Greenhouse Evaluation of Indigenous AMF Inoculums Isolated from High Land and Low Land Areas in Improving Growth and Survival of Seedlings of the Green Legacy Program of Ethiopia

Yonatan Legesse Fole

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 (Specialization in Microbiology)

Office of Graduate Studies
Adama Science and Technology University

June, 2022
Adama, Ethiopia

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DECLARATION

I hereby declare that this Master's Thesis entitled "Greenhouse Evaluation of Indigenous AMF Inoculums Isolated from High Land and Low Land Areas in Improving Growth and Survival of Seedlings of the Green Legacy Program of Ethiopia" 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.

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RECOMMENDATION

We, the advisors of this thesis, hereby certify that we have read the revised version of the thesis entitled "Greenhouse Evaluation of Indigenous AMF Inoculums Isolated from High Land and Low Land Areas in Improving Growth and Survival of Seedlings of the Green Legacy Program of Ethiopia'' prepared under our guidance by Yonatan Legesse submitted in partial fulfillment of the requirements for the degree of Masters of Science in Applied Biology (Specialization in Microbiology). Therefore, we recommend the submission of revised version of the thesis to the department following the applicable procedures.

Major Advisor	Signature	Date
Co-advisor	Signature	Date

APPROVAL PAGE

We, the advisors of the thesis entitled "Greenhouse Evaluation of Indigenous AMF Inoculums Isolated from High Land and Low Land Areas in Improving Growth and Survival of Seedlings of the Green Legacy Program of Ethiopia" and developed by Yonatan Legesse, 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
Co-advisor	Signature	Date

We, the undersigned, members of the Board of Examiners of the thesis by Yonatan Legesse have read and evaluated the thesis entitled "Greenhouse Evaluation of Indigenous AMF Inoculums Isolated from High Land and Low Land Areas in Improving Growth and Survival of Seedlings of the Green Legacy Program of Ethiopia" 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 Biology (Specialization in Microbiology).

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External Examiner	Signature	Date

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).

Department Chair	Signature	Date
School Dean	Signature	Date
Office of Postgraduate Studies, Dean`	Signature	Date

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LIST OF ABBREVIATION AND ACRONYMS

ANOVA	Analysis of Variance
AC	Arbuscular Colonization
AMF	Arbuscular Mycorrhiza Fungi
EBTL	Environmental Biotech Laboratory
Ems	Ectomycorrhiza
ERM	Extraradical mycelium
GLP	Green Legacy Program
HC	Hyphal Colonization
IRM	Intraradical Mycelium
LSD	Least Significant Difference
PVLG	Polyvinyl Lacto-glycerol
RC	Root Colonization
VC	Vesicular Colonization

ABSTRACT

*Ethiopia has been carrying out national tree seedling planting campaigns since its millennium. The low level of seedlings' survival and establishment has been a bottleneck to the realization of the national restoration target. Poor field survival and establishment of trees were caused by low moisture and nutrient content of soils at the planting site. This study is aimed at evaluating the potential of native AMF inoculums isolated from highland and lowland areas in improving the growth and survival of seedlings of the Green Legacy Program of Ethiopia. The study was carried out in three stages. (1) Rhizosphere soil samples were collected from the selected sampling areas, (2) AMF was propagated in trap culture in greenhouse conditions, and (3), the culture of AMF was inoculated onto selected seedlings trees, Delonix regia, Sesbania grandflora, Cassia fistula, and Azodichta indica. Four treatments were set up: AMF inoculum from A.seyal, AMF inoculum from A.tortilis, AMF inoculum from A. abyssinica, and without inoculum (-*ve *control). The growth parameters like shoot height and root height, fresh and dry mass of root and shoot, and the number of leaves; percentage of root colonization, and spore density were measured and analyzed by ANOVA. The results showed that the growth of the trees significantly increased by inoculation of the AMF over that of non-inoculated (control) ($p < 0.05$). AMF spore density and root colonization rate showed the highest in trees treated by AMF from A.seyal among the treatments and followed by A.abyssinica and A.tortilis. Moreover, the highest root colonization and mycorrhizal dependency were observed in S. grandflora and spore density in C. fistula among the selected trees. Furthermore, Pearson's correlation analysis showed that significant positive correlations were observed between the parameters. All of these results suggested that inoculation of native AMF could improve the growth and survival rate of seedlings. However, the application of present findings required to be confirmed by further studies under field conditions.*

Keywords: Arbuscular Mycorrhizal Fungi (AMF), Green Legacy Program of Ethiopia (GLPE), inoculation of AMF, survival of Seedlings.

CHAPTER ONE

1. INTRODUCTION

1.1. Background of the study

Ethiopia has been carrying out national tree seedling planting campaigns since its millennium and planted billion of trees. The Government of Ethiopia envisions covering 22 million hectares of degraded lands with forest by 2030 by significantly enhancing the contribution of forestry to agriculture, water and energy (Kassa, 2018; Mekonnen , 2019). The Ethiopian government had announced a larger goal of planting 20 billion trees during a four-year period. All these development activities have been accomplished under the country's National Green Development program, to combat climate change and environmental degradation. Even if the country has working to rehabilitate degraded lands and restore forests through national tree seedling planting campaigns since 2000 the survival rate of the seedling is very low. According to reports of Adama Woreda Agriculture and Natural Resources Office 2020 and 2021; the survival rate of seed lings is 40% and 50% respectively (repots of AWANRO. 2021).

The low level of seedlings' survival and establishment in the field in Ethiopia had been a bottleneck to the realization of the national restoration target (Eba, 2017; Fisseha *et al*., 2019). In fact, it caused loss of a huge amount of money allocated for this purpose. Poor field survival and establishment of trees in the country were caused by low moisture and nutrient content of soils at the planting site (Fisseha *et al*., 2019; Mahari, 2014). In addition, the selection of suitable tree species for a particular planting site provided the necessary aftercare and utilization of relevant technologies that enhance tree seedlings moisture and nutrient relation were also important. One of such technology could be the inoculation of the seedlings with arbuscular mycorrhizal fungi (AMF) (Asmelash *et al*., 2016).

