TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABSTRACT

Manipulation of the AM fungal symbiosis for minimum input agricultural system provides an alternative to use of chemical inputs which have been associated with pollution and degradation of soil. The diversity, characteristics and distribution of AMF indigenous to the southern Guinea savanna of Nigeria were observed in this study. AMF spores were extracted from four soils from the zone, using the wet-sieving and decanting/density gradient centrifugation method. Thereafter, spores were enumerated with the aid of a stereomicroscope. Characterization of the AMF spores was carried out using reaction to Mezler's reagent and microscopic morphological features. The soil pH, Effective Cation Exchange Capacity (ECEC), Organic Matter (OM), Nitrogen and Available Phosphorus contents of the soil were determined. Characterization of the AMF spores were carried out using morphological (scan electron microscopy and light microscopy). Fungal spores encountered varied in colour (white, orange, reddish brown and black), size (188.16 µm - 412.66µm) and shape (globose, subglobose, and oblong). Thirteen Arbuscular mycorrhizal species: *Scutellospora reticulata, Acalospora laevis,Glomus intraradices, Scutellospora calospora, Gigaspora margarita, Enthrophosphora infrequens, Glomus pansihalos, Glomus tortuosum, Paraglomus brasilianum, Paraglomus occultum, Glomus manihotis, Gigaspora decipien* and *Scutellospora pellucida* were identified in the soil samples. The spores of *Glomus* and *Scutellospora* species were abundant in soils of this zone (30.77% and 23.08% respectively). *S. reticulata* and *G. pansihalos* predominated the soils studied. Significant negative correlations were observed between AMF spore population and soil factors: Soil pH (r = -0.70*); %OM content (r = -0.98*); ECEC (r = -0.79*); % Nitrogen (r = -0.95*) and available Phosphorus (r = -0.74*)

CHAPTER ONE

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts of an estimated 80-90% of vascular plants and some nonvascular plants, such as mosses (Smith and Read, 1997). Compared to other diversity of endomycorrhizal associations, this symbiosis is the most prevalent and is a type of endomycorrhiza in which the fungus penetrates cortical cell walls. (Hennigan, 2009) The hyphae develop mycelium, arbuscules and in most fungal genera, vesicles in roots (Sharif and Moawad, 2006). Arbuscular mycorrhizal (AM) used to be classified as Vesicular Arbuscular Mycorrhizal (VAM) but research uncovered that a major suborder did not form thin-walled, lipid –filled vesicles, so they are referred to as AM association today (Hennigan, 2009). They represent the most widespread and probably most ancient type of association in which the large majority of terrestrial plants must have evolved with compatibility. Both fossil (Remy et al., 1994) and molecular phylogenetic evidence (Simon et al., 1993) support the hypothesis that terrestrial plants evolved with the existing Arbuscular mycorrhiza relationship (Jeffries et al., 2003). AMF receive 3-20% of photosynthate from their host plants in exchange for the transfer of soil-derived nutrient to roots (Treseder and Cross, 2006). Dense AM infections are common in most species of leguminoseae and gramineae. Most of the economically important crops are infected by AMF (Sharif and Moawad, 2006). Morton and Benny (1990) categorized species of AMF in order Glomales encompassing six genera namely; Glomus, Sclerocytis, Entrophosphora, Acaulospora, Gigaspora and Scutellospora and few species about one hundred fifty (150) have also been identified based on the method of spore formation (Mortoa and Benny, 1990; Jeffries et al., 2003).

AMF are probably the most abundant fungi in agricultural soils. Mycorrhizal fungi play important roles in defining the ecological niches occupied by plants and determination of plant composition (Francis and Read, 1995). They have also been reported to regulate

ecosystem responses to environmental change at local to global scales (Treseder and Cross, 2006). Mycorrhizas take over an important role in the survival of plants and additionally enlarge the ecological width of plant species. Cardoso and Kuper (2006) pointed out that Arbuscular Mycorrhizas have the ability to enhance host plant uptake of relatively immobile nutrients in particular P and several micronutrients. The transfer of soil-derived nutrients to roots by AM fungi in exchange for photosynthate from their host plants influence carbon (C) fluxes and nutrient dynamics among plants, soils and atmosphere (Treseder and Cross 2006). Jeffries *et al.* (2003) documented the fact that mycorrhizal plants provide the fungus with photosynthetic C, which in turn is delivered to the soil via fungal hyphae. The extra radical hyphae of AMF therefore act as a direct conduit for host C in the soil and contribute directly to its C pools by passing the decomposition process.

Phosphorus nutrition of plants through Arbuscular Mycorrhiza involves metabolically dependent processes (Smith and Gianinazzi-Pearson, 1988). One enzyme that has been identified as active in Arbuscular Mycorrhiza is fungal alkaline phosphatase (Oliver *et al*., 1983). It has been suggested that this enzyme may somehow be involved in the processes of phosphorus acquisition in mycorrhiza plants (Gianinazzi *et al*., 1992). Tisserant *et al. (*1993) have shown that the quantity of enzymically active fungal biomass increases sharply prior to growth stimulation, and then decreases with age of the infection.

Rhizosphere interactions occur between AMF and other soil microorganisms such as nitrogen fixing bacteria with effects on plant nutrient balances (Paula *et al*., 1993). AM fungi interact with heavy metals/micro nutrients. They can restore the equilibrium of nutrient uptake that is misbalanced by heavy metals (Carneiro *et al*., 2000). Jeffries *et al.*

(2003) reported that Arbuscular mycorrhiza has the ability to ameliorate the toxic effect of heavy metals and organic xenobiotics. AMF can alleviate Aluminium (Al) toxicity (Cardoso and Kuyper, 2006). Furthermore, AMF can accelerate revegetation of severely degraded lands such as coalmines or waste site containing high level of heavy metals, (Marx and Altman, 1979). They can also form an integral component of successful revegetated flue-gas desulphurization sludge ponds (Wilson *et al.*, 1991). Decrease in plants susceptibility to disease as a result of AM infection was reported by Olsen *et al*. (1991) and Cardoso and Kuyper (2006). *Striga* (parasitic weed) infestation on cowpea was delayed by AM (Gworgor, 1992) and the emergence of *Striga* in *Sorghum* was absolutely controlled by AM (Gworgwor and Weber 1992)

AMF aid early establishment and growth of nursery seedlings and prevent soil erosion. These fungi also impart resistance to stress conditions such as drought and high salt concentration. Mycorrhizal colonization of roots has been shown to increase drought tolerance of maize (Subramanian *et al*., 2006); wheat (Al-Karaki, 1998); soybean (Bethlen Falvay *et al.*, 1998); onion and lettuce (Azcon and Tobar, 1998) and redclover (Fitter, 1998). AM symbiosis also improved leaf water potential (Ruiz-Lozano, 2003). Leaf water potential was higher in stressed soybean with AM than that in corresponding non-AM plants. The potential mechanisms include extensive absorption of water by external hyphae (Auge, 2003) stomata regulation through hormonal signals (Goicoechea, *et al.*, 1997), an indirect effect of improved P nutrition upon water relations (Fitter, 1998) and greater osmotic adjustment in mycorrhizal plant (Auge *et al.*, 1994).

Non-mycorrhizal plants occur in habitats where the soils are very dry, saline, water logged, severely disturbed and where soil fertility is extremely high or low (Brundrett, 1991). Climatic factors play important role in the establishment of Arbuscular mycorrhiza since they act on soil characteristics and control the physiology of host plants. Illumination is one of the required factors by mycorrhizal plants to allow optimum photosynthesis. In darkness, the plant may not have extra sugars available to give to the fungus. Inoculation attempts often fail during the winter, even in greenhouses, because the day length is too short to allow optimum photosynthesis (St. John, 1985).

Justification and objectives of the study

The Southern Guinea Savanna (SGS) of Nigeria is generally characterized by coarse textured surface soils which are low in organic matter and chemical fertility. These soils have, therefore, been classified (FAO Classification) as Luvisols, Ferrasols and Lithosols (Salako, 2003). None of the soils in the zone was rated as class 1 with high productivity. Most of the Southern Guinea Savanna soils fall into class 4. Crop productivity in the Southern Guinea Savanna agro ecological zone of Nigeria has reduced over the years dueto some characteristics of the soil. Sanchez *et al.* (2003) identified these characteristics in tropical soils as low soil moisture, low nutrient capital, erosion risk, low pH, high phosphorus fixation, low levels of soil organic matter, Aluminum toxicity and loss of soil biodiversity. Eroarome (2005) also reported that the soils of the zone usually have low productivity due to inadequate moisture retention capacity and low organic matter. They are physically fragile and prone to degradation because the topsoil contains large proportion of sand and with weak aggregation, because of the low level of organic matter

in this layer. The physical constraints are further compounded in gravelly soils or soils with shallow depth overlying plinthic or hardpan layer (Salako *et al*., 2002). Effects of parasitic organisms and weeds are not left out (Gworgwor, 1992 and Gworgwor and Weber 1992).

In the last century, Green Revolution Technology such as the use of pesticides, synthetic fertilizers and high yielding cultivars were used to overcome these constraints (Dalgaard *et al*., 2003). With this technology, food supply in this zone increased, reducing hunger and improving nutrition. Nevertheless, numberless people have no food security (Stocking, 2003). The Green Revolution techniques increased natural resource degradation, raising question about the sustainability of current agricultural practices (Dalgaard *et al*., 2003). Furthermore, these conventional agricultural systems contribute to loss of plant diversity. In conventional high – input systems, for example the addition of chemicals are continual disturbances to the soil system which may affect intrinsic abiotic and biotic soil factors possibly leading to long-term soil degradation (Bethlenfalvay and Linderman, 1992). The use of machines and fertilizers are considered to be responsible for degradation (Rosa Junior, 1984; Gaur and Adholeya, 2004). Chemicals used under these systems pollute both the soil and the atmosphere and also jeopardize the health of both farm workers and consumers. The ecosystem is contaminated with heavy metals. This refers to elements with specific mass higher than 5g cm^{-3}, able to form sulphides (Gaur and Adholeya, 2004). Soil degradation produces change in the diversity and abundance of AM fungal populations (Koomen *et al;* 1990). This is critical because of the role of mycorrhizal fungi in plant

establishment and survival. Such elimination of arbuscular mycorrhizal fungal population can lead to problems with plant establishment and survival.

Even if AM fungi are ubiquitous in terrestrial ecosystem, mechanical or chemical disturbance of the soil can substantially reduce their population, vigor and functioning. The number of spores and root colonization of plants occurring at sites are often reduced by soil disturbance.

The conventional systems of agriculture are too expensive for smallholder farmers in a developing country like Nigeria. The benefits of soil additives are usually short-lived, unless slow release formulations are used and are not sustainable in low input or natural ecosystem. The challenge for the next 50 years is to double food production in a way that does not compromise environmental integrity and public health (Tilman *et al.,* 2002). For better nutrient management in Southern Guinea Savanna of Nigeria, an increase in use of biological potential is important.

The soil of Southern Guinea Savanna (SGS) has been classified by Kogbe and Adediran (2003) to be Typic Paleustalf (USDA), and loamy sand Alfisol with moderate pH and sandy loam texture in some other areas. Total P has been reported to be higher in forest soil than in Savanna (Adepetu, 1970; Adepetu and Corey, 1975) hence cropping requires additional fertilizer input to maintain good yields. However application, of high rate of P fertilizer was reported to be capable of causing nutrient imbalance and consequently yield depression of western yellow maize (Osiname, 1979). Aduloju and Olaniran (2001)

presented experimental evidences that the soil of Southern Guinea Savanna soils of Nigeria is low in organic matter content, available P and effective cation exchange capacity. Obi *et al.* (2008) also reported high infestation of termite in the soil of SGS of Nigeria. The termites feed, build and repair their nest and galleries out of soil fabrics and organic material therefore influencing soil fertility. Poor fertility of SGS soils can be overcome by management strategies. Critical level of Aluminium saturation can be kept deep in the profile by management techniques. Phosphate fixation that also characterized SGS soil needs to be decreased. (Montegomery, 1988). Most of the soils are acid (pH $\leq$5.5) in nature; having high aluminium (Al), iron (Fe) and manganese (Mn) ions levels that readily fix nutrient element in soils (Akinrinde, 2006). He also stated that the use of Arbuscular mycorrhiza fungi can provide possibility for improvement of the use-efficiency of applied nutrient in the face of increasing fertilizer costs, infinite resources, as well as environmental contamination and /or pollution hazards. Low available nutrients, particularly P deficiency, as well as drought and water stress in most tropical soils is one of the important environmental limiting factors for plant growth (Atayese *et al.*, 1993). In these conditions, plant growth is largely reliant upon AM symbiosis for nutrients and water uptake (Querejeta *et al.*,2003), which could significantly reduce the use of conventional fertilizers in soils (Raja, 2006). A more effective method of phosphate fertilization such as the use of Arbuscular mycorrhiza is necessary. The AM association has received attention as part of an increasingly popular paradigm that considers active and diverse soil biological community as essential for increasing the sustainability of agricultural systems (Cardoso and Kuyper, 2006). AMF constitute an important functional component of the soil -plant system that is critical for sustainable productivity in degraded soils. (Gaur and Adholeya,

2004). As suggested by Bethlenfalvay and Lindermann (1992), "The role of AMF may be critical if agriculture is to return to the state where luxury levels of farm inputs of fertilizers, pesticides and or chemicals are decreased to levels that are still economical, yet do not pollute the environment or pose health risk to consumers or handlers".

A thorough understanding of the ecology of species of AM fungi in Southern Guinea Savanna of Nigeria is therefore needed to enable manipulation of the AMF symbiosis for the benefit of minimum-input agricultural system and to obtain sustainable environment for agricultural purpose through organic agriculture. Many biotic and abiotic interactions around roots are probably mediated by AMF. A successful shift in emphasis from chemicals to natural methods, such as crop rotation and the rational use of beneficial soil microorganisms, such as AMF, requires better knowledge on the dynamic relationships between agricultural practices and interactions between cultivated crops, AMF and other soil biota.

There is a dearth of information on the ranges of specific soil variables under which specific AM fungal species occur and thus on conditions, which may be tolerable or optimum for them in the Southern Guinea Savannah. Very limited information is available on the incidence of AM in Southern Guinea Savanna of Nigeria. Redhead (1977) established the occurrence of spores of endogonaceae spp in the soils of Southern Guinea Savanna of Nigeria. Several recent reviews have dealt with role of mycorrhizal associations in soil quality and sustainable agriculture (Dodd, 2002; Barea *et al*., 2002; Harrier and Watson, 2003). These reviews generally focused on temperate soils. However,

Cardoso and Kuyper (2006) reviewed the role of mycorrhizas in tropical soil fertility. No detailed and systematic studies have been conducted on the distribution of mycorrhizal fungi in Southern Guinea Savanna zone of Nigeria. Therefore, this research work is aimed at:

i evaluating the incidence of AMF in soils formed over basement complex and soils formed over sedimentary rock in the Southern Guinea Savanna of Nigeria;

ii assessing the effects of some soil factors on AM fungi in soils of Southern Guinea Savanna of Nigeria;

iii Morphological characterization of indigenous AMF of the soils of Southern Guinea Savanna of Nigeria.

CHAPTER TWO

LITERATURE REVIEW

Characteristics, Occurrence and Distribution of Arbuscular Mycorrhizal Fungi

Arbuscular Mychorizal (AM) aassociation involves primitive fungi in the Glomeromycota. In soil, Arbuscular mycorryzal fungi (AMF) produce a network of hyphae which form thicker hyphae that function as conduits and thin highly branched hyphae which are thought to absorb nutrients. Spores (large for a fungus), asexual spherical structures (20-1000 µm diameters) are formed on hyphae in soil, or in roots. (Brundrett *et al*., 1996). Spores are thick walled multi-nucleate resting structures (Miller *et al.,* 1995). Hyphae (non-septate when young and ramify within the cortex). (Brundrett *et al*., 1996). Arbuscular mycorrhizae are characterized by the formation of unique structures such as arbuscules and vesicules (Wikipedia, the free encyclopedia). Arbuscules (intricately branched haustoria in cortex cell) and vesicles (storage structures formed by many fungi) are structures formed in roots (Brundrett *et al*., 1996).

Mycorrhizal roots and the associated networks of hyphae are a major component of most soils, but cannot normally be seen with the naked eye. Mycorrhizal structures within root are normally not visible, unless roots have been cleared in hot alkali to make them transparent and stained with a dye that binds to fungal hyphae. According to Brundrett. (2008) mycorrhizal associations start when soil hyphae respond to the presence of a root by growing towards it, establishing contact and growing along its surface. Next, one or more hyphae produce swellings called appressoria between epidermal cells. Root penetration occurs when hyphae from the appressoria penetrate epidermal or cortical cells to enter the root. These hyphae cross the hypodermis (through passage cells, if these are present in an exodermis) and start branching in the outer cortex. Spores contain lipids, cytoplasm and many nuclei. They usually develop thick walls with more than one layer and can function as propagules. Spores may be aggregated into groups called sporocarps. Sporocarps may contain specialized hyphae and can be encased in an outer layer (peridium). Spores apparently form when nutrients are remobilized from roots where associations are senescing. They function as storage structures, resting stages and propagules. Spores may form specialized germination structure or hyphae may emerge through the subtending hyphae or grow directly through the wall.

AMF are the most wide spread type and ecologically important root fungal symbionts with more than 90% of higher plant species, including crop and fruit tree species, and are essential to the survival of many tropical plants (Strack et al., 2003). About 95% of the world's plant species belong to characteristically mycorrhizal families (Smith and Read, 1997) and potentially benefit from AM fungus- mediated mineral nutrition due to the fundamental role played by these glomalean fungi in biogeochemical element cycling (Jeffries and Barea, 1994). AM symbiosis occurs in almost all habitats and climates (Barea *et al.*, 1997), including disturbed soils (Enkhtuya *et al.*, 2002) and those derived from mine activities (Bi *et al*., 2003).

AM is the most abundant kind of mycorrhiza described as 'a universal plant symbioses. They occur in a wide variety of hosts, different habitats and also vary in quality and quantity (Bhaskar**,** 2004). Miller and Jastrow (2000) reported that AMF are found in practically every taxonomic group of plants and the list of species not infected is probably far shorter than the infected ones. These microsymbionts occur widely under various environmental conditions with beneficial effects on soil structure. Arbuscular mycorrhizal fungi are recognized as an important, widespread component of most terrestrial ecosystems. The best predictor of AM abundance was standing fine root length. As such, AM abundance tended to be much greater in grasslands than in other biomes (Treseder and Cross, 2006). NIIR Board (2004) stated that AM is found associated with majority of agricultural crops. They are ubiquitous in geographic distribution, occurring with plants growing in arctic, temperate and tropical regions alike.

According to St. John (1985) the fungi that infect more plant tissue than any other kind are the beneficial mycorrhizal fungi. This little appreciated and inconspicuous microorganisms live partly inside the roots. They are allies of both wild and domesticated plants. Mycorrhizae are present in a great variety of cultivated plants. They can be found in almost every soil in the world, except where human activities have suppressed the symbiosis. AMF survive in soil as spores, root fragments and mycelia (Brundrett, 2000).

The majority of herbaceous plant roots in natural ecosystems all over the world are colonised by AMF (Allen *et al.,* 1995). In *Poaceae*, this association is widespread and occurs, with few

exceptions, in both annual and perennial species. Colonization of AM is common in infertile habitats (Newsham and Watkinson, 1998) and typical grassland soils with low phosphorus level (McNaughton and Oesterheld, 1990). Arbuscular Mycorrhizal fungi are major components of rhizosphere microflora in natural ecosystems (Tarafdar and Panwar, 2006). AMF are distributed worldwide (Sharif and Moawad, 2006). On a global basis, mycorrhizae occur in 83% of dicots and 79% monocots, whereas all gymnosperms are mycorrhizal (Wilcox, 1991). They are common in most habitats but AMF are dominant in grasslands and tropical ecosystems.

Most species of plants are capable of associating with fungi of a single family, *Endogonaceae*, to form V-A mycorrhizae (Gerdemann, 1968). According to Leake *et al.* (2004), a substantial part of microbial communities in the soil belong to the AM Fungi, an ancient group of fungi that establishes mutualistic symbiosis with a great majority of plant species. They account for 5-50% of the biomass of soil microbes (Olsson *et al.,* 1999). Biomass of hyphae of Vesicular Arbuscular Mycorrhizal (VAM) fungi may amount to 54 –900Kgha^{-1} (Zhu and Miller, 2003), and some products formed by them may account for another 300Kg (Lovelock *et al.,* 2004). Pools of organic carbon such as glomalin produced by AM fungi may even exceed soil microbial biomass by a factor of 10- 20 (Rillig *et al*., 2001).

The most ancient and widespread mycorrrhizal relationships are formed by AMF. More than 80% of plant species can form AM yet relatively few fungal species (120) from restricted taxon, the Glomales, are involved. This reflects the evolutionary history of the relationship (Jeffries *et al.,* 2003). Almost all tropical crops are strongly responsive to Arbuscular mycorrhizas. A substantial number are also strongly dependent on Arbuscular mycorrhizas (Jaizme and Azcon, 1995).

