Arbuscular Mycorrhizal Fungi Status of Selected Invasive Alien Plants Species in East Showa Zone, Oromia, Ethiopia

By

Woyama Gemado Shararo

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirements for Master of Science Degree in Applied Biology (Applied Microbiology)

Office of Graduate Studies

Adam Science and Technology University

January, 2021

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DECLARATIONS

I declare that the work which is being presented in this research entitled **as "Arbuscular Mycorrhizal Fungi of Selected Invasive Alien Plants Species in East Showa Zone, Oromia, Ethiopia"** in partial fulfillment of the requirement for the award of the degree of MSc in Microbiology. It was authentic record of my original work carried out from October 2019 to September, 2020 under the advisor of Dr: Zerihun Belay, Department of Applied Biology and School of Applied Natural Science, Adama Science and Technology University .The matter embodied in this has not been submitted by other person or me for the award of any other degree. All relevant resources of information used in this body have been duly acknowledged.

Woyama Gemado ---------- ----------

Name signature Date

This thesis has been submitted for examination with my approval as a University advisor.

Advisor: Zerihun Belay

Signature: ----------

APPROVAL SHEET

We the undersigned, members of the board of Examiners of the final open defense by Woyama Gemado Shararo have read and evaluated this thesis entitled "**Arbuscular Mycorrhizal Fungi Status of Selected Invasive Alien Plants Species in East Showa Zone, Oromia, Ethiopia**" and examined the candidate. This is therefore to certify that the thesis has been accepted in the partial fulfillment of the requirement of the degree of Master of Science in Applied Biology.

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LIST OF ACRONYMS AND ABBREVIATIONS

AC	Arbuscular Colonization
AM	Arbuscular Mycorrhiza
AMF	Arbuscular Mycorrhizal fungi
ANOVA	Analysis of Variance
Av P	Available Phosphorus
DTPA	Di ethylene Triamine penta acetic acid
GRSP	Glomaline related soil protein
IAPS	Invasive Alien plant species
ICP-AES	Inductively plasma atomic emission spectrometry
IF	Isolation Frequency
IV	Important Value
OC	Organic Carbon
PVLG	Polyvinyl Alcohol Lacto-glycerol
TN	Total Nitrogen

ABSTRACT

Invasive plant species are alien plant species non indigenous that has adverse effect on the invaded habitat. They are also species that displace native species and have the ability to dominate an ecosystem, or a species that enters an ecosystem beyond its natural range and causes economic or environmental harm. AMF colonization of selected invasive alien plant species was examined in east Showa zone of Oromia, Ethiopia. A field survey was conducted to see the distribution of major invasive alien plants in the different district of East Showa zone. Three replicates of soil and root samples from each plant species were collected during December, 2019 to January, 2020. Physico-chemical analysis of the soil samples, AMF spore extraction and enumeration and AMF root colonization were done. Morphological characterization and identification of AMF spores to species level or morphotype were carried out at Microbiology laboratory, Department of Applied Biology, ASTU. Analysis of variance (ANOVA) and correlation analysis were carried out with the SPSS software package. The results showed that spore density of invasive alien plants species varied between plant species. From Argemon orchrroleula 1418spore//100gm of soil to Senna didymobotry 551 spore/100gm of soil. Fungal colonization showed variation among the roots of alien plant species. It ranged from Nicotinia glauca (32%) to Lantana camara which is 15%. Based up on important value only one dominant AMF species were recorded IV (57) across all plant species. However, majority of AMF species were common with recorded IV (43 to 11.25) and few specie were recorded IV from (9 to 1) which is rare. In general in this study, significant variability in AMF spore density, diversity, and percent of root colonization was observed among the invasive alien species.

Key words: *Arbuscule,* Arbuscular mycorrhizal fungi, Invasive alien plant, Root colonization , Spore, Vesicle.

1. NTRODUCTION

Invasive plant species are also referred to as 'non-native' exotic, non-invaded habitat. They are also termed as species that displace native species and have the ability to dominate an ecosystem, or a species that enters an ecosystem beyond its natural range and causes economic or environmental harm (Colautti and MacIsaac, 2004).

Invasive alien plant species are not native to Ethiopia. According to (Temado *et al*., 2002) exotic plant species are introduced in to Ethiopia in 1976/1977. They cause particular problems on agricultural land, rangeland and biodiversity of the country, National park, water ways, river power dam area, road side, and urban area with great economic and ecological consequence (Temado *et al*., 2002).

In Ethiopia about 35 invasive alien plants species were identified (Gedyon Temiru, 2017). These plants are distributed in different part of the country especially in Tigray, Afar, Somalia, Oromia, Gambela, Benshangul and same south part of Amhara region (Rezene *et al*., 2011). Boy and Witt, (2013) indicated the major invasive alien species of Ethiopia with their botanical name *Senna didymobtrya(l), Argemone orchroleula (L), Nicotinia glauca (Nicotiana), Lantana camara (L), Parthenium hysterophorus (Parthenium (L), Prosopis juiflora C), Eichhornia crassipes (E. crassipes), Xannthium strumarium (L), Ageratum conyzoides (L), Senna occidentalis (L.) and Datura strumarium* (Gedyon Tamiru, 2017).

The invasive alien species are distributed uniformly in the Oromia region mainly in the rift valley area such as Dire Dawa, east Shawa zone, Arsi, Bale, west Arsi, Awash valley, eastern part of Harerghe,Wabeshabelle basin and Borana.These species also distributed in Ilu abbora, Jimma and Wallega (Rezene *et al*., 2011). These invasive alien species are known by their capability to spread fast and competitiveness and ability to colonized area with in short period of time .They have great impact on the society economic life, health and national heritage of global issues (Gedyon Temiru, 2017).

They affect every ecosystem types of plants biodiversity and habitat (Wakshum *et al.*, 2018). Invasive alien plant species have induced impacts on native species directly competing for resource by altering habitat and modify hydrology nutrient cycling and other ecosystem services.

As the study results of Rezene *et al.* (2011) showed that east Showa zone is one of the major zone that located in the rift valley region that colonized by the invasive alien plant species. Beside, these invasive alien species distributed uniformly and enormously in the natural ecosystem, agricultural land of the area like in Koka and Lake Batu, Awash River and the roadside of the national park that is found in zone and urban green areas with economic and social consequence (Wakshum *et al.*, 2018).

AMF are plant symbiotic soil fungi in the phylum glomeromycota that colonize roots of the majority of higher plants. It is estimated that more than 80% of all terrestrial plants form this type of association, that includes many agriculturally and horticulturally important crop species (Smith and Read, 2008). AMF help plants to acquire nutrients such as phosphorus, nitrogen, potassium and zinc from the soil in exchange for photosynthesis (organic carbon) supplied by the host plant (Smith and Read, 2008). AMF also protect their host plants against biotic and abiotic stresses (de Carvalho *et al*., 2010). Other functions of AMF are also a key f actor in maintaining plant diversity (Vander Heijden *et al*.,1998). Since symbiotic association of AMF and plant roots considered to be the oldest and most symbiotic relationship between microorganism and higher plants.

The main aim of this study was to examine the AMF status of selected invasive alien plant specie (*Parthenium hysterophorus (*partenium), *Senna didemobtryka (*fresen), *Argemone orch roleuca(* poppy), *Nicotiana glaua* (tree tobacco), *Lantana camara* (lantana), *Eichlurnia crassi pies* (Water Hyacinth) and *Datura strumarium (jimson weed)* in east Showa zone Oromia, Ethiopia. These plants identified by AAU herbarium.

1.2. Statement of the problem

Ethiopia has great geographic diversity and climatic variability. This has created diverse and suitable ecosystems, which are home to large number of flora, fauna and microbial species. However, there are threats to biodiversity by invasive alien plant species (IAPS). IAS has the ability to establish them, invade, out compete natives and take over the new environment. They pose a global threat to the conservation of biodiversity through their proliferation and spread, displacing or killing native flora and fauna and affecting ecosystem services. They are particularly damaging in geographical or ecological islands, which are rich in endemic species. Invasive plants smother, outcompete and displace indigenous species, changing the composition and function of entire ecosystems including in Ethiopia (Wakshum *et al*., 2018). Invasive Alien Species are great concern in Ethiopia, posing particular problems on agricultural lands, range lands, biodiversity of the country, national parks, water ways, rivers, power dams, roadsides and urban green spaces with great economic and ecological consequences. The great majority of terrestrial plant species are associated with AM fungi (Smith & Read, 2008) and these appear to be important in determining the ecology of plant species and communities (Klironomos *et al*., 2011). The function of the AM fungal symbiosis during the alien plant invasion process has subsequently gained increasing consideration. Therefore, arbuscular mycorrhizal fungi can be expected to have a favorable effect on the process of invasive alien plant species.

Marlers & Zabinski, (1999) reported that AM fungi can increase growth and competitiveness of spotted knapweed (*Centaurea stoebe*) which is one of the most invasive weeds in the inter mountain west of the USA. (Lekberg *et al*., 2013) also showed that leafy spurge (*Euphorbia esula*) support a higher abundance and diversity of symbiotic arbuscular mycorrhizal fungi (AMF) than multi-species native plant communities. Therefore, assessing plant mycorrhizal status and other mycorrhizal related plant functional traits may thus help to provide further understanding of the establishment of alien plant species and their invasion success.

1.3. Objective

1.3.1. General objective

The main objective of this study was to investigate the AMF status of some invasive alien plants species in east Showa zone, Oromia, Ethiopia.

1.3.2. Specific objectives

- To determine the Arbuscular mycorrhizal fungi spore density and diversity associated with the invasive alien species.
- To assess the Arbuscular mycorrhizal fungi root colonization status of the invasive alien plants species.
- To investigate the correlation between arbuscular mycorrhizal fungi parameters and physico-chemical soil parameter of some invasive alien plant species around east Showa zone.

