of Ethiopia. A reconnaissance survey was made across the Bonga coffee forest in October, 2005. Quadrats (20 mX20 m) were identified and the number of coffee and coffee shade tree species was counted. All coffee shade trees in ten sample plots (quadrats) were quantified. All sampled coffee shade tree plants were recorded by their Latin names following standard nomenclature rules (Hedberg and Edwards, 1989; Edward ***et al.,*** 1995).

### 3.2.2 Root and Soil Sampling

Root samples of 14 selected species of shade trees (Table 1) were collected by excavating starting from the trunk and working out towards the fine roots. Roots (20 from each tree) were brought into the laboratory. After carefully washing with tap water, fine roots were cut into 1 cm pieces and maintained in 50% alcohol (FAA).

The area under each tree canopy was partitioned in to six sectors. About one kg of composite soil sample was taken from each sector every time for soil physical and chemical analysis. Six hundred gram of triplicate samples each replica having 200 gm soil was taken for spore extraction to the laboratory. This kind of work was done for about 6 times (October, 2005- March, 2006). The soil was taken from 50 cm depth with in an area of 3 m diameter under the canopy of the trees. The soil samples were analyzed for selected determination: pH, OM%, AVP, TN, Mg, K, Ca, Na, Zn, CEC and EC at Ethiopian Institute of Agricultural Research center, EIAR.

The pH of the soils was potentiometrically measured by using distilled water (Van Reeuwijk, 1993). Organic matter content (OM) of the soil was determined by the wet combustion procedure of Walkley and Black as outlined by Van Ranst *et al*., (1999). Total nitrogen content (TN) of the soil was determined by wet – oxidation procedure of the kjeldahl method (Bremner and Mulvaney, 1982). Available phosphorus content of the soil was determined by Olsen method as outlined by Van Reeuwijk, (1993).

Exchanchangeable cations content and the cation exchange capacity (CEC) of the soils were determined by the 1 M ammonium acetate (pH 7) method according to the percolation tube procedure (Van Reeuwijk, 1993). The effective CEC was calculated as the sum of exchangeable cations extracted by the ammonium acetate buffered at pH 7 plus 1 M KCl extractable Al. The available micronutrient (Zn) was determined by diethylenetriaminepentaacetic acid (DTPA) method (Tan, 1996). The exchangeable acidity was determined by percolating 10 g soil with 100 ml of 1 M potassium chloride solution. It was determined by titrating 25 ml aliquot of the percolate with 0.025 M sodium hydroxide solution after addition of 7 drops of phenolphathalein solution (Van Reeuwijk, 1993).

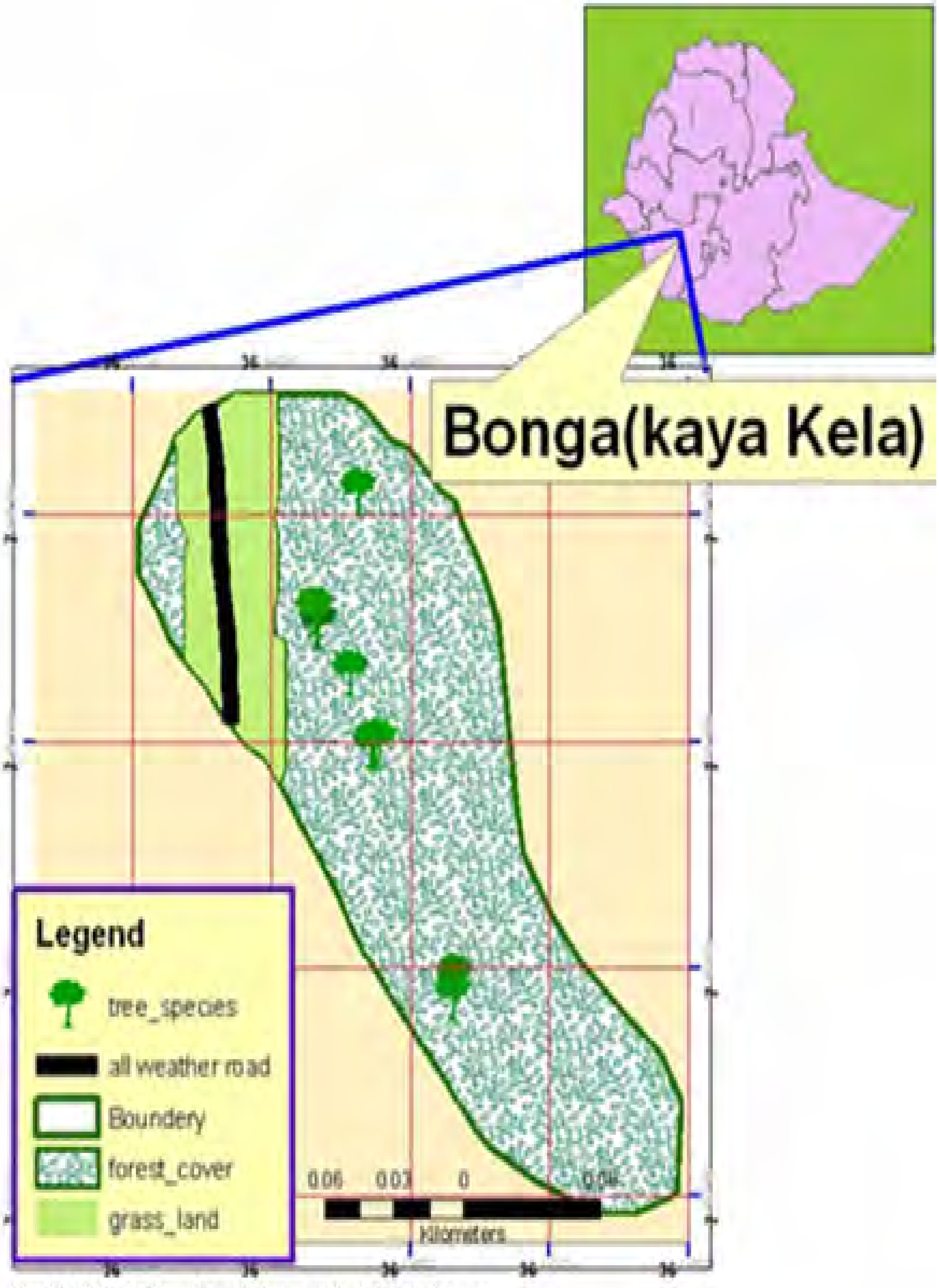

Fig. 1(a) Distribution of sample tree species in the study area.