Biodiversity and conservation of AMF

The diversity of AMF has significant ecological consequences because individual species or isolates vary in their potential to promote plant growth and adaptation to biotic and abiotic factors. Thus, the composition and dynamics of populations of AMF have a marked impact on the structure and diversity of the associated plant communities, both in natural and agricultural ecosystems (Grime *et al.,* 1987; Gange *et al.,* 1990). An important prerequisite to the analysis of populations of AMF in ecological studies is the correct identification of individual isolates. In addition,

physiological studies as well as field inoculation experiments may benefit from genetic and functional analysis of selected isolates.

Until recently, the markers identifying AMF for population and phylogenetic studies were solely morphological or biochemical (Giovannetti and Gianinazzi-pearson, 1994). Studies of the macro- and micro anatomy of fungi yield characters that form the historical bedrock in fungal taxonomy (Kohn, 1992) have been used to build the taxonomy of AMF (Morton and Benny 1990). However, many structures needed for morphological identification and species differentiation are lost during the symbiosis. The arbuscules produced by AMF in plants, for example, are very similar from one species to another. Allozymes have been helpful in providing diagnostic biochemical markers to identify species of AMF, even in colonised roots (Sen and Hepper, 1996).

However, the most powerful tools to study the evolution and population genetics of AMF are molecular techniques that analyse deoxyribonucleic acid (DNA) sequences. These techniques have been used in combination with morphological or biochemical data to investigate specific groups. For example, combined morphological data were used to define relationships among ancient species within Glomales (Redecker *et al.*2000) as well as to provide diagnostic primers important in the classification of these species (Morton and Redecker, 2001). With a similar approach, isolates of uncertain taxonomic position within the family Gigasporaceae could be assigned to known species (Lanfranco *et al.,* 2001). The combination of isozyme profiles and ribosomal gene sequences has also proved useful in defining groups in the genus *Gigaspora* (Bago *et al*.,1998).

A wide variety of techniques can be employed to detect DNA sequence variation in populations of AMF (Lanfranco *et al*., 1998). Polymerase chain reaction (PCR) amplification of targeted genomic sequences followed by restriction fragment length polymorphism (RFLP), allele-specific hybridisation, direct sequencing, or single –strand conformation polymorphisms are increasingly used to detect AMF in natural ecosystems (Sanders *et al*., 1996). Polymerase chain reaction primers based on highly conserved regions of nuclear and mitochondrial ribosomal DNA have been designed (White *et al*., 1990; Sanders *et al*., 1996; Schussler *et al*., 2001). To amplify two variable non-coding regions namely the internal transcribed spacers (ITS) and the intergenic spacers (IGS). Microsatellite-primed PCR, random amplified polymorphonuclear DNA (RAPD) and repeated

DNA probes are highly efficient approaches for the identification of distinct genotypes (Wyss and Bonfante, 1993; Longato and Bonfante, 1997) and have been employed to determine the genetic structure of populations of AMF. DNA markers have been successfully employed to track specific AMF from agricultural and natural ecosystems (Antoniolli *et al*., 2000).

One aspect that has recently received attention in the evaluation of diversity of AMF is their unusual association with endosymbiotic bacteria and the degree of their intraspecific diversity. Bacteria-like organisms in the cytoplasm of AMF were first observed by transmission electron microscopy in the early 1970s (Scannerini and Bonfante, 1991) but confirmation of their prokaryotic nature was impeded by their inability to grow on cell-free media. A combined morphological and molecular approach has now shown that the cytoplasm of *Gigaspora margarita* spores harbours a homogeneous population of bacteria identified, from the sequence of their 16S ribosomal ribonucleic acid (RNA) gene, as close to the genus *Burkholderia* (Bianciotto *et al*., 1996). More recent phylogenetic studies suggest that these endobacteria probably represent a new bacterial taxon (Bianciotto *et al*., 2002).

Polymerase chain reaction assays with oligonucleotides specific for this 16S sequence have revealed these bacteria in all stages of the fungal life cycle (spores and symbiotic mycelia). In addition, isolates of different origin from three Glomalean families (Glomaceae, Gigasporaceae and Acaulosporaceae) display bacteria when observed by confocal microscopy using a fluorescent dye specific for bacterial staining. The endobacteria of *Gigasporaceae* seem to be distinct from those found in other fungal taxa in terms of density, morphology and PCR amplification with specific primers (Bianciotto *et al*., 2000).

It should be noted, however that different situations exist within this genus: *Gigaspora rosea* was the only species to be completely devoid of endobacteria, an observation supported both by morphological observation of several isolates and by PCR experiments whereas *Gigaspora gigantea* harboured genetically and morphologically distinct bacteria (Bianciotto *et al*., 2000). These intracellular bacteria seem therefore to be a genera feature of spores of AMF and not a sporadic component.

Given their importance in ecosystems, conservation of AMF both in situ and ex situ is expedient. Germplasm collections have been developed to acquire, characterise and maintain AMF in living cultures. The two main collections, the BEG (Dodd *et al*. 1994; http://w.w.w.ukc.ac.uk/bio/beg/) and INVAM (http://invam.caf.wvu.edu/) play a crucial role in the preservation and distribution of fungal isolates for research.

Factors Influencing the Distribution of AM Fungi.

In the tropics, germination of AM fungi spores in soil and hyphal penetration of root cortex have been reported to be influenced by certain factors such as climatic, physico-chemical, biological and host genotype. Physico-chemical soil factors affecting the establishment of fungal spores include: soil water content (Cardoso and Kuyper, 2006); organic matter and plant residues (Read, 1991; Treseder and Cross, 2006). Organic soil amendments, such as manure, should be well aged. Fresh organic materials may encourage large microbial populations that can be inhibitory to mycorrhizal fungi. According to Hayman (1974) pesticide treatments also decrease the number of AMF. Also, heavy fertilizer application may lead to faster growth or a larger yield over a short term, and can compensate for a lack of mycorrhizae, but creates an addiction from which there is no easy return (St. John, 1985).

Biological factors that affect fungi establishment include the interaction of AM fungi with other soil organisms such as plant parasitic nematodes (`O` Banno *et al*., 1979). Read (1991) hypothesized that community composition of mycorrhizal fungi would vary as a function of the organic matter in the soil. Specifically, AM plants should be more abundant in ecosystems with smaller pools of organic nutrients in the soil, since this group possesses limited ability to degrade organic matter. However, Treseder and Cross (2006) discovered

that percent root length colonized (RLC), AM abundance and host plant availability were not related to the size, influx, or turnover rate of soil organic matter pools. AM fungal abundance may simply vary in proportion to belowground net primary productivity (BNPP) of AM plants (Harley, 1971). Isotope tracer in laboratory and field studies indicates that AM fungi consistently receive 37-47% of C delivered belowground by host plants. (Johnson *et al*., 2002).

Treseder and Cross (2006) stated that because fine roots provide a substrate for colonization by AM fungi, fine root length determine AM biomass. In their work to examine potential large-scale controls over the distribution of AM, abundance was positively correlated with standing stocks of fine roots. AMF are thought to play a particular important role in P acquisition; hence abundance may be greater where plants are more limited by P, as indicated by high N: P ratios of plant tissue. Treseder and Cross (2006) also noted that regions harbouring the largest stocks of AMF are also particularly vulnerable to anthropogenic nitrogen deposition. Other factors reported by St John (1985) include heavy chemical fertilization, heavy and indiscriminate use of pesticides, disturbance of soil by human activities such as handling and treatment of soil, removal of the plant cover and inversion of soil profiles. According to Jeffries *et al.* (2003), a number of abiotic factors such as climate change, drought stress, pollution and heavy metal contamination can influence the development of mycorrhizal relationships. He reported that excess levels of chemical components in the soil, inefficient mining processes, and treatment of soil with sewage sludge or industrial effluents, overuse of heavy metal containing fertilizers or gas exhausts have among other factors, contributed to the creation

of large areas contaminated by heavy metals, radionuclides and persistent organic pollutants. even though the presence of AMF can alleviate the stresses to plants caused by these external influences. Such areas may jeopardise human health and ecology (Adriano *et al.*, 1998) and therefore should not only be under strict control, but should also be managed in a way which attenuates the possibility of any risk concerning the contamination of the food chain and ground water or air by wind erosion.

Soil degradation produces changes in the diversity and abundance of AM fungal population (Koomen *et al.,* 1990). Variation in soil pH and temperature seem to be the decisive factors in tropical soils influencing distribution of AMF (Mahesh and Selvaraj, 2008). It has been a common experience that although chemical fertilizers have doubled the agricultural productivity, the mycorrhizal infection together with spore production have decreased (Bhardwaj *et al.,* 1997). Studies on the natural occurrence of AMF in Haryana soils showed that the fungal sporulation was more intensive in the rhizosphere of nonlegumes than of legumes. Maximum number of spores (342 spores per 50 g of soil) was observed in the rhizosphere of mustard, followed by chickpea, wheat, pearl millet and pigeon pea (Bhardwaj *et al.,* 1997). Soil pH, total soil P, available P, type of soil, soil moisture and cropping season are all variables that influenced the Arbuscular mycorrhizal population in the natural ecosystem. Numbers of AM spores highly correlated with the presence of total soil P and soil pH indirectly affected the AM population through the total soil P. The spore population was abundant in sandy soils as compared to loamy sands. Drier soils had higher number of AM spores. In summer, the AM population in soil was less as compared to winter season. The jhum fallow contained lower AM fungal population and number of species than the natural forest. Shifting cultivation in the humid tropical

soils causes reduction of AM fungal species. (Singh *et al*., 2003). Kormanik *et al.,* (1982) stated that high-quality seedling stock of hardwood tree species can be obtained in nurseries where cultural practices in the nursery encourage AM development.

Importance of AM Fungi

Effect on plant Nutrient uptake

In most cases of P-deficient soils, the growth of cultivated plants is highly dependent to mycorrhizal status (Diop *et al.,* 2003). Inoculation with AMF usually enhanced growth and shoot biomass of plants (Diop *et al.,* 2003; Ndiaye *et al.,* 2009). St John (1985) reported that mycorrhizae have been clearly shown to improve plant growth over that of non mycorrhizal control. Cassava (*Manihot esculenta* Crantz) is highly dependent on AMF association for plant growth (Howeler and Sieverding, 1983). The main effect of AM is to enhance P uptake of cassava grown on acid soils low in available P (Howeler and Sieverding, 1982). They greatly improve uptake of phosphorus and micronutrients, especially zinc and copper. According to Khalafallah and Abo-Ghalia (2008) AMF have higher capacity to increase growth and yield through efficient uptake in infertile soils. This effect is most marked when crops are grown in phosphorus deficient soils. Prakash *et al.* (2008) recorded increase in fruit yield and enhancement in fruit quality due to higher uptake of nutrients by the plants as influenced by arbuscular mycorrhizae in root zone of the plants.

According to Kormanik *et al., (*1982), AM development increased stem weight of seedlings of eight hardwood tree species by 2- to 80-fold over non mycorrhizal controls. Root weight of all seedlings was increased by 4- to 70-fold by AM. Mycorrhiza is

undoubtedly of extraordinary importance in plant production, plant soil ecology and plays a key role in sustainable agriculture (Bethlenfalvay and Linderman, 1992; Gianinazzi and Scheuepp, 1994). In marginal soils fertilized with rock phosphate, combined application of AMF with N_2 – fixing microorganisms and other organic materials may substantially increase P availability and crop yield (Sharif, 1999, Dey *et al*., 2004). According to Jeffries *et al.* (2003), mycorrhizal symbiosis is a keystone to the productivity and diversity of natural plant ecosystems. AM fungi are of importance as they play vital role in metal tolerance (del Val *et al.,* 1999) and accumulation (Jamal *et al*., 2002). External mycelium of AMF provides a wider exploration of soil volumes by spreading beyond the root exploration zone (Malcova *et al*., 2003), thus providing access to greater volume of heavy metals present in the rhizosphere. A greater volume of metals is also stored in the mycorrhizal structures in the root and in spores, for example, concentrations of over 1200mg kg^{-1} in *G.versiforme* (Chen *et al*.,2001). Another important feature of this symbiosis is that AMF can increase plant establishment and growth despite high levels of soil heavy metals (Enkhtuya *et al.,* 2002), due to better nutrition (Feng *et al.,* 2003), water availability (Auge, 2001) and soil aggregation properties (Rillig and Steinberg, 2002) as associated with this symbiosis.

The stimulation of microorganisms by the plant root system has now attracted attention. Microbial activity has been found to be an important factor influencing metal solubility and an immobilization of soil metals due to precipitation of sulphides and hydrated ferric oxides or by exudation of polysaccharides (Lodenius and Autio, 1989; Ernst, 1996).Organic functional groups on the surface of bacterial cell walls play an important

role in adsorption of metals from the soil solution (Fein *et al.,* 1997). Under other conditions Pb, Zn and Cu may be mobilised from the carbonates and oxides by microbial activity (Bloom-field, 1981). Biological methods to remove pollutants have mainly employed bacteria and saprobic fungi, while the role of mycorrhizal fungi has been almost completely neglected. A well-developed mycorrhizal symbiosis may enhance the survival of plants in polluted areas by better nutrient acquisition, water relations pathogenic resistance, phytohormone production, contribution to soil aggregation, amelioration of soil structure, and thus improved success of all kinds of bioremediation. For example, AMF have been found to decrease caesium uptake by plants (Berreck and Haselwandter, 2001), and thus could be used in the establishment of plant vegetation on soil contaminated with radio nuclides. They therefore have the potential to reduce environmental risks. Effective mycorrhiza may attenuate deleterious soil conditions (Haselwandter and Bowen, 1996).

Arbuscular mycorrhizal fungi also have a potential role in the monitoring of site toxicity (Weissenhorn *et al*., 1993, 1995; Gucwa-Przepiora and Turnau, 2001) and the efficiency of restoration techniques (Orlowska *et al.,* 2002). The use of mycorrhizal parameters as an indicator of changes occurring during soil quality has already been addressed by Lovera and Cuenca (1996), Haselwandter (1997) and Jacquot *et al*. (2000). Levels of colonisation of grasses in polluted field soils have shown to correlate with heavy metal contamination (Mikanova *et al*., 2001). Plants such as *Plantago lanceolata* might be of special value for biomonitoring (Orlowska *et al*., 2002). This species is strongly mycorrhizal, suitable for use in growth chambers and greenhouses where it easily forms mycorrhizal associations (Walker and Vestberg, 1994), and can be vegetatively propagated (Wu and Antonovics,

1975), facilitating the avoidance of genetic variability in response to toxic substances. In addition, it is widespread and tolerant to a broad range of soil types and pollutants (Wu and Antonovics, 1976; Bakker *et al*., 1999; Baroni *et al.*, 2000).

The impact of AMF on the reduction of soil-borne diseases has mainly been evaluated in studies on soil fungal pathogens such as *Phytophthora, Aphanomyces, Fusarium and Verticillium* (Azcon-Agwlar and Barea, 1996) and nematode causing root rots, lesion and galls (Guillemin *et al*., 1993; Pinochet *et al*., 1997). Some studies have shown that mycorrhizal protection could also occur against *Erwinia carotovora* and *Pseudomonas syringae* (Garcia-Garrido and Ocampo, 1989). Again, mycorrhizal symbiosis selectively stimulates the quantity and activities of soil biota having antagonistic activity against soil borne pathogens (Linderman, 2000). Prophylactic effects have been often reported, proving in many situations that AM fungi can act as biological control agents by lessening proliferation and damage caused by pests and soil-borne diseases (St-Arnaud and Elsen, 2005). The mechanism involved in the mycorrhiza induced disease tolerance may be the changed physiological and biochemical nature of the host plant. Increased production and activity of phenolic compounds due to AMF colonization has been reported. Higher phenolic content increases the defence mechanism of host plant and thereby imparts resistance to various diseases.

AM colonization could improve the water absorption of host plant, especially under nutrient limitation (Cardoso and Kuyper, 2006). Rapid root development and efficient root system is observed in mycorrhiza plants (Tisserant *et al*., 1996). The extra radical hyphae

of AMF contribute to soil aggregation and structural stability. Other mechanisms by which mycorrhizal fungi contribute to soil structure include creation of conditions that are conducive for the formation of micro -aggregate enmeshment by external hyphae and roots to form macro-aggregates; and by directly tapping carbon resources of the plant to the soils (Miller and Jastrow, 2000). Hyphae of AMF are more important in this regard than hyphae of saprotrophic fungi due to their longer residence time in soil, because fungivorous soil fauna prefer hyphae of the latter over those of AM fungi (Klironomos and Kendrick., 1996; Gange, 2000).

In addition, AMF produce glomalin (a specific soil protein, whose biochemical nature is still unknown) that has a longer residence time in soil than hyphae allowing for a long persistent contribution of soil aggregate stabilization (Cardoso and Kuyper, 2006). The residence time for hyphae is considered to vary from days to months (Staddon *et al*., 2003) and for glomalin from 6-42years (Rilling *et al*., 2001).Mycorrhizas are thus multifunctional in (agro) ecosystem with potentials for improving physical soil quality (through the external hyphae), chemical soil quality (through enhanced nutrient uptake) and biological soil quality (through the soil food web) (Newsham e*t al*., 1995).

AMF provide efficient nutrient uptake in infertile soils, water uptake and drought resistance in plants (Nowak, 2004; Chen *et al*., 2005). AMF are significant in the ecological improvement of rhizosphere (Medina *et al*., 2003). They contribute to soil C sequestration by producing glomalin, a recalcitrant and abundant soil glycoprotein (Rilling *et al*., 2001). Reynolds *et al*. (2006) reported that AMF restore and improve revegetation. AM fungi have been regarded as a boon for agriculture, forestry and restoration of

disturbed ecosystems. They are effective in overcoming the stress conditions like draught, disease incidences and deficiency of nutrients. Also in forestry, the growth of seedlings and productivity was found to be enhanced by AM fungi.

The impact of AMF in management of disturbed ecosystems

Arbuscular mycorrhizal fungi have been shown to rapidly invade virgin soils at Samphire Hoe in the UK (Dodd *et al*., 2002) and hence accelerate the natural process of plant community development. Samphire Hoe is a newly created land plantform comprising the sub scabbed material excavated during the construction of the channel tunnel. It represents a unique resource where the arrival and establishment of AMF within a sown plant community on a low nutrient substrate can be monitored. Invasion by AMF was monitored in three ways: by assessing the degree of root colonization within the root of plant on the site; by using a successive trap culture technique to determine species richness of AMF and by using sterile substrate bins to determine the extent of wind borne and rain dispersed immigration of propagules of AMF. Levels of colonisation of indigenous plants by AMF were high in May –June (the pre –flowering phase of growth for many plants) reflecting the important role of the mycorrhizal symbiosis in dry, low- nutrient siols. Twelve species of AMF were identified, representing a relatively high diversity for recent deposited subsoil. An on-site experiment indicated that inoculums of AMF could enter the site within eight months and that wind dispersal and rain were possible vectors.

A field experiment compared the outplanting performance of commercially – produced *Elymus pycnanthus* seedlings (in commercial compost with added nutrients) with seedlings

produced in a low-nutrient substrate and inoculated with AMF insulated from the site (a mixture of five species of *Glomus*) or left uninoculated. After fourteen months in field, seedlings inoculated with the indigenous AMF had the same tiller production as the commercially produced plants, despite poorer initial growth. In contrast, non-mycorrhizal controls grew very poorly, with a greater frequency of plant mortality relative to the other treatment. *Elymus* seedlings inoculated with the indigenous AMF ultimately produced approximately seven times the mean number of seed spikes per surviving plant as the commercially produced seedlings and five times greater weight of seed spike. A phytomicrobial approach to the revegetation of nutrient-poor soils has proposed to stimulate plant successional processes as an economically viable sustainable input for land scaping anthropogenic sites (Dodd *et al.,* 2002). A similar conclusion was reached by Greipson and El-Mayas (2001) during soi reclamation in Iceland.

In horticulture and agriculture; The use of AMF in agriculture could lead to a considerable decrease in the amount of chemical pollution in soil water, as recently demonstrated for maize (Giovannetti, 2001). This clearly indicates the potential of AMF for promoting a low chemical input agriculture. A more exhaustive review was made by Atkinson *et al*. (2002). The recent development of molecular probes able to diffentiate AMF within roots and soils (Jacquot-Plumey *et al*., 2001) opens new biotechnological perspective for the defining their population biology and therefore management strategies in the use of these symbiotic microbes in agriculture.