2. LITERATURE REVIEW

2.1. Invasive alien plant

Invasive plant species are among the world's greatest threats to biodiversity and native specie s in protected areas (Lwando, 2009). Invasive species may change the community structure through competition which is categorized into two; exploitation competition whereby there is indirect interactions in the use of resource and interference competition where by invasive plants produce chemicals that affect the native plant (Loeuille, 2017).

Once invasive plants become established, they change the soil chemistry and shift nutrient cycling in an ecosystem. This can have impacts on plant composition, diversity, and succession within a community and also cycling of critical elements like carbon and nitrogen on a larger, potentially even global scale (Rout *et al*., 2013). Both native and invasive plants form some relationships with bacteria and fungi in the soil that facilitate the extraction and conversion of elements to biologically usable forms (Batten *et al*., 2006). Yet it is not well understood whether the changes in soil biogeochemistry are due to an advantage that invasive plants acquire from interacting with their microbiome or when alien species invade and colon -ize the area with other microorganisms. Many plant species are host to a whole group of microorganisms that not only live in plant cells, but also in the soil surrounding the plants' roots (Rout and Callaway, 2012). These micro-organisms often form close mutual relationships with their plant hosts. Some convert atmospheric nitrogen into usable forms that are then exchanged for ;carbon from the plant. Nitrogen in the form of nitrates and nitrites are frequently limiting in soils, yet many invaded ecosystems have shown to have more carbon and nitrogen in plant tissues and soils compared with systems dominated by native plants (Mack *et al*., 2000).

The invasive alien plants reviewed are *Parthenium hystrophorus* L, *Senna didymobotrya* L, *Argemone orchroleuca* L, Nicotiana glauca, *Eichhornia crassipes, Datura strumarium* and *Lantana camara*. *Parthenium hystrophorus* L. known as parthenium weed is of family Asteraceae (Compositae) is distributed in North Central America and native to Mexico. It is erect, perennial herb up to 60cm high, stem repeatedly branched, leaves are alternate (Hedberg *et al*., 2004).

Senna didymobotrya L (fresen) is a species of flowering plants in the fabacceae known by the common name Africa senna, popcorn senna, candelabra tree peanut cassia. It is native to Africa, where it can be found across the continent in several type of habitat. It is bushy shrub 1-5m high. Leaves are 10-30cm long. The stamen are two with large anthers, five medium sized and three small (Edwards, 1989).

Argemone orchroleuca L. is flowering plant in the family papaveraceae commonly known as prickly poppies. It is native to west India and central America; now a cosmopolitan tropical and sub-tropical weed. It is herb to 1 m high, often branching near the base. Stems spiny, Leaves are ternate, blue-green, sessile to clasping, lower leaves deeply lobed, upper more shallowly lobed. Flowers are 25-45 mm in diameter. Petals are white, yellow to orange, 20-30 mm long. Stamens are numerous (Edwards *et al*., 2000).

Nicotinia glauca is shrub or small tree in family solonaceae, of which all parts are glabrous. It is known by the common name tree tobacco, desert tobacco, and wild tobacco. Their leaves are alternate, Flowers may in lax panicles up to 25 cm long and Stamens sub-equal (Hedberg *et al*., 2006).

Datura strumarium L. known by the common names jimson weed, is a plant in the family solanaceae. It is believed to have originated in Mexico, but has now become naturalized on many other regions. *Datura strumarium* is shrub by annual herb up to 2m tall but often much smaller and non-glandular hair. Leave are one or several at the nodes; petiole 1-5 cm long, flowers erect and stamen include; anthers 3-5mm long (Hedberg *et al*., 2006).

Lantana camara L. also known as big-sage; white-sage is fast-growing woody thicket-forming shrub, is native to tropical and sub-tropical South and Central America and currently widely distributed in many countries including Ethiopia (Zalucki *et al*., 2007). It is a species of flowering plant with in the Verbenaceae family. *Lantana camara* L. is small perennial shrub which can grow to around 2 m tall and form dense thickets in a variety of environments (Sharma *et al*., 2005). It has small tubular shaped flowers which each have four petals and are arranged in clusters in terminal areas stems. Flowers come in many different colours including red, yellow, white, pink and orange which differ depending on location in inflorescences, age, and maturity (Ram, 1984).

Eichhornia crassipes commonly known as water hyacinth; common water-hyacinth; free-floating water hyacinth is a perennial aquatic plant (or hydrophyte) native to the Amazon basin (to tropical and sub-tropical South America), and is often considered a highly problematic invasive species outside its native range (Harley *et al*., 1996). It is has broad, thick, glossy, ovate leaves, water hyacinth may rise above the surface of the water as much as 1m in height. The leaves are 10–20 cm across, and float above the water surface. They have long, spongy and bulbous stalks. The feathery, freely hanging roots are purple-black. An erect stalk supports a single spike of 8-15 conspicuously attractive flowers, mostly lavender to pink in colour with six petals. In their native range these flowers are pollinated by long tongued bees and they can reproduce both sexually and clonally (Toft *et al*., 2003).

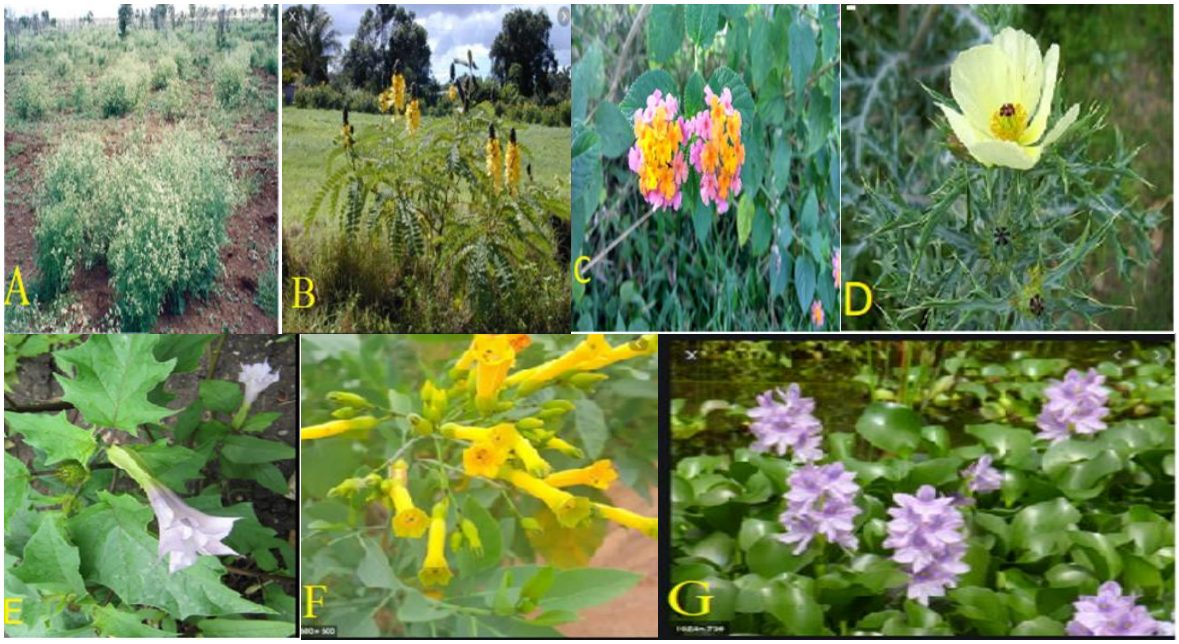

Figure1: (a) *P. hysteropherus (*parthenium*) (b), S. didymobotry* (Africa senna) (C), *Lantana camar* (lantana) (d), A. *orchroleula (e), Datura strumarium (* Jimson weed) (f), *Nicotiana glauca* (Wild tobacco) (g), *Eichhornia crassipe* (Water hyacinth).Some invasive alien plants species in east Showa zone.

2.2. Invasive alien plants and Arbuscular Mycorrhizal Fungi

Invasive alien plants also have AMF as native plants have. AMF are obligate symbiotic fungi which can be found in most species of terrestrial ecosystem and colonized more than 80% all of land plant species. It plays an important role in the colonization of most terrestrial plants in the natural ecosystem (Qin *et al.,* 2015). Two types of mycorrhizal are known: ecto and endo-mycorrhizas (Smith and Read,2008).The ectomycorrhizas are characterized by an extra cellul ar fungal growth in the root cortex whileas the endomycorrhizas are characterized by forming

inters and intra cellular fungal structures called as vesicles and arbuscles. This characteristic growth gives theendomycorrhiza the alternate name vesicular Arbuscular mycorrhizal and these are the structures where the exchange of nutrients between the partners easily takes place (Sahu *et al*., 2010).

The formation of arbsucules greatly increases the contacts area between the plants and the fungus. The exchange of nutrients such as phosphorus, zinic and copper from the soil to the plants took places in arbuscules with the aid of AMF and in return this plant provides carbohydrate to fungi (Franken and George, 2006).

AMF forms a symbiosis relationship with the roots of most terrestrial plants on earth. It improves plant growth through increased nutrient uptake in exchange for photosynthetic carbon from their host. Arbuscular mycorrhizal fungi play an important role in sustainable agriculture as well as agricultural ecosystem management. The important genera of endomycorrhizal fungi reported are *Acaulospora, Entrophosphora, Gigaspora,Glomus, Skclerocystis* and *Scutellospor (*Gianinazzi *et al.,* 2010*)*. The association between AMF and plant benefits the nutrients, growth and survival due to enhanced exploitation of soil nutrients (Moreira *et al.*, 2016).

2.2.1. Taxonomy of Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi (AMF) exist in three forms: spores, soil borne hyphae and colonized roots. The taxonomic identification of AMF species is based upon morphological characteristics of their spore size, colour, and spore wall characteristics, stalk attachment, cytoplasmic structure, wall thickness, structure and ornamentation besides mode of spore germination and presence of secondary spores and sporocarp (Bharadwaj, 2007).

