Evaluation of the potential of selected cereal and legume host plants for using them as trap culture of Arbuscular Mycorrhizal Fungi (AMF) for inoculum production

Bedria Shanko

A Thesis Submitted to the Department of Applied Biology

School of Applied Natural Science

Presented in Partial Fulfillment of the Requirement for the Degree of Master's in Applied Biology (Biotechnology)

Office of Graduate Studies

Adama Science and Technology University

June, 2023

Adama, Ethiopia

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Main Advisor: Zerihun Belay (PhD)

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DECLARATION

I hereby declare that this Master Thesis entitled "**Evaluation of the potential of selected cereal and legume host plants for using them as trap culture of Arbuscular Mycorrhizal Fungi (AMF) for inoculum production** " is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation

_____	_____	_____
Name of the student	Signature	Date

RECOMMENDATION

I, the advisor of this thesis, hereby certify that we have read the revised version of the thesis entitled "**Evaluation of the potential of selected cereal and legume host plants for using them as trap culture of Arbuscular Mycorrhizal Fungi (AMF) for inoculum production**" prepared under our guidance by Bedria Shanko submitted in partial fulfillment of the requirements for the degree of Master's of Science in Applied Biotechnology. Therefore, we recommend the submission of revised version of the thesis to the department following the applicable procedures.

_____ _____ _____

Major Advisor Signature Date

APPROVAL PAGE

I, the advisor of the thesis entitled "**Evaluation of the potential of selected cereal and legume host plants for using them as trap culture of Arbuscular Mycorrhizal Fungi (AMF) for inoculum production**" and developed by **BedriaShanko**, hereby certify that the recommendation and suggestions made by the board of examiners are appropriately incorporated into the final version of the thesis.

_____ _____ _____

Major Advisor Signature Date

We, the undersigned, members of the Board of Examiners of the thesis by **Bedria Shanko** have read and evaluated the thesis entitled "**Evaluation of the potential of selected cereal and legume host plants for using them as trap culture of Arbuscular Mycorrhizal Fungi (AMF) for inoculum production**" and examined the candidate during open defense. This is, therefore, to certify that the thesis is accepted for partial fulfillment of the requirement of the degree of Master of Science in Applied Biotechnology.

_____ _____ _____

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Final approval and acceptance of the thesis is contingent upon submission of its final copy to the Office of Postgraduate Studies (OPGS) through the Department Graduate Council (DGC) and School Graduate Committee (SGC).

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School Dean Signature Date

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ACKNOWLEDGEMENTS

First of all, I would like to thank Allah for everything.

I would like to thank my advisor Dr. Zerihun Belay with deep gratitude for his continuous guidance, constructive advices and support in all cases starting from the beginning to the end of this research work.

I acknowledge the Department of Applied Biology, School of Applied Natural Science,Adama Science and Technology University for their support. I extend my deep gratitude to Melkassa and Holota agricultural research center for their supply of selected seed varieties for my research work.

Next, I would like to thank my families My husband Umer Haji, my son Dr. Ibrahim Umer ,my sisters Genet shanko, Sofiya Shanko and Guuto Ahmed for their continuous support with ideas and finance. I would like to pass my heartfelt thanks to Sara Gebreslassie, Urgessa Enssermu for their continuous advises, brotherly and inspiration and Dr.Mussa Jarso for supporting me when I collected seeds.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

APPENDIX

LIST OF ABBREVIATION

AMF Arbuscular MycorrhizalFungi

ANOVA Analysis of variance

CNM Common Mycorrhizal Network

DGGE Denaturing gradient gel electrophoresis

GRSP Glomalin related soil protein

INVAM International Culture collection of Vascular Arbuscular Mycorrhizal

PVLG polyvinyl-lactic acid-glycerol (PVLG),

SPSS Statistical Package for the Social Sciences

TAG Triacylglycerol

VAM Vascular arbuscular mycorrhizal

SAR Systematic acquired resistance

ABSTRACT

Arbuscular mycorrhizal Fungi (AMF) are soil micro-organisms that increase plant nutrient up take by extending the roots absorbing area. AM fungi helps plants to adapt in different weather condition by creating positive impact on ecosystem. Unfortunately, they cannot be cultured on artificial media and necessitae the search of effective plants to trap them in their roots for inoculum production. The aim of this study was to identify suitable host plants used for mass production of Arbuscular Mycorrhiaza Fungi (AMF). In these processes a trap culture was established under greenhouse to extract spores from soil by wet sieving and decanting methods. Characterization of spores and root colonization were done based on morphological features and the data was analyzed by using statistical software SPSS. The host plant showed high mycorrhization with typical structures (arbuscules, hyphae and vesicles), spore density and diversity. The data showed that spore density was between 23.6 – 11.7 spores' g^{-1} soil and the AMF were identified into 13 genera and 27 AMF species collected from all trap cultures. Sorghum and maize plants induced higher percentage of mycorrhizal response with high spore count (23.36 and 22.06) in spore count and root colonization sorghum 94% and maize 89% than the legume plants respectively. Among host plants the highest species diversity of species was detected from the trap culture of clover. The results showed that sorghum and maize plant were more suitable host plant for AMF spore propagation and that clover was preferable to get more diversity.

Keywords: , Alfaalfa, clover inoculum mass production, maize, and sorghm

1. INTRODUCTION

1.1. Background of the study

Arbuscular rmycorrhizal fungi are obligatory symbiotic soil fungi in association with more than 80% of vascular plants (Akiyama & Hayashi, 2006). The development of AM symbiosis starts with signaling taking place before physical contact between the plant and the fungus (Chabaud *et al*., 2011). The mycorrhization process can be divided into distinct steps, consisting of germinating spores, hyphae differentiation, appressorium formation, penetration of the host root, intraradical hyphae formation, intercellular growth along with developed external mycelium (extra radical hyphae), and arbuscule formation, subsequently exchanging nutrients and carbohydrates between the host and fungus (Goltapeh *et al*., 2008).

Currently, AMF are classified as the phylum Glomeromycota class (Glomeromycetes) which includes four orders (Diversisporales, Glomerales, Archaeosporales, and Paraglomerales), 12 families (Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Claroideoglomeraceae, Diversisporaceae, Geosiphonaecae, Gigasporaceae, Glomeraceae, Pacisporaceae, Paraglomeraceae, Pervetustaceae, and Sacculosporaceae), 34 genera and approximately 316 species (Walker *et al,*2018) .

AMF developed symbiosis with roots to obtain essential nutrients from the host plant and consequently provide mineral nutrients in return, for example, N, P, K, Ca, Zn, and S. Arbuscular mycorrhizal fungi (AMF) facilitate host plants to grow vigorously under stressful conditions by enhancing water uptake, photosynthetic rate and other gas exchange-related traits of the plant. AMF symbiosis improved resistance of plants to a variety of stresses including drought, salinity, herbivory, temperature, metals, and diseases (Smith and Read, 2008).

(Elhindi *et al*,.2017), indicated that arbuscular mycorrhiza fungi inoculation protects *Ocimum basilicum* against salinity stress by improving mineral uptake, chlorophyll synthesis and water use efficiency, and tomato plants inoculated with AMF showed increased leaf area, and nitrogen, potassium, calcium, and phosphorus contents (Balliu *et al*., 2015).

In another study, Selvakuma *et al*.(2016) showed that mycorrhizal inoculation using trap method of maize resulted in longer shoots and roots than the mycorrhizal trap culture with Sudan grass plants. They indicated that, after the first and second plant cycles, maize plants showed a higher percentage of mycorrhizal of colonization and arbuscules than Sudan grass. Maximum spore count was also achieved in the pots of maize plants. A study, based on the DGGE profile using clover (*Trifoliumrepens*), sorghum (*Sorghum bicolor*), and maize (Zea mays) alone or in combination as trap plants, showed that maize exhibited the lowest AM fungal colonization and extraradical hyphae density, but the highest spore number, diversity, and species richness (Yao et al, 2010). They suggest that this is a reliable way to select appropriate trap plants, and a combination of plant species is superior to a single species . The results showed that maize plants are more suitable host plant for AMF spore propagation and trap culture technique and can be used effectively to maintain the AMF culture for long time.

AMF fungi show specific interaction to plants (Sandwrs 2003). This preference includes different colonization levels and growth responses of different plant species to the same AM fungal isolate (Pivato *et al*. 2007). (Liu and Wang, 2003) evaluated the appropriateness of four plant species as trap plants based on the morphological classification of AMF fungi and found that white clover was the best, in terms of spore and species numbers. However, it is still impossible to confirm that all indigenous AM fungi have been isolated because some AM fungi may colonize roots without sporulation.

Therefore, it is vital to select appropriate host plants that support multiplication of the fungi. In this regards, the present study was to identify appropriate host plants that used for inoculum mass production in trap culture using Maize, Sorghum, Clover and Alfalfa as host plants. Therefore, the objective of this study was to evaluate the potential of the host plants that used for inoculums mass production in order to improve crop productivity in Ethiopia.

1.2. Statement of the problem

Ethiopia's economy is dependent on agriculture, so agriculture-based economic growth is the main solution to Ethiopia's chronic poverty. In this case, the soil is the most known resource used for growing plants that provide food for all living things on Earth. There are different types of soil based on the weather condition and land topography. These different types of soil are used to cultivate different crops now days as evidences suggested that soil fertility is decreased due to different activities like intensive agriculture, over-grazing, increasing use of fertilizer, deforestation, soil erosion, accumulation of non-biodegradable wastes and the like.

So it's important to use different methods to improve soil fertility. One best method is using Bio-fertilizers. AMF is commonly known as a bio-fertilizers that provides resistance to various environment biotic and abiotic factors like drought, extreme temperature(hot or cold)hard metals, and pathogens and also provide inorganic nutrient by improve surface absorbing capacity to the host plants. Therefore, this study is aimed to screen and identify the best host plants that produces high spore density, root colonization and spore diversity in order to identify suitable host plans for inoculum mass production of AM Fungi to use these plants for production AMFungi to increase productivity and to restore our degraded environment

1.3. Objectives of the study

1.3.1 General Objective

- To evaluate and identify suitable host plants used for mass production of Arbuscular Mycorrhiaza Fungi (AMF) inoculum for sustainable agricultural system.

1.3.2. Specific objectives

- To compare the AMF root colonization among the host plants.
- To determine the AMF spore diversity among the selected host plants.
- To assess AMF spore abundance among the tested host plants.

2. LITERATURE REVIEW

2.1. Origin and Evolution of AM Fungi

Recent evidence indicates that the evolution of early plants from non-photosynthetic eukaryotes occurred in a fresh water environment by the engulfment and domestication of a photosynthetic cyanobacterium (which subsequently evolved to the chloroplasts) (Ponce-Toledo *et al*., 2017). As other study indicates that, an equally important innovation was required to allow plants to acquire water and nutrients from the substrate in the absence of specialized absorptive organs such as roots, which only evolved later (Brundrett, 2002). At the same time, fungal symbioses were instrumental to allow the colonization of land by descendants of fresh water algae (Rensing, S. A. 2018).