The difficulty in producing a large amount of inoculum of AMF for agricultural practices is less of a problem in horticultural crops, where inoculation could take place in seedling or cutting beds, over a relatively small surface area. Furthermore, the intensive use of i=]i \artificial substrates where AMF are absent facilitate their introduction. The main difficulties relate to the choice of substrates and of horticulture practices compatible with the development of mycelium of AM. The literature regarding AMF and their application in horticulture is extensive (Gianinazzi *et al*., 2001). Successful inoculation is usually achived when AMF are introuduced very early in plant deveplomental process followed by the use of low amount of phosphate fertilisers and selective use of pesticides (Guillemin et al., 1993). By doing so, colonization by AMF will follow root development of the inoculated seedlings or cuttings, with the consequence that plants will already be extensively mycorrhizal when transplanted into the field (Gianinazzi *et al*., 1995). In this respect, the case of micropropagated plants is particularly interesting in that inoculation with AMF can reduce plant losses during the acclimatisation phase, subsequently stimulate plant development (including flowering) and increase productivity after transplantation to the field (Estaun *et al*., 1999)

Following field transplantation, the rhizosphere of a micropropagated plant is usually colonised with a variety of other soil microorganisms, some of which are synergistic but some of which may be antagonistic to AM development. Therefore the study of multi-microbial interaction in the rhizosphere of microplants may be a very useful approach for developing our understanding of managing AMF in plant production systems (Cordier *et al*., 1999).

As a result of the ecosystem degradation processes in desertification-threatened areas, disturbance of natural plant communities is often accompanied or preceded by loss of physico-chemical and biological soil properties, such as soil structure, plant nutrient availability, organic matter content and microbial activity. Therefore, it is becoming critical to recover not only the vegetation but also these biological and physico-chemical soil qualities (Jeffries and Barea, 2001). There is an increasing interest in using AMF to improve revegetation processes for desertified ecosystems, particularly those developed under Mediterranean environments, based on the use of shrub plants belonging to the natural succession (Herrera *et al*., 1993).

A proposed approach to combat desertification includes inoculation with symbiotic microorganisms including AMF. Experiments have been carried out aimed at assessing the long-term benefits of inoculation of shrub legumes with rhizobia and AMF. This has included improving the establishment of target legume species as well as the benefits induced by the symbiotically tailored seedlings in key physico-chemical soil properties (Requena *et al*., 2001). When a field study was carried out within a desertified semi-arid ecosystem in southeastern Spain, the existing natural vegetation was a degraded shrubland where *Anthyllis cytisoides* was the dominant species (Requena *et al*., 1997). This is a drought-tolerant legume able to form symbioses with both rhizobia and AMF. The interaction of these microsymbionts on seedling establishment, survival rates, growth, N-fixation, and N-transfer from N-fixing to non-fixing species associated in the natural succession was studied (Requena *et al*., 2001). In addition, the possible improvement of

soil quality in terms of N content, levels of organic matter, and hydrostable soil aggregates in the rhizosphere of the target plants were also evaluated.

A long-term improvement in the physic-chemical properties was evident in the soil around the *Anthyllis* plants inoculated with an inoculum of AMF based on indigenous taxa. The benefits included an increased N content, and higher amounts of organic matter and soil aggregation in the soil around the roots (*Requena et al*., 2001). It can be assumed that the increase in N content in the rhizosphere of the legume can be accounted for by an improvement in nodulation and N-fixation rates resulting from inoculation of nodulated plants with AMF (Barea *et al*., 1992). The improvement of soil aggregation will matain good water infiltration rates, good tilth and adequate aeration for plant growth, thus improving soil quality (Wright an Upadhyaya, 1998).

Inoculation with native AMF also benefited plant growth, N-fixation and P acquisition by plants. Improved N status of non-leguminous plants grown in association with legumes has previously been described for agricultural crops (Azcon-Aguilar *et al*., 1979), but this was the first demonstration of this phenomenon for natural plant communities in a semi-arid ecosystem. The results support the general conclusion that introduction of target indigenous species of plants, associated with a managed community of microbial symbionts, is a successful biotechnological tool to aid the recovery of desertified ecosystems, suggesting that this represents the initial steps in the restoration of a self sustaining ecosystem (Requena *et al*., 2001).

Ecosystems have been contaminated with heavy metals due to various human and natural activities. The sources of metals in the soil are diverse, including burning of fossil fuels, mining and smelting of metalliferous ores, municipal wastes, fertilizers, pesticides sewage sludge ainingamendments and the use of pigments and batteries (Gaur and Adholeya, 2004). Migration of these contaminants into non-contaminated areas as dust or leachates through the soil is an example of events that contribute towards contamination of our ecosystems. Originally, attention was focused on the potential use of the plant rhizosphere to remediate contaminated soil. Plants were considered technically engineers as solar driven pumps (Adrianoe *et al*. 1998) without considering the associated microbiota. Remediation attempts were directed towards the application of soil amendments to increase the availability of the toxic substances, thus ameliorating the efficiency of phytoremediation (Blaylock *et al*., 1995; Salt *et al*., 1995). Optimisation of the technology included improvement of biomass yield via nutrient supply (Baker *et al*., 1994) and selection of the most efficient plant varieties and genetic engineering (Maiti *et al*., 1991). The capability of plants to produce acid exudates such as carbonic acid (H_2CO_3) and organic substances altering the plant rhizosphere and solubilisation of the adsorbed metals were also considered (Krishnamurti *et al*., 1997). This raised the possibility of using plants effective in organic acid production, such as *Lupinus* spp., as an alternative for chemical amendments stimulating the uptake of metals by plants used in the soil cleaning technologies.

Irrespective of these early successes, it is now evident that the mycorrhizal symbiosis must be taken into account in phytostabilisation. Although initial colonisers of heavily polluted

soils tend to be non-mycorrhizal (Shetty *et al*., 1994), the successful restoration and increase in plant community production, as well as improvement of soil structure, strongly depend upon the appearance of fungal symbionts. Attenuation of stresses is extremely important in sites such as post-flotation wastes, which are often devoid of basic nutrients such as N or P, which have poor water-holding capacity, and are vulnerable to wind erosion (Gucwa-Przepiora and Turnau, 2001). In places lacking AMF, the introduction of inoculums offers an interesting perspective for phytostabilisation techniques. The process may be stimulated by appropriately selected fungal strains.

The source of fungi for production of inoculums is very important, as they should be adapted not only to toxicity but also to climatic conditions. Fungal ecotypes from polluted places appear to be more successful than those from uncontaminated sites (Leyval *et al*., 1995). Extrametrical hyphae developed within the soil may play a role in heavy metal sequestration and thus in alleviation of their toxicity. The mycelium of metal-tolerant *Glomus mosseae* was shown to be several times more efficient in heavy metal adsorption than non-tolerant fungi and over ten times more effective than *Rhizopus orrhizus* used as a biosorption organism (Joner *et al*., 2000). Although the *G. mosseae* strain was shown to transport cabium (Cd) from the soil into the fungal structures within the roots of clover, Cd was restricted (Joner and Leyval, 1997). Similar results were obtained on the Cd and Zn-binding capacity of a fungal strain colonising clover roots isolated from an absolute metallophyte plant, *Viola calaminaria* (Tonin *et al*., 2001).

Inoculation of maize, incerne (alfalfa), barley and others by the fungal isolate obtained from *V. calaminaria* roots has also been shown to attenuate heavy metal stress

(Hildebrandt *et al*., 1999). This demonstrates the contribution of AMF to the accumulation of heavy metals in a non-toxic form within plant roots (Tonin *et al*., 2001). Differences in metal binding capacity exist not only between metal-tolerant and non-tolerant fungi, but possibly also between different species or isolates from the same site. This was shown for native *G. mosseae* in mycorrhizal roots from polish zinc wastes. Mycorrhizal fungi of *Fragaria vesca* were identified using molecular tools (nested PCR with taxon-specific primers performed on spores and trypan blue stained roots) and cytochemical staining was used to visualise the distribution of heavy metals (Turnau *et al*., 2001b).

The diversity in tolerance mechanisms towards heavy metals transferred by the fungus into the root cortical cells also exists between plant species/variety/ecotypes. The plants alone react differently to heavy metals (Antosiewicz, 1992) and may also regulate the effectiveness of mycorrhizal colonisation (Koide and Schreiner, 1992).

Biological methods of removal/degradation of organic pollutants from the soil have mainly employed bacteria and saprobic fungi (Schutzendubel *et al*., 1999). The positive effect of plants on the degradation of polycyclic aromatic hydrocarbons (PAHs) has also been reported (Reilley *et al*., 1996). In such cases plants producing a high root surface area have been the most extensively used. Well-developed associations with soil microbiota are alternative for using chemicals to increase the availability of toxic substances (Schwab and Banks, 1994). Among the organisms that could effectively increase the area of activity are mycorrhizal fungi. A range of mycorrhizal fungi were shown to be involved in the

degradation of organic pollutants and thus they may be also potentially useful in phytodegradation.

Although colonisation by AM was negatively affected by increasing PAH levels in soil (Leyval and Binet, 1998). It can still enhance plant survival and growth by decreasing phosphorus deficiency (Joner and Leyval, 2001) and water stress (Sanchez-Diaz and Honrubia, 1994), improving membrane integrity (Graham *et al*., 1981) or by stimulation of oxidative enzyme production (Salzer *et al*., 1999). These phenomena are all responsible for the attenuation of stress due to pollution.

Arbuscular mycorrhizal fungi can also be helpful in the management of constructed wetlands used for detoxification of a broad range of toxic substances. The importance of mycorrhiza of plants such as *Phragmites communis*, widely used to treat effluents, e.g. containing nitrophenols (Dias,1998), has been neglected. Oliveira *et al*. (2001) reported, however, the presence of the symbiosis when soils had reduced water content. This might play an important role in the initial steps of the establishment of wetland places, and subsequently could influence plant biodiversity in later stages, thus encouraging the re-appearance of mycorrhizal species (Vangronsveld *et al*., 1996).

For successful bioremediation, symbionts must be selected that can withstand the hostile environment of polluted sites. While introduction of new isolates is promising in areas devoid of AMF , the proper management of microbial resources, including mycorrhizal

fungi and a broad range of rhizosphere bacteria, could be more appropriate where they are already present (Jeffries *et al*., 2003).

Interactions with other soil micro-organisms

Arbuscular mycorrhizal fungi are key components of the soil microbiota and obviously interact with other microorganisms in the rhizoshere (Bowen and Rovira, 1999). Formation of AM changes plant physiology and certain nutritional and physical properties of the rhizosphere soil. This, in turn, affects colonization patterns of this region by soil microorganisms by the so called mycorrhizosphere effect (Gryndler, 2000). Arbuscular mycorrhizal fungi thus interact with natural and introduced microorganisms in the mycorrhizosphere, hence affecting soil properties and quality. Conversely, soil organisms are known to affect AM formation and functioning markedly (Barea *et* al., 2002). Deleterious rhizosphere bacteria (Nehl *et al.,* 1996) and mycoparasitic relationships (Jefries, 1997) have been found to interfere with AM development, while many microorganisms can simulate AM formation and or functioning (Barea *et al.,* 2002). Soil microorganisms can produce compounds that increase root cell permeability, thereby increasing root exudation. This in turn, stimulates the growth of hyphae of AMF in the rhizosphere and facilitates root penetration by the fungus. In addition, polysaccharide-producing bacteria may have a synergistic effect on hydrostable aggregate formation by AMF (Miller and Jastrow, 2000).

Rhizosphere microorganisms are also known to affect the pre-symbiotic stages of AM development such as spore germination and germ tube growth (Azcon-Aguilar and Barea, 1992, 1995). Biologically active substances like amino acids, plant hormones, vitamins, other organic compounds and volatile substances {Carbondioxide (Co_2)}, produced by soil microorganisms, can stimulate the growth rates of AMF (Azcon-Aguilar and Berea, 1995; Barea 2000). Detrimental effects of soil microorganisms on spore germination and hyphal growth in soil have also been reported (Linderman, 1992; Azcon-Aguilar and Berea, 1992). Vazquez *et al*. (2000) reported that antifungal compounds produced by *Pseudomonas spp.* did not interfere with AM formation or functioning, nor did a similar molecule produced by *Paenibacillus* sp. (Budi *et al.*, 1999).

The well-known activities of Nitrogen-fixing bacteria and Phosphate-solubilising microorganisms in improving the bioavailability of the major plant nutrients N and P contribute to the AM role in nutrient acquisition (Barea *et al.,* 2002). Management of such interactions is a promising approach either for low-input agricultural technologies (Jeffries and Berea, 2001), or for the re-establishment of natural vegetation in a degraded area (Miller and Jastrow, 2000; Jeffries and Berea, 2001). It is known that certain rhizobial strains improve processes involved in AM formation by *Glomus mosseae, i.e* spore germination, mycelia growth from the mycorrhizal propagules and 'entry point' formation on the developing root system of the common host legume plant (Barea *et al.,* 1996). Measurements of the $^{15}N/^{14}N$ ratio in plant shoots indicated enhancement of the N fixation rates in *Rhizobium*-inoculated mycorrhizal plants, relative to that achieved by the same *Rhizobium* strain in non-mycorrhizal plants (Toro *et al.,* 1998).

Multi-microbial interactions including AMF, *Rhizobium* spp. and PGPR have also been investigated (Requena *et al.,* 1997). In general, the results demonstrate the importance of physiological and genetic adaptation of microbes to environment, and thus the use of local isolates. Several microbial combinations were effective in improving plant development, nutrient uptake, N fixation (^{15}N) or root system quality, showing that selective and specific functional compatibility relationships among the microbial inoculants were evident with respect to plant response. Interactions between AMF and *Azospirillum* may also enhance mycorrhiza formation (Volpin and Kapulnik, 1994).

The interactive effect of phosphate solubilising rhizobacteria and AMF on plant use of soil-P sources of low bioavailability (endogenous or added as rock phosphate) has been evaluated in soil microorganisms using a ^{32}P isotopic dilution approach (Toro *et al.,* 1997). The rhizobacteria behaved as mycorrhiza-helper bacteria, promoting AM establishment. The dual inoculation treatment significantly increased microbial biomass and N and P accumulation in plant tissues and these dual-inoculated plants displayed lower specific activity ($^{32}P/^{31}P$) than their comparable controls, suggesting that the mycorrhizal and bacterized plants were using P sources otherwise unavailable to the plant. It was concluded that these rhizosphere/mycorhizosphere interactions contributed to the biogeochemical cycling of P, thus promoting plant fitness. These effects were further validated under field conditions (Barea *et al.,* 2002).

There is a positive interaction between AM fungi and nitrogen fixing bacteria such as *Rhizobium* in legumes, *Azospirillum* and *Azotobacter* in non legume soils. AMF association remarkably increases the multiplication, persistence and nitrogen fixation rate of these bacteria. Nodulation and N-fixation by legumes in tropical cropping systems show wide variation. This could imply that such legumes may either deplete soil nitrogen or add nitrogen. Insufficient nodulation and fixation may be both due to lack or scarcity of compatible and effective rhizobia, and to nutrient deficiencies coupled with an insufficiency of AM inocula. Hounglnandan *et al*. (2000) demonstrated that the rate of N-fixation of *Mucuna pruriens* (L) DC, a fallow plant to restore soil fertility and control the invasive grass *Imperata cylindrical* (L) Beauv. in the derived savanna of Benin, was often limited by low numbers of effective rhizobia and could be boosted by rhizobial inoculation, except in very P-poor soil.

The authors concluded that farmer's management practices that allow a build up of AM fungal inocula would alleviate P-deficiency and hence increase N-fixation. Similar interactions between AM fungi and rhizobia have been demonstrated for soybean (*Glycine max*) in low –P soils of the savanna in Nigeria (Nwoko and Sanginga, 1999). It was shown that there was a large variation in mycorrhizal responsiveness to soybean inoculation with AM fungi and that this variability should be exploited for selecting legumes for growth on marginal soils. This may be especially relevant because improved soybean cultivars have often been selected under conditions of P-sufficiency, a situation probably not dissimilar from the selection of Al-resistant maize cultivars from Brazil in conditions of P-sufficiency. Cowpea (*Vigna unguiculata*) breeding lines with higher AM colonization showed higher N-fixation in a low P-soil (Sanginga *et al*., 2000).

AM fungal and rhizobial responses might show positive feedback. Rhizobial inoculation increased AM colonization in soybean (Sanginga *et al*., 2000) and mucuna (Houngnandan *et al*., 2001). Marques *et al.* (2001) observed that AM fungi improved the performance of the woody legume, *Centrolobium tomentosum* Benth. Ingleby *et al*. (2001) demonstrated that AM improved nodulation of *Calliandra calothyrsus* Meissn, an agroforestry tree. In a follow-up, both rhizobia and AM fungi were selected from the tree's native range in Central America and from parts of

Africa where the tree was successfully introduced. In both areas, effective microsymbionts were obtained. But the interactions between effective rhizobia and effective AM fungi (and between plant provenances) have not yet been studied (Lesueur *et al*., 2001). This may be important because interactions between AM fungi and rhizobia cannot be predicted from the behaviour of both symbionts individually. It is still not known when the interaction is additive or synergistic, both in terms of costs of the symbioses and in terms of nutrient gains.

A similar positive interaction exists between AMF and phosphate solubilizing micro-organisms which require the complement of AMF for their activities. Ehteshami *et al.*, (2007) reported a significant increase in grain yield , yield components, harvest index grain N and P, soil available P and root colonization percentage when maize was inoculated with *Glomus intraradices* (AMF) *and Pseudomonas fluorescens* under water deficit stress and a comparatively poor response when inoculated with *P.fluorescens* alone under the same condition. Phosphate solubilizing microorganisms interact positively with AMF in promoting plant growth as well as P uptake of maize plant leading to plant tolerance improving under water deficit stress condition. It was found that AM fungi interact synergistically with other microorganisms such as phosphate-solubilizing bacteria and plant growth promoting microorganisms to enhance plant growth and survival. Also a significant effect of bacteria producing plant growth regulators on mycorrhizal development has been reported (Azcon, 1989). In addition, it is known that the soil micro-fauna can also affect development of AM, but the evidence is fragmentary (Gange, 2000; Gryndler, 2000). **Southern Guinea Savanna of Nigeria: Climate, Soil and Vegetation.**

The Southern Guinea Savanna agro ecological zone of Nigeria is characterized with 181-210 days length of growing period (Jagtab, 1995). The major soils have coarse-textured surface soil, and are low in organic matter and chemical fertility. (Salako, 2003). It has 100-150 cm of annual rainfall with wet season lasting 6-8 months. According to Ayansina (2008) rainfall variability for a period of 20 years was very high around Ilorin with values of coefficient of variation ranging between 26 and 49 percent. He also documented that rainfall varies both in time and space. As a result, there is remarkable fluctuation in yield per decade in this ecological zone. The atmospheric climate falls within the group described by Strahler (1970) as low latitude, tropical wet-dry climate.

The distinctive characteristics of this climate are marked seasonal contrast, unreliable precipitation (both in total quantity, intensity and distribution pattern) and dry harmattan of two to four months. Rainfall in this zone is generally between April and October with a break of about two weeks occurring either in July or August. Rainfall distribution is highly unpredictable both in terms of total rainfall and distribution (quantity and intensity) (Ajiboye, 2007). Rainfall anomalies such as decline in annual rainfall, change in the peak and retreat of rainfall and false start of rainfall that are sometimes experienced are detrimental to crop germination and yield, resulting in little or no harvest at the end of the growing season.The false balsam copaiba (*Daniellia oliverii)*, used for carving mortars and pestles for pounding yam, *Terminalia, Lophira, Afzeila, Vitex* and *Khaya senegalensis* (the poor mahogany) are the major plant species found in the Southern Guinea Savanna (Eroarome, 2005).

Ajiboye (2007) reported that soils of Southern Guinea Savanna of Nigeria are formed on either basement complex or upper cretaceous sandstones (Nupe Sandstone). Generally kaolinite and mica have been observed to be the dominant clay present in the moist soils of Southern Guinea Savanna of Nigeria. Although, traces of montmorilonite and vermiculite-smectite interstratified materials were also reported (Adegbite and Ogunwale, 1994). Soils developed on basement complex have been reported by Ojanuga (1979) to be predominated by Kaolinite. He also stated the presence of mica and other 2:1 silicate clays in trace quantity and appreciable quanties of montmorillonite. He therefore concluded that kaolinization was the dominant clay formation process in soils developed over basement complex especially in the Guinea Savanna Zone of Nigeria.

On the other hand, kaolinite and mica had been identified by Adegbite and Ogunwale (1994) to be the dominant clay in soils developed on Nupe Sandstone in the lowland basin of river Niger in the Guinea Savanna Zone of Nigeria, though trace quantities of feldspars, smectite, rutile, goethite, ilmenite and gibbsite were also discovered. Kaolinization was also indicated to be the dominant clay forming process in this soil. They concluded that the dominant pedogenic processes observed in the area of study were, (i) additions of organic matter through flooding and leaf litters, (ii) oxidation-reduction processes resulting in mottling through prolonged water-logging from flooding and fluctuating water table, (iii) clay translocation through lateral and vertical water movement and (iv) transformation of primary minerals into secondary minerals.

CHAPTER THREE

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from soils formed over basement complex (Ilorin and Malate sites,) and soils formed over sedimentary rock (Bacita and Pategi sites) in the Southern Guinea Savanna of Nigeria. Using the random sampling method, auger samples were collected from each of the sampling units at 0-15cm. The soil samples were transported to the laboratory in well labeled polyethylene bags. The core samples were then air dried for 3 days and passed through 2mm sieve in preparation for analysis.