The large diameter and lack of septic and clamp connections distinguish some soil hyphae of AMF from the other fungi (Antal *et al.*, 2001). The fine hyphae are not easily distinguished from other soil fungi. Antibodies to glomalin a recently described glycoprotein produced and abundantly shed by AMF can be used to distinguish AMF hyphae from non-AMF hyphae in soil (Douds and Millner, 1999).

In general as study report (Nichols, 1999), the following are important diagnostic features for preliminary grouping of the AMF into their respective taxa. (1) Vesicles in the root and

formation of chlamydospores (thick wall, asexual spore) borne from subtending hyphae for the suborder Glomineae or (2) Absence of vesicle in the root and formation of auxiliary cells and a zygospores (spores resembling a zygospore but developing asexually from a subtending hyphae resulting in a distinct bulbous attachment) in the soil for the suborder *Gigasporinae*.

The spores of AM fungi are very distinctive. They range in diameter from 10µm for *Glomus tenue* to more than 1000 µm for some *Scutellospora* species. The spores vary in colour from hyaline (clear) to black and in surface texture from smooth to highly ornamental ones. *Glomus* forms spores on the ends of the hyphae. *Sclerocystis* spp. form spores arranged in an orderly manner around a central plexus, *Acaulospora* forms spores laterally from the neck of a swollen hyphal terminus and *Enthrospora* forms spores within the neck of a swollen hyphal terminus. The *Gigasporineae* are divided in to two genera based up on the presence of inner membranous walls and a germination shield for *Scutellospora* or the absence of these structur es for *Gigaspor (*Oehl *et al*., 2011). Phylum Glomeromycota divided in to 3classes, five order , 14 family and 29 genera (Choosanga *et al*., 2019).

The family *Glomeraceae* have 13 species (Vieira *et al.,* 2019*).* They found that the spore formation in 10 genera was exclusively glomoid, one was gigasporoid, seven were scutellosporoid, four were entrophosporoid, two were acaulosporoid, and one was pacisporoid. Whereas, three genera bimorphic spore formation and one genus i.e., *Geosiphon* is associated with cyanobacteria. Taxonomy of AMF from the total number of described AMF species was 318 in early October 2019 (www.amf-phylogeny.com). These species belong to 34 genera and 12 families. They play a key role in natural and agricultural ecosystems due to their capacity to form multifunctional arbuscular mycorrhizae (AM), which are considered to be the oldest, most widely distributed, and most important symbiotic associations in nature Smith and Read,(2008).

2.2.2. Invasive alien plants Species and Arbuscular Mycorrhizal Fungi symbiosis

Invasive alien plants also live with fungi by symbiotic association with plants. In the associati on, fungi colonize the host plant roots intracellular as Arbuscular mycorrhizal fungi and also extracelularly as ectomycorrhizal fungi. They are an important component of soil life and soil chemistry (Sadhana, 2014). The ability of AM fungi to form close associations with most species of plants and significantly improving the up take in mineral nutrients in exchange of photosynthetic decreas the stress causeby the biotic and abiotic factors and huge potential as bio fertilizer have greatly drawn attention to researcher study of AMF on the invasive alien species plants (Ellouze *et al*.,2014). Arbuscular mycorrhizal which is formed between plants and Glomeromycota fungi has the wide distribution in the nature. AM fungi inhabit avariety of ecosystems including agricultural land, forest, grass land and many stressed environments and colonize the roots of most plant including the bryophyte, pteridophyte, gymnosperms and angiosperms (Sadhana, 2014).

When invasive plants are introduced to a healthy ecosystem, they can disrupt fungal mutualistic associations with native plants. Moreover, plant invasions may even prevent these mutualistic associations from occurring by altering soil nutrient dynamics, changing soil food webs, or introducing plant pathogens (Pickett *et al.*, 2018). Smith and Read, (2008) reported that various researches conducted by scientists indicated that many plants do not interact directly with the soil environment; instead, these plants interact indirectly with the soil environment through the arbuscular mycorrhiza fungi. AM fungi possessed the capability of forming symbiosis with plants making the Glomeromycota being organism in most plants (Ghignone *et al*., 2012).

2.2.3. Invasive alien species and ecological significance of Arbuscular mycorrhizal fungi

According to study of (Hartnett and Wilson, 2002);Vander Heijden *et al*., 2003); (Klironomos *et al*., 2011), relationships with mycorrhizal fungi are great importance in shaping the ecology of plant species and communities. Incorporating plant mycorrhizal status and other mycorrhiza related plant functional traits may thus help to provide further understanding of the establishment of alien plant species and their invasion success (Menzel *et al*., 2017). Smith and Read, (2008), Moora, (2014) noted that three groups of plant species

can be distinguished according to their mycorrhizal status: (1) obligate mycorrhizal (OM) plant species that are always colonized by mycorrhizal fungi, (2) facultative mycorrhizal (FM) plant species that are colonized under some conditions but not others, and (3) non-mycorrhizal (NM) plant species that are never found to be colonized by mycorrhizal fungi. It is important to note that plant mycorrhizal status and plant mycorrhizal dependency (or responsiveness) are distinct plant traits, not to be confused studied by (Moora,2014). While mycorrhizal dependency depicts plant species growth responses under given conditions, mycorrhizal status does not give direct information about the functional significance of mycorrhizal colonization for plant individuals. It rather refers to the mere presence/absence of fungal colonization, and can be used as a proxy for estimating the potential importance of mycorrhizal symbiosis for plants at species level (Bueno *et al*., 2017).

2.2.4. Invasive alien plants and Arbuscular mycorrhizal fungi mycelium network

Invasive alien plants form Arbuscular mycorrhizal fungi mycelium network. As study report of (Selosse *et al.,* 2006), AMF are linked to the formation and maintenance of the mycelium network. In order for such a network to exist and be ecologically relevant, it is needed that at least some mycorrhizal species show low selectivity, and hence have the capacity to establish mycelial linkages between different plant species (Helgason *et al*., 2002). Low fungal selectivity may or may not result in comparable responses of different plants to the same fungal species. The existence of such networks allows the movement of carbon, water, and nutrients between plants belonging to different species, genera, or even families (Ferrol *et al*., 2002).

The mutualistic nature of Arbuscular mycorrhizal (AM) symbioses relies on the ability of the fungal mycelium to take up mineral nutrients from the soil solution and to transfer them to the symbiotic roots in exchange for carbohydrates. Plant benefits from AM symbiosis are traditionally recognized as improved access to limiting soil resources, mainly immobile nutrients such as P, Cu, Zn and ammonium. On the other hand, the carbon supplied from the host plant to the fungus is essential to the formation and functioning of arbuscular mycorrhizas and for the completion of the fungal life cycle (Ferrol *et al*., 2002).

Although finding of the (Verbruggen *et al.*, 2012), mutualism of the AM fungi association is based on this bidirectional nutrient exchange. This does not necessarily mean that nutrient transfer from the fungus to the plant is directly linked to C transfer to the fungus. In fact, different AM fungi in symbiosis with the same host plant, can considerably differ in their C–P exchange ratio and, consequently, in their degree of functional compatibility with the plant. Additionally several studies have shown that (Mbodj *et al*., 2018). C transfer can be decoupled from P transfer under certain environmental conditions, as for instance, under conditions of high soil P availability.

According to Ferrol *et al*., (2002), in any case, an indirect link between C and P transfer is evident as C supplied to the fungus will support mycelia growth and, indirectly, P uptake from the soil. This bidirectional transfer of nutrients between the plant and the fungus is believed to take place across the symbiotic interfaces which are bordered by the plant and fungal plasma membranes.

Although as study report of (Hashem *et al*., 2018), current knowledge of the molecular mechanisms underlying transport nutrient in Arbuscular mycorrhizal is still in its infancy, it is generally accepted that such bidirectional transfer of nutrients across the symbiotic interfaces involves passive efflux of solutes from the donor organism into the interfacial apoplast, followed by active uptake by the receiver organism.

As the study report (Allen, 2007), the importance of common mycorrhizal networks for water transfer and redistribution has received remarkably little attention. However, the role of the mycorrhizal network in hydraulic redistribution could be of particular importance in agro forestry systems. After deeply rooted plants have taken up water from profound soil layers, the activity of neighboring shallow rooted plants could be sustained by nocturnal water efflux coupled to water uptake and transfer by mycorrhizal fungi in superficial soil layers. This process of mycorrhizal mediated hydraulic redistribution has been demonstrated for ectomycorrhizal and arbuscular mycorrhizal systems.

As study (Williams *et al.,* 2014), agroforestry systems are particularly conducive to build up and maintain mycorrhizal networks as compared to the annual cropping systems characterized by regular disturbances and bare fallows (de Carvalho *et al*., 2010), noted the existence of such networks can result in faster establishment of the mycorrhizal symbiosis in

seedlings under agroforestry systems because plant colonization tends to be faster from the mycelium than through spore germination.

The role of mycorrhiza in the nitrogen transfer from one plant to another has been studied (Ngwene *et al*.,2013), estimated N transfer of around 25%–35% from the N-fixing tree *Gliricidia sepium* to the grass *Dichanthium aristatum* adjacent to agroforestry plots.

They also observed a positive correlation between *Gliricidia* root density and amount of N in the grass derived from atmospheric sources. They suggested direct N transfer from trees to grass, for instance through root exudates or via common mycorrhizal networks (corresponding to "direct transfer through the mycorrhizal.

Phosphorus can also be moved between plants (with unilateral transfer being more frequent than bidirectional transfer) and can end up in shoots of receiver plants noted by (Selosse *et al.*, 2006). However, P is less mobile and required in lower amounts than N, and the transfer of P is less than that of N. According to study report (Dröge *et al*., 1999), furthermore, the magnitude of P transfer is too small to significantly affect the nutrition of the recipient plant. However, more intense P transfer (and also N transfer) occurs from dying roots with root death occurring, for instance, as a consequence of shoot pruning or root pruning, both regular processes in agro forestry management.