A recent evidence suggest that AM Fungi in the early land plants was a unique event, hence, AM Fungi appear to be a monophyletic innovation that may have enabled the rapid colonization of the continents by vascular plants (Delaux, 2017).Thus, it is conceivable that early rootless plants engaged in various kinds of fungal associations, as they are still observed today in early-diverging plant lineages (Read *et al.,* 2000),and that roots coevolved with AM Fungi in the vascular plants (Brundrett, 2002). So AM Fungi association is the most effective way used to improve plant nutrition and ecosystem management (Min *et al*., 2018).

Arbuscular mycorrhiza (AM), a symbiosis between plants and members of an ancient phylum of fungi, the Glomeromycota, improves the supply of water and nutrients, such as phosphate and nitrogen, to the host plant. In return, up to 20% of plant-fixed carbon is transferred to the fungus (Parniske, M.2008).

2.2. The Definition of Mycorrhiza

According to (OmidAlizadeh, 2011) the term mycorrhiza is rooted from two Greek words myco meaning fungi and Rrhiza meaning root and its' meaning in reality means symbiosis between a fungus and root. Fungiform many types of symbiotic association with the roots or other underground organs of plants. They are estimated to occur in at least 80% of all vascular plants, including angiosperms (the flowering plants), gymnosperms (the cone-bearing plants), many pteridophytes (ferns and their allies), and some bryophytes especially liverworts. Many of these associations are thought to be mutualistic, because the fungus typically absorbs mineral nutrients from the soil and channels these to the plant,

while the plant provides the fungus with sugars. However, there are several different types of mycorrhiza, with different properties and feature that fossils from the Rhynie Chert deposits in Scotland contain fungal structures similar to those of the most common mycorrhiza fungi today the arbuscular mycorrhiza fungi. So, it seems that some of the earliest land plants had already established mycorrhiza associations, and these might even have been a prerequisite for life on land (Ayana, 2020).

2.3. Fungal Organs

The fungi morphology in symbiotic system includes chains outside of root and inside, support cells, vesicle inside the root between the cellular and intracellular Arbuscules (Wang and Qiu, 2006) most of these organs can start a new colonization of plant roots are, a profile for each were described below.

2.3.1. Arbuscules

Arbuscules are usually formed in the inner part of the skin stem cells. The fungus roots, after penetrating into the chain of successive cell divisions, while producing bifurcate branches, become progressively thinner and subtle and in total create an organ which looks like small shrubs which facilitate the exchange of metabolites between the two can coexistent, because of the very large contact surface with the host cell. This shrub looking organs from divergence of successive and bifurcate branches of inner root chains, after passing through the cellular wall and in an enclosed case in plasmatic membrane are formed within the root skin cells with a high level of contact with plant cells, Arbuscule play the role of an exchange organ for limbs and nutrient exchange between fungus and host plants. Studies have shown that in the chain's core of an Arbuscule a lot of mitochondria, glycogen particles, fat cells and dense granules made of poly-phosphate exist in vacuoles(Gosling et al.,2006).

2.3.2. Vesicles

Vesicle are similar looking to bag or sacks, often result in swelling on the end of fungi and form within or between the root and are gradually accumulated by Lipid droplets and form like storage and resting organs(Wang and Qiu, 2006).It has thin to thick wall layer, and contain lipid cells which in terms of swollen chain ends or some of its' middle parts, are created inside or between roots' skin cells, and in the case of intercellular they are confined with plasma membrane similar to Arbuscules.Several AMF species, including in the Glomeraceae and Paraglomeraceae, produce vesicles the outer to middle cortical layers of

root. Vesicles are storage structures contain lipids and glycogen and have a thin to thick wall layer (Smith & Read 2008).

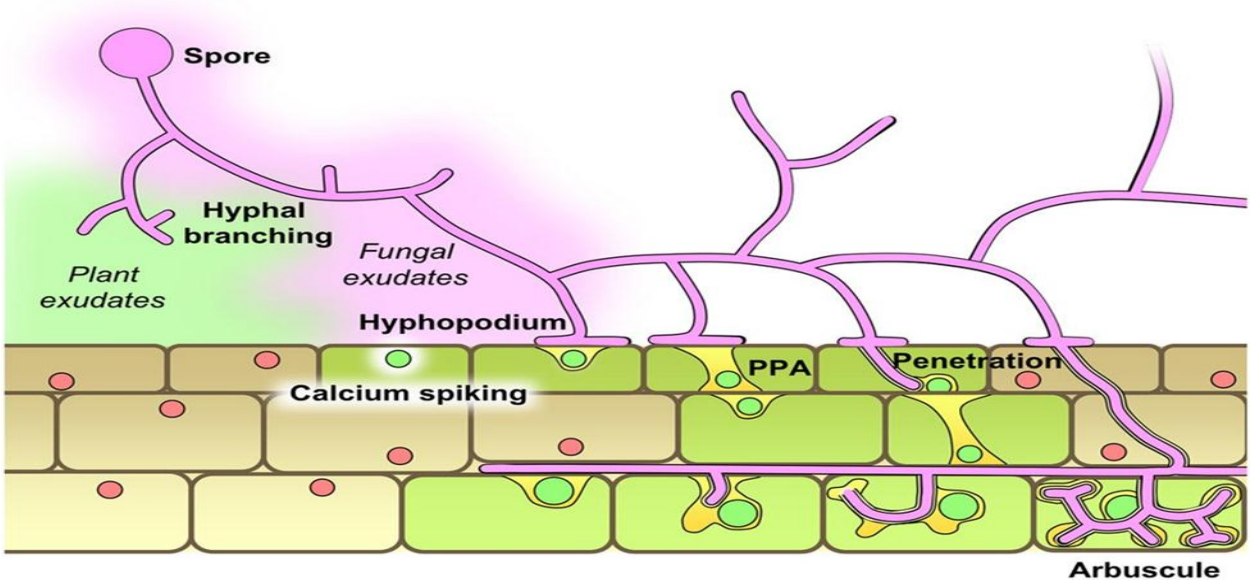


Figure 1 Schematic summary of the root colonization process by AM fungi. Source :- (Bonfante, & Genre, 2010)

2.3.3. Auxiliary Cells

Various forms of bulged cells with thin walls, which are sourced from the outer root chains in funguses under the order of *Gigasprineae*, are called support cells. The level of these cells is in the barbed *Gigasprineae* genus, but in the Schatele spore they are seen as small bulges with an almost flat surface. Before mycorrhiza colonization begins the support cells appear on the Germination tube resulted by the spores. In pot culture, the number of support cells reaches its' peak point shortly after the start of spores, but after four months onwards, their number is reduced or they completely disappear (Smith and Read, 2008).

2.3.4. Spore

AMF spores are produced singly, in loose clusters, tight clusters, or sporocarps in the soil or within host plant roots (Schüßler & Walker 2010). Molecular studies have suggested that spores AMF are multinucleate, and a single spore may contain multiple sequences of the same gene. Moreover, genetic variation of AMF is high not only between species but also nuclei in a single spore, and DNA polymorphism within fungal isolates was identified (Helgason *et al*., 2005).It is thought that spores of all arbuscular mycorrhiza fungi have the ability to colonize the root by forming a germinant pipe (Smith and Read, 2008).

2.4. Type of Mycorrhizal Fungi

Mycorrhizal associations based on the type of fungus, host plant lineages, and structures produced by the root-fungus combination are classified into seven groups: endomycorrhiza (AM) ectomycorrhiza (EC), ecteo-domycorrhiza, ericoid, arbutoid, orchid and monotropoid (Tao *et al*., 2016) .The movement of carbon from plant to fungus is common in all types of mycorrhizal association (Allen *et al*., 2003). Most often, mycorrhizal fungi are beneficial for host plants by improving the nutritional status of the plant (Chen *et al*., 2018).

2.4.1. Ectomycorrhizal Fungus

These fungi are of the class *Basidiomycets* and some are from *Ascomycets* and few imperfect funguses and only of type of *Zygomycetes* called *Andogon*. These funguses do not enter into root cells and that's why they are referred to as Ecto (external). Through the space between the root skins cells the rows of this fungi provide a dense network called the Hartic network for exchange of metabolites with the host plant. In addition by forming a rather thick layer of sheath or a pod on the surface of short and feeder roots, which often by changing the color, the shape of the roots follows frequent branches of two or more. Detection of ectomycorrhizal is easily done through morphological changes of the root sheath (Duponnois and Plenchette, 2003).

Ectomycorrhizal fungi are also found in natural environments, mainly in forests ecosystems. These fungi can form visible reproductive structures (mushrooms) at the feet of trees they colonize. Ectomycorrhizal fungi grow between root cells without penetrating them. Their hyphae grow externally, forming dense growth known as a fungal mantle. These fungi form symbiotic relationships with most pines, spruces and some hardwood 5 to 7% of plants belong to this association (Ramakrishnan and Bhuvaneswari, 2015).

2.4.2. Endo Mycorrhiza Fungus

The association of these kinds of fungi is with in fungus root. Endo Mycorrhizas form associations with most plants approximately 80 percent of all plant species. These fungi are entirely classified as Zygomycets. These types of mycorrhiza are called Endomycorrhize fungi because the fungus penetrates into the root skin cells of host plants (Ramakrishnan and Bhuvaneswari, 2015). The basic principles of naming VAM mycorrhiza is producing specific fungus organs named Arbuscule and Vesicle within the host plants root. In some types of Endomycorrhize fungi, vesicles are not formed; of this type we can refer to the

mycorrhiza fungus which belong to the genus Gigaspora and Scutellospora. Vesicle appear mostly in the mid to late vegetative period, but Arbuscule is the original location for metabolic exchanges between the fungus and plant. Arbuscules are usually formed in the inner part Skin stem cells. The fungus roots, after penetrating into the chain of successive cell divisions, while producing bifurcate branches, become progressively thinner and subtle and in total create an organ which looks like small shrubs which facilitate the exchange of metabolites between the two can coexistent, because of the very large contact surface with the host cell. Vesicle or organs that are similar looking to bag or sacks, often result in swelling on the end of fungi and form within or between the root and are gradually accumulated by Lipid droplets and form like storage and resting organs (Duponnois and Plenchette, 2003)

Endo Mycorrhizas form associations with most plants (approximately 80 percent of all plant species). These fungi cannot be grown in pure culture but must be grown in association with plant roots. They form branched structures called arbuscules within the host's root cells and thus they are known as arbuscular mycorrhiza fungi .The arbuscules are sites of nutrient exchange between the fungus and the host. This manual focuses on arbuscular mycorrhiza fungi. Fungi of the Endo-mycorrhize consist of a septet hyphae are members of the *Phycomycetes* and *Basidiomycetes*. The hyphae of these fungi penetrate the cells of the root cortex forming an internal hyphae network. Some hyphae also extend into the soil (Ramakrishnan and Bhuvaneswari, 2015).