Extraction of AMF spores from soil and enumeration of spores

AM spores were extracted using the wet- sieving and decanting/density gradient entrifugation method of Brundrett *et al.* (1996). One hundred grams (100g) of soil was weighed into 1 liter of water in a beaker. The soil was mixed and washed through series of sieves with different mesh sizes (1000µm, 500µm, 200µm and 53µm). The content of the bottom sieve (53µm sieve) was washed into a small beaker. Ten ml of the soil solution was measured into a 50ml centrifuge tube. Equal volume of 60% (w/v) sucrose in water was added to the soil solution. The mixture was then centrifuged for 2 minutes at 3000rpm. The supernatant in each tube was quickly decanted into a smaller sieve, washed under tap water and transferred into a petri dish. Enumeration of spores was done under the dissecting microscope with magnification X40. The number of spores per gram of soil was calculated and recorded. AMF isolates were characterized morphologically, biochemically and molecularly.

Morphological characterization of isolates

The isolated spores were picked up with a needle under a dissecting microscope and were mounted in both polyvinyl lactoglycerol (PVLG), Meltzer's reagent and PVLG mixed with Meltzer's reagent (1:1 v/v). All spores were examined using a compound microscope. Morphological properties of these spores were determined according to the key proposed by Trappe (1982). The characteristics used include, shape, size, colour, distinct wall layer, attached hyphae, sporocarps, bulbous attachment, clustering and surface ornamentation of spores. Spores were coated with gold to enhance observations under the scan electron microscope (SEM) at X 500. Characterization was made by using the description provided by the international collection of vesicular and arbuscular mycorrhizal fungi (Invam,2001).

Determination of some physic-chemical properties of soils

Physico-chemical analysis of soils samples were carried out. The properties that were determined include particle size analysis, organic matter (OM) content, pH, available phosphorus, nitrogen content and cation exchange capacity (ECEC).

Particle size analysis

Particle size was analyzed using hydrometer method. Fifty grammes (50g) of air-dried soil sample were weighed into one litre beaker. One hundred millilitres of distilled water and 10ml of 30% hydrogen peroxide (H_2O_2) were added to the sample, stirred and allowed to stand for about 2 hour for complete peroxidation. Thereafter, 50ml of 5.05 sodium hexametaphosphate was added. The sample was then stirred with a mechanical stirrer for 15 minutes. The stirred sample was quantitatively transferred into a 1000ml plastic

measuring cylinder and inverted several times in order to mix the content of the cylinder properly. The cylinder was immediately placed on a flat table and the time was noted. A soil hydrometer was slid gently into the suspension until the hydrometer was afloat. The first hydrometer reading was taken 40 seconds after the cylinder was set down. The temperature was recorded. Just before 2 hours elapsed, the hydrometer was placed in the suspension again and a second hydrometer reading was corrected by adding 0.3 for every Celsius that the temperature of the suspension is above caliberation temperature of the hydrometer (usually indicated on the stem of the instrument) or by subtracting 0.3 for every degree Celsius that the temperature of the suspension is below caliberation temperature of the hydrometer.

The first hydrometer reading measured the percentage silt and clay in the suspension. The second reading indicated the percentage clay in the suspension. The percentage sand was obtained by subtracting the sum of the percentage clay and silt from 100 (i.e. % sand = 100 – (% silt + % clay).

Determination of organic matter content

In determining the organic matter content, the wet oxidation method (Shamshudin *et al*., 1995) was used. The soil sample used for this analysis was ground to pass through a 300-mesh sieve. A 0.5g of the finely ground sample was weighed into a 250ml beaker. Ten millilitres (10ml) of 1N potassium dichromate ($K_2Cr_2O_7$) solution were added and the mixture was swirled to mix properly. Twenty mililiters (20ml) of concentrated sulphuric acid (H_2SO_4) was rapidly added to the mixture, swirled for a minute and then allowed to stand for 30 minutes. The mixture was diluted with about 100ml of distilled water and 5

drops of orthophenathrolein indicator was added. The suspension was titrated with 0.5N ferrous sulphate solution to a wine red coloured end point and the volume of ferrous sulphate used was recorded. A blank sample (without soil) was run similar to the above procedures to standardize the dichromate. The milliequivalent of readily oxidizable organic carbon in the soil sample was calculated as follows:

$$\% \text{ Organic Carbon} = \frac{(\text{Blank titre} - \text{Sample titre}) \times AC \times 0.003 \times 100}{\text{Weight of soil used}}$$

$$\text{Where A = Normality of Ferrous Sulphate } (Fe_2SO_4) = \frac{\text{Vol. of } (K_2Cr_2O_7)}{\text{Titre value for blank}}$$

C = Correction factor = 1.33

% organic matter in the soil = % organic Carbon x (100/58)

The pH of the soil was measured at 1:1 (soil: water) using an electronic pH meter. A 20g of soil sample was placed in a 50ml beaker and 20ml of distilled water was added (soil : water ratio of 1:1). The mixture was stirred with a glass rod and allowed to stand for 30 minutes. The mixture was stirred again and the electrode and the pH meter reading was taken. Prior to the reading of the sample, the pH meter was calibrated using solution of known pH (pH 4, 7 and 9)

Determination of Available P content

Available P was determined by Bray-1 extraction method (Bray and Kurtz, 1945). Thirty-seven grammes of Ammonium Fluoride (NH4) was accurately weighed into a beaker and 40ml of distilled water was added. The mixture was stirred vigorously to dissolve. This solution was quantitatively transferred to a 1000ml volumetric flask and the solution was

made up to mark. The resulting solution is 1 Molar Ammonium fluoride solution (1M NH4F). To another 100ml beaker containing 80ml of distilled water, 4ml of concentrated hydrochloric acid (conc. HCl) was added, shaken together and made up to mark with distilled water. The resulting solution is 0.5M HCl. To 15ml of 1M NH4F contained in 500ml volumetric flask, 25ml of 0.5M HCl and 460ml of distilled water were added. The content of the flask was made up to mark with distilled water and mixed thoroughly. The resulting solution is the Bray-1 extractant containing 0.03M NH4F and 0.025M HCl.

Soil – extractant ratio of 1:7 was used for the extraction of available Phosphorous in the soils. Five grammes of soil samples were weighed into extraction bottles and 35ml of Bray-1 extractant was added to each sample. The Mixture was shaken on reciprocating shaker for 30 minutes and the suspension was then filtered through a Whatman No. 42 filter paper. The resulting filtrate was used in determining the available P after the method described by Murphy and Riley (1962). Murphy and Riley is procedure consist of two solutions commonly referred to as stock solution A and B. In preparing stock solution A, 12g of Ammonium molybdate was dissolved in 250ml of distilled water. In another 100ml of distilled water, 0.2908g of Antimony potassium tartrate was dissolved. Both the first and the second solution were mixed together and 1000ml of 5N H_2HO_4 (148ml of conc. H_2HO_4 in 1000ml of distilled water) was added. The solution were well mixed together and brought up to 2000ml with distilled water and stored in a pyrex glass bottle in a cool dark place. Stock solution B was prepared only when required, as this solution does not store more than 24 hours. It contains 1.056g of ascorbic acid mixed with 200ml of stock solution. Available P was determined using spectrophotometry. The soil extract was

coloured using the molybdate blue colouring technique and the absorbance of the resulting solution was read on Spectronic 21D at 882nm wavelength. In colouring the soil extract, 2ml of the extract was pipetted into a test tube, 1.6ml of Murphy and Riley reagent and 6.4ml of distilled water were added. These solutions were thoroughly mixed and were allowed to stand for 30 minutes for full colour development before reading the absorbance. A calibration curve for the instrument was prepared by reading the absorbance of coloured standard solutions of known P concentration at the same 882nm wavelength on the Spectronic 21D. The standard solutions contained 0, 0.1, 0.2, 0.4 and 0.5 $mgkg^{-1}$ P. The Spectronic 21D absorbance readings were plotted against the standard P concentrations to obtain a standard calibration curve. This curve was used in determining the P concentration in the soil extracts and consequently the calculation of available P.

Estimation of Total Nitrogen

Total nitrogen was estimated by macro Kjeldahl method. Air dried soil sample (0.5g) was weighed into dry 500ml macro Kjeldahl flask and 20ml of distilled water was then added. The flask was swirled for a few minute and then allowed to stand for 30 minutes. This was followed by the addition of 1 tablet of Kjeldahl digesting tablets. 0.05, K_2SO_4, 0.25g, $CuSO_4$, few quantity of selenium powder. Concentrated H_2SO_4 (30ml) was then added. When the water has been removed and the frosting has ceased, the heat was increased until the digest cleared. The mixture was then boiled for 5 hours. The heating, during the boiling was regulated so that H_2SO_4 condensed about half way up the neck of the flask. The digest was carefully transferred into another clean macro-kjedahl flask (750ml). All sand particles was retained in the original digestion flask because sand can cause severe bumping during

kjeldahl distillation. The sand residue was washed with 50ml distilled water four times and the aliquot was added into 50ml of H_3BO_3 indicator was added into 500ml erlenmeyer flask which was the placed under the condenser of the distillation apparatus. The end of the condenser was about 4cm above the surface of boric acid solution.

A 750ml-kjeldahl flask was attached to the distillation apparatus. NaOH (5ml of 405) was poured through the distillation flask opening the funnel stop lock. Distillation was then commenced. The condenser was kept cool (below 30^0C), sufficient cold water was allowed to flow through and the heat was also regulated to prevent sucking back. Fifty millilitres of distillate was collected and distillation was stopped.

To determine the NH_4N in the distillate, the distillate was titrated against 0.01N HCl using a 25ml burette graduated at 0.1ml intervals. The colour at the end point was from green to pink.

The % N content of the soil was then calculated using formula below,

$$\% N = \frac{0.01x\ 0.014x\ 100x\ 100)\ x\ t}{5ml\ x\ \text{weight of sample}}$$

t = sample filtration (ml)

Determination of exchangeable cation exchange capacity (ECEC)

To determine exchangeable acidity, 5g of air dried soil (sieved through 2mm sieve) were weighed into a 250ml conical flask. A 50ml of 1N potassium chloride (KCl) solution was added to the soil sample in the conical flask. The flask was shaken on a reciprocating shaker for 1 hour and the content was filtered through Whatman No. 42 filter paper. Twenty five mls of the filtrate was pipetted into a 100ml conical flask and 50 ml distilled

water was added along with 5 drops of phenolphthalein indicator. The resulting solution was titrated with 0.01N sodium hydroxide (NaOH) to a permanent pink end point. The volume of the base used was recorded and used in the calculation of total exchangeable acidity (H+Al).

Exchangeable cations (calcium, magnesium, potassium and sodium) were extracted with neutral normal ammonium acetate (NH_4OA_C at pH 7.0). Calcium and magnesium content in the ammonium acetate extract were determined titrimetrically using the versenate (EDTA) method. For the determination of calcium and magnesium, 20ml of the soil extract was pipetted into a 250ml conical flask and 50ml of distilled water was added. Thereafter, 20ml of concentrated ammonia solution was added along with 10 drops of 2% sodium cyanide and 5ml of 10% hydroxylamine hydrochloride. The sample was titrated with 0.02N EDTA solution using 5 drops of erichrome black T as indicator with colour changes from wine red to pure blue end point. This titre value was used for the calculation of the concentration of calcium and magnesium present in the soil.

To determine the amount of calcium present, 20ml of soil extract was placed in 100ml conical flask. Twenty millilitres of 20% potassium hydroxide solution was added to the extract along with 10 drops of 2% sodium cyanide, 5ml of 10% hydroxylamine hydrochloride and 5 drops of calgon indicator. The resulting solution was titrated against 0.02N versenate (EDTA) until the end point was reached. The titre value obtained here was used for calculating the cationic concentration of calcium present in the soil. The difference between the titre value obtained here and that obtained from the initial titration gives the cationic concentration of magnesium present in the soil. Potassium and sodium

contents were determined by flame photometry. Standard solutions containing 0, 2, 4, 6, 8 and 10 mg/L (ppm) were prepared for sodium using sodium chloride and normal neutral ammonium acetate (1N NH_4OAc) were used in the preparation of these standard solutions.

In calibrating the flame photometer, the meter reading was set to zero using the blank solution (Normal neutral ammonium acetate and the maximum reading of 100 was set using the highest standard solution of 10mg / L (for sodium) or 20 mg / L (for potassium). Other standard solutions were then read (values were between 0 and 100). The samples were then aspired and the photometer readings were recorded. Where the sample reading exceeded 100, further dilution of the sample with normal neutral ammonium acetate was carried out to bring the sample reading to between 0 and 100. The dilution ratios in such cases were noted. After every ten readings, the calibration of the flame photometer was checked again using the lowest and the highest concentration of the standard solutions. A standard calibration curve was plotted each for sodium and potassium and the concentrations of each element (Na and K) in the sample was read from their respective calibration curves.

The Effective Cation Exchange Capacity (ECEC) was determined by the summation of the exchangeable bases (Ca, Mg, Na and K) and exchangeable acidity expressed in $cmolkg^{-1}$.

Statistical Analysis:

Percentage frequency of occurrence of varying AM fungal species in the different soil sample was estimated. Correlation analysis was used to determine the relationship between AM spores population and some soil parameters such as pH, Organic content, Nitrogen content ECEC and available P.

CHAPTER FOUR

RESULTS AND DISCUSSION

Occurence and distribution of AMF spores in selected soils of Southern Guinea Savanna

The wet sieving and decanting/density gradient centrifugation method of Brundrett *et al.* (1996) used in this study effectively screened out the spores of other types of fungi. In a preliminary study, when the supernatant obtained from the wet sieving was plated out and incubated on Potato Dextrose Agar (PDA), no fungal isolate was detected. Some bacteria colonies were however observed on the plates after two days (Plate 1). Levy *et al.* (2003) and Roesti *et al.* (2005) reported the occurrence of bacteria spp on the surface of AMF spores. The bacteria were discovered to be feeding on the hyaline outer spore wall layer of the AMF spores. One aspect that has received attention in the evaluation of diversity of AMF is their unusual association with endosymbiotic bacteria and the degree of their intraspecific diversity. Bacteria-like organisms in the cytoplasm of AMF were first observed by transmission electron microscopy in the early 1970s (Scannerini and Bonfante, 1991), but confirmation of their prokaryotic nature was impeded by their inability to grow on cell-free media.

A combined morphological and molecular approach has now shown that the cytoplasm of *Gigaspora margarita* spores harbours a homogeneous population of bacteria identified, from the sequence of their 16S ribosomal RNA gene, as close to the genus *Burkholderia* (Bianciotto *et al.*, 1996). More recent phylogenetic studies suggest that these endobacteria probably represent a new bacterial taxon (Bianciotto *et al.*, 2002). Polymerase Chain Reaction (PCR) assays with oligonucleotides specific for this 16S sequence have revealed these bacteria in all stages of the fungal life cycle (spores and symbiotic mycelia). In addition, isolates of different origin from three Glomalean families (Glomaceae, Gigasporaceae and Acaulosporaceae) were reported to display bacteria when observed by confocal microscopy using a fluorescent dye specific for bacterial staining. The endobacteria of *Gigasporaceae* seem to be distinct from those found in other fungal taxa in terms of density, morphology and PCR amplification with specific primers (Bianciotto *et al*., 2000). It was pointed out, however that different situations exist within this genus: *Gigaspora rosea* was the only species to be completely devoid of endobacteria, an observation supported both by morphological observation of several isolates and by PCR experiments whereas *Gigaspora gigantea* harboured genetically and morphologically distinct bacteria (Bianciotto *et al*., 2000).

A mixed population of AMF spores encountered in the soil of Southern Guinea Savanna of Nigeria is shown in Plate 2. The spores varied in colour, size and shape suggesting that the isolates belong to different genera and species. The morphological properties of the AMF spores are shown in Tables 1-4. The shapes of the AMF spores varied from globose to oblong; some were cream, white or hyaline, while some other ones were brown to orange brown in colour. By comparing the features with that from Invam data base, these spores were identified to be spores of *Glomus, Gigaspora, Acaulospora, Entrophospora, Scutellospora* and *Paraglomus species.* The occurrence of the various species varied considerably among the different sampling locations (Tables 1-4). A

total of 13 isolates were encountered in all the soils. Genus *Glomus* had the highest number of isolates. Invam data base has reported the genus *Glomus* to be the most diverse in glomales. In a study from Senegal, the presence of diverse AMF communities in sand dunes including *Scutellospora* and *Acaulospora* with *Glomus* having the highest species of isolates was reported (Diallo *et al.,* 1999). Likewise, AMF community in the Namibian desert were exclusively composed of *Glomus* and *Acaulospora* spp (Stutz *et al*., 2000). Ilorin soil had the highest number of isolates (13) (Table 4) while Bacita soil had the least (8) (Table 1). Pategi and Malete soils had 10 and 11 isolates respectively (Tables 2 and 3). *Scutellospora reticulata* was found to be the predominant AMF species colonizing all the study sites, followed by *Glomus pansihalos*. The population pattern of AMF varies greatly and their diversity is affected by various factors including soil environmental conditions, host plant and agricultural practices (McGonigle and Miller, 1996). According to Bever *et al.* (1996) every phase in the life cycle of AMF (spore germination, hyphal development, colonization and sporulation) is influenced by plant roots. AMF colonisation pattern is affected by soil pH (Wang *et al*., 1985) and interaction with other microorganisms (Bagyaraj, 1984).

Figure 1a shows the mean total AMF spores per kilogram of soil and species variation in soils developed over basement complex (Ilorin and Malete soils) and those formed over sedimentary rock (Pategi and Bacita soils), while Fig. 1b shows the biodiversity index of AMF in all study sites. Soil formed over basement complex had significantly ($F_{1,2} = 57.1$; $p < 0.05$) higher mean AMF spore kg^{-1} soil and higher isolate variation (602 kg^{-1} soil and 13 species respectively) than soil developed over sedimentary rock (213 kg^{-1} soil and 10 species respectively). The variation in number of spores/kg of soil recorded among the study sites could be as a result of many factors, some of which may include differences in crops that were cultivated in these sites and the previous state of those sites before collection of soil samples. Soil pH, total soil P, available P, type of soil, soil moisture and cropping season.

In a study reported by Sharif and Moawad (2006) the number of mycorrhizal spores was markedly affected by the crop types and sites. Agricultural management practices might affect AMF communities both qualitatively and quantitatively (Miller *et* al., 1995). Oehl *et* al. (2003) also reported that crop rotation, fertilization, and tillage affect the composition and diversity of AMF communities as well as spore and mycelium densities in temperate and tropical agro-ecosystems. In the present study, Pategi soil was cropped with *Sorghum bicolor* intercropped with cassava (*Manihot esculenta* Crantz). Rotation with groundnut (*Arachis hypogaea)* was also practiced while Bacita soil was cropped majorly with sugarcane (*Saccharum officinarum*), rice (*Oriza sativa*) and tomatoes (*Lycopersicum esculentum* Mill.) in some other parts. Ilorin soil on the other hand was cropped with maize (*Zea mays*) intercropped with cassava while Malete soil was cropped with okro (*Abelmoschus esculentus*), maize and cowpea (*Vigna unguiculata*Walp.). Cassava for example has been discovered to be highly dependent on a vesicular-arbuscular (VA) mycorrhizal fungi association for growth (Howeler and Sieverding, 1983).

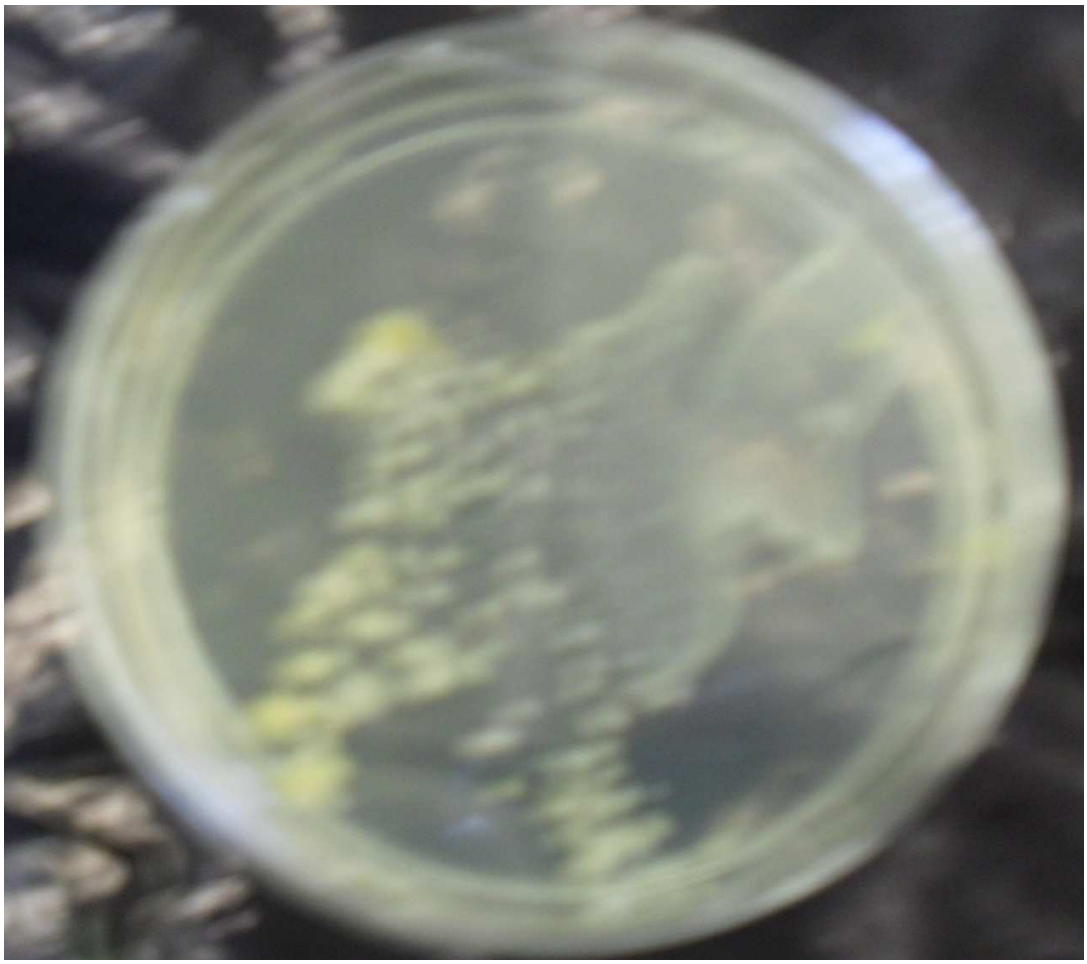


Plate 1: Supernatant from net sieving screened for spores of other fungi showing bacteria colonies.