2.3. Arbuscular mycorrhizal fungi of plants and soil structure and chemical quality

AMF improves soil structure for the stability of the agro-ecosystems which in turn determines the flow of water, gas, and nutrients in the soil (Rehman *et al*., 2019). The AMF cover a range of spatial scales, and include (1) directing clay particles around the hyphae, (2) producing polysaccharide secretions that connect clay particles, (3) performing a "packing" effect of particles by hyphae, leading to a new microstructure (Dorioz *et al*., 1993) and (4) directly transferring the carbon from plants to soil, which promotes aggregation.

Mycorrhizal effects do not depend only on the live mycelium. They also produce and subsequently deposit a substance known as glomalin. For analytical purpose, this glycoprotein complex is often referred to as "glomalin related soil protein" (GRSP). It is a very recalcitrant glycoprotein with high cementation capacity which remains in the soil for

longer time periods up to 6 to 42 years than the hyphae, thus contributing more persistently to the stabilization of aggregates (Thies and Rillig, 2012). (Lovelock *et al.*, 2004) estimated that approximately 3.2% of soil total C and 5% of soil N in tropical forests was in the form of glomalin.

(de Carvalho *et al.*,2010) suggested that as a rule of thumb around 10% of soil organic carbon is constituted by GRSP. These values suggest that the contribution of AMF to soil C sequestration is substantial.

The mycorrhizal symbiosis implies carbon costs for the plants ranging from10% to 20% of photo assimilates. If one scales up such levels (assuming that15% of photosynthate are consumed by fungi) to the global scale, about 10 Pg9 (10×10^9 g) of Care annually used by fungi and (at equilibrium) returned to the atmosphere (Schuur, 2016).

Chapman *et al.*(2006) estimated that the annual C flux through AMF amounts to 10^{12} g, most certainly are far too low. Considering that tropical ecosystems are mainly composed of AMF dominated vegetation the role of AMF in the regulation of global carbon balance is substantial. By producing recalcitrant compounds such as GRSP, mycorrhizal agro forestry systems make a major contribution to the rehabilitation of degraded land sequestration of carbon, and possibly reduction of emissions of other greenhouse gases such as N_2O(Mutuo *et al.*, 2005).

2.3.1. Arbuscular mycorrhizal fungi of plants and acquisition of soil phosp horus

AMF found in soil and roots of plants. Root hairs are important absorptive features extending a few millimeters to a few centimeters beyond the root surface, but we must not ignore the fact that they also excrete organic materials into the soil. As study report (Chantal, 2004) , In fact, root hair absorption of mineral nutrients decreases with age and old root hairs tend to be more excretory than absorptive. Absorption is greatest by root hairs although it also occurs in non-hairy root cells, and absorption rates are higher close to the root apex. Arbuscular mycorrhizal fungi typically penetrate roots at the level of root hairs (Smith and Read 1997). Treseder, (2013) noted that some of the hyphae that grow along roots spread AMF colonization to new root tissues. Extra radical hyphae extend away from the roots into bulk

soil, but also into the zones of root hair senescence and root turnover hence complementing root uptake capacities and minimizing the impact of root exudation losses (Chantal, 2004).

Roots influence microbial populations and community structure and most likely influence microbial processes. For example, N mineralization of alfalfa stoves was enhanced by the presence of living roots presumably because of the plant derived C input to the rhizosphere stimulated the populations of saprotrophs as reported(Pare *et al*., 2000).

Roots can also mobilize P directly from the soil mineral and organic P pools. According to (Chantal, 2004), the organic acids released by roots (more accurately called organic anions) solubilize unavailable soil phosphates, mobilizing P that can then be taken up by plants.

According to the (Parfitt, 1978), organic anions chelate metal cations associated with P in minerals; this destabilizes minerals and releases P. Furthermore, organic acids strongly bind to P sobbing sites making these sites unavailable for reaction with P. Organic anions also react with free Ca, Fe and Al ions. This competing with the precipitation reactions of P with the cation (Stevenson,1967). On the other hand, roots release phosphatase enzymes which cleave phosphate groups out of soil organic matter when P supply becomes low. Plants mobilize nutrients indirectly by releasing molecules into the soil that stimulate specific soil organisms such as symbiotic N2-fixers and AMF (Lum and Hirsch, 2002). By the provision of a C source to rhizosophere microorganisms, plants also stimulate a large number of microorganisms with P solubilization or mineralization capabilities (Richardson, 2001).

2.3.2. Arbuscular mycorrhizal fungi of plants and protection against heavy metals and aluminum

The host plant provides the fungus with soluble Carbon sources and the fungus provides the host plant within an increased capacity to absorb water and nutrients from the soil. 90-95% of all land plants form some type of mycorrhizal associations so that mycorrhizae not roots are the chief organs of nutrient uptake by plants (Smith and Read, 1997).

According to Andrade *et al. (*2003) and Silva *et al.(*2006), besides their effects on the chemical properties of the soil and the ability to supply nutrients to the plants, AMF may mitigate phytotoxic effects caused by elements such as heavy metals and aluminium. Heavy

metal concentrations in host plant tissues may decrease as a result of fungal association, and reductions of Pb concentrations were noted in the aerial parts of soybean.

Similarly, the concentration of Al^{3+} is reduced in mycorrhizal banana plants as compared to non mycorrhizal banana (Rufyikiri *et al.,* 2000). Tolerance to Al may be conferred as a result of increased P acquisition or through other mechanisms. A study of P uptake and Al resistance in relation to the dynamics of GRSP would be helpful. On the other hand, acting through mechanisms similar to those that are involved in enhanced P uptake, the mycorrhizal association can enhance Zn and Cu uptake when concentrations of these metals are limiting.

The collective effects of AM fungi on soil qualities also results in higher water retention capacity, which benefits plant growth in addition to improved nutrient supply. The benefits of AM fungi are particularly critical for plants in dry sandy soils in arid regions. These soils often show low fertility and are highly vulnerable to erosion by wind and rain. In such cases, plantings with mycorrhizal plants can be a sustainable way to counteract erosion and improve soil fertility (Min *et al.,* 2018). As study report of (Nakmee *et al*., 2016), apart from the improved soil structure, AM fungi reduce nutrient leaching from the soil. Nutrient leaching is a serious problem since it results in loss of soil fertility and pollution of ground water and surface water (rivers, lakes).

According to the study (Cameron *et al*., 2013), intact ecosystems exhibit a good nutrient retention capacity due to efficient adsorption and retention of nutrients by roots and soil microorganisms (including AM fungi). However, agricultural soils are by definition disturbed by agricultural practice (in particular plowing) and they receive large amounts of fertilizer mainly N, P, K. These in particular the highly mobile nitrate are prone to be washed out from the soil due to the lack of a good nutrient retention system.

The beneficial effects of AM fungi against nutrient leaching operate at different levels. First, improved soil structure allows for increased nutrient sequestration to the micro and macro aggregates in mycorrhizal soil. AM fungi take up nutrients from the soil solution and final mycorrhizal soils exhibit better retention capacity of thesoil solution (Querejeta, 2017). They are benefitting at the same time the availability of nutrients and water to the plant. Adetailed documentation of the beneficial effect of AMfungi on plants under drought stress was reporte d for tomato (Bitterlich *et al*., 2018).

Reduced leaching from mycorrhizal soils has been documented in particular for P and N, but it conceivably also involves other mineral nutrients. AM fungi integrate the nutrient fluxes in the soil by generating closed nutrient cycles there by promoting long term soil fertility (Min *et al*., 2018).

3. MATERIAL AND METHODS

3.1. Description of Study Area

The study sites were located in the districts of east Showa zone in Oromia Reginal State, Ethiopia. East Showa is located in the Rift valley region connecting the western zone to the eastern parts of Oromia. It is bordered in the south by west Arsi zone, in the southwest by the southern Nations, Nationality and People Region (SNNP), in the west by south west Shewa and Oromia special zone surrounding Finfinne, on the northwest by north Showa, on the north by the Amhara region, on north west by Afar region and on the south east by Arsi and Bishoftu, Metahera, and Batu town. It covers an area of 9547 Km^2 of land. Its latitude and longitude between 8°9′N 38°49′E and 8.150°N 38.817°E. The temperature of the east Showa zone ranges between 30.8ºC in May and 12 ºC in December with an average annual temperature of 21ºC (east Showa zone Agricultural office). The rain fall distribution is uni modal and the rainy season lasts for three months from mid-May (741.43 mm) to July (219.28) and September (104.45mm). The soil texture of the study area is classified as sand soil, clay soil, sandy loam and loam soil(east Showa zone Agricultural office). Specifically, the sampling locations are found in east Showa in Adama worede and Ademi tulu jido kombolcha (ATJK). Sampling of invasive alien plant species was carried out by purposive sampling method.