Arbuscular mycorrhizal fungi (AMF) are among soil microorganisms that greatly impact plant nutrition and growth under nursery and field conditions as they establish the arbuscular mycorrhizal association with their hosts. These fungi colonize the plant root cortex and spread their hyphae into the surrounding soil where they scavenge for low mobility nutrients like phosphorus, which they translocate back to the plant host; in turn,

they receive from the plants carbon compounds to grow and complete their life cycle. They also confer the plants resistance against pathogen, and improvement in water relations, as well as impacting soil structure (Goetten *et al*., 2016; Smith & Read, 2008).

AMF are considered as natural growth regulators of a majority of terrestrial flora. 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).AMFs promoted a significant increase of the area of root absorption of plants colonized, maximizing the use of water and nutrients (dos Santos *et al*., 2017). These fungi enhance plant resistance to water stress, high temperatures, conditions of toxicity and acidity of soil and pathogens, in addition to soil stabilization in the form of aggregates (Peng *et al*., 2013).

It is found that a wide variation among undisturbed and disturbed sites in the occurrence of AMF. For example, Ortega *et al*., (2004), reported that mycorrhizal association has been estimated to occur in 95% native vegetation from undisturbed sites, whereas it occurs in vegetation from disturbed sites in less than 1%. It was also confirmed that fine non woody roots (<2cm), which were responsible for most mineral and water absorption, were located in the upper 20 to 30 cm of soil (Marx *et al*., 2002), which signifies the importance of edaphic and phenologic factors in the establishment of AMF. Therefore, the lack of mycorrhizal fungi on root systems is a leading cause of poor plant establishment and growth in a variety of disturbed forest, restoration, agricultural, suburban and urban landscapes.

At afforestation sites devoid of mycorrhizal propagules seedlings never develop fully and often die (Ortega *et al*., 2004). Thus, the selection and inoculation of mycorrhizal fungi with beneficial functions can contribute to the establishment of seedlings and the recovery of degraded or disturbed areas, since they easily accelerate plant development and increase the tolerance to transplant stress and to the restoration of nutrient cycling (dos Santos *et al*., 2017). In spite of their potential and benefits, the large-scale use of AMFs is still limited, mainly due to the lack of availability of inoculant in high quantities, low cost and high quality (IJdo *et al*., 2011).

However, studies on the role of AMF in rehabilitation and regeneration of degraded lands are very limited or nil. Recently, the urgent need for low-growth and survival of plant seedlings in national re-vegetation program is necessitated alternative approaches that involve soil microorganisms. These alternatives should take into consideration the role of AMF a beneficial soil organism to potentially enhance the establishment and survival of seedlings. Considering all these benefits of the mycorrhizal association, inoculation of woody species' seedlings under nursery conditions is a strategy to reduce costs of chemical fertilizers and to produce seedlings with good vigor which would translate into high survival and growth at the field.

Therefore, the objective of this study was to evaluate the potential of native AMF inoculums isolated from highland and lowland areas to improve the growth and survival of seedlings of the Green Legacy Program of Ethiopia.

1.2. Statement of the problem

In Ethiopia forests were highly declined due to high population pressure resulting in cutting trees for growing food crops, shelter and road construction. In order to combat climate change, the Ethiopian government has started to mobilize the population with the goal of planting 20 billion trees during a four-year period. Despite the recent effort of the government forest seedling plantation across the country, the survival rate of these seedlings is very low 40-50% according to the reports of Adama Woreda Agriculture and Natural Resources Office (2020 and 2021). The low level of seedling survival rate could not only be a result of low nutrient, but also due to a shortage of the water availability in the soil (Eba, 2017; Fisseha *et al*., 2019).

A lot of works have been conducted regarding to the abundance and diversity of the AMF in different land use systems in Ethiopia (Zerihun *et al*., 2013, 2020). In addition, several other studies have been done in related to the identification and characterization of symbiotically effective indigenous rhizobia strains isolated from *faba bean*, field pea, chickpea and lentil and etc. (Adamu *et al*., 2001; Argaw, 2013; Belay & Assefa, 2011; Girma *et al*., 2019; Mulissa *et al.*, 2016). Attempts were made in mass-production and distributing selected strains of rhizobia to farmers in various parts of the country (Alemayehu, 2020). Moreover, the most research activities on the production of bio-

fertilizer have also been focusing mainly on symbiotic nitrogen fixation of major legume crops (Mulissa *et al*., 2016).

They easily accelerate plant development and increase the tolerance to transplant stress and to restoration of nutrient cycling. However, the potential, benefits, and the large-scale use of AMF in Ethiopia was nil, mainly due to the lack of availability of inoculant in high quantities, low cost and high quality.

To overcome these problems the selection and inoculation of mycorrhizal fungi with beneficial functions could be contributed to the establishment of seedlings and the recovery of degraded or disturbed areas. AMF inoculation could significantly improve tree seedlings nutrients and water relation to potentially improve their field survival, establishment and growth thereby improving forest restoration success (Asmelash *et al*., 2016).

1.3. Objectives of the Study

1.3.1. General Objective

- To evaluate the potential of indigenous AMF inoculums isolated from highland and lowland areas to improve the growth and survival of the seedlings of the Green Legacy Program of Ethiopia

1.3.2. Specific Objectives

- To compare the potential of the native AMF inoculums isolated from low land and high land areas.
- To evaluate effects of native AMF inoculums on the growth of the seedling plants GLP of Ethiopia.
- To assess the AMF percent of root colonization, spore abundance and diversity of seedling plants the GLP of Ethiopia.
- To evaluate the physcio-chemical analysis of soil samples
- To compare the plant growth parameters of inoculated and non-inoculated

1.4. Research question

This research will address the following research questions.

i. Are the native AMF inoculums isolated from low land and high land areas are effective?
ii. What is the effect of native AMF inoculums on the growth of the seedling plants GLP of Ethiopia?
iii. How effective was the AMF percent of root colonization, spore abundance and diversity of seedling plants the GLP of Ethiopia?
iv. What are the physcio-chemical properties of the soil samples?
v. What is the difference between the plant growth parameters of inoculated and non-inoculated?