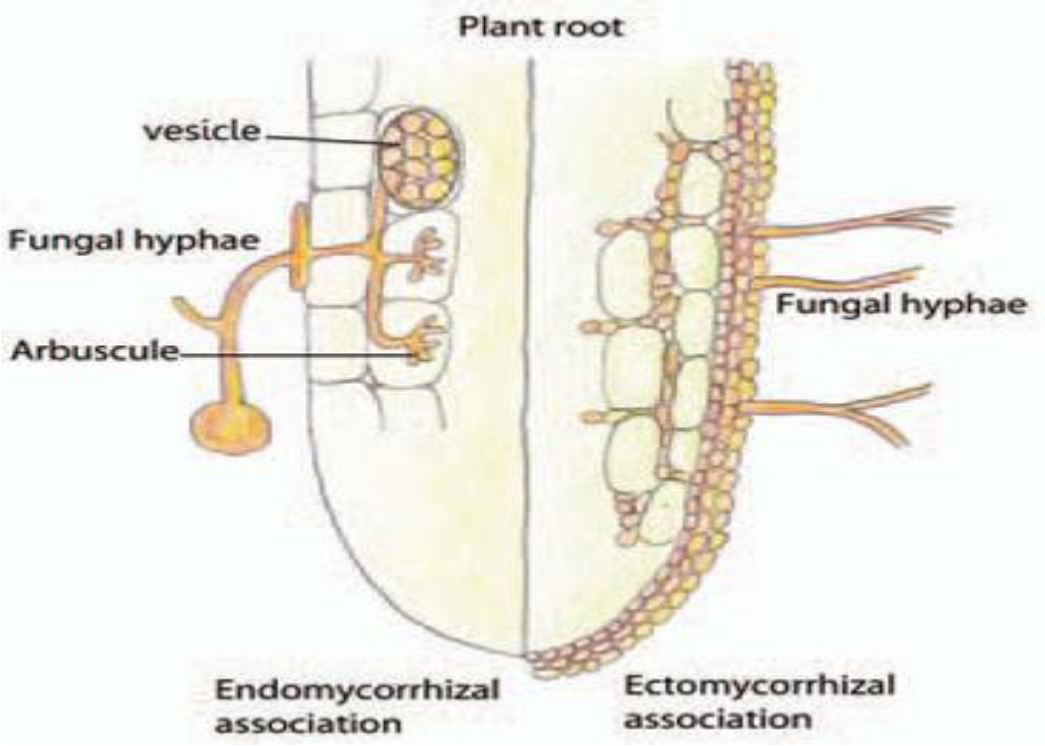


Figure 2 Schematic representation of endo- and ectomycorrhizal association between plant and fungus. Source:- (L Taiz and E Zeiger, Plant Physiology, 2002.)

2. 4.3. Ericoid Mycorrhizas

The cold, nutrient-poor, acidic upland soils of the northern hemisphere tend to be dominated by heath land plants of the family *Ericaceae*, such as *Calluna* (ling), Erica (the bell heathers), and *Vaccinium* (bilberry, cranberry, etc.). Equivalent soils in the southern hemisphere support a different family the *Epacridaceae*. All these heath land plants have a distinctive type of mycorrhizal association with Ascomycota that produce coils of hyphae in the thin lateral roots termed "hair roots. "The coils develop within the root cells but outside of the host plasma membrane and nutrient exchange is thought to occur primarily through this interface. The fungi that produce ericoid mycorrhizas are unusual because they seem to be free-living saprotrophs in soil. They grow in laboratory culture, producing septet hyphae with a fragmented, zigzag growth form, but only one of them (Hymenoscyphuericae) has been studied in detail. DNA and RNA profiles indicate that there is considerable genetic diversity between isolates that are similar in colony appearance (Brundrett, 2004).

2.4.4. Orchid Mycorrhizas

Orchid mycorrhizas are entirely different from any of those above, because orchids are parasitic on a fungus for at least the early part of an orchid's life. The seeds of orchids are extremely small, consisting of an embryo and only a few nutrient reserves. When orchid seeds are triggered to germinate they produce a few root hairs and these must be colonized by a fungus at an early stage or the seedling will die(Rasmussen, 1995).

Orchid seeds are very small and do not have sufficient nutrients to support germination. Therefore the early seedling stage is obligatately mycorrhizal and in the natural environment the seeds cannot germinate without a suitable fungus. However, in the laboratory seedlings can be germinated *in vitro* through using artificial media with or without fungi and this technique is widely used for propagation of orchids Zettler & Piskin, (2011).

2.4.5. Monotropoid mycorrhizas

The monotropoid plants are simply parasitic on ectomycorrhizal fungi, which in turn draw their sugars from the tree. This transfer of nutrients has been demonstrated by supplying $14CO_2$ to the leaves of trees and following the label as it moves down to the roots as sucrose before entering the mycorrhizal sheath as labeled sugar alcohols or trehalose, and thence through the mycelia cordsand ultimately into the flowering spikes of Monotropa. The sheath that develops around the roots of Monotropa is typical of an ectomycorrhizal sheath, with a Hartignet. But the fungus also produces small pegs that project into the root cells and then expand, surrounded by the plant cell membrane. The three membered symbiosesinvolve a direct nutritional connection between a tree host, mycorrhizal fungus, and parasitic higher plants (Bonfant and Genre, 2010).

2.4.6. Ectendo Mycorrhiza Fungi

This type of fungus the fungal sheath is reduced or it doesn't exist, the Hartic network has not expanded, but the roots penetrate inside (Brundrett, 2004). Ecto mycorrhiza fungi can form Ectendo mycorrhiza mode on different hosts and in good conditions. In the first step of classifying, we can find the type of fungi related to each mycorrhiza according to the transverse wall. Those that have no walls are Fecomistic End fits and those that have fungi with transverse walls are Ascomycets or Basidiomycetes. In the first grown where Fecomistic fungi have no transverse walls, they belong to Endogonaceae and are Zygomycetes genus .These fungi are rarely seen freely. They are dispersed around the

roots and they penetrate in the spaces between cells and into the host plant cells and have particularly formed split suction organs (Arbuscular) inside the cell and the vesicle are inside or outside the host tissue, and mostly form spores or sporocarps with complexstructures (Bedini *et al*., 2008).

2.4.7. Arbutoid mycorrhiza

Arbutoid mycorrhiza is like those of ericoid and monotropoid mycorrhizas, found in the plant order Ericales. Arbutoid associations are found in the *pyrolaceae* family in order Ericales. The fungi of arbutoid mycorrhizas are basidiomycetes, often the same fungal species that form the ectomycorrhizal associations. A major difference between the arbutoid and ectomycorrhizal association is that the hyphae of the former do actually penetrate the outer cortical cells, and fill them with coils, which the hyphae of ectomycorrhizal do not. The intracellular coils, along with the mantle sheath and hartig net are the diagnostic features of arbutoid mycorrhiza (Brundrett, 2002).

2.5. **The Mutual Benefits of Arbuscular Mycorrhiza Fungi with the Partners**

Plants provide carbon to their fungal partners. The photosynthetic product hexose is transported to the arbuscular part of fungal cytoplasm and gets converted into glycogen and TAG (triacylglycerol). These are suitable firms of carbohydrates that are easily transported to long distances within the fungal network. In the case of plants, mycorrhiza increases the surface area of roots for improved uptake of water and nutrients. Immobile nutrients are absorbed by the plants through diffusion. In nutrient depleted, tropical regions with excessive rainfall where essential nutrients are leached from soil surfaces, mycorrhizal fungi can extend their external hyphae beyond the depleted zones. As a result, more volume of soil becomes accessible to plant roots. Therefore, plants with mycorrhizal associations are more efficient in the absorption of nutrients like nitrogen, phosphorus, potassium, and calcium (Brundrett, 2004).

There are several ways by which AM fungi help plants to absorb water from the soil. AM fungal hyphae grow into the soil matrix, and create a skeletal structure to hold primary soil particles together by physical enlargement. Soil structure and its porosity are important factors for water retention, especially during the dry season. AM fungi can also change the hormonal flow of information from plant roots to shoots, and affect stomata responses when soil water potential is lowered. It has been reported that mycorrhizal associations

help plants increase nutrient uptake during water-stressed conditions by increasing hydraulic conductivity in roots (Al-Karaki, 1998).

The rhizosphere is the site where microorganisms interact with both plant roots and soil constituents. The higher carbon demand of AM fungi competitively inhibits the growth of plant pathogens. Furthermore, the mycorrhizal fungal partner can also improve the nutrient status of the host plant by compensating the loss of root biomass due to pathogen attack by increasing its tolerance. With AM Fungi production of plant defense chemicals like phenolic substances, phytoalexins, and chitinases are increased. Symbiotic processes are not affected by these chemicals but systemic plant defense mechanisms are turned on. Mycorrhizal associations also protect plants against heavy metal toxicity which in turn defend host plants from other harmful pathogens. Competitive inhibition of pathogens by endo and ectomycorrhizal fungi is demonstrated to protect host plants from diseases like root, root collar disease, etc. (Askar and Rashad, 2010).

2.5.1. Symbiotic association of AM Fungi with plants

Studies described that, the symbiosis of AMF with plants had been reported 400 million years ago (Selosse *et al.,* 2015). Such types of links are established as a succession of biological processes, which lead to a variety of useful effects in both natural ecosystem and agricultural biotas (Van der Heijden *et al*., 2015).AM fungal associations were so successful relation that still the majority of land plants in most ecological niches use in this symbiotic association. Therefore, AMF are vital endosymbionts playing an effective role in plant productivity and the functioning of the ecosystem. They are of key importance for sustainable crop improvement (Gianinazzi *et al*., 2010).

As studies indicated that AMF association improve plant tolerance to biotic and abiotic factors (Plassard and Dell, 2010). They have the ability to improve characteristics of soil and consequently encourage plant development in normal as well as in stressful circumstances (Begum *et al*., 2019). AMF colonization improves tolerance of plants to stressful cues by bringing about several changes in their morpho-physiological traits(Begum *et al*., 2019)

2.5.2. AMFungi used as Manager of the Ecosystems

Arbuscular mycorrhiza fungi found in all ecosystem have been observed in virtually all major ecosystems worldwide (Öpik *et al*., 2006), from arctic regions (Varga *et al*., 2015), to tropical forests (Lovelock *et al*., 2003), from the deserts in the Arabic peninsula (Al-

Yahya'ei *et al*., 2011) to the high Himalayans. While some AM Fungal isolates show only restricted distribution in natural communities, others appear to be true cosmopolitans (Rosendahl *et al*., 2009).

The occurrence of truly cosmopolitan AM fungal species (Rosendahl *et al*., 2009) suggests that these fungi are extremely adaptable, both, in terms of environmental conditions, and in terms of a wide host range. Since AM fungi play an instrumental role in the protection against abiotic stresses such as nutrient starvation, heat (Bunn *et al*., 2009), and drought, they can benefit their hosts in the wild and in agriculture (Wu, 2017). Consequently, AM fungi are thought to have a great impact in natural environments (Smith and Read, 2008), as in managed conditions in agriculture, horticulture, and forestry.

2.5.3.Relation between AM Fungi and mineral Nutrition

As several reports described that symbiotic relation of AM Fungi and the plants increasing nutrient up take of the plants in addition to this increase soil fertility and ecosystem functions. A prominent role of such symbiotic relationship is to transfer nutrients, for example, organic carbon (C), in the form of lipids and sugars. AMF colonization is widely believed to stimulate nutrient uptake in plants. AMF have the capability to boost the uptake of inorganic nutrients in almost all plants, specifically of phosphate (Smith *et al*., 2003; Nell *et al*., 2010). AMF are also very effective in helping plants to take up nutrients from the nutrient-deficient soils (Kayama and Yamanaka, 2014).