Key information about the map of study area shown by arrow on map

Adama worede

Adami tulu jido komblcha (ATJK) worede

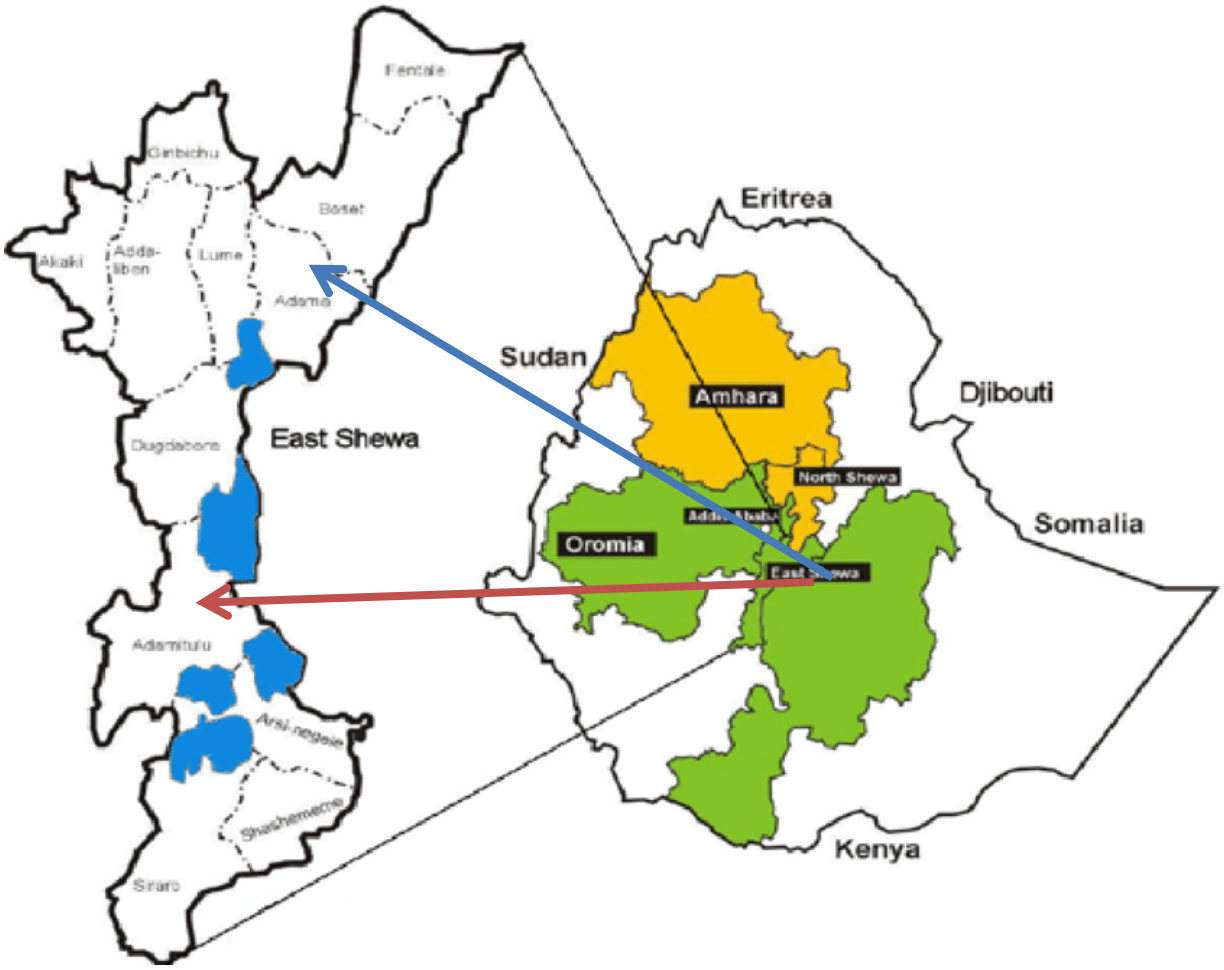

Figure2. Map of the study area from Ethiopia, Oromia and East Shawa (internet).

3.2. Study Design and Field Sampling Method

A survey was performed to evaluate the distribution of major invasive alien plants in the districts of east Showa zone. A descriptive cross sectional study design was employed and purposive sampling technique was used to collect the roots and rhizosphere soil of plant samples to analyze mycorrhizal status of the selected invasive plants. A total of the seven different species of alien plants species were selected for this study including, *Senna didy mobotrya* (African senna), *Argemone orchroleula* (prickly popies), *Nicotianaglauca* (wild tob acco), *Lantana camera* (lantana), *Parthenium hysterophorus* (parthenium weed), *Eichhornia rassipes* (water hyacinth), *Datura strumarium* (jimson weed).

Three replicate of soil and root samples were collected from each plant species. In total the 3 x7 = 21 soil and root samples were collected during December, 2019 to January, 2020. From each plant species about 500 g of composite soil sample was collected and sealed in sterile plastic bags. Similarly, 3x7 =21 fine root samples from the invasive alien plant species were collected washed with tap water and cut in to2-3cm pieces and placed inside plastic tubes and preserved in 50% ethanol. Finally, samples were taken to Adama Science and Technology University, Microbiology laboratory for further analysis.

3.3. Physco-chemical Analysis of Soil Sample

The physical and chemical properties of soil samples were done at the Melkasa agricultural Research Center (MARI) following the methods described below. The pH was measured by using soil to water suspension ratio 1:2.5 (w/v), total N was determined by the Kjeldahl meth-od (Hinds *et al.*,1980), organic carbon by oxidation procedure(Walkley *et al*.,1934), available P done by Olsen method(Olsen *et al*, 1954) and soil texture was measured by Boyacuse hydrometer methods (Gee *et al.*,1986) using the textural triangle at Melkasa Agricultural Research center.

3.4. AMF Spore Extraction and Enumeration

AM fungal spores were extracted from soil by wet sieving and decanting method (Gerdeman and Nicolson, 1963) and in sucrose (50% w/v) solution after (Brundrett *et al.,* 1996). Hundred grams of soil sample was suspended in 1L of tap water by gentle stirring. Heavier particles were allowed to settle for a few seconds and the liquid is decanted through a 500µm

sieve to remove large particles of organic matter. The suspension was then, passed through 180 µm, 90 µm and then through a 63 µm sieves successively. The contents of 180 µm, 90 µm and 63 µm sieves were poured into a centrifuge tubes and centrifuged for 5 minutes at 2000 rpm. After pouring off the upper solution, 50% of sucrose solution was added in to the pellet and the mixture was centrifuged for 1 minute at 2000 rpm. Finally, spores, spore clusters and sporocarps were carefully washed and transferred to a Petri dish to prepare for counting under the dissecting microscope (ISO 1006) at ×40. Enumeration of spore numbers per 100g of dry soil was under taken according to INVAM (2013),http://invam.caf.wvu.edu.

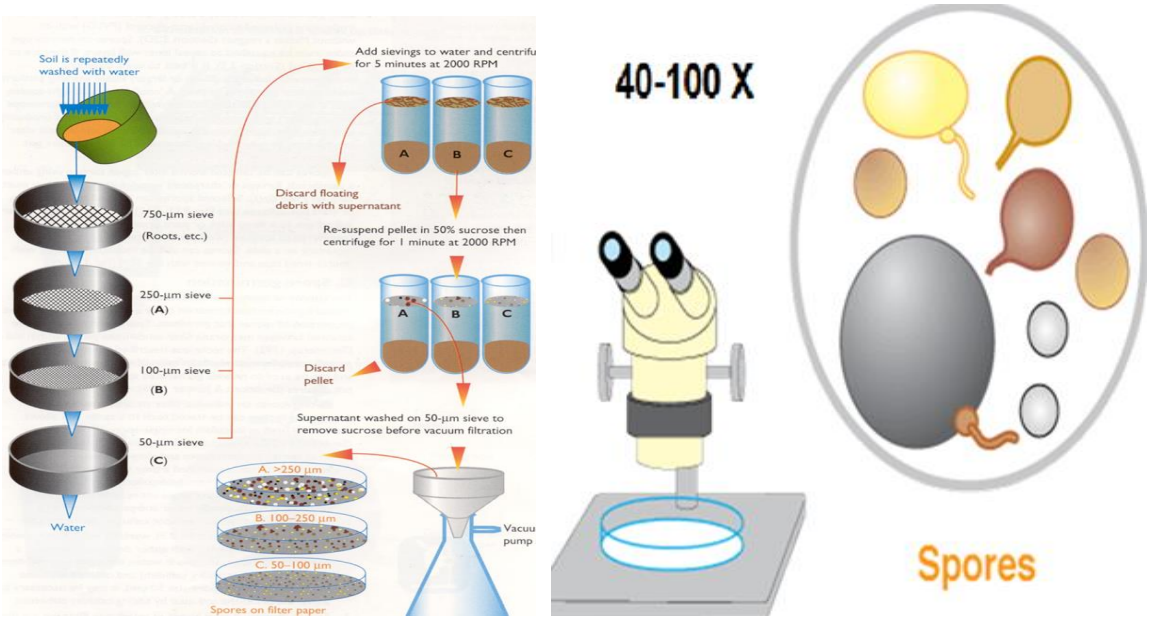

Figure3: Extraction of AMF spore from soil and enumeration (Brundrett *et al*., 1996).

3.5. Identification and Characterization of Spores

The AMF spores were morphologically characterized and identified to species level or morphotype at Microbiology laboratory, Department of Applied Biology, ASTU. About 70% of healthy looking spores were picked with forceps and mounted on slides in polyvinyl-lactic acid-glycerol (PVLG),(Omar *et al*.,1979) or in PVLG mixed with Melzer's reagent (1:1 v/v) (Morton, 1991). Spores were examined under a compound microscope (OLYMPUS BX51) at a magnification of ×100-x40 and identified to the species level or to a specific morphotype based on online references of species description INVAM http://invam.caf.wvu.edu, West Vir ginia University,USA, University of Agriculture in Szczecin, Poland http://www.zor.zut.edu.pl/Glomermycota/, Schüßler and Walker,(2010) and the Schüßler AMF phylogeny website.

3.6. AMF Root Colonization

The stored root samples were taken from 50% w/v alcohol and washed several times in tap water. The root segments were immersed in 10% (w/v) KOH solution and heated in autoclave at121^{0}C for15minutes depending on the structure of the root and then, roots were rinsein tap water and whitened in a solution of alkaline hydrogen peroxide (10% H_2O_2) for 30 minute at room temperature (Brundrett *et al*.,1996). Then after, the roots were treated with 10% HCl(v/v) for15-30 minutes at room temperatureand finally stained in 0.05% w/v trypan blue in lacto glycerol (1:1:1) lactic acid,glycerol andwater) at 121^{0}C for 10 minutes in an autoclave and rinsed with tape water and stored in distaining solution of lactoglycerol (lactic acid, glycerol and distilled water(1:1:1) for more than two days(Brundrett *et al*.,(199).