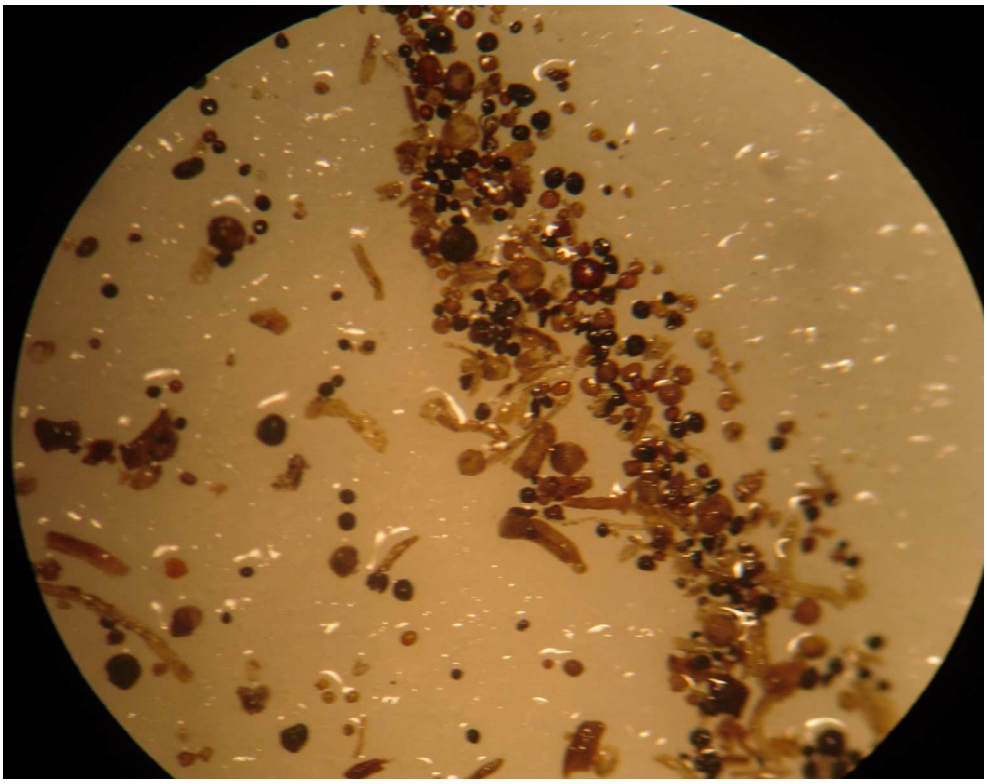
- 

Plate 2: Mixed population of AM spores from soil of Southern Guinea Savanna

Table 1: Morphological features of AMF spores isolated from Bacita soil

Features	**AMF ISOLATES**							
	BC1	**BC2**	**BC3**	**BC4**	**BC5**	**BC6**	**BC7**	**BC8**
Colour	**Black**	**Brown**	**Reddish brown**	**Orange**	**Pale cream**	**Black**	**Orange brown**	**Yellow brown**
Size (X 500µm)	412.66	369.67	256.08	188.16	198.97	232.24	225.42	210.08
Edge	Smooth	Smooth	Watty	Smooth	Smooth	Watty	Smooth	Smooth
Shape	Globose	Globose	Globose	Globose	Subglobose	Subglobose	Subglobose	Globose
Special feature	Occurring Singly	Occurring singly	Occurring singly	Occurring singly	Occurring singly with hyphal/filament	Occurring singly	Occurring singly	Occurring singly with hyphal/filament
Identity	*Scutellospora reticulate*	*Enthrophospora infrequens*	*Glomus pansihalos*	*Glomus tortuosum*	*Paraglomus occultum*	*Scutellospora pellucid*	*Acaulospora laevis*	*Glomus intraradices*

Table 2: Morphological features of AM spores isolated from Pategi soil

Features	AMF ISOLATES									
	PG1	**PG2**	**PG3**	**PG4**	**PG5**	**PG6**	**PG7**	**PG8**	**PG9**	**PG10**
Colour	**Cream**	**Orange brown**	**Black**	**Yellowish brown**	**Reddish brown**	**white**	**Brown**	**Black**	**Orange brown**	**Pale yellow**
Size (X 500µm)	247.76	188.16	455.26	351.19	256.08	305.50	389.67	175.84	225.42	259.93
Edge	Smooth	Smooth	Smooth	Smooth	Smooth	Watty	Smooth	Watty	Smooth	Smooth
Shape	Globose	Globose	Globose	Oblong	Globose	Globose	Globose	Subglobose	Globose	Globose
Special features	Occurring Singly	Occurring singly	Occurring singly	Occurring singly	Occurring singly	Occurring singly	Occurring singly	Occurring singly	Occurring singly	Occurring singly with hypha/ filament
Identity	*Paraglomus brasilianum*	*Glomus tortuosum*	*Scutellospora reticulata*	*Scutellospora calospora*	*Glomus Pansihalos*	*Gigaspora margarita*	*Enthrophospora infrequence*	*Scutellospora pellucida*	*Acalospora laevis*	*Glomus manihotis*

Table 3: Morphological features of AM spores isolated from Malete soil

Features	AMF ISOLATES										
	MT1	**MT2**	**MT3**	**MT4**	**MT5**	**MT6**	**MT7**	**MT8**	**MT9**	**MT10**	**MT11**
Colour	**White**	**Brown**	**Black**	**Orange brown**	**Pale yellow**	**Cream**	**Orange brown**	**Black**	**Yellowish brown**	**Reddish brown**	**Cream**
Size (X 500µm)	305.50	389.67	175.84	225.42	259.93	247.76	188.16	455.26	351.91	308.30	308.30
Edge	Smooth	Smooth	Watty	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Shape	Globose	Globose	Subglobose	Subglobose	Globose	Globose	Globose	Globose	Oblong	Globose	Globose
Special features	Occurring Singly	Occurring singly	Occurring singly	Occurring singly	Occurring singly hyphal/ filament	Occurring singly	Occurring singly	Occurring singly	Occurring singly	Occurring singly	Occurring singly
Identity	*Gigaspora margarita*	*Enthrophosphora infrequens*	*Scutellospora pellucid*	*Acalospora laevis*	*Glomus manihotis*	*Paraglomus brasiliamum*	*Glomus tortuosum*	*Scutellospora calospora*	*Scutellospora reticulata*	*Glomus pansihalos*	*Gigaspora decipien*

Table 4: Morphological Features of AM spores isolated from Ilorin soil

Features	**AMF ISOLATES**												
	IL1	**IL2**	**IL3**	**IL4**	**IL5**	**IL6**	**IL7**	**IL8**	**IL9**	**IL10**	**IL11**	**IL12**	**IL13**
Colour	**Black**	**Orange brown**	**Yellow brown**	**Yellow brown**	**White**	**Black**	**Brown**	**Reddish brown**	**Orange brown**	**Pale cream**	**Cream**	Pale yellow	Cream
Size (X500µm)	232.24	225.42	210.08	351.91	305.05	412.66	369.67	256.08	188.16	198.97	247.76	259.93	308.30
Edge	Watty	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Shape	Subglobose	Subglobose	Globose	Oblong	Globose	Globose	Globose	Globose	Globose	Subglobose	Globose	Globose	Globose
Special features	Occurring Singly	Occurring singly	Occurring singly	Occurring singly	Occurring singly	Occurring singly	Occurring singly	Occurring singly	Occurring singly	Occurring singly with hypha filament	Occurring singly	Occurring singly	Occuring singly
Identity	*Scutellospora pellucida*	*Acalospora laevis*	*Glomus intraradices*	*Scutellospora calospora*	*Gigaspora margarita*	*Scutellospora reticulata*	*Enthrophosphora infrequens*	*Glomus pansihalos*	*Gloomus tortuosum*	*Paraglomus occultum*	*Paraglomus brasilianum*	Glomus manihotis	Gigaspora decipien

The ability of some crop types to support high AMF population may be attributed to the root exudates of these plants, which stimulate the germination of mycorrhizal spores and increase the infection percentage. According to Osonubi *et al.* (1994), cassava responded to Vam inoculation significantly. Mathimaran *et* al. (2007) also discovered that spore densities of AMF were significantly affected by plant species identity. Mahesh and Selvaraj (2008) reported 82% Vam colonization in *Sorghum* compared to 20% in *Saccharum.* Bacita site for example had been under intensive use in the previous three to five years. There was a record of the use of heavy machine such as tractor, mounting plough and harrow. Substantial use of both urea and NPK fertilizer was also on record. Overhead sprinkler and underground irrigation together with the use of chemicals such as gramaxone, (paraquat$^{(R)}$) and Pedimenthalin (stomp$^{(R)}$) were practiced. Brundrett (2000) observed lower diversity of AMF in disturbed sites compared to undisturbed site. While all identified AMF species in this study were common to both soil types, *Scutellospora calospora* and *Gigaspora decipien* were found only in soils formed over basement complex as shown in Tables 1-4.

Davies *et al.* (1994) stated that high levels of available soil nutrients may also affect the species of AMF present as a result of differences in their sensitivity to high nutrient, notably P availability. Soil and environmental management factors may also be responsible for this variation. According to Sharif and Moawad (2006), soils, plants and environmental management factors mainly affect the mycorrhizal fungi and their development in an ecosystem. Figures 2-5 show the percentage frequency of occurrence of various AMF species encountered in Ilorin, Malete, Pategi and Bacita soils respectively. The soils formed over Basement complex (Ilorin and Malete) had 13 and 11 species respectively while those formed over sedimentary rock (Bacita and Pategi) had 8 and 10 species respectively. Indigenous AM fungal communities generally contain several fungal species (Sharif and Moawad, 2006). Panwar and Tarafdar (2006) also reported variation in percentage of AMF spores in different location. Sieverding (1989) stated that normally 5-15 VAM species may be found in an agro-ecosystem. The spatial distribution of AM fungal species can vary and even when the number is the same at two different sites, the species composition of the fungal population can be completely different. The differences observed in species composition may be due to agricultural management practices embarked upon on those sites. The predominant AM species in the soils from the four sites were *Scutellospora reticulata* and *Glomus pansihalos.* In a study reported by Mathimaran *et al.* (2007), AMF communities in tropical ferrasol under simple crop rotation were also dominated by *Scutellospora* spp. Variation in spore population may be attributed to soil edaphic characters particularly to the acidity, high moisture and organic carbon in soil (Mahesh and Selvaraj, 2008). Another factor, according to Treseder and Cross (2006) that could influence distribution of AM fungi is fine root length. This is because fine roots provide a substrate for colonization by AM fungi.

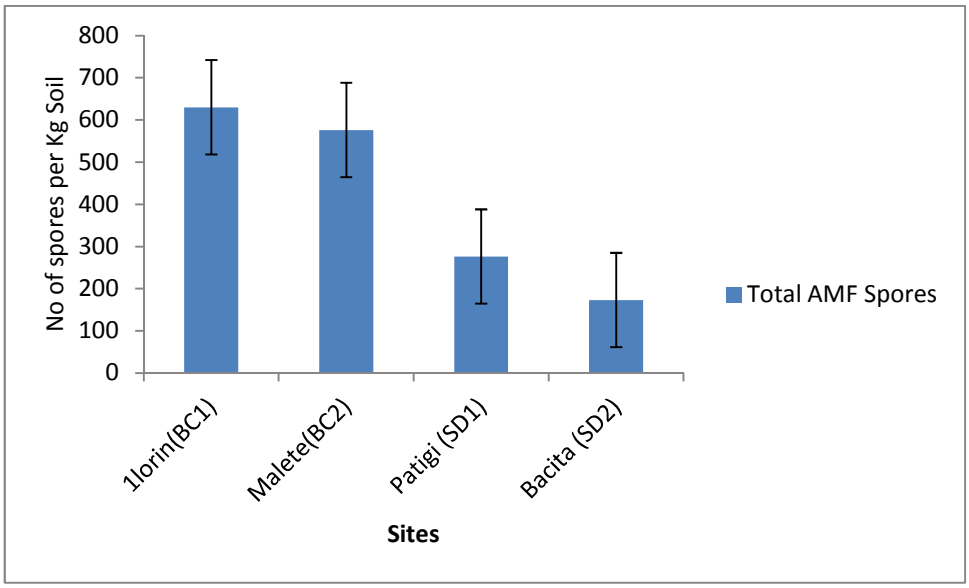


Fig 1a: Average total AMF spores kg^{-1}soil from different sites. Bars are standard error.

BC1=Soil formed over Basement Complex 1, BC2 = Soil formed over Basement Complex 2, SD1= Soil formed over Sedimentary rock 1 and SD2= Soil formed over Sedimentary rock 2.

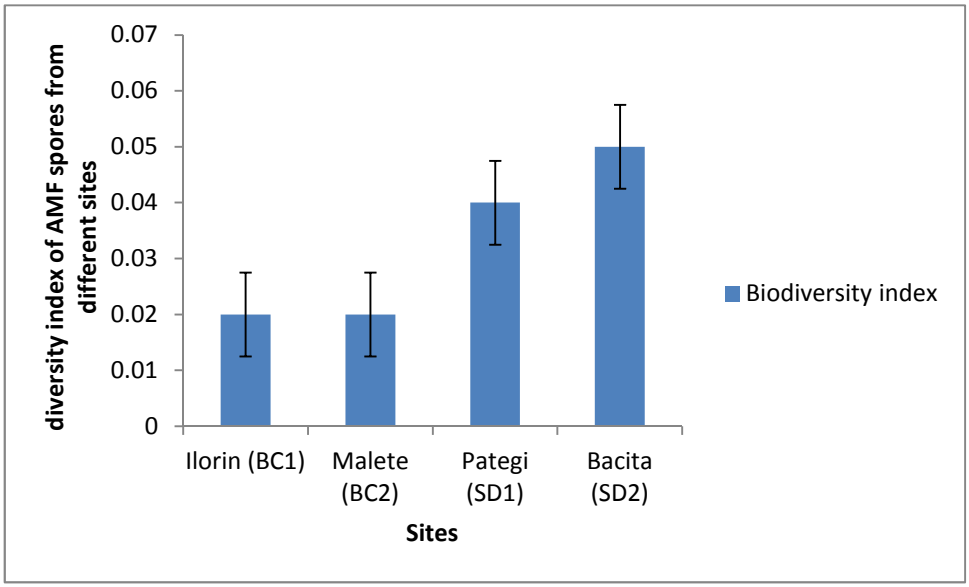


Fig 1b: Biodiversity index of AMF in different sites. Bars are standard error

BC1=Soil formed over Basement Complex 1, BC2 = Soil formed over Basement Complex 2, SD1= Soil formed over Sedimentary rock 1 and SD2= Soil formed over Sedimentary rock 2.

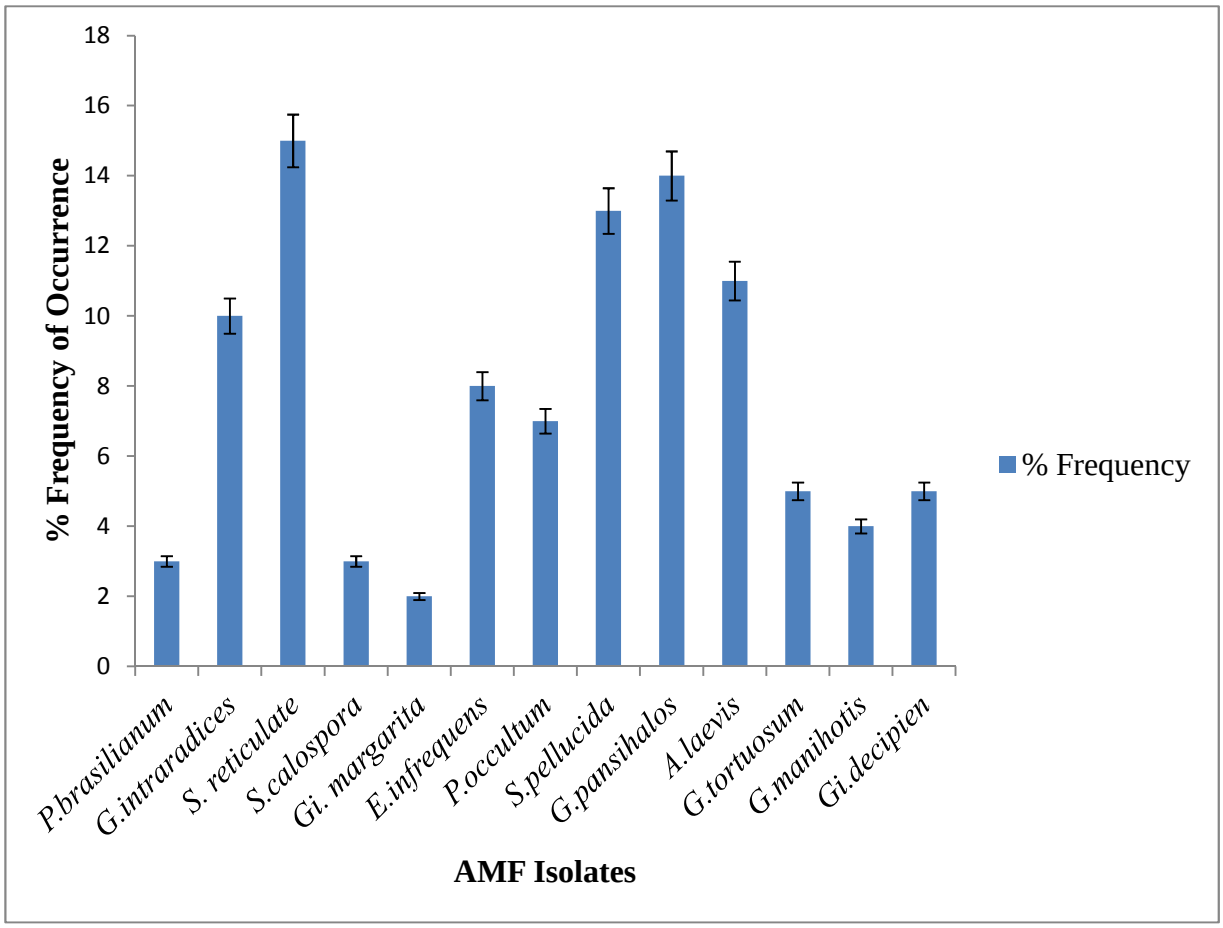


Fig 2: % Frequency of occurrence of AMF isolates from Ilorin soil. Bars are % error.