1.5. Scope of the study

This study was concentrated on collecting the sample of soil from highland and lowland areas then developed AMF inoculums in greenhouse pots using sorghum crops as a trap for production of AMF inoculants and then evaluate the prepared inoculums of AMF inoculants by using the soil and root that AMF colonized as AMF inoculants. The mycorrhizal soil and roots were used immediately as inoculum source for the next experiment on selected seedlings used for Green Legacy Program (GLP) of Ethiopia. Finally, there would be assumptions on the potential of the native of AMF by evaluating plant growth parameters (plan height, plant leaves number, dry mass, fresh mass and root colonization) with controls without inoculums seedling used in GLP of Ethiopia.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Forest

Forests are important and grow in many places around the world; an ecosystem includes many plants and animals. Many animals live in forests and need them to survive. The development of forest policy in Ethiopia is strongly intertwined with the evolution and vicissitude of its state structure. Although some accounts claim the beginning of modern Ethiopian state as early as the second-half of the 19th century, it is generally acknowledged that an organized and elaborated state structure only emerged after the Second World War Bahiru, 1991; Teshale, 1995). Parallel to changes in policy and politics, the principal economic policy also shifted from a kind of 'laissez-faire', to a command economy, and to a free-market (Keller, 2002; Vaughan, 2003; Dessalegn, 1994, 2004). Those fluxes have had significant implication for the development of forest policy.

Whereas, Ethiopia's forest which formerly covered most of the country has been depleted by the defunct feudo-bourgeois for selfish interest of the aristocracy and the nobility; Whereas, immediate and decisive action must be taken in order to avert this disasters situation by agitating and coordinating the broad masses to plant, conserve, develop and administer the country's forest and wildlife resources. Ethiopia has been carrying out national tree seedling planting campaigns since its millennium and planted billion of trees. The Government of Ethiopia envisions covering 22 million hectares of degraded lands with forest by 2030 by significantly enhancing the contribution of forestry to agriculture, water and energy (Kassa, 2018; Mekonnen , 2019).

2.1.1. *Azodichta indica* (Neem)

Neem is a member of the Mahogany family. Order *Rutales*, Suborder *Rutinae*, Family *Meliaceae*, Subfamily *Melioideae*, Tribe *Melieae*, Genus *Azadirachta*, Species *indica*. Neem is an omnipotent tree and a sacred gift of nature. Neem tree is mainly cultivated in the Indian subcontinent. Today it is known by the botanical name *Azadirachta indica* (Figure: 1*)*. Neem has been used extensively by humankind to treat various ailments before the availability of written records which recorded the beginning of history. Since prehistoric times, Neem has been used by humankind (Kumar & Navaratnam, 2013).

Azadirachta indica (Neem) is growing in most of the tropical and subtropical countries. The tree has adaptability to a wide range of climatic, topographic and edaphic factors. It thrives well in dry, stony shallow soils and even on soils having hard calcareous or clay pan, at a shallow depth. Though not well known by name among the society, Neem is one of the common trees that grow in various parts of Ethiopia. Benishangul-Gumuz State is one of the regions where Neem grows widely. A study conducted by the states investment bureau indicates that while Ethiopia has the potential for Neem tree production, Neem oil is not currently produced either due to lack of proper information on its uses or available technologies. However, every part of the Neem tree roots, leaves, flowers, seeds, trunks and branches has multiple uses (Ahmed *et al*., 1989).

The plant debris is potential source of organic manure and leaves could be used as a source for the preparation of compost having both fertilizer and pesticide a potential (Gajalakshmi & Abbasi, 2004). Anti-feedant properties found in Neem compounds helps to protect the plants when applied on them and also it is being used to manufacture bio-insecticide that is environmental friendly and do not have any side effects on plants and soil (Adhikari *et al.*, 2020).

The plant is distributed widely in the world providing a source of inspiration for novel drug compounds, as plant derived medicines which have made large contributions to human health and well-being. Presently it can be seen growing successfully in about 72 countries worldwide, in Asia, Africa, Australia, North, Central and South America. The tree was introduced into West Africa at the beginning of this century (Ahmed *et al*., 1989).

Figure 1: *Azodichta indica* (Neem) tree seedlings (Taken from Global Academy, Adama)

2.1.2. *Sesbania grandflora* (Vegetable hummingbird, katurai, agati, or West Indian pea)

Sesbania (Figure: 2) consists of about 50 species of fast growing trees, perennial shrubs and herbaceous annuals. Some thirty-three species are found in Africa, 20 of which in eastern Africa. Tanzania, where 15 of these are found, is considered a major center of diversity of these species (Lugenja, 1988; Otieno, 1987). However, Sesbania species have not been fully exploited as multi-purpose plants in Ethiopia. Sesbania species are among the useful multipurpose trees that could have an increasing role as a home-grown feed supplement (Tessema, 1988). Due to their ability to grow in a wide range of soils, Sesbania species are increasingly expanding their range of adaptability and utility compared to many other legumes (Evans & Rotar, 1987).

One of the most serious constraints to the sustainability of agriculture in sub-Saharan Africa is soil nutrient depletion (Bindraban *et al*., 2012). Many factors cause the decline of soil fertility and carbon stocks of African dry lands including deforestation, overgrazing, unsustainable agriculture, and climate change (Bakhoum *et al*., 2018). Planting nitrogen fixing trees such as *S. grandflora* is an effective solution for increasing soil productivity (Bakhoum *et al*., 2018). The International Center for Agroforestry Research (ICRAF) is interested in the role of *S. grandflora* in improved fallows especially in savannah woodland region (Anon, 1992; Kwesiga *et al*., 1999; Ndungu & Boland, 1994).

Sesbania Adanson (*Papilionoideae*, *Leguminosae*) is a genus comprising about 85 trees, shrubs, and perennial or annual herbs that are extensively dispersed in tropical and subtropical regions of the world. About 30 species are found in Africa and Madagascar, 16 in Asia, Australia, and Pacific islands, and nine in the Neotropics (southern USA to northern Argentina, notably North and Central America). Approximately five species have a pantropical range (Evans, 1990).

Sesbania species are frequently utilized and cultivated in agroforestry as green manures and other products for soil improvement (Evans, 1990). Shade plants, windbreaks, cover crops, ornamentals, fish poisons (e.g. source of isoflavones), and fiber supplies, construction materials, and food for both humans and cattle are all examples of *Sesbania's* Extensive economic value, particularly in Africa and Asia (Hoffmann & Moran, 1991).

Several species have been proven to be highly successful at removing lead, zinc and copper from industrial waste sites and other contaminated soils (Branzini *et al.*, 2012).