Finally, the roots were mounted in (PVLG) polyvinyl lactoglycerol mountant on microscopic slide and covered with 40x22 mm cover slips.Then, slides were observed under a compound light microscope and each fragment were checked for its entire length for the presence of the mycorrhizal structures: hyphae,arbuscles and vesicles(intracellular and intera-cellular,hyphae and extra matrix).

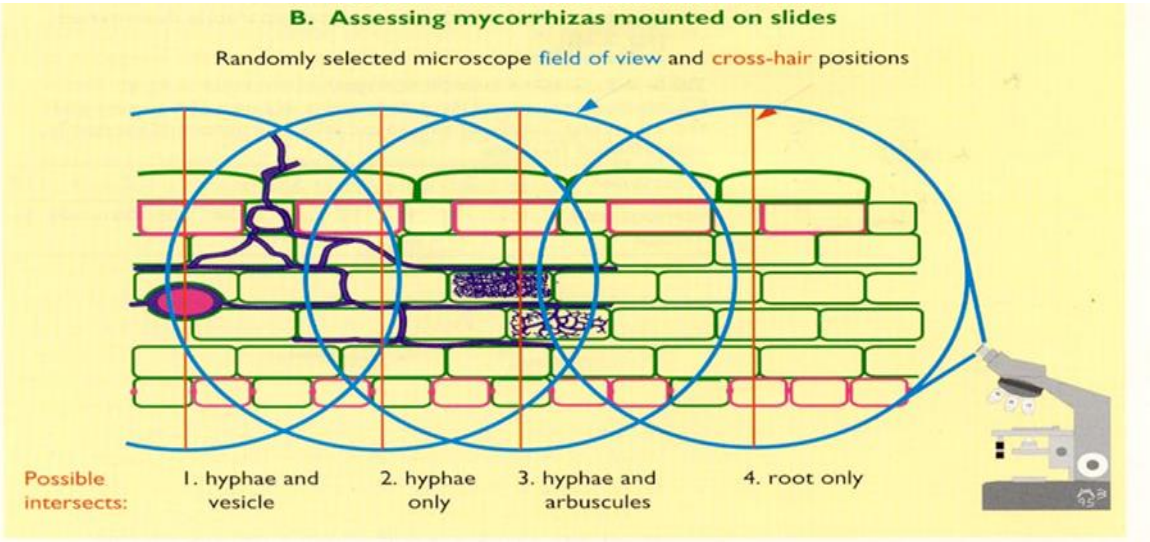

Figure4: Quantification root colonization using the magnified intersections method (Brundett *et al*., 1996).

The percent of root length colonized by anyAMF structure were quantified by using the magn ified intersection method after (McGonigle *et al.,* 1990).

The percentage of root colonization was calculated as follows:

RLC % = Total Root Colonization (G)− Negative Colonization (N) X 100
Total Root Colonization (G)

AC % = Number of Arbuscle x 100
Total Root Colonization (G)

VC % = Number of V;esicle X 100
Total Root Colonization (G)

Where, RLC % = percentage of Root Length Colonization
AC % = percentage of arbuscle Colonization
VC% = percentage of vesicle Colonization

3.7. AMF Spore Analysis

The AMF spore diversity and density of fungi were calculated by the following method such as: Isolation frequencies (IF), relative abundance of spores (RA) and the importance value (IV) following formula:

IF (%)=Number of samples in which a given species of AMF was isolated / the total number of samples×100
RA (%) =Number of spores in a given species / total number of spores ×100
IV (%) = (IF + RA)/2. The importance value (IV) was used to evaluate the dominance of AMF species based on IF and RA and was calculated as IV = (IF + RA)/2. An IV ≥ 50% indicates that the genus or species is dominant; 10% < IV< 50% applies to common genera or species; an IV ≤ 10% indicates that the genus or species is rare (Chen *et al.*, 2012).

3.8. Data Analysis

Analysis of variance (ANOVA) and correlation analysis were carried out with the SPSS software package (version 24.0). Significance differences in AM fungal spore abundance and percentage of root colonization between the plants were tested using Fisher's least significant difference (HSD) at P≤ 0.05 level.

4. RESULT AND DISCUTION

4.1. **Soil physical and chemical analysis**

Data of soil physical and chemical parameters of the invasive alien plant species showed variation (Table1). The rhizophere soil pH of the of plant species was neutral to slightly alkaline, with significantly highest mean pH values of 7.55 in *Nicotinia glauca* and lowest mean value of 7.12 in *Lantana camara*. The soil organic carbon concentration varied between (1.62 up to 0.44), Where significantly higher in *Eichhonia crassipes* ($P<0.05$) compared to all plant samples. Statistically significant differences were observed among all plant samples for both TN and Av.P values ($p<0.05$, Table1).

Table 1: Soil physco-chemical characteristics in east Showa zone, Oromia, Ethiopia.

Sample	Soil texture	pH	Av p(ppm)	TN %	OC %
1. *Parthenium hysterophorus*	Sandy loam	7.38±0.07ab	7.8±0.07d	0.15±0.01bc	0.57±0.1c
2. *Senna didymobotry*	sandy loam	7.32±0.03b	15.9±0.29a	0.13±0.01dc	0.58±0.05c
3 .*Lantana Camara*	sandy loam	7.12 ± 0.017^{c}	9.4±0.13c	0.13±0.00 dc	0.9367±0.1b
4. *Argemon orchroleuia*	Loam	7.35±0.029b	12 ±0.1b	0.19±0.00b	1.0800±0.07b
5. *Datura strumarium*	sandy loam	7.25 ± 0.029^{bc}	7.25±0.18d	0.067±0.14d	0.44±0.03c
6. *Nicotinia glauca*	sandy loam	7.55±0.029a	7.4±0.52d	0.13±0.0dc	0.55±0.05c
7. *Eichhonia crassipes*	sandy loam	7.35±0.029b	12.4±0.16b	0.52±0.02a	1.62±0.07a

Av p: available phosphorus, TN: total nitrogen, OC: organic carbon.

4.2. AMF spore abundance and root colonization

Total AMF spore abundance varied among the invasive alien plant species. AMF spore density recorded from rhizosphere of *Argemon orchroleuia* was significantly higher than all the invasive alien plant species (P< 0.05, Table 2). Spore densities recorded in *Nicotinia gauca* and *Eichhonia crassipes* were significantly higher than the three plant species including *Parthenium hysterophorus*, *Senna didymobotry* and *Lantana camara* (P< 0 .05).

Table 2: Spore density and root colonization of AMF in selected invasive alien plants in east Shawa zone Oromia, Ethiopia.

Invasive plant	Spore density/100gm	Hyphae (%)	Arbuscules (%)	Vesicules(%)
1.*Parthenium hysterophorus*	686.6±21.6c	28±4.16	37.3±5.8	36.3±9.87
2.*Senna didymobotry*	551.6±27.5c	20.6±3.28	42.6±4.1	33.3±4.7
3.*Lantana camara*	659.6±25.5c	15.6±1.6	29±5	20.3±0.88
4.*Argemon orchroleuia*	1418±49.54a	19.3±5.17	23±3.8	21±5.68
5.*Datura strumarium*	691±39.39c	19 ±3.05	31.6±8.68	24.3±3.38
6.*Nicotinia gauca*	1057±31.7b	32±5.7	50.3±8.4	40.6±3.28
7.*Eichhonia crassipes*	1141.6±88.2b	22±9.38	34±5.6	27.3±8.98

AMF root colonization occurred with typical structures such as arbuscules, vesicles and hyphae in all invasive plants. The AM fungi colonization pattern shown homogenies among root of invasive plants. The highest hyphal colonization 32% was recorded from the *nicotinia gauca* followed by *Parthenium hysterophorus* 28%, *Eichhonia crasspies* 22.%, *Senna didymobotry* 20.6, *Datura strumarium* 19%, *Argemon orchroleula* 19.3% and *Lantana camara* 15.6% which

is least root colonization(Table.2).

4.3. AMF community composition

A total of 23 AMF morpho species, were identified from rhizosphere soil of the invasive plants species (Table.3). Four morphospecies, from *Gigaspora,* followed by three species each from *Claroideoglomus Acaulospora*, and *Funneliformis*, and two species each from *Rhizophagus* and *Glomus.* However, *Diversispora, Paraglomus, Septoglomus, Entrophosphora, Scutellospora* and *Racocetra* were each represented only by one species. Two morphspecies from *Glomus* and one from *Racocetra* were unidentified to species level (Table 3).

Table 3: Number of morphotypes per genus characterized in soil sampled from the rhizophere of Selected invasive alien plant species in east Showa zone Oromia, Ethiopia.

	Genus	**Number of morphotypes characterized**
1	*Acaulospora* Trappe & Gerd (1974)	3
2	*Claroideoglomus* C. Walker & A. Schüßler (2010)	3
3	*Diversispora* C. Walker & A. Schüßler (2004)	1
4	*Funneliformis* C. Walker & A. Schüßler (2010)	3
5	*Rhizophagus* P.A. Dang. (1896)	2
6	*Glomus* Tul. & C. Tul. (1845)	2
7	*Gigaspora* Gerd & Trappe (1974)	4
8	*Paraglomus*	1
9	*Septoglomus*	1
10	*Entrophosphora*	1
11	*Scutellospora* C. Walker & F.E. Sanders (1986)	1
12	*Racocetra* Oehl, F.A. Souza & Sieverd. (2008)	1
Total		23

The isolation frequency (IF) and relative abundance (RA) of AMF species varied greatly among the invasive plants species (Table 4). *Claroideoglomus claroideum* were distributed across all rhizophere soil of plant species

Table 4: AMF species distribution among the selected invasive alien plants in east Shawa zone Oromia, Ethiopia.