Therefore, they can significantly boost the phosphorus concentration in both root and shoot systems (Al-Hmoud and Al-Momany, 2017). Under phosphorus-limited conditions, mycorrhizal association improves phosphorus supply to the infected roots of host plants (Bucher, 2007). According to (Sun *et al*., 2018), AMF are soil-borne fungi that can significantly improve plant nutrient uptake and resistance to several abiotic stress factors. AMF-mediated growth promotion is not only by improving water and mineral nutrient uptake from the adjoining soil but also by safeguarding the plants from fungal pathogens (Jung *et al*., 2012).

2.5.4. AM Fungi used as disease defense mechanisms

As study indicate that plant roots colonized by AM Fungi internally or externally and release molecular signal that recognized by plants and this signaling is so important to increase disease resistance of AM Fungi that have shown to trigger defense responses in various plant species(Wan *et al*., 2008).Defense mechanisms in the host have to be

attenuated to allow AM fungal infection and colonization of the roots, general defense needs to remain active to cope with rhizospheric pathogens. Indeed, general disease resistance of_mycorrhizal plants is not decreased. In contrast, mycorrhizal_plants often exhibit increased disease resistance (Borowicz, 2001;Cameron D.D.*et al*., 2013).

Experiments with split root systems revealed that_this effect is often systemic, i.e., the entire plant is protected_against pathogens. This can involve generally improved plant health due to better nutrition, or a systemic induction of the defense status, known as systemic acquired resistance (SAR). Mycorrhizal plants may be prepared to react faster stronger to pathogen attack, a phenomenon known as induced systemic resistance (ISR), or priming (Conrath *et al.*, 2006). In addition, AM fungi,or other microbes associated with their mycelium, can directly interfere with rhizospheric pathogens either by the release of antimicrobial compounds, or by direct competition for space and resources(Jacott *et al*., 2017)

2.6. AM Fungi inoculation and plant response

As study indicates that Soil phosphorus is a critical factor in plant response and responses are generally better under low phosphorus levels. Host genotypes and fungal strains seem to influence the response of plants to inoculation. The worldwide field experiment has provided evidence to show that under marginal P-deficiency soils lacking in effective AM fungal endophytes increase in yield of wheat, maize, barley, potatoes, and cowpea.Increased uptake of zinc has also been shown in AM fungus inoculated peach, maize, wheat and potato in zinc deficiency soils(Yuvaraj *et al*.,2020). The AM associations related to increased uptake of sulfur and calcium, improved water absorption and tolerance of plants to water stress in citrus and avocado seedlings have also been noticed. There are also reports of increased levels of cytokinins and chlorophyll by AM fungus- inoculated plants (Lands *et al*., 2004). AMF inoculation, represented by Rhizophagus clarus and Acaulospora colombiana, increased plant growth, P content, photosynthetic rate, and mycorrhizal colonization of I. paraguariensis seedlings harvested at 90 and 180 days after inoculation. Phosphorus addition did not affect mycorrhizal colonization and AMF inoculation suppressed the needs of P fertilization (Tomazelli *et al*., 2022)

Therefore, many researchers were trying to use alternative approaches based on either manipulating or adding microorganisms to enhance plant protection against pathogens. The useful microorganisms (e.g., bacteria genus, *Bacilli subtilis*) and fungi contend with plant

pathogens for nutrients and by attacking them with antimicrobial agents and by parasitizing pathogens (Tarafdar *et al*., 1994).

2.7.AM Fungi and Abiotic stress

2.7.1. Drought

Drought stress affects plant life in many ways; for example, shortage of water to roots reduces rate of transpiration as well as induces oxidative stress (Hasanuzzaman *et al*., 2013). Drought stress imparts deleterious effects on plant growth by affecting enzyme activity, ion uptake, and nutrient assimilation (Ahanger *et al*., 2017a). However, there is a strong evidence of drought stress alleviation by AMF in different crops such as wheat, barley, maize, soybean, strawberry, and onion (Moradtalab *et al*., 2019). Plant tolerance to drought could be primarily due to a large volume of soil explored by roots and the extra-radical hyphae of the fungi (Ouledali*et al*., 2016).

Rice has its mechanisms to drought stress, and they are also assisted by living soil organisms. Arbuscular mycorrhizal (AM) fungi are among one of the soil microorganisms that may enhance drought resistance of rice. It assists plants in uptake water and nutrients. It also plays roles in regulating plant hormones, as well as stomatal behavior under drought stress. Apart from that, intercropping is likely contributing to the improvement of drought resistance and AM fungi activity. Intercropping can enhance AM fungi colonization and improve the root morphology of rice which beneficial for drought resistance (Yuvaraj , and Ramasamy,2020). The mycorrhizal development still strongly stimulate the improvement of plant growth and increased plant survival under drought stress. AMF had shown to reinforce drought tolerance in numerous plants (Berg *et al*., 2009).

2.7.2. Temperature

Soil temperature increases, plant community reaction and may be dependent on AMF interactions for sustainable yield and production (Bunn *et al*., 2009). Heat stress significantly affects plant growth and development by imparting loss of plant vigor and inhibition of seed germination, retarded growth rate, decreased biomass production, wilting and burning of leaves and reproductive organs, abscission and senescence of leaves, damage as well as discoloration of fruit, reduction in yield, cell death and enhanced oxidative stress (Hasanuzzaman *et al*., 2013).

AMF can increase plant tolerance to cold stress (Birhane et al., 2012. Moreover, a majority of reports state that various plants inoculated with AMF at low temperature grow better than non-AMF-inoculated plants (Zhu et al., 2010b). AMF support plants in combating cold stress and eventually improve plant development (Gamalero et al., 2009). Moreover, AMF also can retain moisture in the host plant (Zhu et al., 2010a), increase plant secondary metabolites leading to strengthen plant immune system, and increase protein content for supporting the plants to combat cold stress conditions (Abdel Latef and Chaoxing, 2011b).As study indicates that during cold stress, AMF-inoculated plants showed an enhanced water conservation capacity as well as its use efficiency (Zhu et al., 2010b). AMF improve the synthesis of chlorophyll leading to a significant improvement in the concentrations of various metabolites in plants subjected to cold stress conditions (Zhu et al., 2010a). The role of AMF during cold stress has also been reported to alter protein content in tomato and other vegetables (Abdel Latef and Chaoxing, 2011b).

Generally, AMF-inoculated plants show better growth under heat stress than do the non-AMF inoculated ones (Gavito *et al*., 2005). As evidences indicated that cultivating different plants in very hot area by inoculating AMF is effective and increase productivity due to those plants grow in hot, dry and desert areas by resisting the weather condition. The association of AMF *Glomus fasciculatum* with plant growth and development leads to positive changes in growth under conditions of high temperature (Maya and Matsubara 2013).

2.7.3. Salinity

It is widely known that the soil salinization is an increasing environmental problem posing a severe threat to global food security. Salinity stress is known to suppress growth of plants by affecting the vegetative development and net assimilation rate resulting in reduced yield productivity and promotes the excessive generation of reactive oxygen species (Ahanger *et al*., 2017a,). Attempts are being made to explore potential means of achieving enhanced crop production under salt affected soils. One such potential means is the judicious use of AMF for mitigating the salinity-induced adverse effects on plants (Santander *et al*., 2019). Several research studies showed the efficiency of AMF to impart growth and yield enhancement in plants under salinity stress (Abdel and Chaoxing, 2014).

As study described by (Ruiz et al.1996) showed that the addition of AM fungi to lettuce and onion plants resulted in increased accumulation of phosphorus under conditions of

salinity stress. Furthermore, AM can affect the ionic balance of plants, especially about Na+ and Cl− (Garg and Manchanda ,2009).Furthermore, the addition of AM to tomato (*Lycopersicon esculentum*) under conditions of salinity improved anti-oxidant enzyme production, thus protecting cell membranes from damage. AM fungi can also improve the secretion of different types of hormones, one of them being abscisic acid. Mycorrhizal effects on hormones are important, as these hormones can enable plants to overcome many environmental stressed (Husband *et al*.,2002). For example, inoculation of lettuce (*Lactuca sativa*) with Glomus intraradices induced enhanced levels of hormones in these plants under conditions of salinity stress and this, in turn, affected the regulation of stomatal closure. Salinity may also induce drought conditions for plants, so AM fungi may also help plants increase water uptake. The addition of mycorrhizas to leek (*Alliumporrum*) increased the surface area of the roots, thereby increasing water absorption by the plants. The efficiency of water use in lettuce plants improved significantly with the addition of mycorrhizas under salt stress (Johnson *et al*.,2011).

(El-Nashar, 2017) reported that AMF enhanced growth rate, leaf water potential, and water use efficiency of the *Antirrhinum majus* plants. Recently, (Ait *et al*., 2019) reported the beneficial effects of AMF symbiosis on physiological parameters such as photosynthetic rate, stomatal conductance, and leaf water relations under saline regimes. AMF significantly alleviated the deleterious effects on photosynthesis under salinity stress (Sheng *et al*., 2011). Mycorrhizal inoculation markedly improved photosynthetic rate, and other gas exchange traits, chlorophyll content, and water use efficiency in *Ocimum basilicum L*. under saline conditions (Elhindi *et al*., 2017). AMF-inoculated Allium sativum plants showed improved growth traits including leaf area index, and fresh and dry biomass under saline conditions (Borde *et al*., 2010). Recently, (Wang *et al*., 2018) have reported considerable enhancement in fresh and dry weights, and N concentration of shoot and root due to mycorrhizal inoculation under moderate saline conditions.

2.7.4. Heavy metals

AMF are widely believed to support plant establishment in soils contaminated with heavy metals, because of their potential to strengthen defense system of the AMF mediated plants to promote growth and development. Heavy metals may accumulate in food crops, fruits, vegetables, and soils, causing various health hazards (Yousaf *et al*., 2016). AMF association with wheat positively increased nutrient uptake under aluminum stress (Aguilera *et al*., 2014). Plants grown on soils enriched with Cd and Zn exhibit considerable

suppression in shoot and root growth, leaf chlorosis, and even death (Moghadam, 2016).

There are many reports in the literature on uncovering the AMF-induced effects on the buildup of metals in plants (Souza *et al*., 2012). Heavy metals can be immobilized in the fungal hyphae of internal and external origin (Ouziad *et al*., 2005) that have the ability to fix heavy metals in the cell wall and store them in the vacuole or may chelate with some other substances in the cytoplasm (Punamiya *et al*., 2010) and hence reduce metal toxicity in the plants. The strong effects of AMF on plant development and growth under severe stressful conditions are most often due to the ability of these fungi in increasing morphological and physiological processes that increase plant biomass and consequently uptake of important immovable nutrients like Cu, Zn, and P and thus reduced metal toxicity in the host plants (Miransari, 2017).