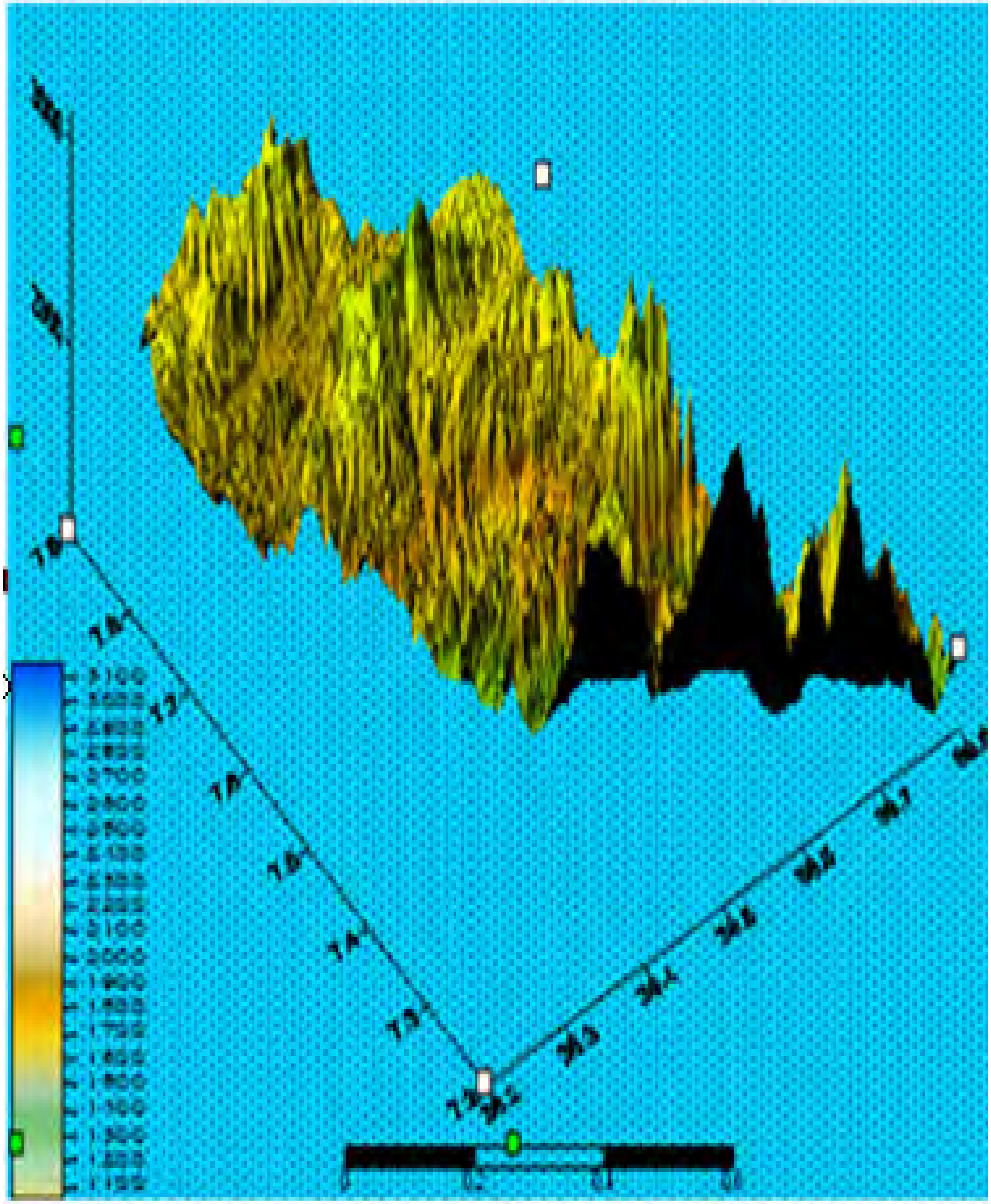

Fig. 1(b) The three dimensional view of the study area and its surroundings.

Table1. Coffee shade tree species and their respective geographical locations in Bonga coffee forest studied for AMF colonization

| **Scientific name** | **Family name** | **Location** |
|---|---|---|
| *Milletia ferruginea* | Fabaceae | 07$^0$19’.376’’N<br>036$^0$14’.947’’E |
| *Schefflera abyssinica* | Araliaceae | 07$^0$19’.383’’N<br>036$^0$14’.899’’E |
| *Croton macrostachyus* | Euphorbiaceae | 07$^0$19’.430’’N<br>036$^0$14’.874’’E |
| *Ficus vasta* | Moraceae | 07$^0$19’.389’’N<br>036$^0$14’.900’’E |
| *Albizia gummifera* | Fabaceae | 07$^0$19’.466’’N<br>036$^0$14’.870’’E |
| *Olea capensis spp.* | Oleaceae | 07$^0$19’.465’’N<br>036$^0$14’.868’’E |
| *Cordia Africana* | Boraginaceae | 07$^0$19’.505’’N<br>036$^0$14’.846’’E |
| *Ehretia abyssinica* | Boraginaceae | 07$^0$19’.509’’N<br>036$^0$14’.845’’E |
| *Pouteria adolfi-friederici* | Sapotaceae | 07$^0$19’.509’’N<br>036$^0$14’.845’’E |
| *Pavetta oliveriana* | Rubiaceae | 07$^0$19’.509’’N<br>036$^0$14’.845’’E |
| *Ficus sur* | Moraceae | 07$^0$19’.465’’N<br>036$^0$14’.867’’E |
| *Prunus Africana* | Rosaceae | 07$^0$19’.509’’N<br>036$^0$14’.845’’E |
| *Phoenix reclinata* | Palmae | 07$^0$19’.489’’N<br>036$^0$14’.859’’E |
| *Polyscias fulva* | Araliaceae | 07$^0$19’.482’’N<br>036$^0$14’.862’’E |

## 3.3 Arbuscular Mycorrhizae (AM)

### 3.3.1 Spore extraction

Spores were separated from the bulk of the soil by the wet sieving and decanting method, followed by sucrose centrifugation described by Walker, (1991). Soil samples were collected from around the selected sample trees. A known amount of fresh soil was thoroughly wetted and then dried to constant weight at $70^{o}$c. After having constant dry weight, 100 gm of dry soil was reweighed for calculation of number of spores per unit of dry soil.

The soil suspension was passed through 710 µm Endicott's sieve just to remove stones and roots. After that the soil suspension was strained through a fine sieve (32 µm) and transferred to four 50 ml centrifuge tubes. Adding water and balancing, the soil samples were resuspended and centrifuged at 2000 rpm for about 5 minutes. The supernatant which contains floating organic material including dead spores was discarded. Then the pellets were resuspended in sucrose solution (50%) and centrifuged up to 2000 rpm for about one minute. Rapidly the supernatant was sieved (32 µm) and washed thoroughly (at least for one minute) to replace the sucrose and alleviate osmotic stress on spores. The pellets in the centrifuge tubes were discarded. Finally all of the solid material was carefully washed from the sieve in to a Petri Dish to prepare for spore counting under a Stereoscope Microscope.

### 3.3.2 Enumeration of spores

Enumeration of spores was done according to INVAM, (2006) as follows. First using a fine ruler, the diameter of the ocular field of the stereoscopemicroscope at a magnification of 4X was determined (where spores can be easily distinguished from mineral particles and organic debris).

The area of the spherical field was calculated at that magnification (4X). It was having a diameter of 5mm that the area of the field was 19.6mm$^2$(radius 2.5mm).

Plastic Petri dishes were used to count spores because the base of the plate is flat. Because the dish also is hydrophobic, enough water was added to have complete coverage of the base. Those dishes which are 85mm across were used. That is, the area of the base was calculated to be 5672mm$^2$. From this datum, and the area of the ocular field (19.5mm$^{2)}$**,** total number of fields in the dish was: 5672/19.6= 289.

After that, the extracted spore suspension was added to a Petri dish and then the dish was rotated randomly to spread out spores as evenly as possible.

Finally, spores were counted in 40 fields randomly chosen over the area of the dish. Average number of spores per field was calculated and multiplied by 289(# fields/dish).

### 3.3.3 Staining Root samples

Root staining (hot staining) was adopted from Kormanik and McGraw, (1982); Brundrett ***et al.***, (1994). Root samples were washed and transferred to labeled tube. Having cleaned samples, clearing was done adding 10% KOH solution in a water bath at 90$^o$c in a fume cupboard for about 4-5 hrs. (Clearing removes cytoplasm from host root cells, leaving root structure and fungal elements intact). Darkly pigmented roots were bleached with hydrogen peroxide at room temperature for about 30 minutes and acidified with 1% HCl for 2-3 minutes. Then the cleared root samples were stained in 0.05% trypan blue in water bath at 90$^o$c in a fume cupboard for about 30 minutes. Samples were drained and washed thoroughly with distilled water at the end of every action. Afterwards the samples were left in de staining solution (14:1:1 lactic acid: glycerol: water) for more than two days in a dark room just to remove colouration from empty root cells. Finally, roots were mounted in de staining solution as parallel lengths on a microscope slide beneath a 50 x 24 mm cover slip and prepared for quantification.

### 3.3.4 Quantification of AMF colonization

AM colonization was assessed on cleared and stained root. Percent AM root colonization was estimated using, a magnified intersection method, a hair line graticule inserted in to eyepiece acted as the line of intersection with each root at x 200 magnification under the compound microscope (MC Gonigle et al., 1990). At each intersection there were six possible mutually exclusive outcomes. The line might intersect at (**p, q, r, s, t** and **u**). where:

**G**_ (**p+q+r+s+t+u**) intersections inspected,
p _ no fungal structures,
q _ arbuscules,
r _ mycorrhizal vesicles,
s _ arbuscules and mycorrhizal vesicles,
t _ mycorrhizal hyphae but no arbuscules or mycorrhizal vesicles,
u _ hyphae not seen to be connected to arbuscules or mycorrhizal vesicles.