		IF (%)							RA (%)							IV (%)							AV	Status
	Genera	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7		
1	*Acaulospora* Trappe & Gerd (1974)																							
	A. denticulate (Sieverd &S.Tora)	43	0	43	0	0	0	43	16.6	0	33.3	0	0	0	50	29.8	0	38.2	0	0	0	46.5	16.4	C
	A. tuberculata (Janos &Trappe)	29	0	29	0	0	0	0	50	0	50	0	0	0	0	39.5	0	39.5	0	0	0	0	11.3	C
	A. rehmi (Sieverd &S.Toro)	0	0	0	0	0	0	0	0	0	0	0	0	16.6	83.3	0	0	0	0	0	22.8	56.2	11.3	C
2	*Claroideoglomus* Walker & A. Schüßler (2010)																							
	C. claroideum	100	100	100	100	100	100	100	6.15	29.23	15.38	10.76	6.15	29.23	3.07	53.3	64.6	57.7	55.4	53	64.6	51.5	57.2	D
	C. etunicatum	71	71	0	71	71	71	0	1.96	31.4	0	25.5	5.9	353	0	36.5	51.2	0	48.2	38.4	53.1	0	32.5	C
	C. latum	14	0	0	0	0	0	0	100	0	0	0	0	0	0	57	0	0	0	0	0	0	8.14	R
3	*Diversispora* C. Walker & A. Schüßler (2004)																							
	D. tortuose	0	0	0	0	0	0	14	0	0	0	0	0	0	100	0	0	0	0	0	0	57	8.14	R
4	*Entrophosphora*																							
	E. nevadenesis	0	0	14	0	0	0	0	0	0	100	0	0	0	0	0	0	57	0	0	0	0	8	R

		IF (%)							RA (%)							IV (%)							AV	Status
	Genera	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7		
5	*Funneliformis* C. Walker & A. Schüßler (2010)																							
	F. geosporm	29	29	0	0	0	0	0	50	50	0	0	0	0	0	39.5	39.5	0	0	0	0	0	11.3	R
	F. coronatum	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	1	R
	F. mossae	71	71	71	71	0	71	0	3.7	26	22.2	37	11.1	0	0	37.4	48.5	46.6	54	0	41	0	32	C
6	*Glomus* Tul. & C. Tul. (1845)																							
	Glomus sp.1 red brown geosporum like	57	57	57	0	0	57	0	30	10	30	0	0	30	0	43.5	3.5	43.5	0	0	43.5	0	16.3	R
	*Glomus sp.*2 sporocarpic, thick wall, smooth	29	0	0	0	29	0	0	25	0	0	0	75	0	0	27	0	0	0	52	0	0	11.3	C
7	*Gigaspora* Gerd & Trappe (1974																							
	Gigaspora giganta	29	29	0	0	0	0	0	28.6	71	0	0	0	0	0	28.8	50	0	0	0	0	0	11.25	C
	Gigaspora alibida	0	29	29	0	0	0	0	25	25	75	0	0	0	0	0	27	52	0	0	0	0	11.3	C
	Gigaspora margarita	0	14	14	0	0	0	0	0	100	0	0	0	0	0	0	57	7	0	0	0	0	9.14	R
	Gigaspora rosea	0	0	29	0	0	29	0	0	0	23.5	0	0	76.4	0	0	0	26.3	0	0	52.7	0	11.3	C
8	*Rhizophagus* P.A. Dang. (1896)																							
	Rhisophagus aggregatum	86	86	86	86	86	86	0	11.4	29.5	25	2.3	20.5	11.4	0	48.7	57.7	55.5	44.1	53.2	48.7	0	43.9	C
	Rhisophagus	42	43	0	0	43	0	0	14.	42.9	0	0	42.9	0	0	28.1	42.	0	0	42.	0	0	16.3	C

		IF (%)							RA (%)							IV (%)							AV	Status
	Genera	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7		
	diaphanous								3								9			9				
9	*Paraglomus*																							
	P. occultum	0	43	43	43	0	0	0	0	6.25	75	18.8	0	0	0	0	24.6	59	30.9	0	0	0	16.4	C
1 0	*Septoglomus*																							
	S. constrictum	0	0	57	57	0	57	57	0	0	22.2	22.2	0	11.1	44.4	28.5	0	39.6	39.6	0	34	50.7	27.5	C
1 1	*Scutellospora* C. Walker & F.E. Sanders (1986)																							
	S. calaspora	0	14	0	0	0	0	0	0	100	0	0	0	0	0	0	57	0	0	0	0	0	8.1	R
1 2	*Racocetra* Oehl, F.A. Souza & Sieverd. (2008)																							
	Racocetra sp.	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	57	0	0	0	0	0	8.1	R

C: common, D: dominant, R: rare

On the basis of IV (impotence value) *Claroideoglomus claroideum* was categorized in to dominant species with IV 57.2% (Chen *et al*., 2012). However, the different species under the different AMF genera were categorized into "common" and "Rare" groups with 10<IV< 50 and IV<10), respectively (Table.4). *Acaulospora denticulate, Acaulospora tuberculata, Acaul ospor rehmi, claroideoglomus etunicatum, Funneliformis geosporm, Funneliformis mossae, Gigaspora alibida,Gigaspora giganta, Gigaspora rosea, Glomus caladonium,Glomus mossae ,paraglomus occultum, Rhisophagus aggregatum, Rhizophagus diaphanous and Septoglomu s constrictum* species were found to be common with IV value of 43.9, 32.49, 32.49, 27.49, 1 6.35, 16.35, 16.28, 16.28, 11.28, 11.28, 11.28, 11.28 and 11.25 respectively. However, specie s such *as Claroideoglomus lutam, Diversispora tortuosa, Entrosphora nevadenesis, Gigaspor a margarita, Funneliformis coronatum* and *sctellospora calaspora* were rare with the IV 9.14 , 8.14, 8.14, 8.14, 8 and 1 respectively.

Table 5: AMF diversity of selected invasive alien plants in east Shawa zone, Oromia Ethiopia.

No	Invasive plants	No of Genera	Name of Genera	No of species
1	*Parthenium hysterophorus*	6	*Claroideoglomus, Funneliformis, Glomus, Rhisophagus Acaulospora, Gigaspora*	12
2	*Senna didymobotry*	7	*Claroideogolmus, Funneliformis , Glomus, Rhisophagus, Gigaspora, Paraglomus, Scutellospora*	13
3	*Lantana camara*	9	*Claroideoglomus, Funneliformis, Glomus, Rhisophagus, Acaulospora, Gigaspora, Septogiomus,Entrosphera,Paraglomus*	11
4	*Argemon orchroleula*	5	*Clariodeoglomus, Funneliformis, Rhisophagus, Septoglomus, Paraglomus*	6
5	*Datura strumarium*	4	*Claroideoglomus, Funneliformis, Glomus, Rhisophagus*	6
6	*Nicotinia glauca*	7	*Claroideoglomus, Funneliformis, Glomus, Rhisophagus Acaulospora, Septeglomus, Entrosphera*	8
7	*Eichnonia crassipes*	4	*Claroideoglomus, Acaulospora, Septoglomus, Diversispora*	5

The maximum number of species was detected in *Senna didymobotry* (13), followed by *Parthenium hysterophorus(12), Lantana Camara* (11), *Nicotinia glauca,* (8) *Argemon Orchroleula* and *Datura strumarium (*6) each. The least number of species (5) was recorded.

in *Eichhonia crassipes*. However, maximum number of genera was detected in *Lantana camara* (9) and minimum number was identified 4 each from *Datura strumarium* and *Eichho nia crassipes.*

4.4. The correlation between AMF and physco- chemical soil parameter

Table 6: The correlation between AMF parameters and physic chemical soil parameter of selected invasive alien plant species around east Showa zone.

	A	V	H	SD	pH	AvP	TN	OC
A	1							
V	0.781**	1						
H	0.731**	0.865**	1					
SD	-0.151	-0.073	0.117	1				
pH	0.436**	0.563**	0.509*	0.396	1			
AV.P	-0.105	-0. 127	-0.256	0.053	-0.101	1		
TN	-0.119	-0.126	-0.057	0.459*	0.110	0.356	1	
OC	-0.283	-0.351	-0.187	0.559**	-0.124	0.378	0.881**	1

**. Correlation is significant at the 0.01 level (2-tailed) *. Correlation is significant at the 0.05 level (2-tailed). A: arbuscule, V: vesicule, H: hyphae, SD Spore density, pH, AvP: available phosphorus, TN: Total nitrogen, OC: organic carbon. Correlation between physco - chemical and AMF parameters can be written as the following by using table below:

The correlation between AMF and physico- chemical soil parameter was explained as follow: arbuscule positively significantly correlated with vesicule and hyphae ($P<0.01$) and however, no significant correlation was observed between AMF colonization and spore density ($p>0.05$). The result also indicated that soil pH formed significantly positive correlation with AMF colonization such as arbuscules, vesicles and hyphae ($P<0.05$; $P<0.01$). AMF spore

density was positively correlated with soil total nitrogen and organic carbon ($P<0.05;0.01$)(Table.6)

5. Discussion

Invasive plant species are among the world's greatest threats to biodiversity and native specie s in protected areas (Lwando, 2009). Invasive species may change the community structure through competition which is categorized into two; exploitation competition whereby there is indirect interactions in the use of resource and interference competition where by invasive plants produce chemicals that affect the native plant (Loeuille, 2017). AMF are obligate symbiotic fungi which can be found in most species of terrestrial ecosystem and colonized more than 80% of land plant species. It plays an important role in the colonization of mostter restrial plants in the natural ecosystem (Qin *et al*., 2015).

In this study most of mycorrhizal fungi were extracted from selected invasive alien plants, spore characterization and identified base on morphological character. The sampling sites were characterized by the soil type with sandy loam and loam soil, total nitrogen is Medium available phosphorus and organic carbon with the significant difference between the invasive plants.