It is also believed that enhanced growth or chelation in the rhizospheric soil can cause metal dilution in plant tissues (Audet, 2014). AMF reportedly bind Cd and Zn in the cell wall of mantle hyphae and cortical cells, thereby restricting their uptake and resulting in improved growth, yield, and nutrient status (Garg and Chandel, 2012). There is support for the idea that AMF plants may have an important role in enhanced P uptake and dry-matter production in plants. The present study was undertaken to evaluate the response of *Medicago sativa* L. (alfalfa) to different AMF species. Alfalfa is one of the perennial Leguminosae. Alfalfa has rapid growth rate, high biomass production, and deep root system distribution in soil. Moreover, alfalfa is a perennial plant that establishes permanent coverage on the soil surface. Establishment of such continuous soil coverage can be effective in permanent cleanup of contaminated soil. We were looking to differentiate among different AMF species with regard to their P uptake and biomass allocation. Furthermore, we tested the effects of selected AMF species on the ability to absorb P and partition of heavy metals in alfalfa plants growing on contaminated soil (Zaefarian et al.,2013).

Mycorrhizae can disturb the uptake of different metals into plants from the rhizosphere and their movement from the root zone to the aerial parts (Li *et al*., 2015). Mycelia of various AMF have a high cation-exchange capacity and absorption of metals (Takács and Vörös, 2003). Metal non-adapted AMF settle the polluted soils and reduce uptake and accumulation of heavy metals, as observed in perennial ryegrass (*Lolium perenne*) in artificially polluted soil with various elements like Cd, Ni, and Zn (Takács and Vörös,

2003). AMF are believed to regulate the uptake and accumulation of some key inorganic nutrients.

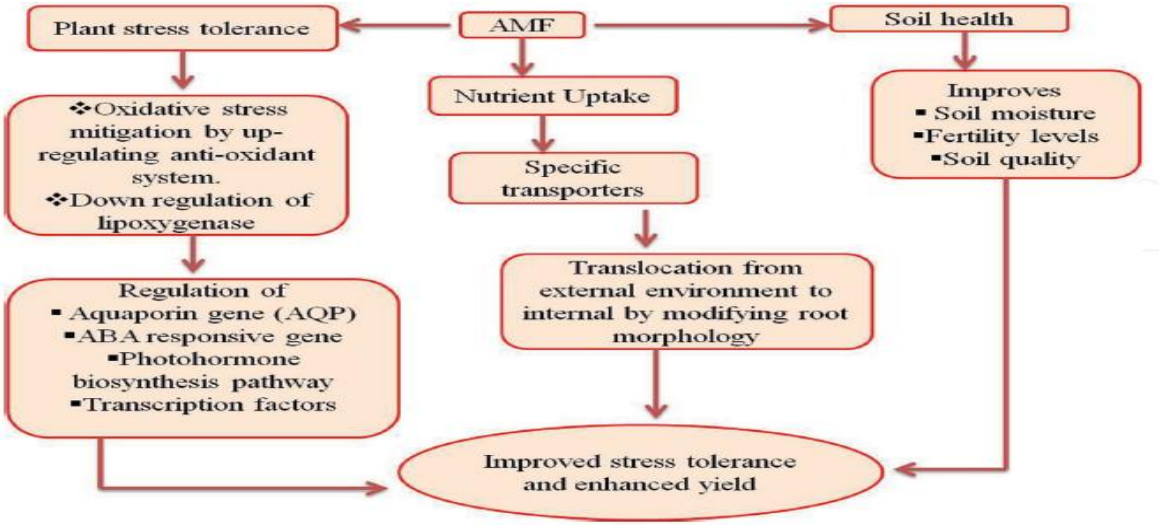


Figure 3 A diagrammatic illustrations of mycorrhizal roles in regulating numerous ecosystem processes and promoting plant development under abiotic stress conditions. Source: (Aziz *et al*. 2023).

2.8. Taxonomy of AM Fungi

Before molecular techniques, the only way to identify AM fungi was by a careful microscopic examination of the spores. (Redecker *et al*., 2000) used both morphological and molecular data and transferred *S. coremioides* to the genus *Glomus*. (Morton and Redecker, 2001) erected two new families in the order *Glomales* (now *Glomerales*), i.e., *Archaeosporaceae* and *Paraglomaceae* (now *Paraglomeraceae*). This was based on the data from molecular, morphological and biochemical investigations.

As evidences indicated that Taxonomy and classification of AMfunga takes place continuously for many years. (Walker *et al*, 2018), classified the phylum Glomeromycota into a single class (*Glomeromycetes) which includes four orders (Diversisporales, Glomerales, Archaeosporales, and Paraglomerales)*, 12 families (*Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Claroideoglomeraceae, Diversisporaceae, Geosiphonaecae, Gigasporaceae, Glomeraceae, Pacisporaceae, Paraglomeraceae, Pervetustaceae, and Sacculosporaceae*), 34 genera and approximately 316 species.

2.9. Characteristics of AMF

As study indicates that one characteristic of AM fungi is their spore structures. AMF spores are the major survival units that have multi-nucleate, heterokaryotic structures (Bever JD *et al*.2005), and are formed singly, in clusters or sporocarps in the soil, and within root tissue in some mycorrhiza species. The development of symbiosis starts with signaling taking place before physical contact between the plant and the fungus. Both partners produce molecular signals triggering preparative responses in the other (Chabaud *et al*., 2011).

As evidence indicates that the other characteristics of AM Fungi is the mycorrhization process which follow different steps, consisting of germinating spores, hyphae differentiation, appressorium formation, penetration of the host root, intraradical hyphae formation, intercellular growth along with developed external mycelium (extraradical hyphae), and arbuscule formation, subsequently exchanging nutrients and carbohydrates between the host and fungus (Goltapeh *et al*., 2008).

As another evidence indicate that, different AM Fungi species show different characteristics based on plant species, nutrient use, plant development and plant defense. The same plant is colonized by different AMF species, which may colonize different plant species and create networks of communication and channels for solute transport between distinct plants at variable distances (Gollotte *et al*.,*2004*). Some AMF have more influence on nutrient use efficiency, others on plant development and others on plant defense (Smith & Read, *2008*).

In addition to these AMF are used as bio-inoculants, and researchers encourage their use as prominent bio-fertilizers in sustainable crop productivity (Barrow, 2012). Furthermore, AMF-inoculated soil forms more constant masses and significantly higher extra-radical hyphal mycelium than do the non-AMF-treated soils (Syamsiyah *et al*., 2018). Glomalin-related soil protein (GRSP) is believed to maintain water content in soils exposed to different abiotic stresses (Shen *et al*., 2014), which later on regulates water frequencies between soil and plants, automatically triggering plant development. Glomalin contains 30–40% C and its related compounds that safeguard soil from desiccation by enhancing the soil water holding capacity (Sharma *et al*., 2017). Growth-related functions, for example, stomatal conductance, leaf water potential, relative water content (RWC), PSII efficiency, and CO2 assimilation are affected by AMF inoculation (He *et al*., 2017; Chandrasekaran *et al*., 2019).

3. MATERIAL AND METHODS

3.1. Description of the Study area

The study was conducted at Adama Science and Technology University (ASTU). It is located in Oromia Regional State, East Shoa zone, Ethiopia and 100 km away from Addis Ababa situated between 8^0 33' 43.56", latitude (N) and 39^0 17' 23.28" longitude (E) with an elevation 1620 m.a.s.l . Mean annual rainfall of Adama was 928.8 mm, with the highest mean monthly rainfall recorded in July (443.2mm) and the lowest in December (48.6mm). The mean monthly maximum temperature recorded was 30.7^0C in May and the mean minimum was 23^0C in August and maximum was 32^oC. Rainfall was erratic and uneven in distribution. The soil type is dominated by Loam and clay loam soil textures (Dejene and Birhanu, 2020).

3.2. Research design

The experiment was conducted in a pot under green house to evaluate suitable host plants that could be used for AMF inoculum mass production. Selected seed varieties of host plants, two from cereals; sorghum (*Sorghum bicolor*) and maize (*Zea mays*) and two from forage legumes; alfa alfa (*Medicago sativa*) and white clover (*Trifolium repens*) were collected from Awash Melkasa agricultural research center and transported to Adama Science and Technology University. All AMF morphological analyses of the soil samples were done in microbiology lab of the Applied Biology Department, ASTU.

3.3. Soil sample collection

Soil samples for the trap culture were collected randomly from the rhizosphere of (*Acacia seyal)*, located in the ASTU compound. The acacia tree species were characterized by relatively high AMF colonization and very high AMF abundance and diversity according the report of (Zerihun Belay *et al.* 2013). From randomly selected different areas of the acacia species, about 18kg of rhizosphere soil were taken into a depth of 30 cm and that was pooled into a composite. The samples were collected in alcohol sterilized plastic bags and spore extraction and identification from soil and the assessment of spore density also was done by wet sieving and decanting method according to (Brundrett *et al.*, 1996). Counting of spores also was done according to INVAM (2020).

3.4. Trap culture

In order to obtain a suitable host plant to produce infective AMF spores and propagules for evaluating of the host plants in inoculum production, a trap culture was established in green house according to INVAM (2020). Seeds of sorghum(*Sorghum bicolor*), Maize (*Zea mays),*white clover (*Trifolium* repens), and alfalfa (*Medicago sativam*) were surface sterilized by immersing them in a 0.5%v/v sodium hypochlorite solution for 15 minutes, and washing them with sterile distilled water 7-10 times.

Then, they were sown at 2 cm depth in each plastic pot and covered with sterilized sand. They were grown in 15 cm plastic pots filled with thoroughly homogenized field soil samples that contained root sections, AMF spores, and hyphae and colonized root from the field mixed with sterile sand (1:1; v/v) in a greenhouse for at least 8 weeks. The pots were arranged in a randomized block design with three replicates. Pots were irrigated every day as needed. Finally, watering was reduced during the final three weeks to promote sporulation. At the end of two months the plants were cut near the base, and the cultures has been air-dried and checked for the presence and identification of spores, spore diversity and density.

3.5. AMF spore extraction and Enumeration

AM fungal spores were extracted from substrates of trap culture by wet sieving and decanting according to Brundrett *et al*. (1996). Hundred gram soil sample from each pot was suspended in 1L of tap water by gentle stirring. Heavier particles then allowed settling for a few seconds and to decant the liquid through four stacked sieves of decreasing pore size: 500, 180, 90, and 63 µm. The first sieve was used to catch large pieces of floating organic debris, and the second sieve was used to capture larger spore and some AMF sporocarps, and the third and fourth sieves were used to capture AMF spores and fine particles. The contents of 180 µm, 90 µm sieves and 63 µm were then poured into a centrifuge tube containing water and centrifuged for 5 minutes at 2000 rpm. After pouring off the supernatant solution, the contents were mixed with 50% g/v sucrose and then centrifuged at 2000 rpm for 1 min. The supernatant was carefully poured through a 63 µm sieve and carefully washed with tap water to remove the sucrose. Finally, spores, spore clusters, and sporocarps were carefully washed and transferred to plastic Petri dishes for

examination under the stereomicroscope. Enumeration of spore numbers per gram of dry soil was undertaken according to INVAM, http://invam.caf.wvu.edu.

3.6. Spore identification and characterization

AMF spores were picked up with forceps and mounted on slide in polyvinyl-lactic acid-glycerol (PVLG), (Brundrett *et al.*,1996) or PVLG +Melzer's reagent (1:1 v/v) (Gerdemann and Nicolson, (1963). Spores were examined under compound microscope (OLYMPUS-BX51) at a magnification of 400x and identified to the species level or specific morphotype based on a current species descriptions and identification manual by online references of species description INVAM http://invam.caf.wvu.edu, The main known AM Fungi features identified were, spore development, spore arrangement, spore shape, spore size, spore color and subtending hyphae.