A reasonable estimate of percentage of root length colonization (%RLC) was done from 100 or more intersections for each root sample. Where a total of **G (= p+q+r+s+t+u)** were inspected, the percentage of root length colonized by hyphae of all types was calculated as: hyphal colonization **(HC) =100[(G-p)/G].**The percentage of root length colonized by mycorrhizal hyphae was calculated as: **MHC=100[(q+r+s+t)/G]**.The percentage of root length colonized by arbuscules, and the percentage of root length colonized by mycorrhizal vesicles, were calculated as: arbuscular colonization **(AC) = 100(q+s/G)** and vesicular colonization **(VC) = 100(r+s/G).**

### 3.3.5 Identification and Characterization of spores

Spores were grouped in to respected genera according to morphological characteristics. Permanent slides were prepared for each different spore morphotype with polyvinyl-alcohol and polyvinyl-alcohol plus Melzer's solution ( 1.5 g KI, 0.5 g I, 100 g chloral hydrate and 100 ml water) (1:1) (INVAM, 2004; Merryweather, 2004).

After the uniformity of the morphological groups was confirmed under the phase contrast illumination microscope, the different morphotypes were tentatively identified to the genus level. Spore identification was based mainly on spore size, shape, color, wall structure, hyphal attachment (simple, swollen and bulbous) and Melzer's solution reaction (INVAM, 2004; Merryweather, 2004).

The diameter of the spores was measured using phase contrast illumination microscope having connected with analysis program. Color of the spores was assessed by comparison with a color chart given by INVAM. Slides were prepared with spores crushed to different degrees in PVLG and Melzer's PVLG (1:1 ratio) especially for wall structure and Melzer's solution reaction investigations (INVAM, 2004; Merryweather, 2004).

Statistical analyses were performed with the program SPSS V.11.0 package (SPSS Inc., Chicago, IL., USA).

# 4. Result

## 4.1 Floristics

In tested ten quadrants, various types of shade tree species were identified and listed (Table 3). The species composition of coffee shade trees at Bonga coffee forest is quite heterogeneous.

Nineteen species of woody shade trees falling in to 13 families were recorded from Bonga forest (Table 3). The family Fabaceae, Araliacea, Boragnaceae, Moraceae and Rutaceae were represented by two species, where as the rest had only one representative species.

In terms of both density and diversity, the coffee shade trees were dominated by species of Milletia *ferruginea*(22%) followed by *Olea capensis* (15%)*, Schefflera abyssinica* (11%), *Phoenix reclinata* (11%), *Prunus africana* (10%) and *Dracaena steudneri* (8%) (Table 3).

Although these species accounted for less than 40% of the species diversity, they contributed more than 80% to the density of species and families of coffee shade trees. The common shade trees like *Cordia*, *Croton* and *Albizia* were rarely encountered at the study site.

The understorey vegetations are mainly dominated by the legume shrub *Desmodium* species (Table 4). Sometimes *Dracaena* species abundantly occurred as members of major under storey plants. Some bryophytes (mosses) were found to heavily cover both shade trees and coffee plants.

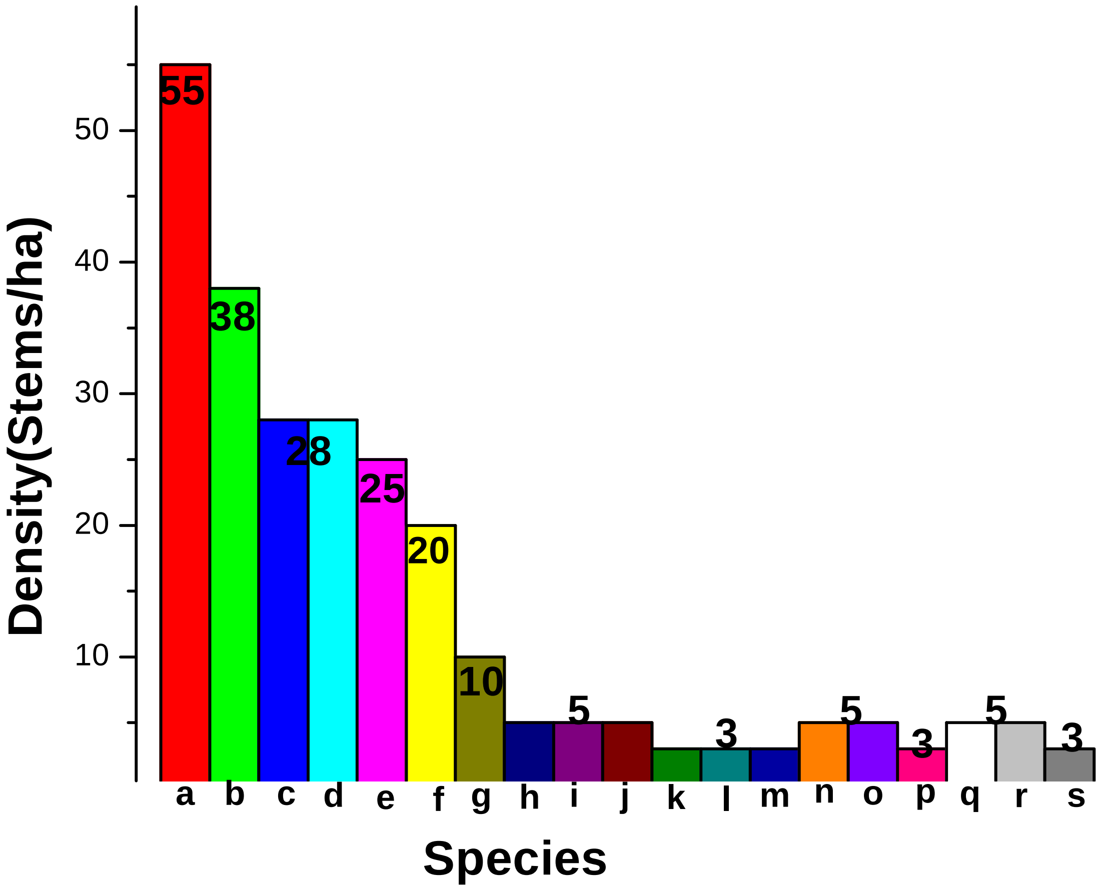

Fig. 2 Frequency distribution of dominant shade trees in Bonga coffee forest.

Where: **a**=*Milletia ferruginea,* **b**= *Olea capensis* ssp., **c**= *Schefflera abyssinica,* **d**= *Phoenix reclinata,* **e**=*Prunus africana,* **f**= *Dracaena steudneri,* **g**= *Croton macrostachyus,* **h**= *Albizia gummifera,* **i**=*Cordia africana,* **j**= *Ficus vasta,* **k**= Bersama *abyssinica,* **l**= *Ehretia abyssinica,* **m**=*Teclea nobilis*, **n**= *Vepris dainellii,* **o**= *Pouteria adolfi-friederici,* **p**=*Polyscias fulva,* **q**=*Pavetta oliveriana,* **r**= *Sapium ellipticum* and **s**= *Ficus sur*

**Table 2**. Characterstics of rhizospher soil under the canopy of each tree species.

| | Species | pH | O.C | T.N | P | EC | CEC | Na | K | Ca | Mg | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | *Milletia ferruginea* | 5.10 | 2.366 | 0.261 | 3.60 | 0.049 | 27.70 | 0.21 | 0.07 | 6.41 | 2.93 | 1.298 |
| 2 | *Schefflera abyssinica* | 4.90 | 2.111 | 0.231 | 1.33 | 0.025 | 29.20 | 0.19 | 0.10 | 3.63 | 2.34 | 0.511 |
| 3 | *Croton macrostachyus* | 4.70 | 1.89 | 0.176 | 1.05 | 0.027 | 22.25 | 0.15 | 0.12 | 2.64 | 1.91 | 0.265 |
| 4 | *Ficus vasta* | 4.83 | 2.274 | 0.206 | 0.91 | 0.021 | 26.15 | 0.14 | 0.12 | 3.22 | 2.05 | 0.133 |
| 5 | *Albizia gummifera* | 4.93 | 2.119 | 0.233 | 1.25 | 0.029 | 28.25 | 0.15 | 0.25 | 5.23 | 2.25 | 0.374 |
| 6 | *Olea capensis spp* | 4.93 | 2.604 | 0.277 | 1.17 | 0.046 | 32.10 | 0.14 | 0.50 | 5.98 | 3.42 | 0.644 |
| 7 | *Cordia africana* | 5.75 | 2.333 | 0.27 | 2.65 | 0.064 | 31.15 | 0.12 | 0.22 | 9.33 | 3.52 | 2.542 |
| 8 | *Ehretia abyssinica* | 5.50 | 3.048 | 0.337 | 4.00 | 0.061 | 30.05 | 0.14 | 0.11 | 9.68 | 2.55 | 2.838 |
| 9 | *Pouteria adolfi-friederici* | 5.10 | 2.525 | 0.306 | 1.40 | 0.059 | 29.65 | 0.18 | 0.08 | 6.50 | 3.37 | 1.591 |
| 10 | *Pavetta oliveriana* | 5.20 | 2.707 | 0.348 | 1.31 | 0.082 | 30.05 | 0.15 | 0.06 | 7.42 | 2.78 | 1.963 |
| 11 | *Ficus sur* | 5.25 | 2.289 | 0.291 | 1.82 | 0.051 | 29.45 | 0.11 | 0.33 | 6.69 | 3.13 | 0.857 |
| 12 | *Prunus africana* | 5.38 | 2.654 | 0.291 | 3.40 | 0.051 | 29.45 | 0.55 | 0.14 | 7.22 | 2.19 | 2.159 |
| 13 | *Phoenix reclinata* | 4.45 | 2.814 | 0.312 | 2.60 | 0.076 | 26.80 | 0.10 | 0.21 | 5.35 | 2.33 | 1.062 |
| 14 | *Polyscias fulva* | 5.63 | 2.449 | 0.304 | 3.05 | 0.06 | 32.25 | 0.13 | 0.71 | 7.52 | 2.96 | 1.853 |