The AMF spore density between the rhizophere of invasive alien plants was ranging from 55 1 spores/100g(*Senna didymobotry*) to 1418 spores/100g soil (*Argemon orchroleuia*)(Table.3). This is comparable with AMF spores recorded in the rhizosphere soil of acacia species found in Bishoftu and, Zeway (Belay *et al*., 2013). They found that rhizosphere soil harbored AMF fungal spores ranging from 370 spores g-100 soil in *A. nilotica* to 1500 spores g-100 in *A. seyal* from open grazing field (OGF) at Zuway. This is indicating that the invasive alien plant species are moderately mycorrhizal dependent organisms.

The colonization of different AM structures varied greatly among plant species. The mean percentage of hyphal colonization across all invasive alien plant species was between 15.6 % and 32 %. This result is much lower than hyphal colonization (27.3-71.7%) recorded in fruit crops from low-input cropping system in Showa Robit, Ethiopia (Belay *et al*., 2014). However, the result is comparable to hyphal root colonization recorded in *Acacia saligna* an exotic plant species from woodland area in rift valley region, Ethiopia (Belay *et al.*, 2013). A total of 12 AMF genera and 23 species were identified from the rhizosphere of different

Invasive plant species. Amongst the AMF genera, the highest number of species. recorded from *Funneliformis*(3) species), *Acaulospora* (3species),*Claroideoglomus*(3 species) followed by the genera *Glomus* (2species), *Rhisophagus* (2 species), *Gigaspora* (4 species) and *Diversispora, Scutellospora, Septoglomus*, *Entrophosphora* and *Paraglomus* with one species(Table.4). Based on the IV%*Clariodeoglomus clarodeum* species *was* dominant which have important value of 57%. A similar trend was reported from Ethiopia by (Belay *et al*., 2014), that 32 species representing 12 genera were detected from soil samples under the rhizosphere of the fruit crops. The dominant genera were *Acaluspora, Funliiformis* and *Glomus* which were diversified into 8 species.

AMF species richness was comparatively variable between the invasive alien plants. The highest number of AMF species richness was recorded in *Senna didymobotry* (13), *Parthenium hysterophorus* (12) and *Lantana camara* (11). This result is similar to that observed in different acacia tree species from woodland area in rift valley region (Belay *et al*., 2013) and fruit crops (Belay *et al*., 2014) from different land use systems in Ethiopia. They identified nearly 17 and 14 AMF species in average from different species of acacia trees and fruit crops, respectively. However, the AMF species diversity from *Nicotinia glauca, Eichnonia crassipes, Datura strumarium* and *Argemon orchroleula* in this study was 3 times lower than the AMF morphotypes isolated from *A. abyssinica* (19), *A. saligna* (19*) A. seyal* (17) in acacia trees and Mango (18) and Banana (16) in fruit crops. It showed that arbuscular mycorrhizal fungal abundance and diversity in this study did not differ between native and invasive plants. Similarly, report done by (Bunn *et al*., 2015) showed that AM fungal abundance (measured by percentage colonization, extraradical hyphal and spore densities, as well as neutral lipid fatty acid and glomalin concentrations) did not differ between native and invasive plants, but invasive plants hosted different AM fungal communities in 78% of studies.

Moreover, the soil total nitrogen and organic carbon have statistically significantly correlated with arbuscules and vesicles $P<0.05$(Table.6). Alguacil *et al*., (2016) studied soil characteristics driving AM fungal communities in Semiarid Mediterranean soils and concluded that three soil properties including pH and nutrients such as manganese and zinc levels were the main factors triggering the distribution of AMF. These results contribute to a better understanding of the ecological factors that can shape AMF communities, an important soil microbial group that affects multiple ecosystem functions.

6. Conclusions and Recommendations

6.1. Conclusions

In this study, significant variability in AMF spore density, diversity, and percent of root colonization was observed among the invasive alien species. Maximum AMF spore density, diversity and mycorrization was recorded in *Argemon orchroleuia*, *Nicotinia gauca* and *Senna didymobotry* respectively. In general, a comparable result of AMF spore density & diversity and percent of root colonization was recorded between the native plant species such as acacia and fruit crops and the invasive alien species. There was a strong association between mycorrhizaion and soil pH and, sporulation and soil total nitrogen and organic carbon.

6.2. Recommendations

Based on the findings the following recommendations were forwarded.

- This study depends on morphological character to identify AMF genera and the specie Therefore, molecular characterization is vital to identify species of the fungi properly.
- Controlled studies will be needed whether AMF symbiosis is the key factor or driver for the invasive alien plant species to distribute aggressively.
- More species of invasive plant trees need to be included in the future for investigation of their mycorrhizal status from other areas of the country.

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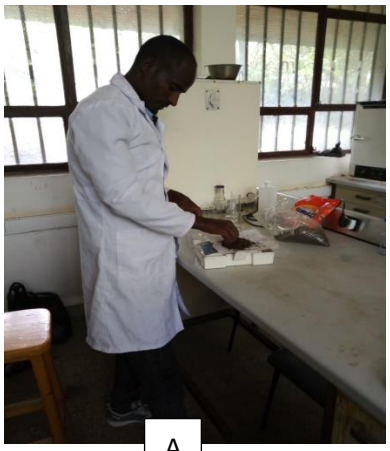
8. APPENDIXES

Appendix 1: Step of extraction of AMF from soil was showed from letter A to G



A

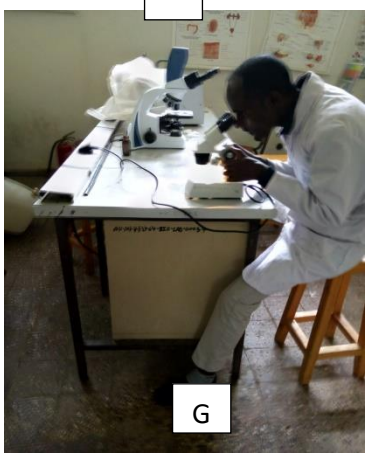
B

C

D

E

F



G

Appendix2 Pouring of AMF spore in to the slide and identification

A Pouring of spore

B Identification of spore

Appendix 3: Same Species of extracted AM fungi from soil after identification.

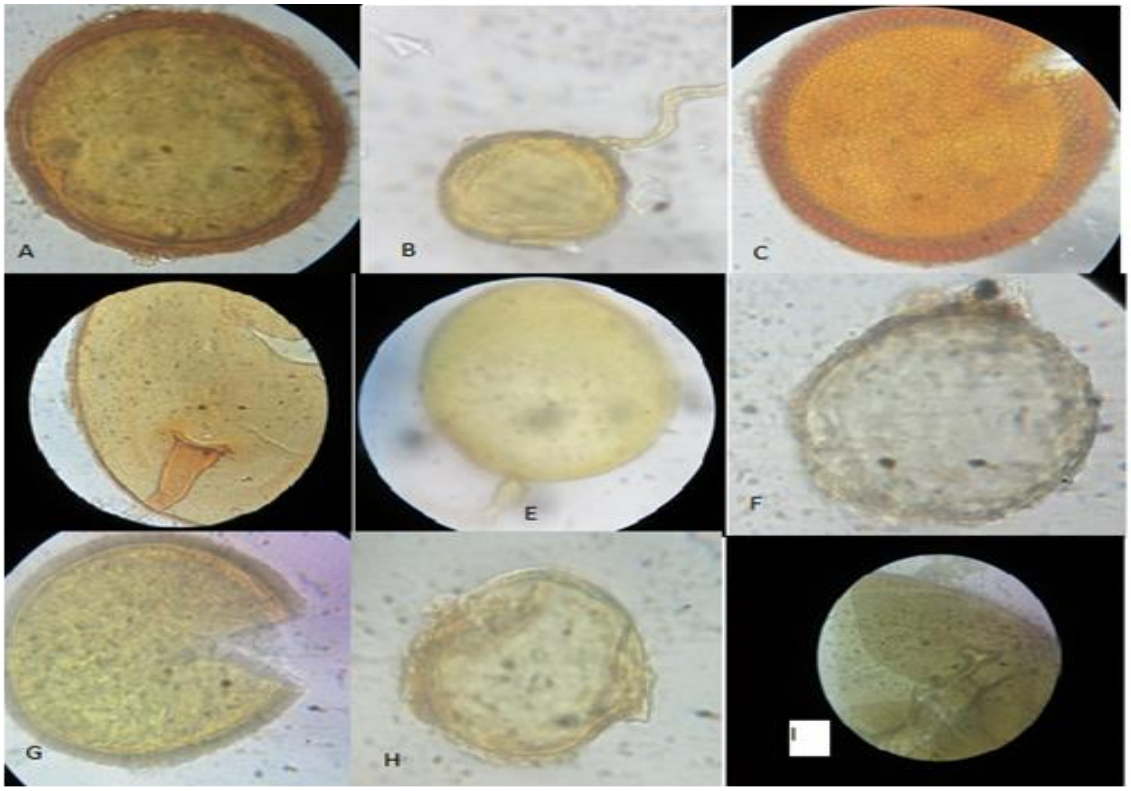


Fig. A. Claroideoglomus etunicatum, B. Glomus hoi C. Acaulospora tuberculata D. Funneliformis mosseae E. Gigaspora rosa F. Paraglomus occultum G. Claroideoglomus claroideum H. Rhizophagus aggregate.

Appendix 4: Significance difference between root colonization of AMF

	Sum of Squares	mean Squares	Significant
Arbuscle	1441.143	240.190	0.121
Within group	1616.0	115.42	
Total	3057.143		
Vesicles	1116.992	186.150	0.194
Within group	1534.0	109.57	
Total	2650.52		
Hyphae	578.476	96.413	0.365
Within group	1130.667		
Total	1709.143		
Spore density	1855178.571	309196.429	0.00
Within group	188130.66	6295.048	
Total	1943309.238		