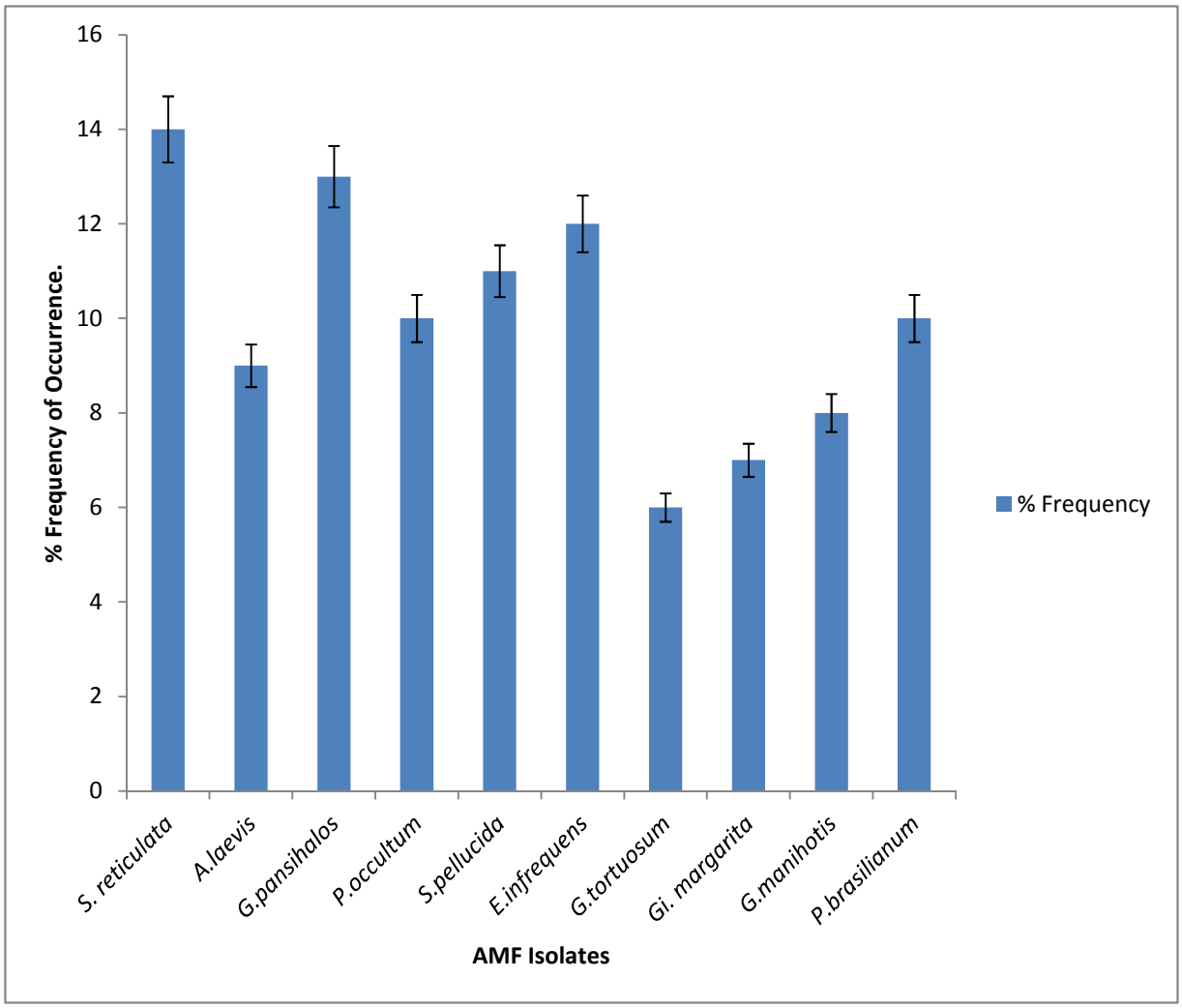


Fig 3: % Frequency of occurrence of AMF isolates from Pategi soil. Bars are % error.

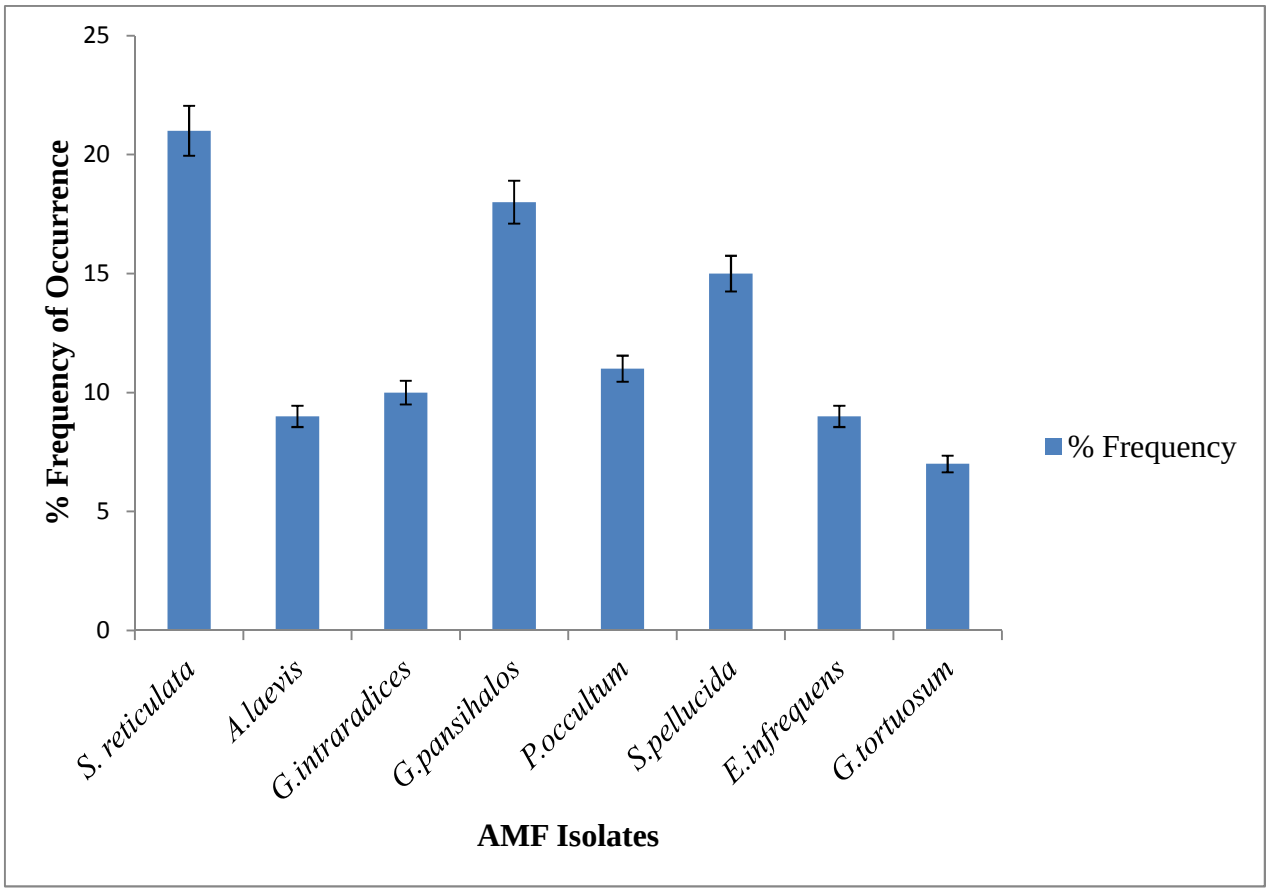


Fig 4: Frequency of occurrence of AMF isolates from Bacita soil. Bars are % error.

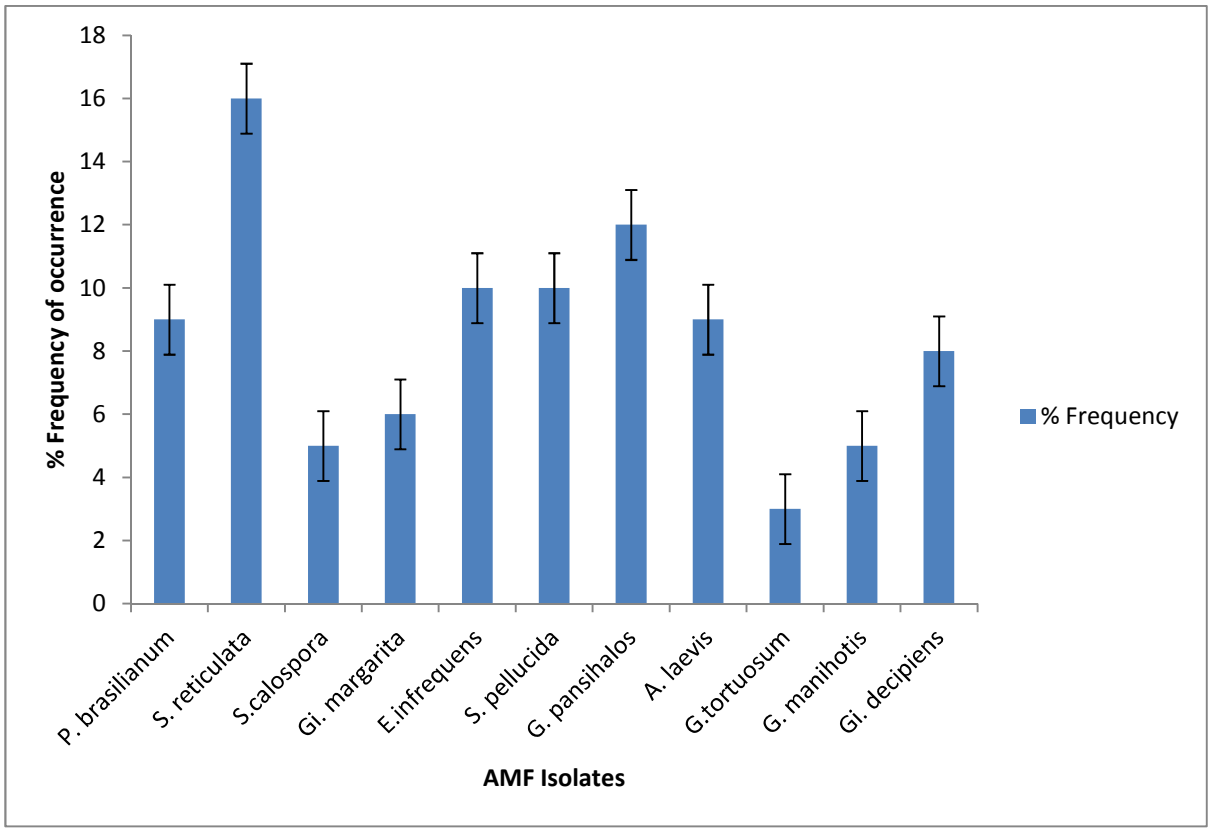


Fig 5: % Frequency of occurrence of AMF isolates from Malete soil. Bars are % error.

Physicochemical properties of soils

Table 5 shows some physicochemical properties of soils from the four study sites. Soils formed over basement complex were more acidic (pH 5.85, 5.88) than those formed over sedimentary rock (pH 6.10, 6.21). The sedimentary based soils had higher % organic matter(1.10-1.47), higher Phosphorus (2.9, 14.20), higher % nitrogen (3.5, 11.2) higher ECEC (4.68, 4.97) than those formed over basement complex which had % organic matter value of (0.48, 0.48) soil P (1.88, 2.15) % nitrogen (2.10, 2-80) and ECEC (4.08-4.53) The sedimentary based soils were sandy in texture while the soils formed over basement complex had loamy sand texture.

Correlations of some soil factors with AMF spore population in soil

Figures 6-10 show the correlations between some soil factors and AMF spore population in soil. Significant negative correlations were observed between AMF spore population and soil factors studied: soil pH, r = - 0.70* (fig. 6); % O.M content, r = -.97* (fig. 7); available P, r = -0.74*(fig. 8); % Nitrogen, r = - 0.95* (fig. 9) and ECEC, r = - 0.79* (fig.10). Eason *et al*. (1999) and Panwar and Tarafdar (2006) also reported negative correlation between soil P and AMF population of soil. This finding in this study is in line with the report that P application to soil and high available P content resulted in a decreased spore number, and suggests that available soil P content affects the production, survival and germination of AM fungal spores (Isobe *et al.,* 2007). These authors also reported a decrease in spore density in acid or alkaline soil. The density of AM fungal spores in soil is known to vary with the soil environment, such as P content, presence of plant roots and crop species (Isobe *et al.* 2007*)* However, Panwar and Tarafdar (2006), reported a positive correlation between soil pH and spore population. Also, according to these authors, spore densities peaked during summer and reached minimal values during raining season. When soil conditions were suitable for spore germination, mycorrhizal colonization increasesd and spore abundance decreased (Mason *et al.,* 1992; Ragupathy and Mahadevan, 1993). Fontenla *et al*. (1998) postulated that when number of spores in soil is high, the frequency of colonization is low, and vice versa.

Treseder and Cross (2006) also recorded a negative correlation between AM abundance and soil organic matter pools. This agrees with Read's hypothesis (1991) that the community composition of mycorrhizal fungi would vary as a function of the organic matter in the soil. Specifically, AM plants should be more abundant in ecosystems with smaller pools of organic nutrients in the soil, since this group possesses limited ability to degrade organic matter. AM abundance may be greater where plants are more limited by P, as indicated by high N:P ratios of plant tissue (Treseder and Cross, 2006).

TABLE 5: SOIL PHYSICOCHEMICAL CHARACTERISTICS.

SOIL CHARACTERISTICS	**STUDY SITE**			
	ILORIN	**MALETE**	**PATEGI**	**BACITA**
pH (1.1)	5.85	5.88	6.61	6.10
% OM	0.48	0.48	1.10	1.47
P mg/kg	1.88	2.15	2.90	14.20
% N	2.10	2.80	3.50	11.20
$Ca^{(2+)}$ (Cmol kg^{-1})	3.20	2.80	3.50	3.60
$Mg^{(+)}$ (Cmol kg^{-1})	0.60	0.40	0.50	0.80
$K^{(+)}$ (Cmol kg^{-1})	0.39	0.34	0.49	0.43
$Na^{(+)}$ (Cmol kg^{-1})	0.14	0.14	0.19	0.14
Total Acidity (Cmol kg^{-1})	0.20	0.40	0.40	0.40
ECEC (mg kg^{-1})	4.53	4.08	4.68	4.97
Sand %	84.8	88.8	90.8	84.8
Silt %	8.0	4.0	2.0	6.0
Clay %	7.2	7.2	7.2	9.2
Soil Texture	Loamy sand	Loamy sand	Sandy	Sandy

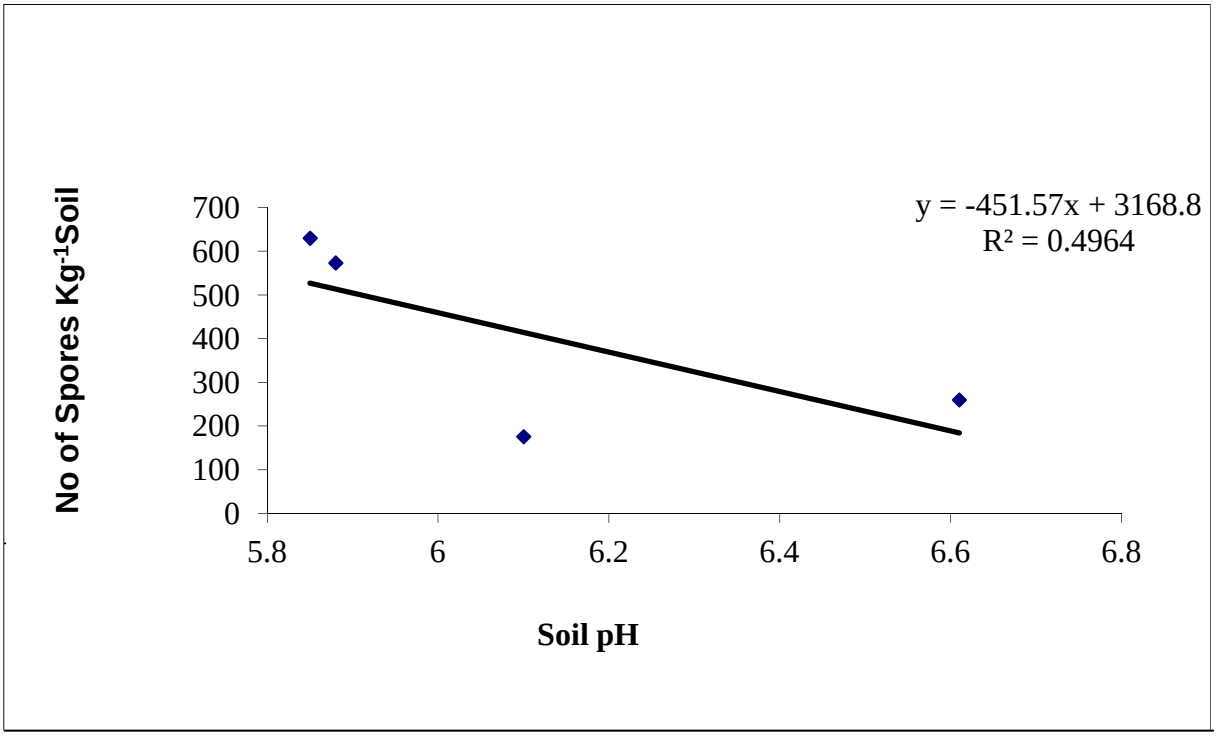


Fig 6: Correlation between soil pH and total number of AMF spores kg^{-1} soil

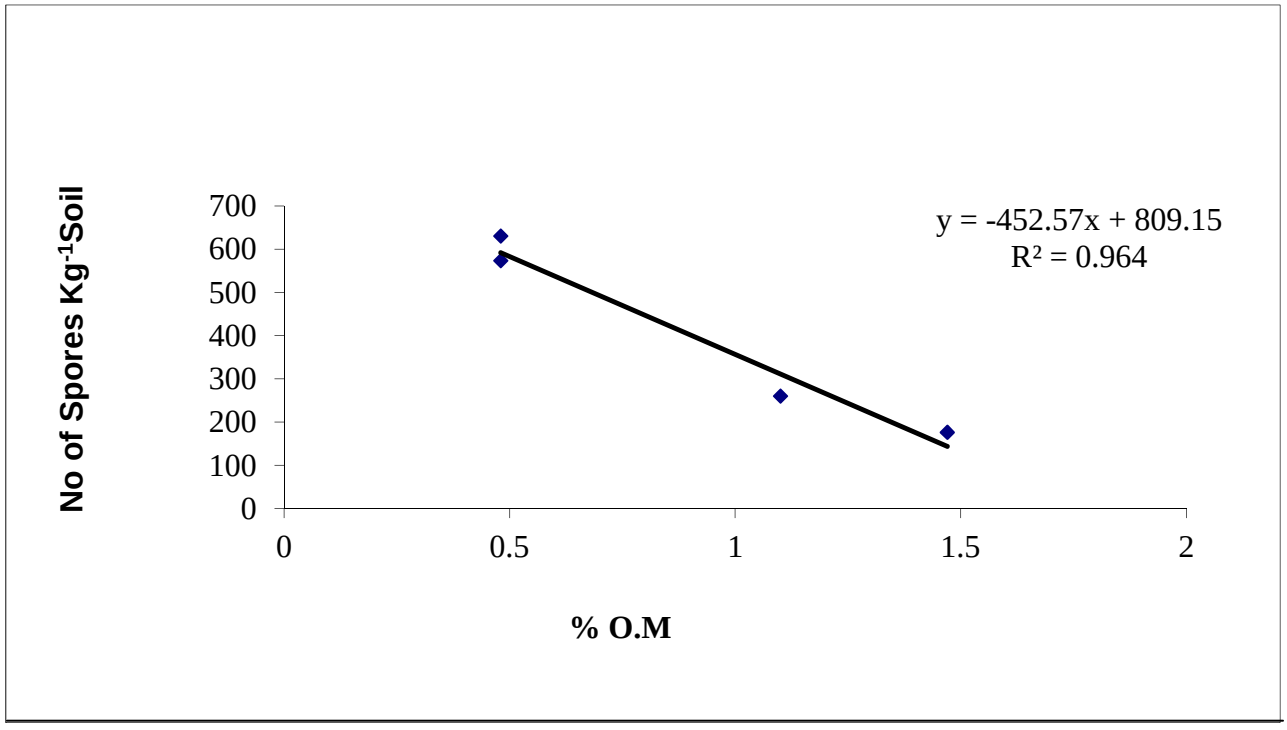


Fig 7: Correlation between % O.M. of soil and total number of AMF spores kg^{-1} soil