pH (H2o, 1:2.5): E C-ds/m: Na, K, Ca, Mg & CEC –Cmol(+)/kg: T.N, O.C –(%): Av. P, Zn – (ppm).

Table 3. Percentage composition of species and families of dominant coffee shade trees of Bonga forest.

| Family name | Species name | *%Species count | *%Family count |
|---|---|---|---|
| Fabaceae | *Milletia ferruginea* | 22 | 24 |
| | *Albizia gummifera* | 2 | |
| Oleaceae | *Olea capensis* ssp | 15 | 15 |
| Araliaceae | *Schefflera abyssinica* | 11 | 12 |
| | *Polyscias fulva* | 1 | |
| Palmae | *Phoenix reclinata* | 11 | 11 |
| Rosaceae | *Prunus africana* | 10 | 10 |
| Dracaenaceae | *Dracaena steudneri* | 8 | 8 |
| Euphorbiaceae | *Croton macrostachyus* | 4 | |
| | *Sapium ellipticum* | 2 | 6 |
| Moraceae | *Ficus vasta* | 2 | 3 |
| | *Ficus sur* | 1 | |
| Rutaceae | *Vepris dainellii* | 2 | 3 |
| | *Teclea nobilis* | 1 | |
| Boraginaceae | *Cordia africana* | 2 | 3 |
| | *Ehretia abyssinica* | 1 | |
| Sapotaceae | *Pouteria adolfi-friederici* | 2 | 2 |
| Rubiaceae | *Pavetta oliveriana* | 2 | 2 |
| Melianthaceae | *Bersama abyssinica* | 1 | 1 |

* Percentages of total count in 10 quadrats

Table 4. Coffee counts and Desmodium population under shade trees in different quadrats.

| **Shade tree species and authority** | **Coffee count**[+] | **Desmodium population** |
|---|---|---|
| 1. *Milletia ferruginea*(Hochst.) Baker. | 21 | + |
| 2. *Ehretia abyssinica* R.Br.ex Fresen. | 22 | +++ |
| 3. *Olea capensis* ssp *welwitsdhii* Friis & P.S. Green. | 16 | ++ |
| 4. *Albizia gummifera* (Gmel.) C. A.Sm. | 19 | - |
| 5. *Schefflera abyssinica*(Hochst.ex A.Rich.) Harms. | 13 | +++ |
| 6. *Croton macrostachyus* Del. | 49 | +++ |
| 7. *Ficus vasta* Forssk. | 21 | +++ |
| 8. *Pouteria adolfi-friederici* (Engl.) Baehni. | 10 | ++ |
| 9. *Phoenix reclinata* Jacq. | 17 | + |
| 10. *Dracaena steudneri* Schw.ex Engl. | 15 | - |
| 11. *Prunus Africana* ( Hook.f.) Kalkam. | 12 | - |
| 12. *Vepris dainellii* (Pich.-Serm.) Kokwaro. | 10 | + |
| 13. *Cordia africana* Lam. | 20 | + |

[+] Total coffee count (stems/quadrat)

+++ = Densely populated *Desmodium* (> 20 stems/$m^2$ near sampled coffee plant)

++ =moderately populated *Desmodium* (10-19 stems/$m^2$ near sampled coffee plant)

+ = Very few *Desmodium* (1-9 stems/$m^2$ near sampled coffee plant)

- = no *Desmodium* around sampled coffee plant

## 4.2 AMF Spores

### 4.2.1 Identification and Characterization of spores

In this study, based on major differences in spore morphological appearance, four different types (genera) of spores were detected. These were: type I, II, III and IV. The first three spore types were found in all rhizosphere soil samples but the last type(IV) was absent in 36% of the rhizosphere soil samples under ***S. abyssinica, C. macrostachyus, F. vasta, O. capensis*** and ***P. reclinata*** (Table 6). These types were found under plant rhizosphere of lower spore densities (Table 6). Characteristics exhibited by the different types of spores are indicated (Table 5)

Table 5. Arbuscular mycorrhizal fungal spore characteristics

| TYPE | COLOR | SHAPE | DIAMETER( µM) | SUBTENDING HYPHAE | HYPHAL ATTACHMENT | TENTATIVE GENERA |
|---|---|---|---|---|---|---|
| **I** | Light yellow (Honey) to Brown (even Black) | Spherical | 150-175 | + | Simple | Glomus |
| **II** | White to Gray | Globose | 350-600 | + | Bulbous /Swollen | Gigaspora |
| **III** | Brown | Globose to Ellipsoidal | 100-150 | + | Bulbous /Swollen | Scutellospora |
| **IV** | Brown to Black | Round to Oblong | > 400 | - | - | Acaulospora |

Where: + = presence of subtending hyphae

- = absence of subtending hyphae (sessile)

### 4.2.2 AMF Spore density

Table 6. presents results from measurements of spore density (number of spores per 100 g dry soil) of 14 selected shade tree species. All rhizosphere soil samples generally displayed high spore densities. *Ficus vasta, C. macrostachyus and S. abyssinica* showed the largest number of spores while *E. abysinica*, *P. africana* and *P. fulva* exhibited the smallest spore density. Significant spore number variation was also displayed by different members of the same family of shade trees. In Araliaceae, *S. abyssinica* and *P. fulva* showed spore counts of 997 and 638/100g soil, whereas the family Moraceae was represented by *F. vasta* and *F. sur* with spore counts of 1313 and 753/100g of soil, respectively. Similarly the family Boraginaceae that contains *C. africana* and *E. abyssinica* showed spore count of 760 and 578/100g soil, respectively (Table 6).