These plants (*leguminosae*) also symbiotically fix atmospheric nitrogen with soil bacteria from the genera *Rhizobium*, *Azorhizobium*, *Bradyrhizobium*, and *Synorhizobium* (Mahamat *et al*., 2021).

Soil nutrient depletion is one of the most important restrictions to agriculture's sustainability in Sub-Saharan Africa (Bindraban *et al*., 2012). Deforestation, overgrazing, unsustainable agriculture, and climate change are all factors that contribute to the reduction of soil fertility and carbon reserves in African dry lands (Rao *et al*., 1997). Mineral fertilizers are one of the options; nevertheless, they are expensive and unsustainable. Scientists have tested low-cost agroforestry solutions for soil fertility replenishment to solve these issues (Bindraban *et al*., 2012).

Leguminous species from arid areas, in general, play an important role in reforestation projects and the fight against desertification (Mahamat *et al.*, 2021). Furthermore, incorporating legume trees into cropping systems improves yield stability and reduces land degradation (Sileshi *et al*., 2012).

Figure 2: *Sesbania grandflora* trees seedlings (Taken from Google Sesbania picture)

2.1.3. Cassia fistula (golden shower, purging cassia, Indian laburnum)

C. fistula (Figure: 3) native to the tropical regions of Asia is widely distributed across Pakistan, India, Indochina, and Malaysia. Due to use of *C. fistula* as an ornamental and shade tree around houses, on the edges of roads and in the streets, parks, and gardens, now it has also been introduced to the tropical regions of Africa and America (Hanif *et al*., 2010).

Presently *C. fistula* is gaining popularity all over the world, due to its ornamental, medicinal and water purifying attributes (Hanif *et al*., 2010). Previously *C. fistula* was not considered as an important food commodity. But now a report on the use of *C. fistula* meal as a replacement for soybean meal in practical diets of Oreochromis niloticus fingerlings represents that beyond its medicinal potential. *C. fistula* can also be used as a food commodity (Kwesiga *et al*., 1999). However, there is a number of article describing the medicinal potential of *C. fistula*. Antitumor (Systems, 2006).

Figure 3: *Cassia fistula* tree (Taken from Google)

Ornamental plants are grown for their aesthetic look, with no particular concern for their utility. This does not preclude any particular type of plant being grown for both of these attributes. Thus *C. fistula* was found as suitable candidate for planting it along roadside, lane, streets, schools, institutions, office complexes, residential areas and parking spaces for controlling, air and noise pollution in addition to its ornamental and medicinal use. The sap of *C. fistula* leaves have a purgative action on the digestive organs of grazing animals

like goats, cows and buffalo, and thus it is also well suited for planting on wastelands (Asif *et al*., 2007).

2.1.4. *Delonix regia* (Royal Poinciana)

Delonix regia (Royal Poinciana) (Figure: 4) is a genus of flowering plants in the family *Fabaceae*, sub family *Caesalpiniodeae*. It contains trees that are native to Madagascar and East Africa. (The Legume Phylogeny Working Group (Azani *et al*., 2017). Large ornamental tree with fern-like bipinnately compound leaves and attractive red peacock flowers and native to Madagascar (Sedrez dos Reis *et al.*, 2014). This tree some say is the world's most colorful tree (Raf *et al*., 1994), the individual flowers are striking: they have four spoon shaped spreading scarlet or orange-red petals about 7.6 cm long, and one upright slightly larger petal (the standard) which is marked with yellow and white. According to (Azani *et al*., 2017), this tree is deciduous in climates that have a marked dry season, but in other areas, it is known as a semi-evergreen tree.

Very rare in the wild of its native Madagascar, this deciduous tree is grown throughout the lowland tropics prefers sandy soil. Widely planted in the 'Bereha', 'dry and Moist' Kolla' agro climatic zones, especially at Dire Dawa Town. The flowers, leaves and barks contain most of the active constituents. The flowers possess insecticidal (Narayanasamy, 2001). The free radical scavenging activity of various sections of *D. regia* was investigated using various in vitro methods, as well as the identification of powerful molecules or bioactive principles (Salem MZM, 2013).

Figure 4: *Delonix regia* (Royal Poinciana) trees A- leaves parts and B flower parts (Taken from ASTU compound)

2.2. General overview on Arbuscular mycorrhizal Fungi

The word mycorrhiza is given the mutualistic association between fungus (myco) and roots (rhiza) of the plants. This association is a symbiotic because the relationship is advantageous for both organisms. This was given by Frank in 1885 (Trappe, 2005), mycorrhizal fungi appear to have coevolved with plants for over 400 million years to become part of the root system as evidenced by fossil mycorrhiza found in carbonaceous deposits. These fungi in soil are ubiquitous throughout the world and form symbiotic relationships with the roots of most terrestrial plants. In natural ecosystems, it is exceptional for a plant not to possess a mycorrhizal root system. Therefore, it could be said

that mycorrhizal association is very common or almost universal phenomenon in plant kingdom (Bagyaraj, 2014). These are macro-symbiotic and micro-symbiotic. The macro-symbiont gained increased exploration of soil with a net of hyphae that increase the uptake of water and nutrient from the soil interphase. The micro-symbiont uses the carbon provides by plants for its physiological functions, growth and development (Habte, 2000).

Mycorrhizae form a network of filaments that associate with plant roots and draw nutrients from the soil that the root system would not be able to access otherwise. This fungus-plant alliance stimulates plant growth and accelerates root development. It also makes the plant less susceptible to soil-borne pathogens and to other environmental stresses such as drought and salinity (Goetten *et al*., 2016). In return, the plant provides carbohydrates and other nutrients to the fungi. They utilize these carbohydrates for their growth and to synthesize and excrete molecules like glomalin (glycoprotein). The release of glomalin in the soil environment results in better soil structure and higher organic matter content (Rillig & Mummey, 2006). However, in soil that has been disturbed by human activity, the quantity of mycorrhizae decreases drastically so that there are not enough of them to produce a significant benefit on plant growth and soil health (Soka & Ritchie, 2014). Tree-based multilayer intercropping (agroforestry) systems are sometimes shown to support a more abundant and diverse AMF community than conventionally managed systems (Asmelash *et al*., 2021).