3.7.Assessment of AM Fungi root colonization

Root samples were preserved in 50% ethanol prior to cleaning. To ensure uniform staining, the roots were chopped in to small (1-2 cm) segments. Then, the roots were washed and the root specimens were stored in capsules or tubes. Finally, the root samples were placed in beaker containing 10% KOH (w/v) solution and heated in a water bath at 90^{o}C for 1 hour, and treated with KOH solution and washed with tap water until no brown color appeared.

The root samples were put in beaker with alkaline H_2O_2 at room temperature for 10 min. or until the roots were bleached. Finally, the root samples were rinsed thoroughly using at least three complete changes of tap water to remove the H_2O_2. Then the roots were covered with acidified dilute HCL (1-3.5%) for 3-4 minutes. The treated root samples were stained in 0.05% w/v trypan blue in lacto glycerol (1:1:1 lactic acid, glycerol and water) at 90^{o}C for several hour in water bath. Samples were drained and washed thoroughly with distilled water at the end of every step except HCl treatment. The samples were left in distaining solution (lactoglycerol) for more than two days in a dark room. Stained root fragments were arranged in glass slides and observed under the microscope for the presence of hyphae, vesicles and arbuscules. Finally, roots were mounted in PVLG mountant on microscopic slides and covered with 24×24 mm coverslips (Brundrett *et al*., 1996).

AMF colonization was assessed from cleared and stained roots according to McGonigle *et al.*(1990). A total of 150 intersections were taken for each subsample to estimate percent AMF root colonization under a compound microscope at a magnification of 100X. The presence of arbuscular colonization (AC) and vesicular colonization (VC) were calculated by dividing the count for the 'arbuscules' and 'vesicles' categories, respectively by the total number of intersections and times by 100. Hyphal colonization (HC) was calculated by dividing the proportion of non-negative intersections to total number of intersections.

3.8. Data Analysis

All data were subjected to one-way ANOVA to test the differences in AM colonization and spore density among plant species. Multiple mean comparisons were performed using Tukey's HSD post hoc test at the 0.05 level of probability with one-way ANOVA. Spore density (SD) was expressed as the number of AMF spores per gram of soil. Species richness (SR) was measured as a number of AMF species per sample. Analysis of variance (ANOVA) was carried out with the SPSS software package (version 26. 0).

Isolation frequency (IF) was calculated as (the number of samples in which a given species was isolated/ the total number of samples) ×100%. Relative abundance of spores (RA) was calculated as (the number of spores in a given species / total number of spores) ×100%. 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 a genus or species is dominant; 10% < IV < 50% applies to common genera or species; an IV ≤ 10% indicates that a genus or species is rare (Chen *et al.*, 2012).

4. RESULT AND DISCUSSION

Trap cultures, using host plants grown in soil diluted with sterile sand, are most commonly used to isolate AM fungi (Brundrett *et al*., 1999). It provides additional information on fungal diversity that complements spore occurrence data obtained using the same soil samples and may provide valuable new information about the biology of AM fungi at times there is a possibility to get species that could not be recovered with trap methods (Brundrett *et al*., 1999). In this study, high AMF spore density, diversity and root colonization were obtained from trap host plants (Table 1 figure 4,5 and 6).

4.1. AMF root colonization

A total of four trap cultures (maize, clover, sorghum, and Alfa alfa) were established from field soils originating from *Acacia seyal*. The host plant showed high mycorrhization with typical structures (arbuscules, hyphae and vesicles). AMF root colonization (hyphal colonization) varied from 83.3% to 94.6% (Figure 4). The highest Hyphal colonization (Hc) was found on sorghum (94.6%) which was followed by 89.3 %, 87.3% and 83.3% hyphal colonization on maize, alfaalfa and clover, respectively. The difference between maize and Alfaalfa was not statistically significant ($p < 0.05$), with arbuscule colonization highest in the roots of maize (18.6%) and followed by clover (11.5%), sorghum (10.3%), and Alfa alfa (9.6%).Vesicle colonization was very high in the roots of clover (18.0%), followed by alfalfa (16.3%), sorghum (15.3%) and maize. respectively.

The variation in percent colonization clearly supported by the observation of (Yao *et al*, 2010) in the mono-cropping treatments root colonization differed significantly between plant species, with colonization of maize roots lower than that of sorghum. In the mono-cropping treatments, sorghum plants supported the highest extraradical hyphae density, followed by maize and clover plants. The same result has been reported by (Artib *et al.* 2019), They reported the results of the roots of the plant species studied on (maize, sorghum, wheat, barley, and beans) were found to be mycorrhizal and recorded significantly highest mycorrhizal colonization frequencies in the roots of sorghum, Similarly they reported that the arbuscular contents were high in maize; and Vesicular contents were very low in maize.

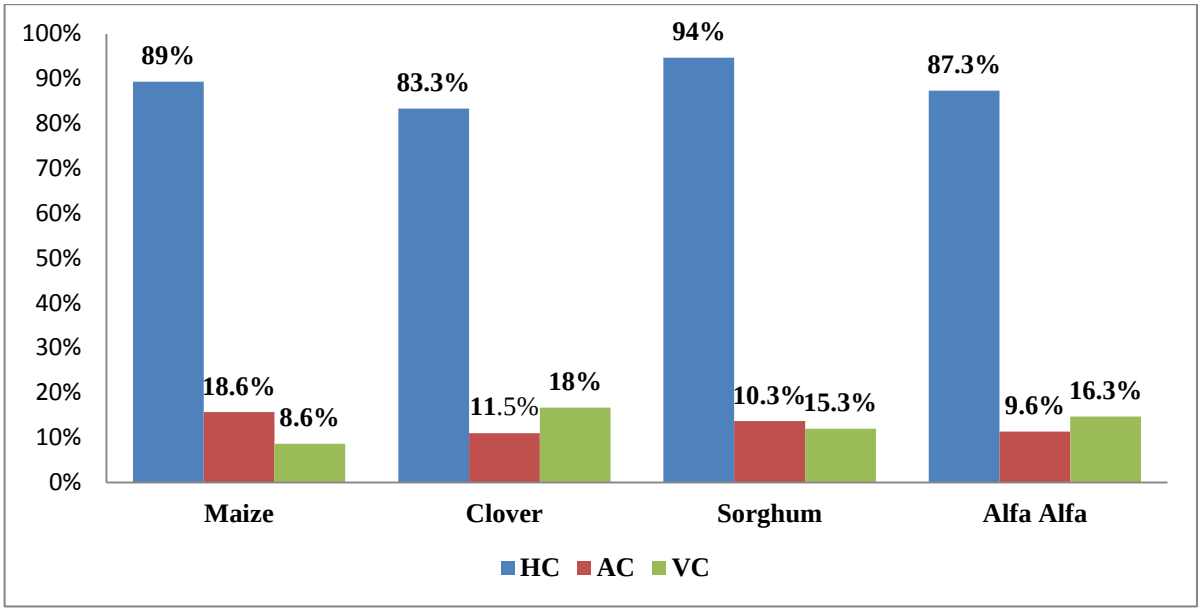


Figure 4 AMF root colonization on maize, clover, sorghum, and alfalfa. HC: Hyphal Colonization; AC: Arbuscule Colonization; and VC: Vesicle Colonization.

4.2. AMF spore density

Rhizosphere soils from *Acacia seyal* high numbers of AMF spores 15.0 spores' g-1 soil The average number of AMF spore density enumerated in the trap culture ranged from 11.7 to 23.6 g^{-1}. There was a significant difference in AMF spore density among the type of host plants. The highest average spore density of 23.6 spores' g^{-1} soil was observed for sorghum (*Sorghum bicolor*), followed by maize (*Zea mays*) (22.1 spores' g^{-1} soil), Alfalfa (*Medicago sativa*) (16.9 spores' g^{-1} soil) and the lowest of 11.7 spores' g-1 of soil for white clover (*Trifolium repens*). Significant difference ($p < 0.05$) in spore density was observed among host plants. The differences in spore numbers produced with different trap plants might be due to the variation in host plant root type and morphology, carbon biomass, nutrient and endogenous hormone level(Liu *et al*, 2003). According to (Yao *et al*, 2010) in the mono-cropping treatments, maize plants supported the highest spore number, followed by sorghum and clover plants.

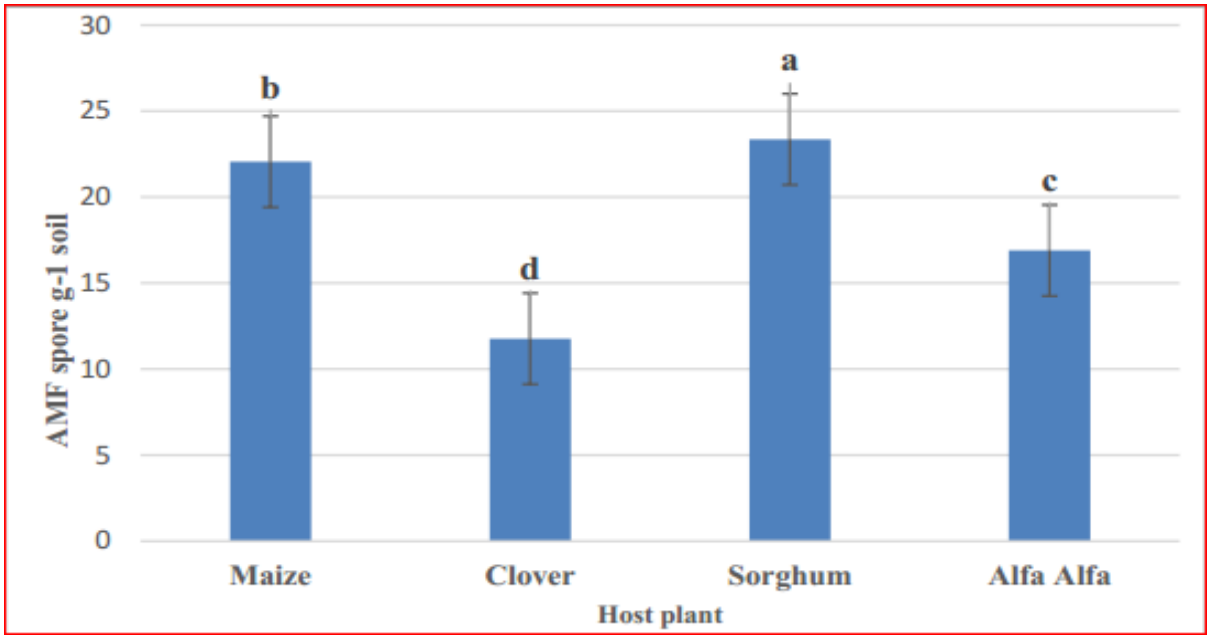


Figure 5 AMF spore density of maize, clover, sorghum, and Alfalfa

4.3 AMF spore diversity

A total of 27 AMF species belonging to 13 genera were identified from all trap cultures (Table 2). Five species belonged to *Gigaspora*, four to *Funneliformis*, three each to *Dentisculata*, Glomus, and *Rehizophagus*, two each to *Acaulospora*, *Clarideoglomus*, and one each to *Corymbiglomus*, *Scutellospora*, *Pacispora*, *Paraglomus*, *Racocetra*, *Scutellospora*, and *Septoglomus* . The highest species frequency (82) was detected from the trap culture of *Rhizophagus aggregates* , whereas the lowest (1) was recorded from *Dentiscutata nigra* and *Scutellospora species* . Among host plants, the highest number of genera (10) and species (15) was detected from the trap culture of clover, followed by alfa alfa plant with 10 genera and 14 species. . The lowest diversity was recorded from sorghum with seven genera and 12 species and from maize with 6 genera, and 12 species, respectively.