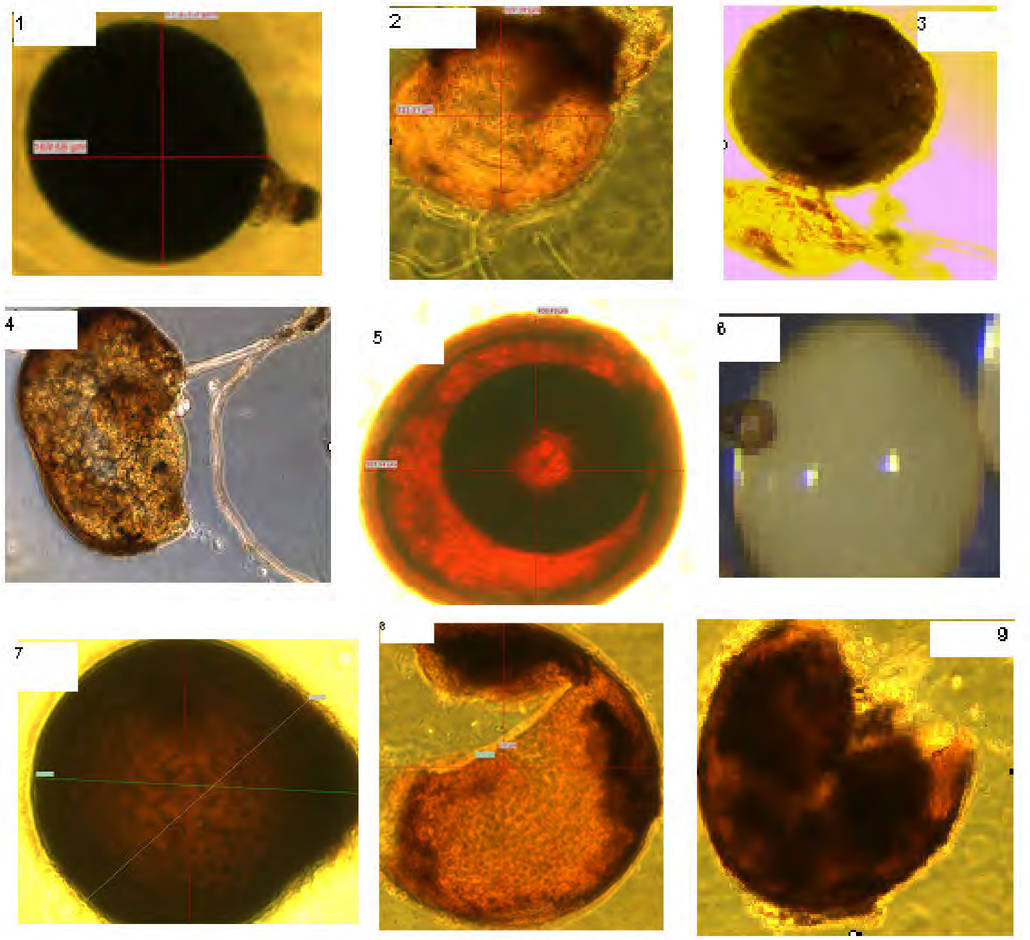

**Fig. I.** ***1–9***, Photomicrographs of arbuscular mycorrhizal fungi spores.***1&3***, Spores of Glomus *spp*. ***2&6***, Spores of *Gigaspora spp*. ***4&5,*** Spores of *Scuttelospora spp*. ***7***, Spores of *Acaulospora spp*. ***8***, Fractured spore of Gigaspora *spp*. in Melzer's reagent. Note the purple reaction of the innermost wall. ***9***, fractured spore of Scuttelospora spp. in Melzer's reagent. Note here Melzer's reagent reaction is deeper than ***8.*** (Scale of magnification: ***1, 3, 4, 5,*** and ***9*** = X400; ***2, 6, 7***, and ***8*** = X100)

**Table 6**. Spore density (number of spores per 100 g dry soil) of all spore types (mean of 6 replicates).

| Species | Total | Type 1 | Type 2 | Type 3 | Type 4 | pH |
|---|---|---|---|---|---|---|
| *Milletia ferruginea* | $670 \pm 17^{f}$ | $521 \pm 14^{h}$ | $86 \pm 5^{f}$ | $56 \pm 3^{bc}$ | $7 \pm 2^{c}$ | 5.10 |
| *Schefflera abyssinica* | $997 \pm 11^{c}$ | $807 \pm 12^{c}$ | $129 \pm 4^{c}$ | $61 \pm 4^{bc}$ | - | 4.90 |
| *Croton macrostachyus* | $1098 \pm 31^{b}$ | $892 \pm 15^{b}$ | $149 \pm 8^{b}$ | $57 \pm 2^{bc}$ | - | 4.70 |
| *Ficus vasta* | $1313 \pm 36^{a}$ | $1089 \pm 17^{a}$ | $171 \pm 7^{a}$ | $53 \pm 2^{c}$ | - | 4.83 |
| *Albizia gummifera* | $805 \pm 17^{e}$ | $631 \pm 5^{f}$ | $106 \pm 2^{d}$ | $63 \pm 2^{ab}$ | $5 \pm 2^{c}$ | 4.93 |
| *Olea capensis spp.* | $910 \pm 15^{d}$ | $728 \pm 15^{d}$ | $123 \pm 4^{c}$ | $59 \pm 2^{bc}$ | - | 4.93 |
| *Cordia africana* | $760 \pm 17^{e}$ | $576 \pm 10^{g}$ | $101 \pm 8^{de}$ | $68 \pm 3^{a}$ | $15 \pm 2^{ab}$ | 5.75 |
| *Ehretia abyssinica* | $578 \pm 11^{h}$ | $396 \pm 4^{j}$ | $125 \pm 2^{c}$ | $51 \pm 2^{c}$ | $6 \pm 2^{c}$ | 5.50 |
| *Pouteria adolfi-friederici* | $787 \pm 13^{e}$ | $623 \pm 8^{f}$ | $93 \pm 3^{ef}$ | $60 \pm 2^{b}$ | $11 \pm 2^{bc}$ | 5.10 |
| *Pavetta oliveriana* | $827 \pm 22^{e}$ | $654 \pm 4^{e}$ | $102 \pm 2^{d}$ | $54 \pm 3^{c}$ | $17 \pm 2^{a}$ | 5.20 |
| *Ficus sur* | $753 \pm 8^{e}$ | $592 \pm 9^{g}$ | $103 \pm 3^{d}$ | $49 \pm 3^{c}$ | $9 \pm 2^{bc}$ | 5.25 |
| *Prunus africana* | $635 \pm 9^{g}$ | $483 \pm 9^{i}$ | $96 \pm 3^{e}$ | $50 \pm 3^{c}$ | $6 \pm 1^{c}$ | 5.38 |
| *Phoenix reclinata* | $674 \pm 19^{f}$ | $563 \pm 17^{g}$ | $73 \pm 3^{g}$ | $38 \pm 2^{d}$ | - | 4.45 |
| *Polyscias fulva* | $638 \pm 14^{g}$ | $487 \pm 6^{i}$ | $81 \pm 3^{f}$ | $58 \pm 2^{bc}$ | $12 \pm 2^{b}$ | 5.63 |

Means followed by the same letter in the same column are not significantly different at 0.05 level.

## 4.3 Root length Mycorrhizal colonization

All shade tree species exhibited colonization by AM fungi (Table 7). Typical structures like arbuscules and vesicles were observed in all tree species. Shade tree species also showed similarity and differences in % colonization between members of the same family. In Fabaceae, *M. ferruginia* and *A. gummifera* presented a non significant difference in colonization (68% and 71%, respectively). However, in Araliaceae, *S. abyssinica* and *P. fulva* (76% and 56 %); in Moraceae, *F. vasta* and *F. sur* (81% and 65%); in Boraginaceae, *C. Africana* and *E. abyssinica* (67% and 52 %) exhibited significant difference in colonization percentages (Table 7).

***F. vasta, C. macrostachyus, S. abyssinica and O. capensis*** showed the highest level of colonization (Table 7). ***E. abyssinica, P. africana and P.fulva*** exhibited the lowest level of colonization.

Root length colonized by AMF for coffee was found to be generally higher as compared to tree species with in the same rhizosphere soil characteristics except that VC% is generally lower coffee than in shade trees. (Table 7&8).

**Table 7**. Different types of root colonization percentages of shade trees (means of 6 replicates

| Species | HC (%) | MHC (%) | AC (%) | VC (%) |
|---|---|---|---|---|
| 1. *Milletia ferruginea* | 69 | 51 | 5 | 11 |
| 2. *Schefflera abyssinica* | 76 | 57 | 6 | 12 |
| 3. *Croton macrostachyus* | 81 | 60 | 4 | 14 |
| 4. *Ficus vasta* | 81 | 62 | 3 | 16 |
| 5. *Albizia gummifera* | 71 | 51 | 6 | 12 |
| 6. *Olea capensis spp.* | 73 | 55 | 5 | 12 |
| 7. *Cordia africana* | 67 | 47 | 7 | 10 |
| 8. *Ehretia abyssinica* | 52 | 39 | 6 | 6 |
| 9. *Pouteria adolfi-friederici* | 69 | 52 | 5 | 11 |
| 10.*Pavetta oliveriana* | 72 | 53 | 6 | 12 |
| 11. *Ficus sur* | 65 | 45 | 9 | 8 |
| 12. *Prunus africana* | 54 | 40 | 3 | 7 |
| 13. *Phoenix reclinata* | 61 | 43 | 8 | 8 |
| 14. *Polyscias fulva* | 56 | 42 | 5 | 8 |

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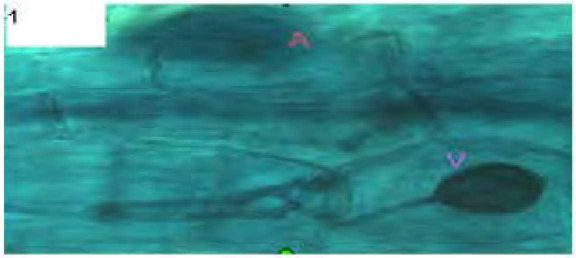