AMFs promote a significant increase in the area of root absorption of plants colonized, maximizing the use of water and nutrients (dos Santos *et al*., 2017). These fungi enhance plant resistance to water stress, high temperatures, conditions of toxicity and acidity of soil and pathogens, in addition to soil stabilization in the form of aggregates (Peng *et al*., 2013). However, wide variations have been seen among undisturbed and disturbed sites in the occurrence of AMF. For example, Ortega *et al*., (2004) reported that mycorrhizal association has been estimated to occur in 95% of native vegetation from undisturbed sites, whereas it occurs in vegetation from disturbed sites in less than 1%. It was also confirmed that fine non-woody roots (<2cm), which are responsible for most mineral and water absorption, are located in the upper 20 to 30 cm of soil (Marx *et al*., 2002), which signifies the importance of edaphic and phenologic factors in the establishment of AMF. Therefore, the lack of mycorrhizal fungi on root systems is a leading cause of poor plant establishment and growth in a variety of disturbed forest, restoration, agricultural, suburban and urban landscapes. At afforestation sites devoid of mycorrhizal propagules (e.g., cutover lands,

treeless areas, mining spoils), non mycorrhizal seedlings never develop fully and often die (Ortega *et al*., 2004). Thus, the selection and inoculation of mycorrhizal fungi with beneficial functions can contribute to the establishment of seedlings and the recovery of degraded or disturbed areas, since they easily accelerate plant development and increase the tolerance to transplant stress and to the restoration of nutrient cycling (dos Santos *et al*., 2017).

2.2.1. Different Types of Mycorrhiza

Currently, mycorrhiza is defined as a symbiotic relationship between most plant species and intimate fungi of mostly, the root and rarely, the subterranean stems and thallus of bryophytes (Brundrett *et al*., 2004). Mycorrhiza facilities the bidirectional movement of nutrients whereby carbon and inorganic nutrients respectively, are supplied to the fungi and the host plant (Asmelash *et al*., 2016). However, about 500 plant species are "myco-heterotrophic" that obtain carbon from the fungal hyphal networks in the soil *(*Gomes *et al*., 2019).

There are three main types of mycorrhizas namely; endomycorrhiza, ectomycorrhiza, and ectendomycorrhiza (Asmelash et al., 2016). These are further divided into seven, (Table: 1) ectomycorrhiza, monotropoid mycorrhiza (a kind of ectomyccorrhiza), arbutoid mycorrhiza (a kind of ectomyccorhiza), ericoid mycorrhiza (a kind of endomycorrhiza), orchid mycorrhiza (a kind of endomycorrhiza), arbuscular mycorrhiza (a kind of endomycorrhiza) and ectendomycorrhiza is a kind of mycorrhiza that has both the characteristics of ectomycorriza and endomycorriza (Finlay, 2008).

Table 1: Summary of main characters of mycorrhizal association (Taken from Fitter and Moversoen, 1996, updated by Smith and Read, 2008)

	Ecto mycorrhizas	Arbuscular mycorrhizas	Ericoid mycorrhizas	orchid
Taxa Fungi involved	Basidiomycota, Ascomycota	glomeromycota	ascomycota	Basidiomycota
Plant involved	Perennial, typically woody	Taxonomically diverse, many species (2×10^5)	Ericales	Orchidales
Morphology Diagnostic features	Hartig net	Arbuscules	Intracellular hyphal, complexes in epidermis	Hyphal coils (pelotons) in cortical cells
Other structures	Sheath	Vesicles, coils		
Functionality P & N acquisition	Important	Important	Important	Dominant
Water relations	May increase flux by mycelia transport	May increase flux by binding soil to root	Unknown	Unknown
Pathogen protection	?Important unknown	?Important	Unknown	Unknown
others	Protection from ionic toxins	Micronutrients	Protection from ionic toxins	Carbon supply to plant

Arbuscular mycorrhiza (AM) and Ectomycorrhizal (ECM) are the most important types of mycorrhiza. AM is formed in more than 80% of the terrestrial plants, while ECM is formed in only 3% of plant species which, however, are most of the very important forest tree species of the temperate and boreal regions (Fisseha *et al*., 2019). ECM fungi develop sheath or mantle around the feeder roots, the mycelium penetrates between the cells (intercellularly) of the root and forms the Hartig net but do not form intracellular

penetrations. The fungi involved are mostly Basidiomycota and Ascomycota. Arbuscular mycorrizal fungi (AMF), on the other hand, colonize the root cortex both intercellularly and intracellularly and with no sheath formation (Editor, 2013). Inside cortical cells, special tree-like symbiotic structures called the arbuscules, peculiar features of AMF, are formed (Figure: 5). Formerly, AM fungi used to be called Vesicular-Arbuscular Mycorrhiza fungi due to the presence of a storage body called vesicles.

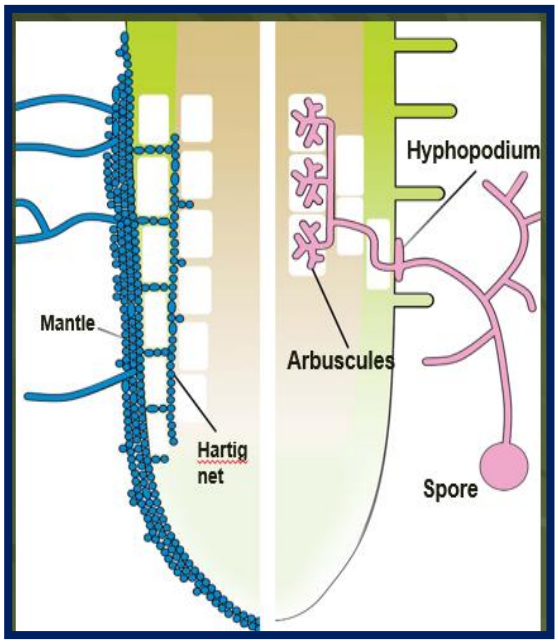


Figure 5: Left side ECM and right side AMF (Adopted from (Bonfante & Genre, 2010)).

Ectomycorrhizal fungus surrounds the root tip with a thick mantle of closely apprised hyphae, whereas the Hartig net develops around epidermal cells (green). In the case of arbuscular mycorrhizas, the root tip is usually not colonized. Hyphae develop from a spore and produce a hyphopodium on the root epidermis. Intraradical colonization proceeds both intra and intercellularly and culminates with the formation of arbuscules, little fungal trees, inside inner cortical cells (brown).