Liu & Wang (2003) conducted a study on selection of appropriate host plants from maize, tobacco, white clover and silverweed cinquefoil for trapping arbuscular mycorrhizal fungi and reported that white clover was generally the optimal host plant to detect diversity of AM fungi. In addition, Zerihun Belay *et al*.2015 reported that white clover was the optimal host plant to detect species diversity of AM fungi compared to the other hosts tested.

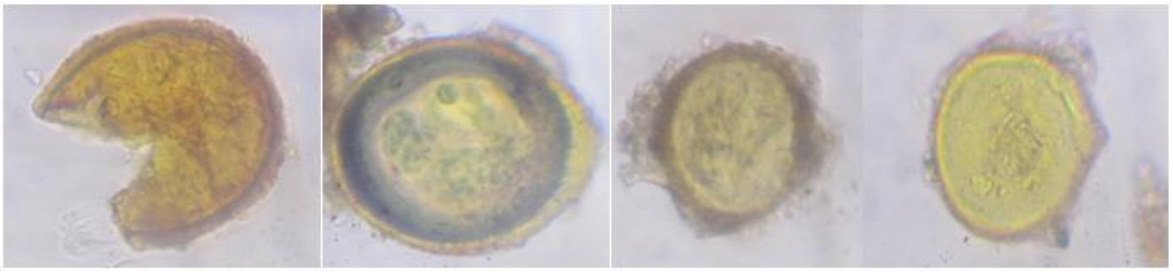
Arbuscular mycorrhizal symbiosis does not show clear host specificity (Selosse et al., 2006); some species of AM fungi have a wide range of host plants, while others have been found only in the rhizosphere of a single host plant, such as *Acaulospora splendida* found in the rhizosphere of *Quercus costaricensis* (Sieverding, 1991). Newman *et al*. (1994) showed that the same AMF is capable of affecting simultaneously two adjacent plants of different species which can be linked with a common underground mycelial network.

In this study the distribution and dominance of different species observed in all of the different rhizospheres of the four plants showed that *Clarideoglomus claroideum, Clarideoglomus etunicatum,Funneliformis mosseae, Gigaspora albida, Gigaspora gigantia, Gigaspora margarita, Paraglomus occultum* and *Rhizophagus aggregatum* were the dominant species among the host plants with variable abundance according to the calculation of (Chen *et al*. 2012), while the other species were specific and common. This result is similar to previous reports conducted in Iran, the genera belonging to the family Glomeraceae, particular, *Glomus* and *Funneliformis*, are present in most areas of Iran (Sedaghati et al., 2021). The dominant species are *Rhizophagus aggregates*, *Clarideoglomus claroideum, Funneliformis mosseae, Funneliformis geosporum*, and *Clarideoglomus etunicatum*. *Glomus intraradices* is among the dominant species.

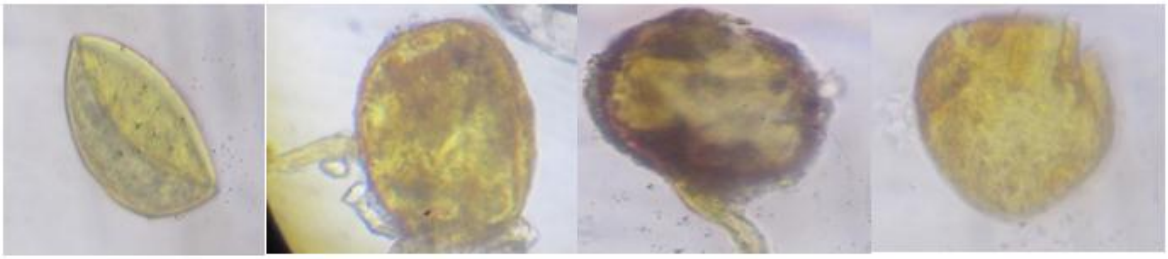
In another study, Zerihun Belay *et al*. (2015) showed that *Claroideoglomus* and *Funneliformis* were the dominant genera in all land use types in both trap culture and field soil in Ethiopia. The authors also showed that trap culturing enhanced spore abundance but caused a loss of AM Fungi species richness.

In this study, trap plants that give high spore density(Sorghum) exhibited low number of species diversity , and the same result was reported in that trap culturing enhanced spore abundance with reduced AMF species richness compared with the field soil samples (Chaturvedi*et al.* 2012). Plants that used as trap culture in this study indicated that different number of species specificity by the same AMFungi as example *Clarideoglomus claroideum* recorded different number specificity on all four host plants

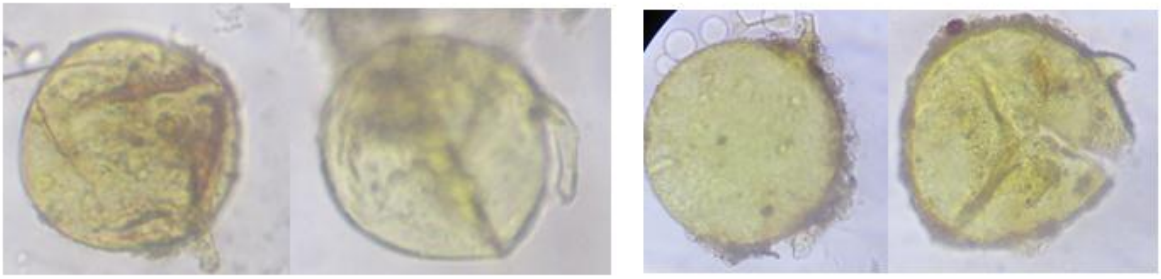
Figure 6. Dominant AMF species that indicates AMFungi spore diversity



Claroideoglomus claroideum

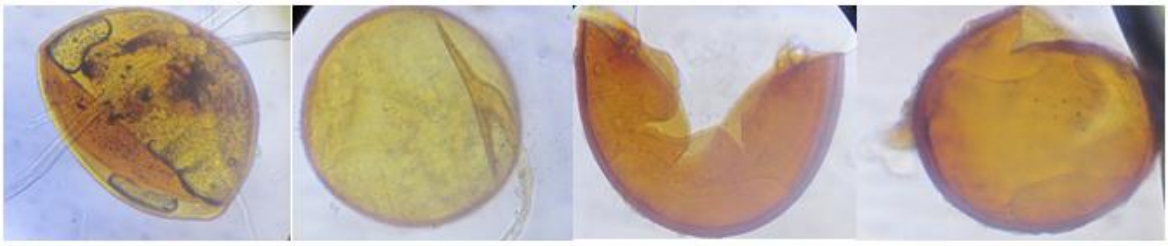


Rizophagus agregatus



Paraglomus occultum

Funneliformis mossea



G albida G.gigantia G. margarita F.geosporum

Table 1 Arbuscular mycorrhizal fungi, their frequency of occurrence and relative abundance in rhizosphere soil of host plants.

AMF species	Host plants spore number				% IF	%RA	%IV	Status according to Chen et al2012
	Alfa alfa	Clover	Maize	Sorghum				
Acaulospora								
A. scrobiculata (Berch, S.M. 1985)	1	1	-	-	50	0.51	25.51	C
A. spinosa (Walker & Trappe (1981))	-	-	-	2	25	0.51	12.76	C
Clarideoglomus								
C. claroideum (Morton, J. B. and Redecker. 2001)	34	27	1	3	100	16.7	58.35	D
C. etunicatum (Morton, J. B. and Redecker. 2001)	2	23	-	1	75	6.68	40.84	D
Corymbiglomus								
C. corymbiform (Błaszk. & Chwat (2012))	-	-	-	6	25	1.54	13.27	C
Dentiscutata								
D. nigra (Redecker, and Walker. 2013)	1	-	-	-	25	0.25	12.63	C
D. heterogama (Redecker, and Walker. 2013)	-	-	-	2	25	0.51	12.76	C
Funneliformis								
F. mosseae (Walker & Schuessler (2010))	14	26	3	27	100	17.99	58.99	D
F. geosporum(Walker & Schuessler (2010))	33	-	2	-	50	8.99	29.49	C
F. coronatum (Walker & Schuessler (2010))	10	-	2	-	50	3.08	26.54	C
F. caledonium (Walker & Schuessler (2010))	-	-	2	3	50	1.28	25.64	C
Gigaspora								
G. albida (Bentivenga, S.P. and Morton, J.B. 1995)	-	4	3	1	75	2.05	38.52	D
G. gigantia (Bentivenga, S.P. and Morton, J.B. 1995)	8	3	2	1	100	3.59	51.8	D
G. margarita (Bentivenga, S.P. and Morton, J.B. 1995)	2	-	2	4	75	2.05	38.52	D
G. rosea (Nicolson & N.C. Schenck (1979))	-	-	1	-	25	0.26	12.63	C
Gigaspora sp. (Morton, J.B. and Benny, G.L. 1990)	-	-	-	1	25	0.26	12.63	C
Glomus								
G. caledonium (Walker & Schuessler (2010))	-	1	-	-	25	0.26	12.63	C
G. badium (Walker & Schuessler (2010))	-	1	-	-	25	0.26	12.63	C
G. rubiforme (Walker & Schuessler (2010))	3	-	-	-	25	0.77	12.88	C
Pacispora								
P. scintillans (Walker, Vestberg & Schuessler (2007))	1	4	-	-	50	1.28	25.64	C
Paraglomus								
P. occultum (Morton & Redecker (2001))	3	3	2	2	100	2.57	51.28	D
Racocetra								
R. gregaria (Redecker, and Walker. 2013)	-	6	-	-	25	1.54	13.27	C
Rhizophagus								
R. aggregates(Walker & Schuessler (2010))	22	58	2	1	100	21.33	60.66	D
R. diaphanous (Walker & Schuessler (2010))	-	7	-	-	25	1.79	13.39	C
R. fasciculatus (. Walker & Schuessler (2010)	-	3	-	-	25	0.77	12.88	C

Scutellospora								
Scutellospora spp. of uncertain (Walker & F.E. Sanders (1986))	-	-	1	-	25	0.25	12.63	C
Septoglomus								
S. constrictum (Oehl, and E. Sieverding. (2011))	6	5	-	-	50	2.82	26.41	C
Total	14	15	12	12				

5. CONCLUSION

In this study sorghum displayed the highest spore density and root colonization than maize, alfa alfa and clover. Therefore, it is possible to recommend sorghum plants as a potential host plant for mass production of Arbuscular Mycorrhiaza Fungi (AMF) inoculum . In terms of spore diversity clover harbored high Arbuscular Mycorrhiaza Fungi species. *Clarideoglomus claroideum, Clarideoglomus etunicatum,Funneliformis mosseae, Gigaspora albida, Gigaspora gigantia, Gigaspora margarita, Paraglomus occultum* , , and *Rhizophagus aggregatum* were the dominant species distributed on all these plants with variable abundance. The highest species frequency (82) was detected from the trap culture of *Rhizophagus aggregates* , whereas the lowest (1) was recorded from *Dentiscutata nigra* and *Scutellospora species*.The efficiency of mycorrhizal propagation and mycorrhizal interaction with host should be checked in field trial in several plant cycles to verify AMF establishment.