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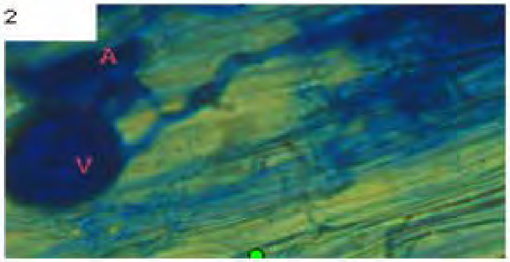

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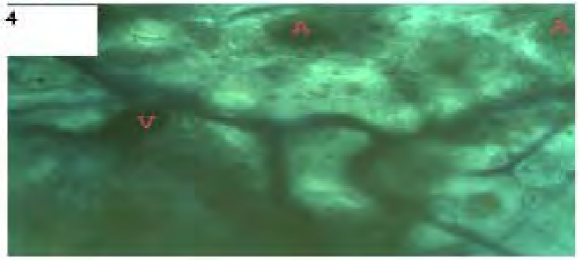

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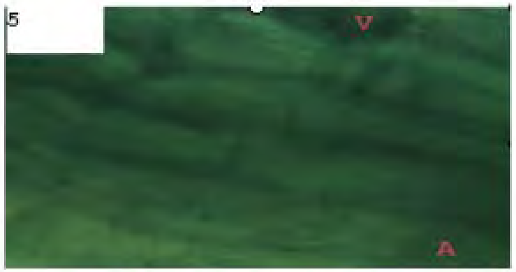

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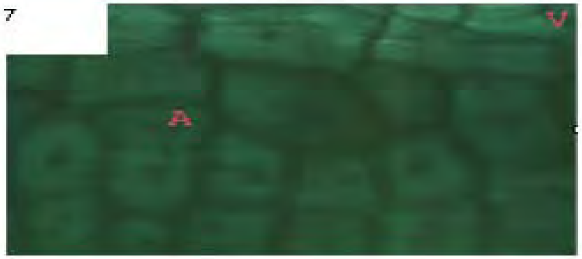

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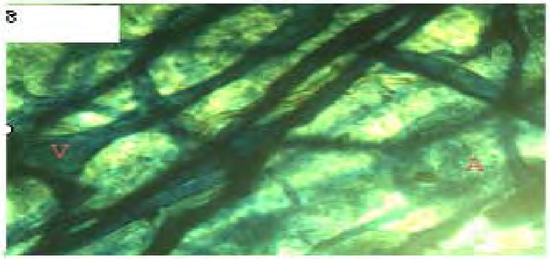

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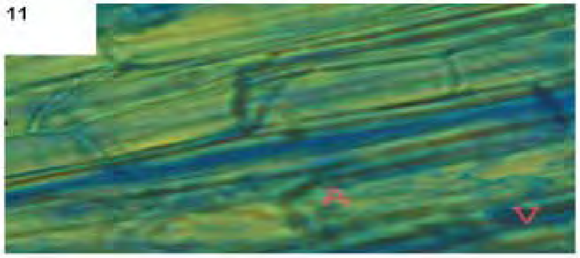

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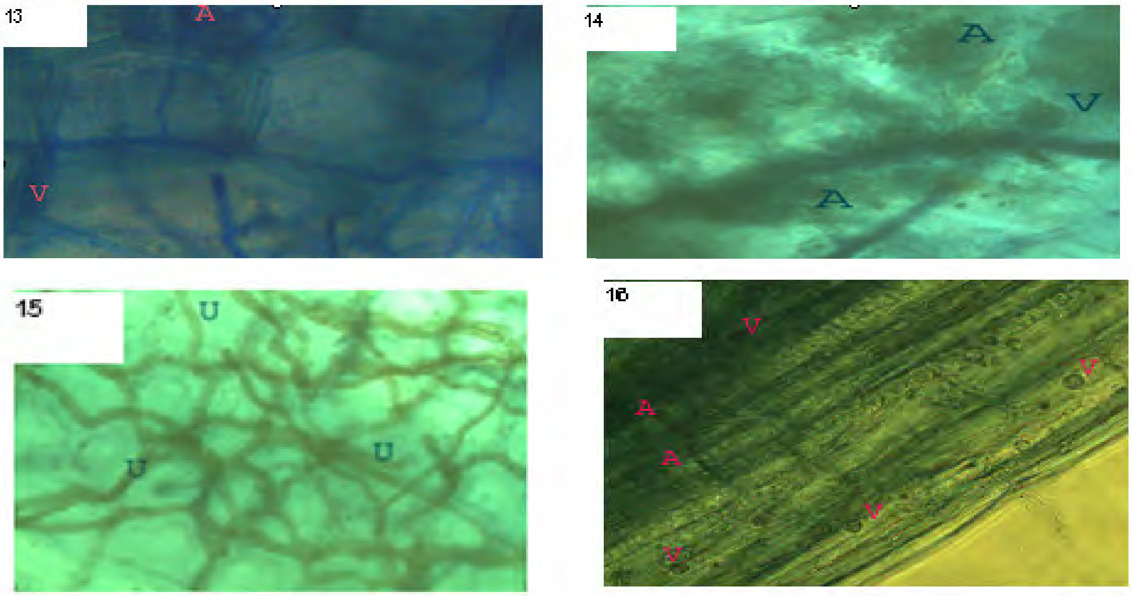

**Fig.II**. ***1-16,*** Photographs of colonized roots of tree species with hyphae, arbuscules and vesicles indicated: ***1,*** *Milletia ferruginea;* ***2,*** *Schefflera abyssinica;* ***3,*** *Croton macrostachyus;* ***4,*** *Ficus vasta;* ***5,*** *Albizia gummifera;* ***6,*** *Olea capensis spp.;* ***7,*** *Cordia africana;* ***8,*** *Ehretia abyssinica;* ***9,*** *Pouteria adolfi-friederici;* ***10;*** *Pavetta oliveriana* ***11,*** *Ficus sur;* ***12,*** *Prunus africana;* ***13,*** *Phoenix reclinata;* ***14,*** *Polyscias fulva;* ***15,*** *Prunus Africana.* Note here peculiar structures like arbuscules and vesicles were not observed; ***16,*** *Coffee tree.* (Scale of magnification = X100)

Coffee trees under the canopy of *F. vasta*, *C. macrostachyus, S. abyssinica, O. capensis spp., P. oliveriana, P. adolfi-friederici and A. gummifera* (87%, 83%, 80%, 80%, 79%, 78% & 78%, respectively) presented the highest colonization percentage. Coffee trees under *E. abyssinica*, *P. Africana, P. fulva and P. reclinata* (57.9%, 58.7%, 59.8% & 67%, respectively) exhibited the lowest colonization percentages (Table 8). Counter example of intensity of colonization of coffee root by AMF is indicated pictorially (Fig.II, ***16***).

**Table 8**. Different types of root colonization percentages of coffee plants under shade trees (means of 6 replicates).

| Coffee trees under shades | HC (%) | MHC (%) | AC (%) | VC (%) |
|---|---|---|---|---|
| 1. *Milletia ferruginea* | 76 | 56 | 13 | 5 |
| 2. *Schefflera abyssinica* | 80 | 60 | 15 | 4 |
| 3. *Croton macrostachyus* | 83 | 61 | 16 | 2 |
| 4. *Ficus vasta* | 87 | 64 | 18 | 4 |
| 5. *Albizia gummifera* | 78 | 57 | 19 | 2 |
| 6. *Olea capensis spp.* | 80 | 57 | 18 | 3 |
| 7. *Cordia africana* | 73 | 55 | 12 | 7 |
| 8. *Ehretia abyssinica* | 58 | 39 | 8 | 9 |
| 9. *Pouteria adolfi-friederici* | 78 | 57 | 14 | 6 |
| 10. *Pavetta oliveriana* | 79 | 56 | 14 | 7 |
| *11 .Ficus sur* | 71 | 57 | 10 | 8 |
| *12 .Prunus africana* | 59 | 36 | 9 | 7 |
| *13 .Phoenix reclinata* | 67 | 47 | 9 | 10 |
| 14. *Polyscias fulva* | 60 | 42 | 8 | 8 |

## 4.4 Soil characteristics and Mycorrhization

Table 9 presents correlation between soil characteristics, AM colonization and spore density. Spore densities were inversely correlated with O.C, total N, available P, EC and Zn (Table 9).