The germination of a resting spore is followed by the production of a short explorative mycelium. The perception of plant exudates, released by the host root, induces recursive hyphal branching, increasing the probability of a direct contact between the symbionts. Signal transduction leads to the activation of cellular and transcriptional responses (green cells and nuclei). The contact between the plant and fungus is followed by the adhesion of a hyphopodium to the root surface. This triggers the assembly of a broad aggregation of cytoplasm named the prepenetration apparatus (PPA) in the contacted epidermal cell and underlying outer cortical cell. Subsequent intracellular fungal colonization strictly follows

the route of PPAs from the epidermis to the inner cortex. Here, intercellular hyphae can develop along the root axis. Eventually, a highly branched arbuscule occupies most of the cell volume, forming an extensive surface for nutrient exchange.

Hyphae Within root (intraradical) and outside root (extraradical) Arbuscules Highly branched, thin-walled structures within host cell formed in soil or roots; asexual, variable in size and color between taxa, may contain hundreds of nuclei. Formed singly or in spore balls Vesicles Ovoid, ellipsoid to irregular in shape, thin-walled, formed from hyphae in root cortex. Auxiliary Cells, Clusters of thin-walled cells formed on hyphae outside the root only in suborder Gigasporinae (Lin *et al*., 2008).

2.2.2. Features of AM Fungi

The fungal accommodation process Although the regulatory mechanisms leading to fungal branches filling most of the host cell lumen are poorly understood, the process of arbuscule accommodation, which requires a substantial remodeling of the cortical cell, has been long described (Parniske, 2008). Similar to any intracellular hypha, all the thin arbuscule branches are enveloped by the perifungal (now periarbuscular) membrane. This invagination of the host plasma lemma does not simply surround the arbuscule as a whole, but closely follows the surface of each branch, molded to the arbuscule itself (Fig.2), The arbusculated cell is where the interface acquires its maximum spatial complexity, with a composition very similar to that of plant primary cell wall (Nanjundappa *et al*., 2019). The assembly of this extensive amount of membrane and cell wall material requires the recursive involvement of PPA-like structures, organized both in advance of fungal penetration through the cell wall and along the ‘ trunk ’ hypha, as smaller aggregates anticipating the formation of fungal branches (Wang *et al*., 2017).

Perception of the host plant by the pre-symbiotic mycelium is mediated by root exudates. The responsible compounds that are released by the root and diffuse a short distance, before being washed away or degraded, have been identified as strigolactones (Besserer *et al*., 2006). They stimulate AM fungal metabolism and branching, a change in mycelia growth pattern that is thought to increase the chances of an encounter with the host. Strigolactones also have a hormonal role in inhibiting lateral branching of shoots (Gomez-Roldan *et al*., 2008). This direct effect on plant development is considered to be the main function for this class of molecules, which are in fact produced by many plant taxa (H. Akiyama et al., 2010), including those not associated with mycorrhizal fungi. The leakage

of strigolactones from roots into the soil and their rapid hydrolysis have in any case made them ideal for signaling root proximity in the rhizosphere (Akiyama *et al*., 2010). These multiple functions recall the pleiotropic effects of auxins, and promote consideration of strigolactones as hormones (Figure: 6).

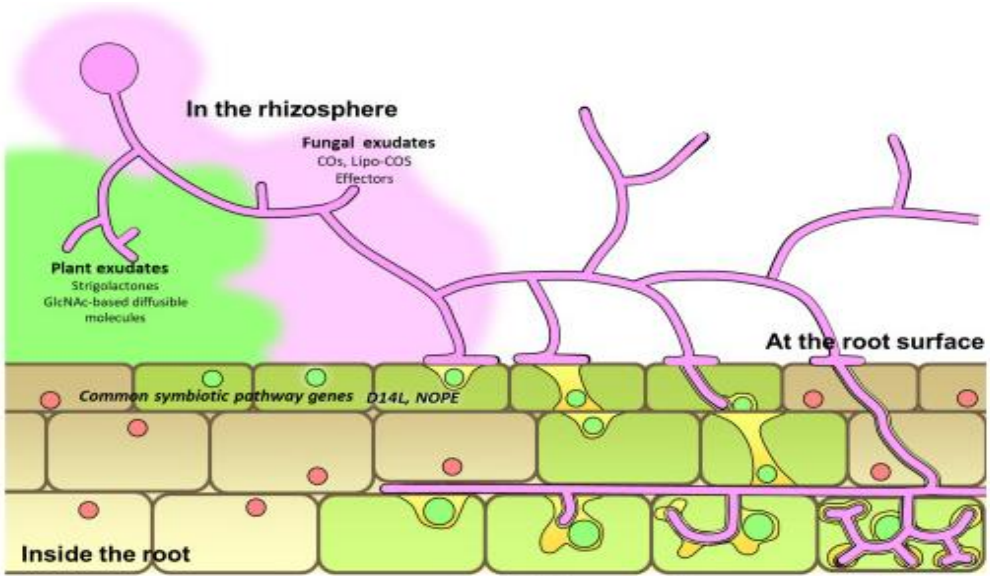


Figure 6: AM colonization (Adopted from Keymer et al., 2017)

2.2.3. Unique characteristics of AMF

AMF are one of the most abundant organisms in the rhizosphere and the relationships can be found within a broad range of more than 200,000 species of host plants. Despite their abundance and wide range of relationship with plant species, AMF have been known to show low species diversity and only approximately 341 species have been described within a fungal phylum, Glomeromycota, on the basis of morphology until Nov.2021 (http://amf-phylogeny.com).

AMF, is obligate biotrophs, unculturable in the absence of a host strictly depend on their green hosts for growth and reproduction and their large asexual spores contain thousands of nuclei, making classical genetic approaches unsuitable. Due to non-septate nature of the hyphae AM fungi are considered to be asexual, although the hyphae of genetically distinct strains can join and exchange genetic material. However, molecular studies have suggested that diversity of these fungi may be much greater. In addition, high genetic variation of AMF has been reported within species, even within a single AMF spore (Sanders, 2002).