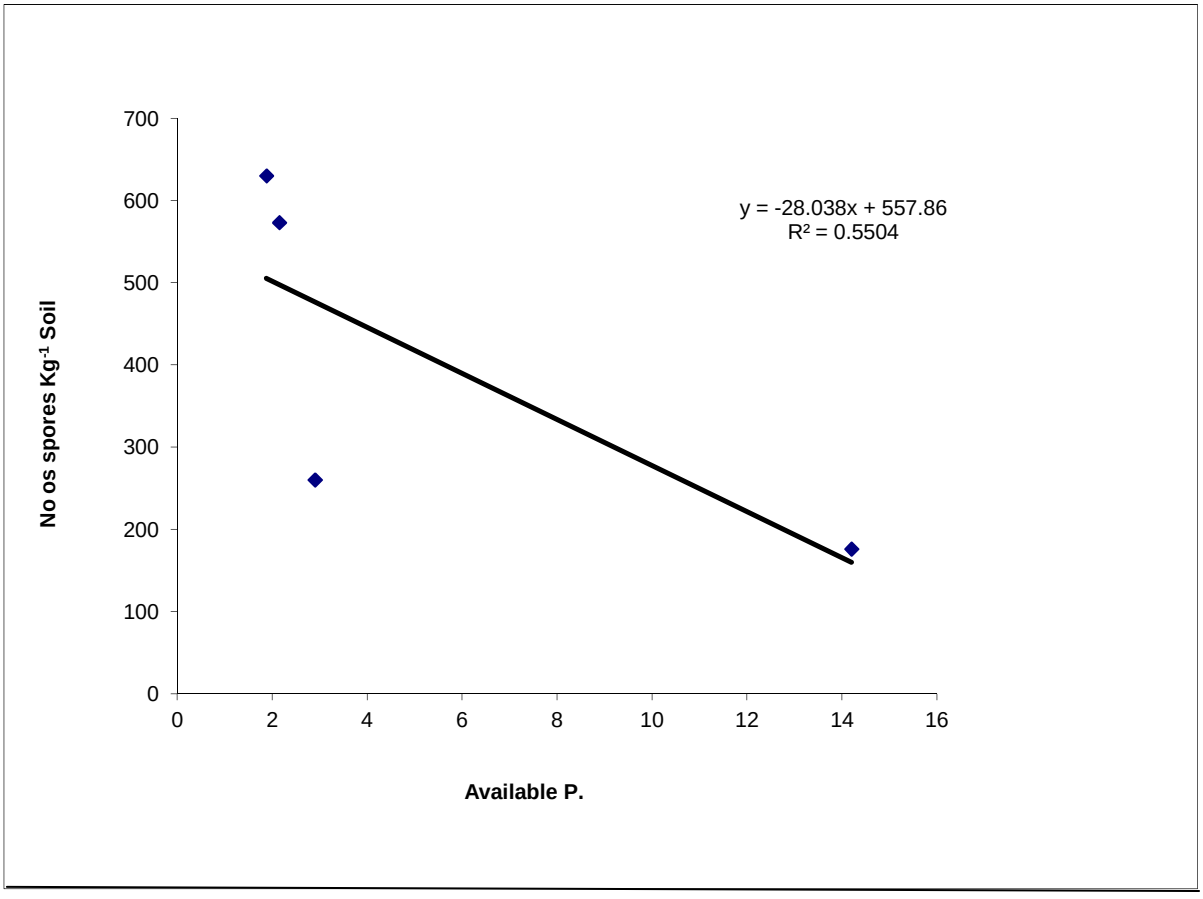


Fig 8: Correlation between soil Available P. and total number of AMF spores kg^{-1} soil

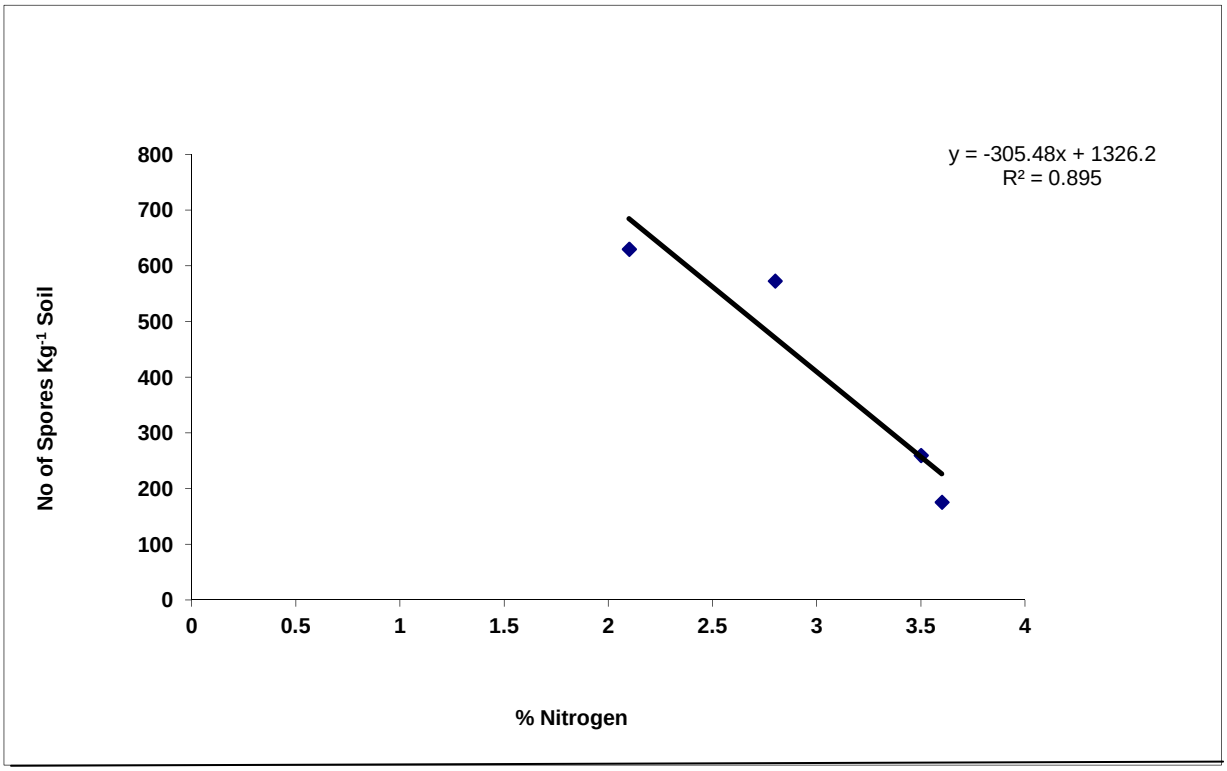


Fig 9: Correlation between % N. and total number of AMF spores kg^{-1} soil

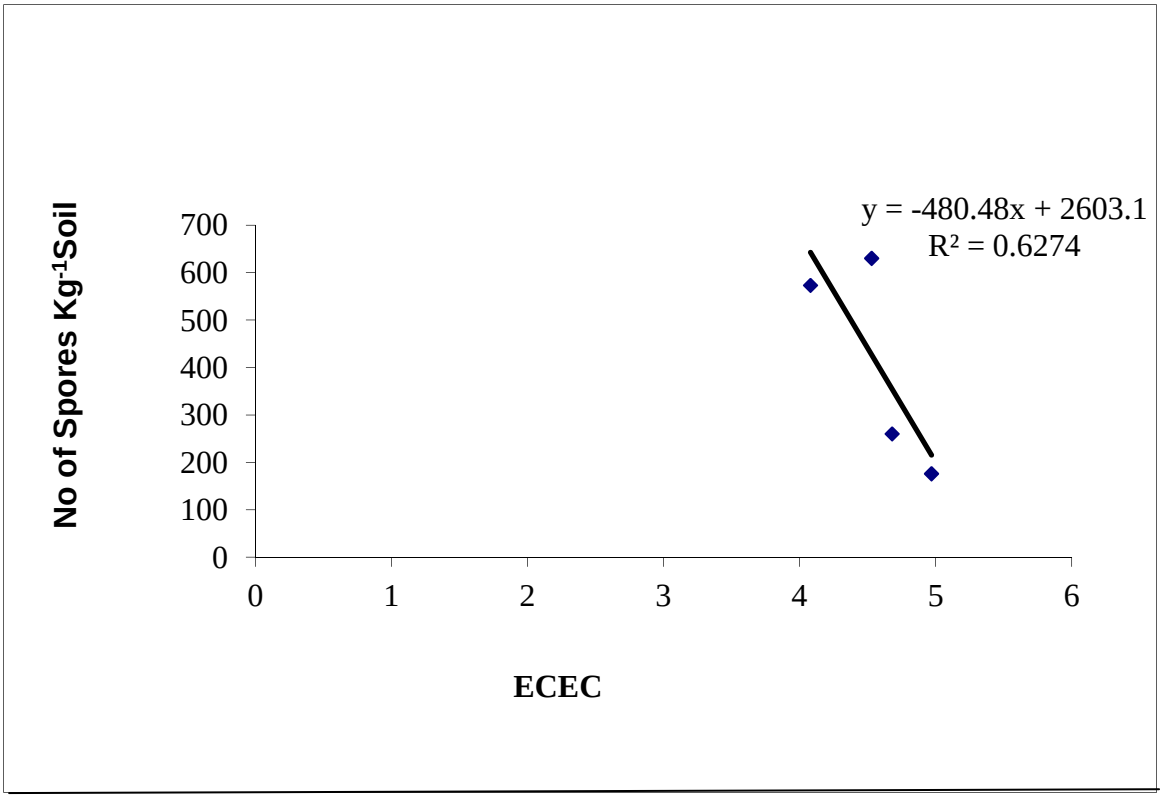


Fig 10: Correlation between Soil ECEC and total number of AMF spores kg^{-1} soil.

Morphological characterization of AMF spores

Plates 3-14 show the Scan Electron Microscopy (SEM) and subcellular structure of the AM spores isolated from study sites. The sizes of AMF spores indigenous to Southern Guinea Savanna of Nigeria vary from 188µm - 413 µm. Some of the spores obtained were globose while some were subglobose and some other ones were oblong in shape. Subcellular structures show spores with one, two and some with 3 internal layers designated as L1, L2 and L3.

Paraglomus occultum Morton and Redecker (2001)

Plate 3 shows a Glomus spore with a long hyphal attachment. L1 had a thin coating of organic debris on it's surface. This layer had no reaction in melzer's reagent and produced a light yellow reaction in melzer's reagent continuing to the wall of subtending hypha. L3 increased in thickness in the region of hyphal attachment and had a yellow reaction in melzer's reagent. This was identified as *Paraglomus occultum* based on description in Invam data base.

Glomus tortuosum Scheck and Smith

The spore shown in Plate 4 is globose with only one layer of spore wall. A subtending hypha is evident. This agrees with the description of *Glomus tortuosum* reported in Invam data base.

Scutellospora calospora Nicolson and Gard

Plate 5 reveals a spore with prominent germination shield. Two spore wall layers are evident L1 gave pale yellow colour with green tint adhering to L2 which gave a pale yellow colour and almost equal to L1 in thickness. According to reports from Invam data base and Brundrett (1999) only genus *Scutellospora* produces germination shield in spores. Hence, this spore was identified as *S. calospora spore* based on description in Invam data base.

Entrophospora infrequens

Plate 6 shows a globose shaped spore, 389.67µm in size at magnification x500µm. It showed 2 distinguished spore wall layers; L1 covered by a thin sublayer L2 which had orange brown colour in PVLG. According to Wu and Lin , this is typical of *Entrophospora infrequens.* The species is known to produce globose or subglobose with a zygospores swollen tips which are reddish brown to brown at maturity and are produced singly in soil.

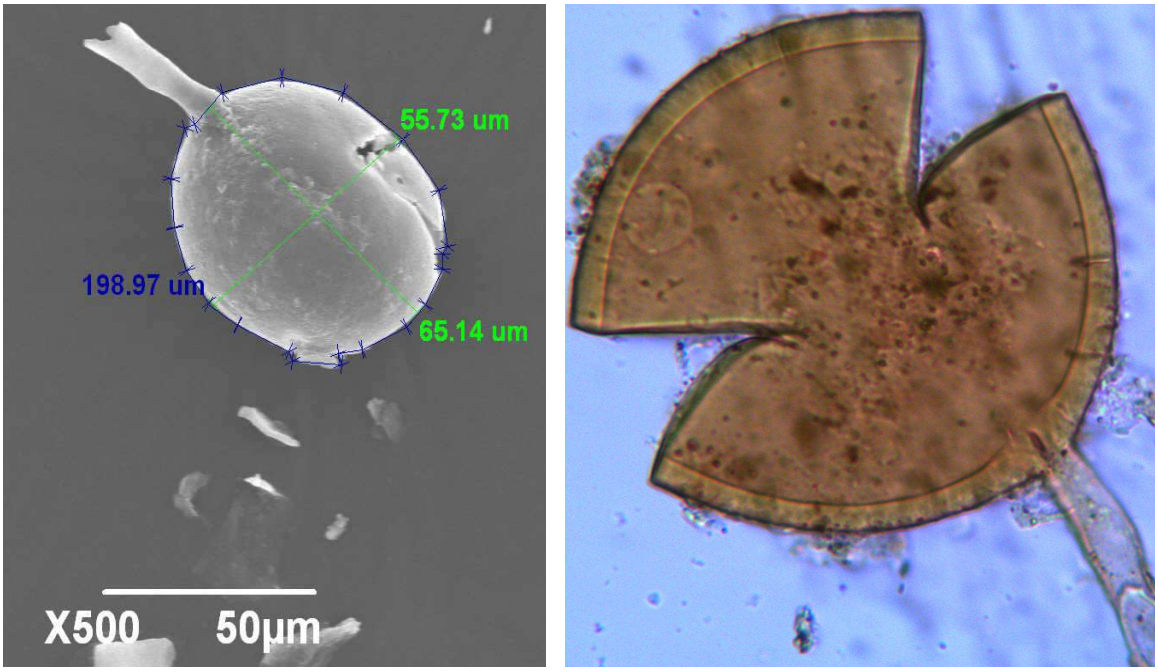
Acaulospora laevis Gerd and Trappe

Plate 7 shows a globose, orange brown spore. The first layer L1 was smooth L2 showed no reaction in Melzer's reagent and had dark orange brown colour. The third layer, L3 was yellow-brown and

showed no reaction in melzer's reagent. No hyphal attachment was observed. These morphological characterization are consistent with characteristics of *Acaulospora* laevis in Invam data base.

Gigaspora margarita Becker and Hall

The spore of *G. margarita* was 3 layered (Plate 8). The L1 was smooth brownish yellow. L2 stained dark red brown in Melzer's reagent while L3 was adherent with the laminate layer. A point of hypha attachment was also revealed. This is in harmony with the description of *Gi. margarita* given in invam data base.