Correlation analyses also indicated that HC, MHC and AC were inversely correlated with O.C, total N, available P, EC and Zn. Vesicular arbuscular was not significantly correlated with any one of the soil characteristics tested. Only HC % was negatively correlated with pH (with r = 0.540) at 0.05 level of confidence.

Similar trend of correlation analysis was observed between coffee trees and soil characteristics. Pearson's correlation coefficients for all parameters measured for coffee were presented in table 10.

**Table 9**. Correlation (r) between soil parameters and colonization percentage of AMF and spore density of shade trees.

| | **Soil parameters** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **pH ($H_2O$)** | **O.C** | **T.N** | **Av. P** | **EC** | **CEC** | **Zn** |
| **HC** | -0.540* | -0.647* | -0.704** | -0.825** | -0.627* | -0.472ns | -0.736** |
| **MHC** | -0.528ns | -0.691** | -0.741** | -0.823** | -0.642* | -0.495ns | -0.74** |
| **AC** | -0.503ns | -0.645* | -0.712** | -0.791** | -0.621* | -0.510ns | -0.703** |
| **VC** | -0.021ns | 0.062ns | 0.292ns | 0.37ns | 0.357ns | 0.176ns | 0.038ns |
| **SD** | -0.509ns | -0.599* | -0.742** | -0.795** | -0.688** | -0.514ns | -0.727** |

**- Correlation is significant at the 0.01 level.
* - Correlation is significant at the 0.05 level.
ns- non significant at 0.05 level.

**Table 10.** Correlation (r) between soil parameters and colonization percentage of AMF for Coffee

| | **Soil Parameters** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **pH(H2O)** | **O.C** | **T.N** | **Av. P** | **EC** | **CEC** | **Zn** |
| **HC** | -0.555* | -0.696** | -0.708** | -0.853** | -0.592* | -0.486ns | -0.76** |
| **MHC** | -0.54* | -0.647* | -0.704** | -0.823** | -0.627** | -0.472ns | -0.738** |
| **AC** | -0.503ns | 0.645ns | -0.712** | -0.791** | -0.621* | -0.510ns | -0.703** |
| **VC** | -0.021ns | 0.062ns | 0.292ns | 0.037ns | 0.357ns | 0.176ns | 0.038ns |

**- Correlation is significant at the 0.01 level.
* - Correlation is significant at the 0.05 level.
ns- non significant at 0.05 level.

# 5. Discussion

The results of this investigation revealed that Bonga coffee forest is composed of various types of dominant shade tree species. Although the coffee forest is represented by 19 species falling in 13 families of shade trees, in terms of density only six species covered 80% of the population of the sampling site. In general, this forest is characterized by high number of stems (254 stems/ha) of dominant coffee shade trees.

In the present study, *O. capensis* , *S. abyssinica*, *P. reclinata* and *P. africana* were encountered as dominant shade tree species with percentage occurrences of 15, 12, 11 and 10 %, respectively. These trees were not reported in other studies (FAO, 1968; Demel and Tigeneh, 1991). Nevertheless, our results strongly agree with investigation conducted at Yayu national forest priority area (Taye, 2001).

*Milletia ferruginea, Albizia gummifera, Cordia africana, Croton macrostachyus* and *Dracaena steudneri* comprise 40 % of the total number of plant species. At Bonga coffee forest, 24% of the dominant shade trees were principally represented by leguminous trees such as *M. ferruginea* and *A. gummifera*. On the other hand, investigation made at Yayu national priority area revealed exceedingly higher percentage of leguminous plant including Acacia spp. which had not been found in Bonga forest 40% ( Taye, 2001).

In terms of shade tree species composition, this coffee forest is by far more heterogeneous than what has been reported from both south western (FAO, 1968b) and eastern (Demel and Tigeneh, 1991) part of the country. The highest stem counts (55 stems/ha) of ***M. ferruginea***, the frequent abundance of ***Desmodium*** sp. and other legume seedlings and saplings in this forest, could not underestimate the remarkable role of legume plants. ***Desmodium*** spp. was encountered in 10 of the thirteen sampling tree species with varying degrees of abundance (Table 4). Enormous number of nodules on roots of *Desmodium* species were observed. This may indicate that they may be responsible for replenshment of removed nitrogen by larger non-nitrogen fixing nearby plants.

During the reconnaissance survey extremely large number of coffee seedlings and saplings were observed in the study area. Evidently, higher density (71 %) of *Coffea arabica* L. was recorded in Bonga coffee forest which is by far greater than what has been reported elsewhere (Taye, 2001). Furthermore, this high stem density (613 stems/ha) of coffee recorded in this forest may suggest the coffee forests' potential for genetic resources conservation of coffee and for promotion of globally renewed interest in cultivation of organic coffee.

Different genera of arbuscular mycorrhizal fungi were recovered from soil samples obtained from Bonga coffee forest indicating diversity among populations. Based on spore morphological characters observed and comparing the features, four groups of AMF have been identified.

Among these groups, Glomus (type1) was the predominant taxonomic group which showed the highest counts in all rhizosphere soils under each tree species. Its dominance was more significant under the rhizosphere of F. vasta, C. macrostachyus, S. abyssinica and O. capensis which showed 83%, 81%, 81% and 80% of total spores, respectively. The dominancy of Glomus was also supported with report from dry afromontane forests of Ethiopia (Tesfaye *et al.*, 2003b), the tropical rain forest of Xishuangbanna, China (Zhao *et al., 2001*), tropical rain forest in Mexico (Guadarrama and Alvarez-Sanchez, 1999), and arid and semi arid lands of North Jordan (Mohammad *et al.,* 2003). Glomus species were also the most frequently encountered fungi in the fecal samples collected from terrestrial and arboreal small mammals in a Panamian cloud forest with 87% frequency of occurrence in the samples (Mangan and Alder, 2000). This has a positive consequence in dispersal of AMF in a given habitat/ecosystem. The rhizosphere soil pH of different shade trees was slightly acidic to acidic (Table 2) that may have favored this genus as reported by Frioni *et al*., (1999).

The second dominant spore type encountered was Gigaspora type AMF. This genus was also found to be abundant at lower pH (Table 6). It was also present in all soil samples. The presence of conspicuous swelling directly below the spore on the subtending hyphae is a typical feature of the genus (Brundrett *et al.*, 1996; INVAM, 2004; Merryweather, 2004). No sporocarps were seen and spore surface was smooth (Fig.I, *6*).

The 3rd dominant was genus Scutellospora. It is also known for its bulbous subtending hyphae and strong Melzer's reagent reaction (Brundrett *et al*., 1996; Merryweather, 2004; INVAM, 2004). Although it was found in all rhizosphere soil samples, it was dominat on *Cordia africana* and *Shefflera abbysinica*. However, its distribution on other shade tree species was not as significant as Glomus and Gigaspora. This genus mimics with Gigaspora (type 2) except slight shape variation and response to Melzer's reagent reaction. Scutellospora exhibit strong response to Melzer's reagent reaction (Merryweather, 2004; INVAM, 2004) (Fig. I, *8&9*).

The least dominant spore type was the genus Acaulospora. It is large black sessile spore (Trappe, 1982; Shenck and Pereze, 1988 cited in Frioni et al., (1999); Gerdemann and Nicolson, 1963). It was absent in 36% of rhizosphere soils in ***S. abyssinica***, ***C. macrostachyus***, ***F. vasta***, ***O. capensis ssp***. and ***P. reclinata*** (Table 6). These types were found under plant rhizosphere of lower spore densities. This could be due to reduction of competition between spore types for colonization because the number of other types of spores was decreased.

In addition to that this taxonomic group was encountered in rhizosphere soils of relatively lower pH. Such a distribution is expected on slightly acidic to acidic rhizosphere soils. This spore type was found to be dominant in soils with high pH (Frioni *et al*., 1999).

Million, (2002) reported that more than 80% spore extracted beneath *Acacia tortilis* was Glomus. Similarly Munro *et al*., (1998) found out that Glomus spores were the dominant beneath the host tree *Acacia tortilis*. On the other hand Shasho, (2002), reported that 50.7% of the extracted spores from the rhizosphere of *Erythrina brucei* were Gigaspora and 38.8% were Glomus. This contrast may be attributed to host preference, changes in abiotic factors or seasonal variations.