2.2.4. Development of Arbuscular Mycorrhizal (AM) Association

In the absence of host plants, the life cycle of the AMF cannot be completed and unlike certain classes of fungi, they do not reproduce sexually. Their life cycle begins with an asexual spore being germinated and forming hyphae with a limited life span in the soil; and it begins with an asymbiotic process in the response to physical influences, such as humidity, temperature and pH (Giovannini *et al*., 2020). But the spores respond with an increase in hyphal branching and metabolic activity to root exudates. This constitutes the Pre-symbiotic phase and both AMF species and host plants start to contact and exchange chemical and molecular signals (Lies *et al*., 2018). Its success is very dependent on two conditions: soil properties (e.g., pH, moisture, and temperature); and the host plant (e.g., root exudates, like flavonoids, CO_2, and unknown ramification factors) (Souza, 2015). Plant roots release for example strigolactones, which results in the branching of the hyphae. In response, the branched hyphae secrete a diffusible signal to the host roots, which initiates the expression of symbiotic-related genes (Nayak, 2008).

A complex process and is characterized by distinct developmental stages in the growth of the AM fungus (figure: 7). Spore germination, hyphal differentiation, appressorium formation, root penetration, intercellular growth, intracellular arbuscule formation and nutrient exchange (Akiyama K. *et al*., 2005).

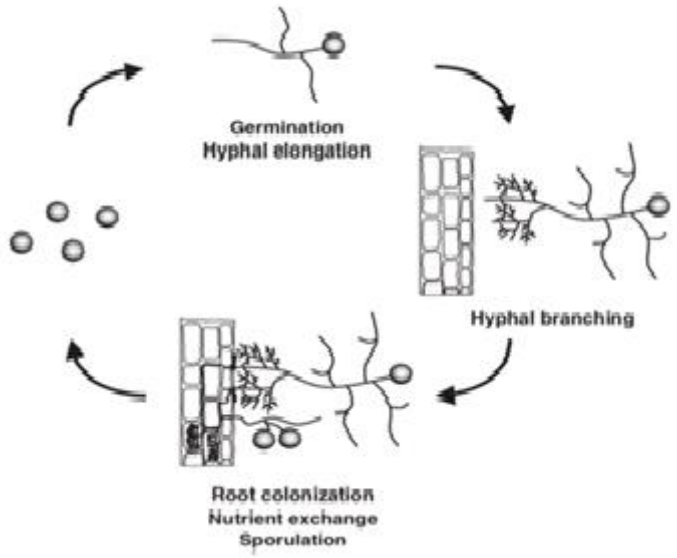


Figure 7: shows the life cycle of an AM fungus and the different steps during AM (adopted from (Akiyama et al., 2005).

2.2.5. Formation of AM symbiosis

Mycorrhizae are symbiotic relationships that form between fungi and plants. The association between plants and AMF is one of the most important symbioses on earth, linking the root and the soil system, and represents an ancient symbiosis (Smith & Read, 2008). Hyphae and arbuscules have been reported in fossils of Aglaophyton isolated from the Rhynie chert, and this evidence has established the existence of AM symbiosis in the early Devonian (Giovannini *et al*., 2020). Furthermore, molecular works based on the nucleotide sequence divergence of 18s rDNA suggests that the Glomales arose 350–460 million years ago and that the symbiosis was instrumental in the successful colonization of land by plants. In early October 2019, the taxonomy of AMF from the total number of described AMF species was 318. (www.amf-phylogeny.com) (Schussler & Walker, 2010). There are 34 genera and 12 families among these species (Zerihun *et al.,* 2020). Some of the important genera are Glomus, Acaulospora, Gigasporinae, Scutellospora, Enterophospora and Sclerocystis (Aggarwal *et al*., 2011).

The symbiosis between AMF and autotrophic plants is regarded as mutualistic. With the exception of a few achlorophyllous species, AM host plants are autotrophic and, although normally colonized by mycorrhizal fungi in the field, they are usually capable of satisfactory growth in the absence of mycorrhizal colonization, provided that mineral nutrient supplies are adequate (Smith & Read, 2008). In contrast, the Glomeromycota fungi are obligate symbionts, as their extraradical hyphae are unable to take up carbohydrates. Thus, they depend on photosynthates supplied by the plant and utilize a considerable proportion of its assimilated carbon probably 20 % of net photosynthates (Rybczyński *et al*., 2014). In return, the fungi improve the supply of water and nutrients, such as phosphorus (P), and nitrogen (N) to the host plant through extraradical and intraradical hyphae, arbuscules, and the root apoplast interface. And AMF benefit the plants by alleviate biotic and abiotic stress from their host by inhibiting the growth of pathogenic organisms and increasing plant resistance to drought and other unfavorable conditions such as tolerance to drought, high soil temperatures, toxic heavy metals, extremes in pH and transplant shock (Millar & Bennett, 2016). Fungus recognition by the host plant are mediated by a partially characterized signalling pathway partly shared with *Rhizobium* legume symbiosis (figure: 8).

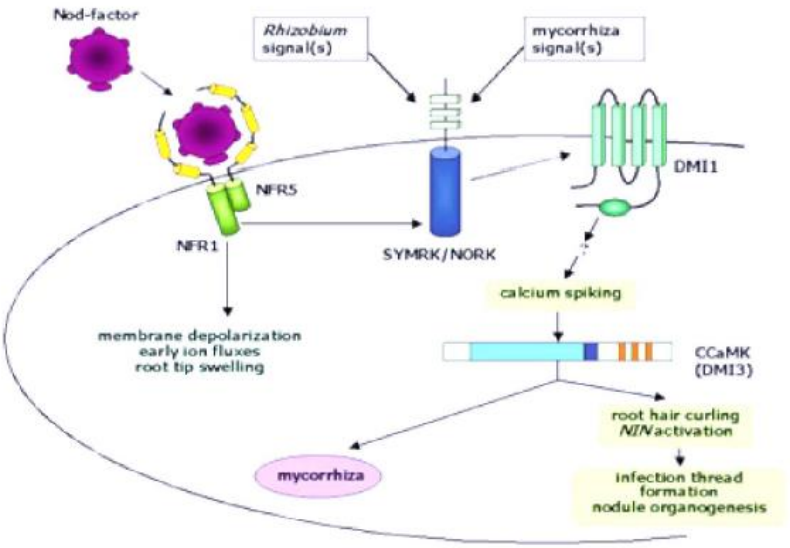


Figure 8: Model of signaling pathways (Adopted from Hause and Thomas 2005).