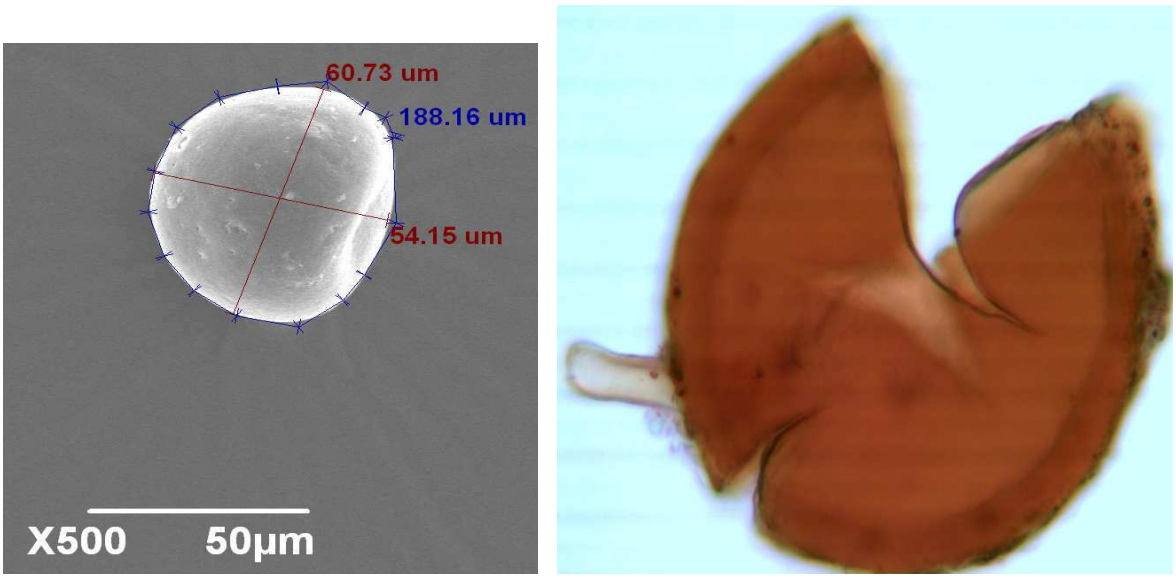


a **b**

Plate 3: Spore of *Paraglomus occultum*

a: SEM morphology

b: Subcellular structure

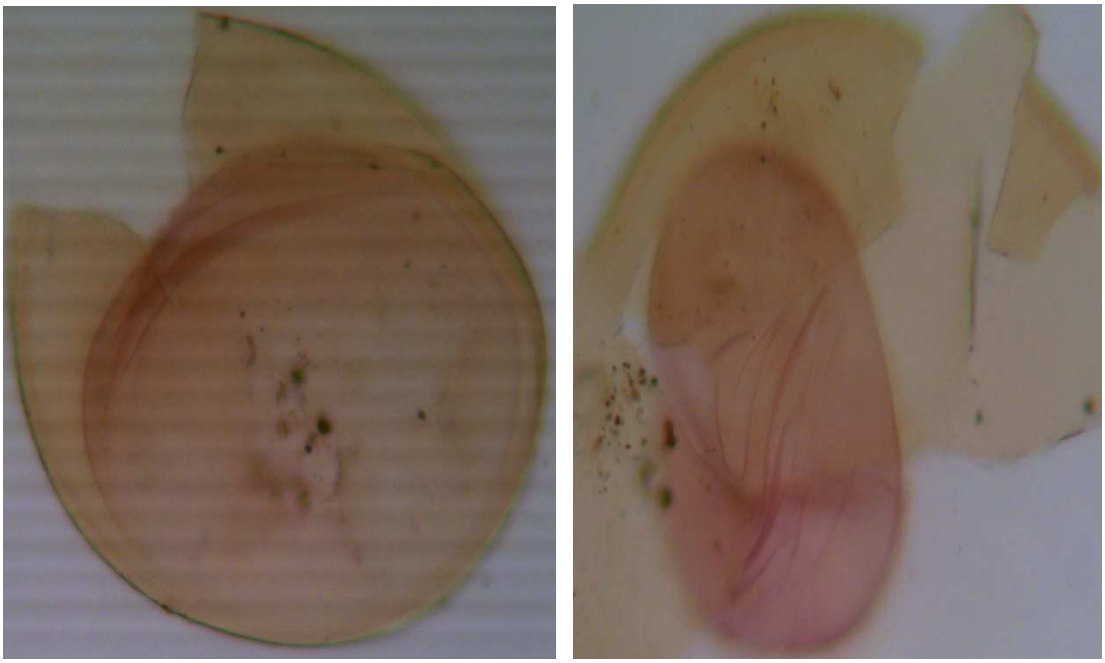


a **b**

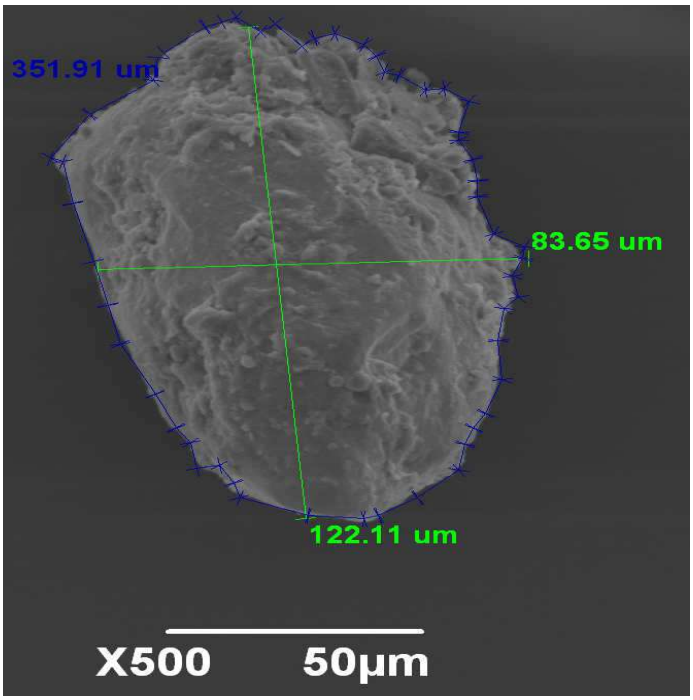
Plate 4: Spore of *Glomus tortuosum*

a: SEM morphology

b: Subcellular structure



a b



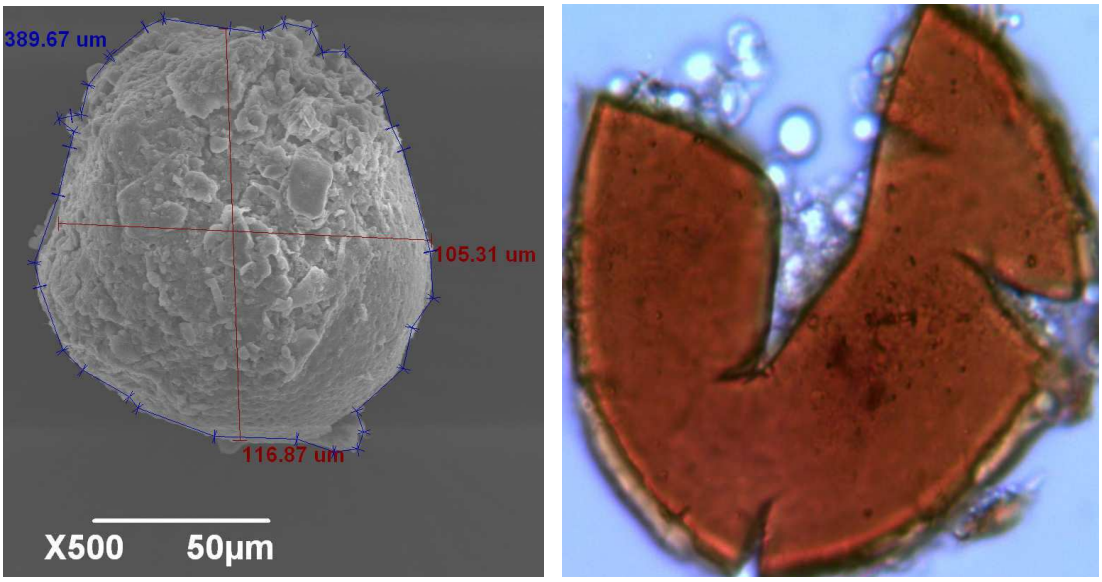
c

Plate 5: Spore of *Scutellospora calospora*

a: Subcellular structure

b: Subcellular structure of spore showing germination shield

c: SEM morphology

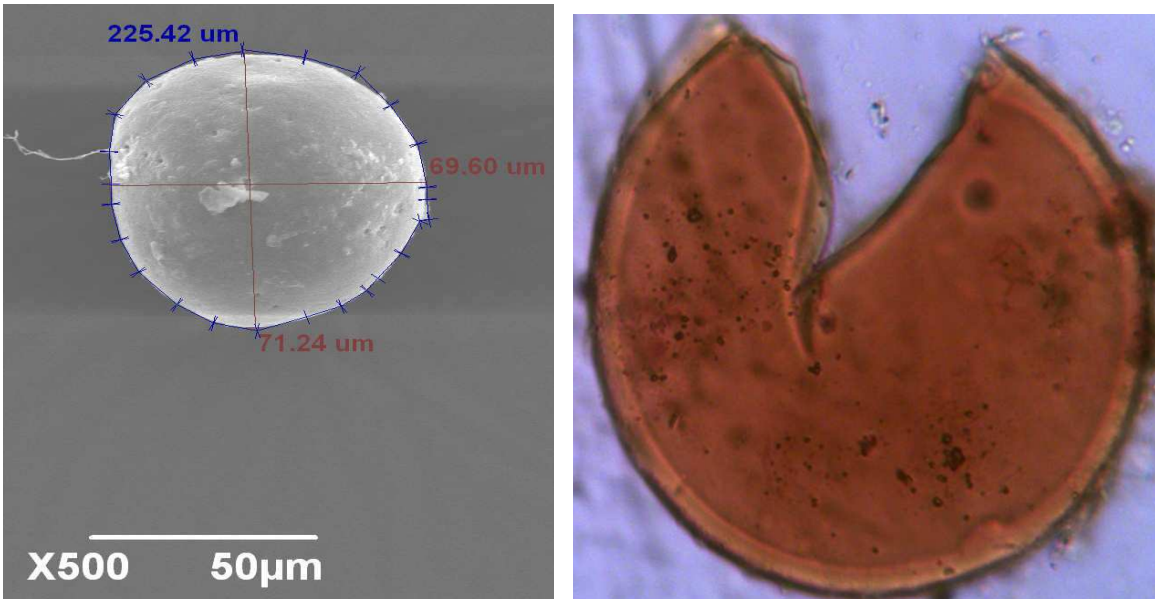


a b

Plate 6: Spore of *Entrophospora infrequens*

a: SEM morphology

b: Subcellular structure

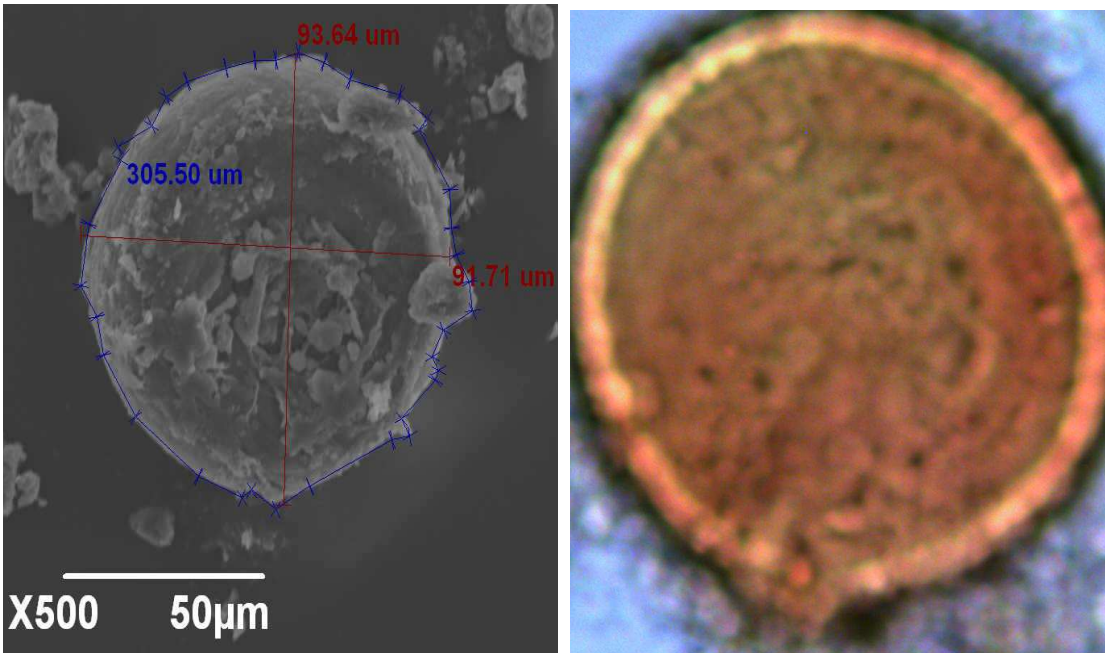


a b

Plate 7: Spore of *Acaulospora laevis*

a: SEM morphology

b: Subcellular structure



a b

Plate 8: Spore of *Gigaspora margarita*

a: SEM morphology

b: Subcellular structure

Paraglomus brasilianum Morton and Redecker

Plate 9 shows a globose spore with 3 layers of spore wall; a thin L1 almost sloughing and coated with organic debris which had no reaction in Melzer's reagent, L2 which turned dark yellow in Melzer's reagent and a thin third layer, L3. The identification of this species was based on properties reported for *Paraglomus brasilianum* in Invam data base.

Glomus intraradices Schenek and Smith

Plate 10 shows a spore of Glomus intradices. The spore was globose, had 3 spore wall layers and an hypha filament attachment. L1 stained pinkish in Melzer's reagent, L2 was thinner and adherent to L1 while L3 was pale yellow-brown. Invam data base gave this same description for *Glomus intraradices*.

Glomus manihotis Howeler

A spore of *G.*. manihotis is shown on plate 11. The subcellular features revealed a globose spore with spore wall layers. The first layer L1, was thin and light purple in Melzer's reagent while L2 was thicker. Spore appeared squashed rather than cleanly broken. L3 was dark yellow almost of the same thickness as L1. The point of hyphal attachment was revealed in SEM structure. This isolate was identified as *Glomus manihotis* based on the description in Invam data base.

Glomus pansihalos

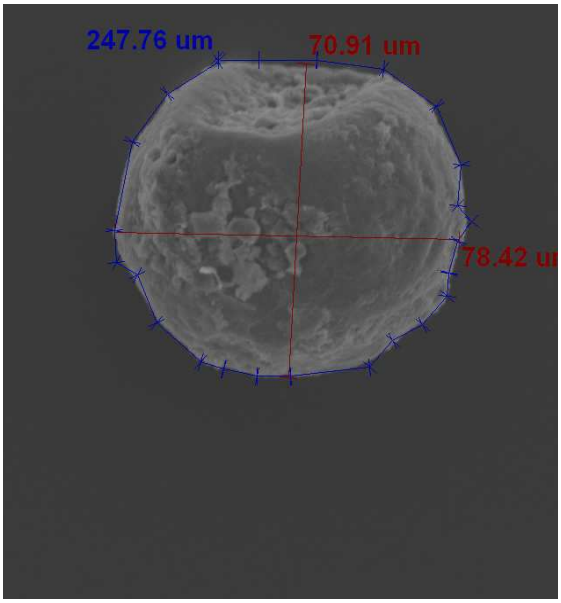
Plate 12 shows microscopic features of a spore of *Glomus pansihalos.* The spore had a globose shape and 2 distinctive spore wall layers. L1 was ornamented with cerebriform folds and spins while L2 was reddish brown and laminated. None of the wall layers reacted to Melzers reagent. SEM shows point of attachment of hyphal filament. This corresponds with the description for *Glomus pansihalos* by Wu in the Glomales of Taiwan. According to this author, the spore of *G. pansihalos* are borne singly in soil. There are globose, (60-) 90- 120 (-180) µm diam, occasionally subglobose, obovoid, ellipsoid, 70-130x80-140 µm. spores hyaline to pale yellow becoming reddish brown or dark brown with age.

Gigaspora decipiens Hall and Abbott

The spore of Gi. decipiens shown in plate 13 had 3 spore wall layers. L1 was smooth and adhered to L2 which was pale yellow while L3 was thin and darker. A hyphal filament attachment was evident. This typifies a *Gi. decipiens* spore as described in Invam data base. Readhead (1977) also described species of *Gigaspora* from Nigeria soil to be characterized by bulbous based spores.

Scutellospora reticulata

Plate 14 shows the subcellular structure and scan electron microscopy of a spore of *S. reticulata*. The spore was globose with no distinct inner wall layer. Its outer wall was laminated. It showed no reaction with Melzer's reagent. Brundrett (2008) identified such a spore to be long to *Scutellospora reticulata*

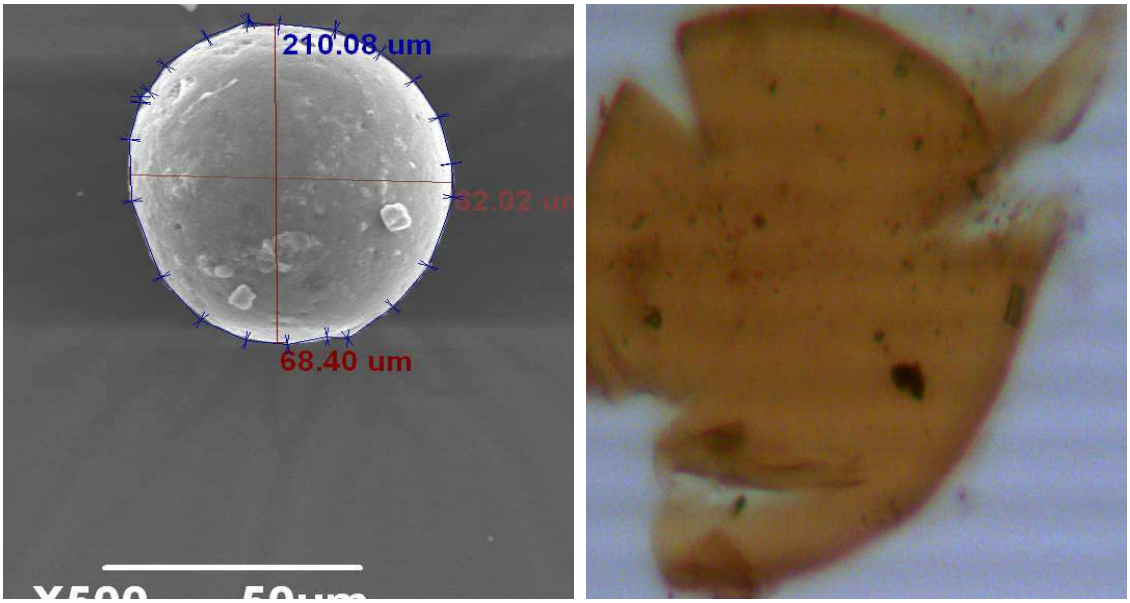


a b

Plate 9: Spore of *Paraglomus brasilianum*

a: SEM morphology

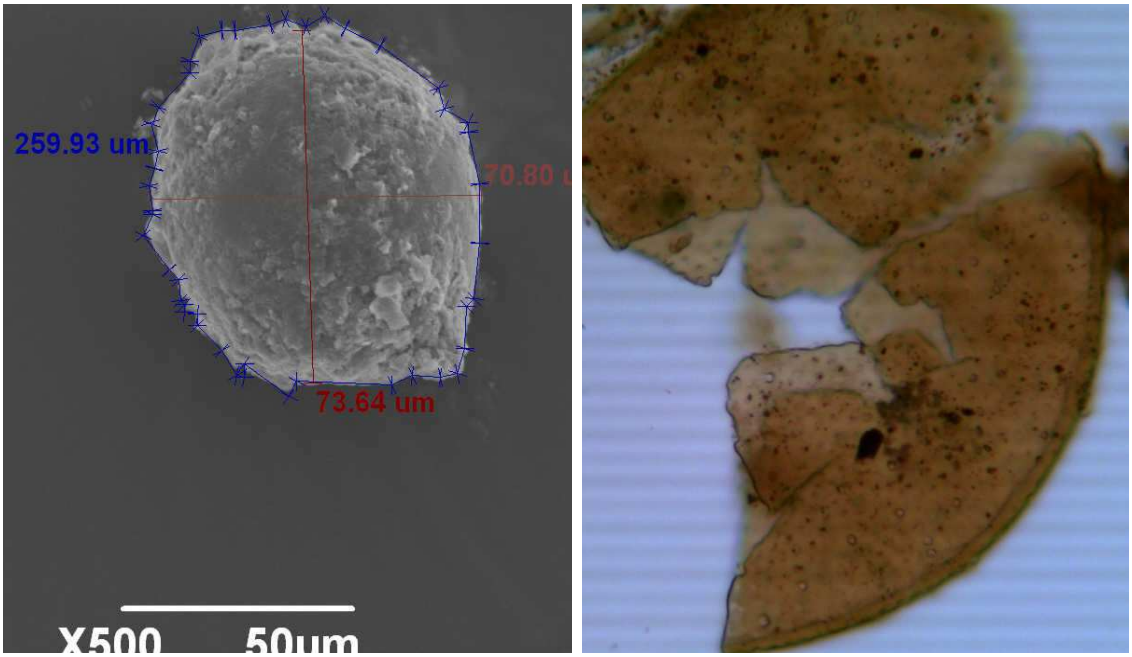
b: Subcellular structure



a b

Plate 10: Spore of *Glomus intraradices*

a: SEM morphology
b: Subcellular structure

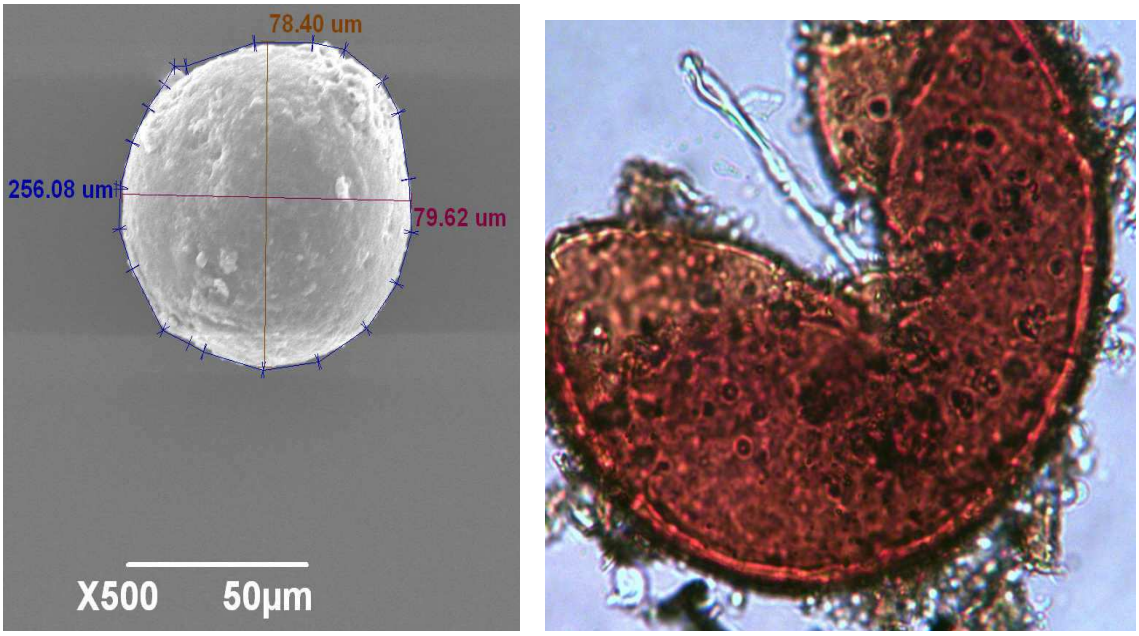


a b

Plate 11: Spore of *Glomus manihotis*

a : SEM Structure

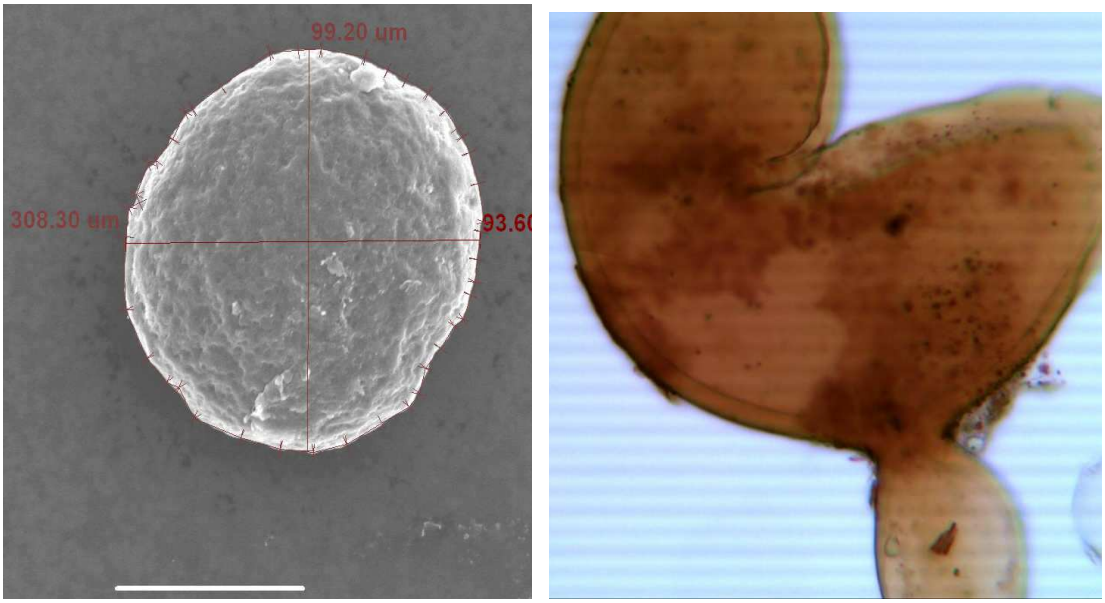
b: Subcellular Structure



a b

Plate 12: Spore of *Glomus pansihalos*

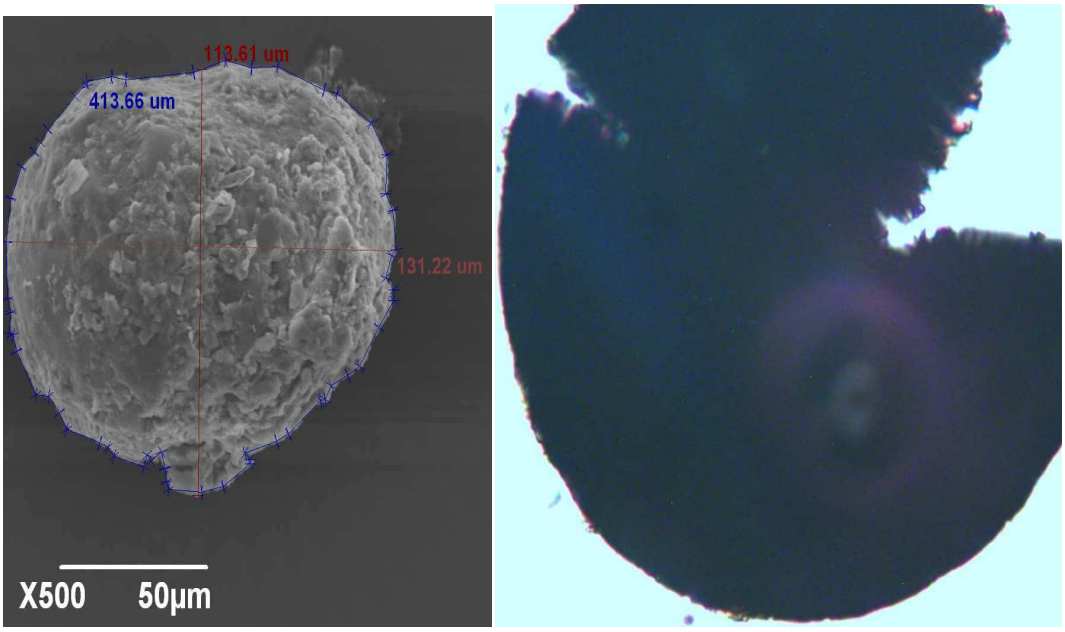
a: SEM morphology b: Subcellular structure



a b

Plate 13: Spore of *Gigaspora decipiens*

a: SEM morphology **b: Subcellular structure**



a b

Plate 14. Spore of *Scutellospora reticulata*

a: SEM morphology

b: Subcellular structure

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