6. RECOMMENDATIONS

During this study, the potential of each AMF species on plant growth, development and reproduction was not evaluated . Therefore, it is necessary in the future to evaluate effect of each AMF species inoculation on different plant growth development and reproduction based on their root morphology, soil type and weather conditions.

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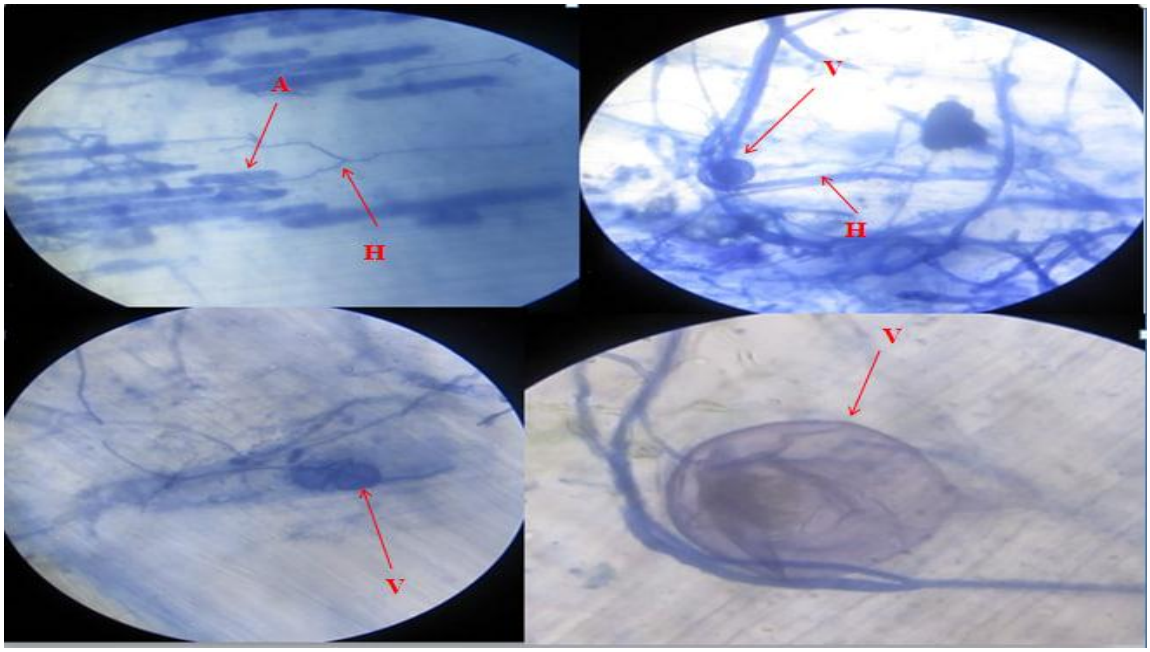
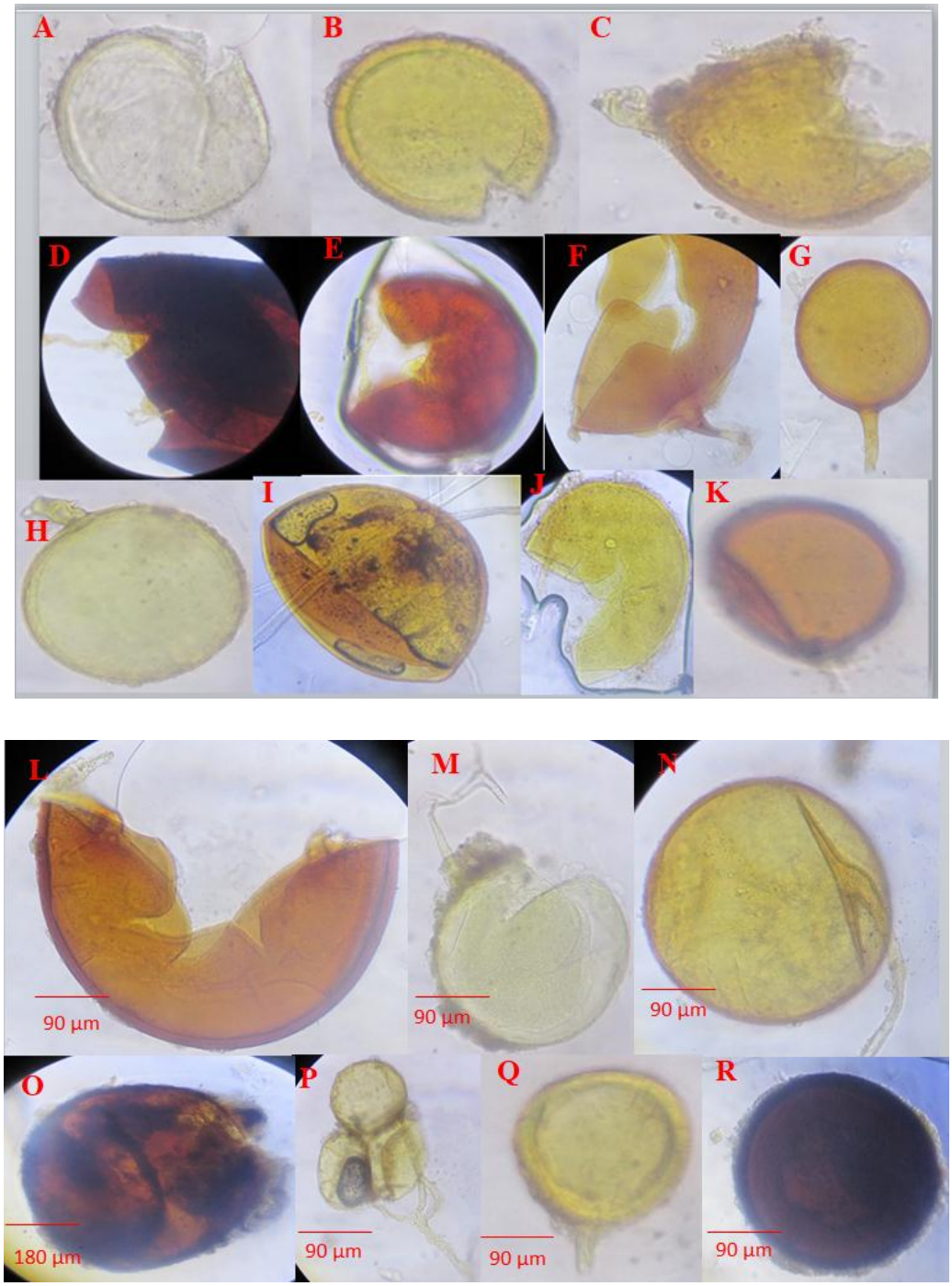
8. APPENDIXES

Appendix 1. Microscopic photos of some morphotypes extracted from the roots of trap culture host plants . All photos are from slides made in PVLG.



AMF root colonization in maize, clover, sorghum, and Alfalfa. HC: Hyphal Colonization; AC: Arbuscule Colonization; and VC: Vesicle Colonization. The same letter in the row indicates not significantly different values based on one-way ANOVA and Tukey's honestly significant difference (HSD) post hoc test ($P < 0.05$).

Appendix 2. Microscopic photos of some morphotypes extracted and identified spores from trap cultures plant soil.



A) A*caulospora scrobiculata*; B) *Claroideoglomus claroideum*; C) *Claroideoglomus etunicatum;* D)*Dentiscutata rubra*; E) Dentiscutata nigra; F) *Funneliformis coronatum*; G) *Funneliformis geosporum*H)*Funneliformis mosseaei;* I)*Gigaspora albida*;J)Gigaspora gigantean; k)Glomus badiumL) *Gigaspora margarita*; M) *Pacispora scintillans*(180); N) *Paraglomus occultum*; O)Racocetra gregaria;P)Rhizophagus aggregatus and Q)Rhizophagus fasciculatus; R) *Septoglomus constrictum.*

Appendix 3. Representative pictures to show growth of host plants.

Aa (alfa alfa), Cl (clover), So (sorghum), and Ma (maize) .

Appendix 4. Representative pictures to give further illustration of all practical works beginning from trap culturing to the end of laboratory session.

a) Weighiting 100 gram soil sample, b) decanting through four stacked sieves, c)Centrifugation in water and in a 60% sucrose solution, d) and e) Enumeration and extraction of AMF spore under stereomicroscope, f) morphological Identification of AMF spore under a compound microscope.,

Appendex 5. Output SPSS data analysis, Descriptive; Hyphal colonization, Arbuscule colonization, Vesicle colonization and Spore Density

Mychorrizal structures		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean Lower Bound	Upper Bound	Minimum	Maximum
Hyphal colonization	Maize	3	89.3333	1.52753	0.88192	83.8721	91.4612	86.00	89.00
	Sorghum	3	94.6666	1.73205	1.00000	89.6973	98.3027	93.00	96.00
	Clover	3	83.3333	3.51188	2.02759	74.6093	92.0573	80.00	87.00
	Alfa Alfa	3	87.3333	1.00000	0.57735	85.5159	90.4841	87.00	89.00
	Total	12	88.2500	4.37191	1.26206	85.4722	91.0278	80.00	96.00
Arbuscule colonization	Maize	3	18.6667	9.01850	5.20683	-3.7365	41.0699	10.00	28.00
	Sorghum	3	10.3333	4.16333	2.40370	-0.0090	20.6756	7.00	15.00
	Clover	3	11.3333	1.52753	0.88192	7.5388	15.1279	10.00	13.00
	Alfa Alfa	3	9.6667	4.16333	2.40370	-0.6756	20.0090	5.00	13.00
	Total	12	12.5000	5.97723	1.72548	8.7022	16.2978	5.00	28.00
Vesicle colonization	Maize	3	8.6667	4.04145	2.33333	-1.3729	18.7062	5.00	13.00
	Sorghum	3	15.3333	4.04145	2.33333	5.2938	25.3729	11.00	19.00
	Clover	3	18.0000	4.00000	2.30940	8.0634	27.9366	14.00	22.00
	Alfa Alfa	3	16.3333	2.30940	1.33333	10.5965	22.0702	15.00	19.00
	Total	12	14.5833	4.85159	1.40053	11.5008	17.6659	5.00	22.00
Spore density	Maize	3	22.0603	0.65558	0.37850	20.4318	23.6889	21.46	22.76
	Sorghum	3	23.3608	0.18183	0.10498	22.9092	23.8125	23.19	23.55
	Clover	3	11.7762	0.38265	0.22092	10.8256	12.7267	11.49	12.21
	Alfa Alfa	3	16.9065	0.21675	0.12514	16.3681	17.4449	16.69	17.12
	Total	12	18.5260	4.80022	1.38570	15.4760	21.5759	11.49	23.55