The distribution of arbuscular mycorrhizal fungi on the basis of spore density showed that there existed significant spore density differences among the tree species tested (Table 6). The highest spore counts were observed under *F. vasta,* C. *macrostachyus* and *S. abyssinica.* This might be due to the cumulative effect of low level of P, favorable shade condition and the largest population of Desmodium (legume shrub) encountered under these trees (Table 2 and 4). In this context, Colozzi and Cardoso, (2000) demonstrated that the legume intercropping cultivation increased the concentration of AMF spores in the soil. The least spore counts were encountered under *Ehretia abyssinica*, *Prunus africana* and *Polyscias fulva.* This could be due to the relatively higher level of P concentration in the soil, lower population of Desmodium and genetic variations among the tree species.

In this work the least average number of AMF spores extracted (578/100g) (Table 6) was larger as compared with the previous findings of Yonas, (2005) who reported 57.9 spores per 100g dry soil of *Acacia polyacantha* in a dry savannah wood land ecosystem. Similarly Shasho, (2002) reported more than 300 spores per 100g soil beneath *E. brucei* from a high land wood land ecosystem. Musoko *et al*., (1994) estimated 200-500 spores per 100 g soil which are closer to the minimum average number of AMF spores extracted per 100g of soil observed in the present study (578 spores/100g soil, Table 5).

The pattern of number of spores in coffee rhizosphere soil was similar to respective shade tree species. Generally, the density of spores obtained in the present study was higher. This may be due to the fact that the land is under the natural forest where there was no disturbance on the vegetation cover.

It has been demonstrated that the translocation of part of the assimilated nitrogen in the non-nitrogen fixing plants originates from the legume partner in particular habitat, is believed to be mediated by mycorrhizal fungi. Under such situation, nitrogen is transferred from the legume to the non-legume through mycelia of mycorrhizal fungi without entering the soil solution (Martensson *et al*., 1998).

Therefore, the dominance of *M. ferruginea*, the presence of *A. gummifera*, the dense population of *Desmodium* and other legume plant seedlings and saplings greatly contribute to such essentially important interactions to support the normal growth of coffee and others in that natural forest.

The level of AM colonization was high in all tree species. *F. vasta*, *C. macrostachyus, S. abyssinica* and *O. capensis spp.* presented the highest percentage with 81%, 81%, 76% and 73% of root segments invaded, respectively (Table 7). The lowest levels (52%, 54% and 56%) were presented by *E. abyssinica, P. african* and *Polyscias fulva*, respectively (Table 7). This situation may be explained by differences in abiotic factors in the rhizosphere soil and genetic variations among the tree species.

The pattern of % root infection was also found to vary between members of the same family. In Fabaceae, *M. ferruginia* and *A. gummifera* presented a non significant difference in colonization (69% and 71%, respectively). However, the other showed significant difference with the highest in Moraceae, *F. vasta* and *F. sur* (81.28% and 65.33%); in Araliaceae, *S. abyssinica* and *P. fulva* (76% and 56 %) and the least in Boraginaceae, *C. africana* and *E. abyssinica* (67% and 52 %)(Table 6).The variations in some species with relation to the degree of colonization and presence/absence of AMF in the same or different collecting places, seem to indicate that environmental factors linked to the host influence the presence and mycorrhizal colonization level (Alexander 1989). Similarly it was observed such a variation of root colonization at genus and family levels (St. John 1980).

A direct relationship between colonization percentage and spore density in soil was found in each tree species (Table 6 and 7). Jasper *et al*., (1993) and Frank and Morton (1994) concluded that sporulation was directly correlated with AM colonization.

Root length colonization of coffee grown under different shade tree species showed the same pattern of colonization with higher percentage than tree species with in the same rhizosphere soil (Table 8). This may be because of ecosystem functioning of the increased plant diversity around the coffee tree. Grime *et al*., (1987) showed that the transfer of assimilates from one plant to understorey component is facilitated through a common mycorrhizal network that may necessitate more AM fungal colonization. Apart from this some edaphic and environmental factors may contribute to the variation in root length colonization of coffee under different shade trees.

Correlation analyses indicated that HC, MHC, AC and spore density were negatively correlated with O.C, total N, available P, EC and Zn (Table 9). Bohrer *et al*., (2004) concluded that aboitic factors had minimal influence on AMF colonization variation, so AM colonization dynamics was in response to plant phenology. While the present result indicated that correlations were very significant between edaphic factors and AMF colonization except for vesicular colonization. This strongly supported the opinion that climatic factors and edaphic factors could influence the AMF colonization (Udaiyan *et al*., 1996; Staddon *et al*., 2003). AMF could enhance plant uptake of P and other nutrients, especially in nutrient deficient environment (Gupta *et al*., 2002).

The inverse correlation between Zn and SD and Zn and AMF colonization might be due to the toxic nature of Zn. When $Zn^{2+}$ and $Cd^{2+}$ present in excess, these ions are generally assumed to be the chemical species that are taken up by and are toxic to soil microbes (Giller *et al*., 1998). On the contrary, in previous studies on AMF and Cu (Griffioen *et al*., 1994) and Zn and Cd (Weissenhorn and Leyval, 1994), no correlation was found between the concentration of these metals in sludge-amended agricultural soils and AMF populations/colonization. The same trend was also observed on coffee trees (Table 10).

The importance of mycorrhiza to coffee has been reported by several investigators indicating that coffee plants are heavily mycorrhizal under natural conditions inferred that coffee plants are heavily mycorrhizal under natural conditions. These benefits include enhanced growth and increased P and Zn uptake of young coffee seedlings in nursery conditions (Lopes *et al*., 1985; Siqueira *et al*., 1998); enhanced tolerance to nematodes (Vaast *et al*., 1998) and increased survival of coffee plants after field transplanting and in agroforestry systems (Sieverding and Toro, 1986 cited in Vaast and Zasoski, 1992). Agroforestry systems can increase soil nutrient availability and accelerate P cycling because the deeper tree roots can (1) retrieve nutrients from lower soil horizons (Young, 1997), (2) enhance the chemical and physical quality of soils and (3) increase soil microbial activity (Cooper *et al*., 1996).

# 6. Conclusion and Recommendations

This work recognizes the diversity and population density of coffee shade trees and AMF versus nutrient dynamics in a coffee agroforestry system in Bonga, South western part of Ethiopia. The results of this investigation revealed that Bonga coffee forest is composed of various types of dominant shade tree species. Although the coffee forest is represented by 19 species falling in 13 families of shade trees, in terms of density only six species covered 80% of the population of the sampling site. Extremely large number of coffee seedlings and saplings were observed in the study area. Evidently, higher density (71 %) of *Coffea arabica* L. was recorded in Bonga coffee forest which is by far greater than what has been reported elsewhere.

Arbuscular Mycorrhizal fungi are commonly considered to be non specific with respect to their host and their ability to colonize particular plant species. Conversely, it is investigated that plant species differ in their ability to form and benefit from mycorrhizal relationship. In general *Ficus vasta* was totally displayed the highest number of spore count and colonization, and also showed highest number of the three spore types followed by *Croton macrostachyus.* Similarly, the shade trees with low spore density were found to harbour all morphological types. AMF also vary in the manner in which they assist their host in different conditions through promotion of nutrient uptake.

Research on the plant–mycorrhizal fungal interaction will always be hindered by a basic asymmetry. While plants are easily counted and measured, measurements of the fungal community are elusive. Nonetheless, the observation of high diversity within a fungal community, as well as the diverse approaches required to detect them, give a glimpse of the complexity within the fungal community and dramatically illustrate the limits of understanding of mycorrhizal fungal community processes and dynamics.

As knowledge of the distinct ecologies of individual fungal species grows, simple assumptions about the influence of mycorrhizae on plant communities need to be reevaluated. Such a reevaluation will enhance the appreciation of belowground organisms as dynamic participants in plant community processes, and of belowground biodiversity as an essential component of ecosystem health.

This study didn't investigate the whole range of organisms in the rhizosphere which could give benefits to trees. So, it is difficult to draw conclusion that the well being of the shade trees and coffee plants in the natural forest is due to only AMF colonization.

However, AMF could be considered to be a future tool in agriculture, i.e. fertilizer substitute and as biocontrol agent. So, it is recommended that-

a) studying the ecology, diversity and host range of AMF is of enormous importance before application.
b) their relation with nutrient dynamics and other soil characteristics should be evaluated before use .
c) these organisms should receive the proper management.
d) the impact of litter fall on AMF spore density and diversity should be evaluated in the future.

Finally, this symbiosis command great interest as the subject of future research with relation to food security.

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