2.2.6. AMF Taxonomy and classification

Nearly 300 species of the phylum Glomeromycota (Schussler & Walker, 2010) comprising arbuscular mycorrhizal fungi (AMF), only 57 were originally described to form spores at the tip of sporogenous hyphae in epigeous or hypogeous sporocarps, i.e., fruit bodies, with or without a peridium and with a gleba. Currently there are about 318 in early October 2019 described AMF species (*Glomeromycota*species list at (www.amfphylogeny.com).Consensus classification of the Glomeromycota (Redecker *et al.*, 2013). *Glomeromycota,* Class-*Glomeromycetes,* Four orders (*Archaeosporales, Diversisporales, Glomerales* and *Paraglomerales),* 11 families and 24 genera (Picture: 9). Unfortunately, the molecular phylogenetic position remained unknown for many other sporocarpic species within the Glomeromycota, which Schussler & Walker, (2010) retained in their original genera, as species of uncertain position.

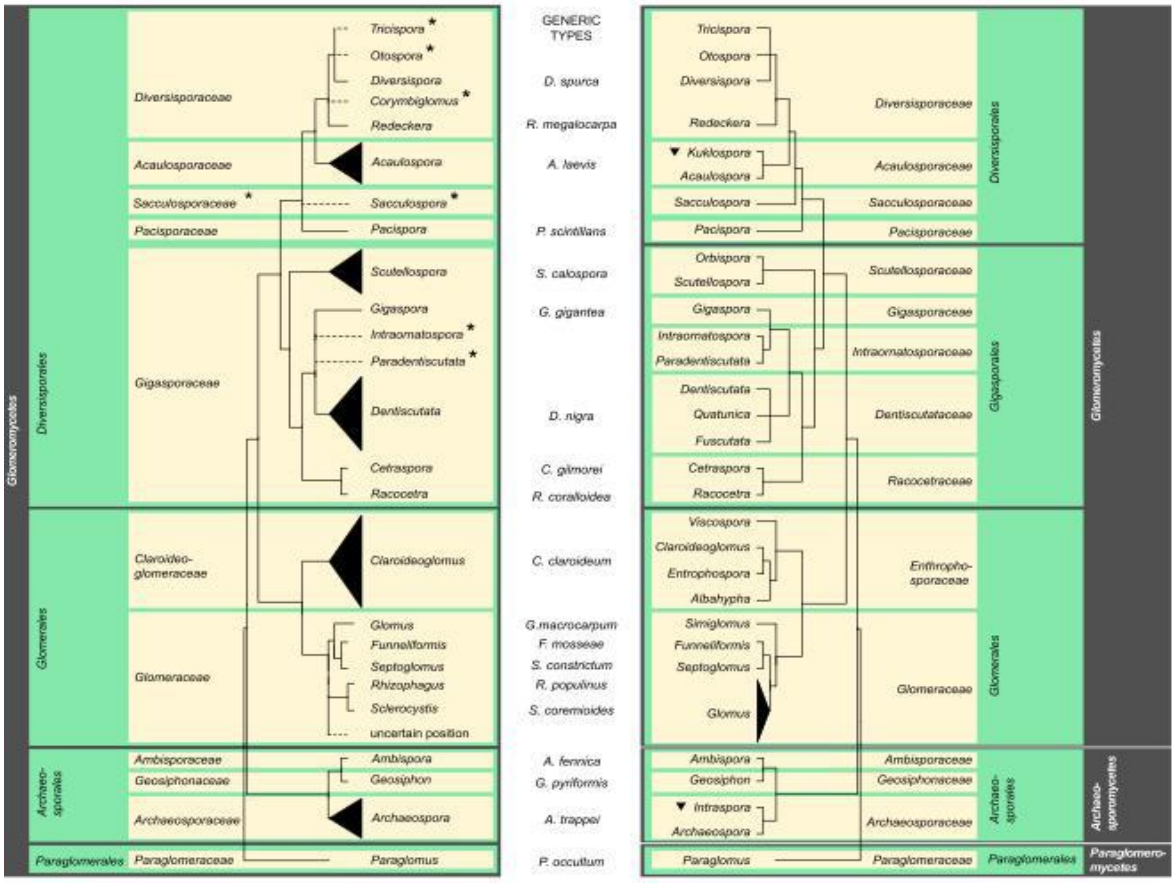


Figure 9: Consensus classification of the Glomeromycota (left panel) in comparison to the system summarized by Palenzuela *et al.*, (2013) (right panel).

2.2.7. AMF and ecosystem functioning

AMF are communities exhibit a great deal of practical diversity among species, which is contributed to scheme productivity (Argaw, 2013; De Vries & Bardgett, 2015; Powell & Rillig, 2018). Completely different AMF isolates may possess distinct functional traits; some are higher at protective their hosts from pathogens and others are better at up phosphorus uptake. The mixture of those AMF isolates in an exceedingly a single community may lead to larger improvement of plant productivity (Maherali & Klironomos, 2007). Magnified AMF species richness may scale back competitive difference among plant species, maintaining a constant level of plant productivity even below unsteady soil conditions (Powell & Rillig, 2018). Thus, scheme productivity and stability may increase with increasing AMF species richness. The useful effects of skyrocketing diversity of AMF on individual or community-wide plant productivity will doubtless be explained by practical complementarily of the fungi (Maherali & Klironomos, 2007).

The functional complementarity hypothesis was subsequently proposed to explain mycorrhizal diversity effects on individual plant and community productivity (Koide & Kabir, 2000), and direct evidence of such was obtained in an experiment with wild leek (*Allium ampeloprasum L.*) in which the plant acquired more P with two AMF species than with either species alone (Schnepf *et al.*, 2016). While distinct taxa of AMF may clearly be functionally complementary, the relationship between AMF taxonomic diversity and functional complementarity is likely to be dependent on the level of taxonomic resolution (Maherali & Klironomos, 2007). Increasing the number of isolates of a particular species, or the number of species of a given genus, may have some impact on function. However, more functional variation may occur by increasing the number of genera or families (Van Der Heijden *et al.*, 2004). Functional trait conservatism among AMF isolates at the family level may thus be more likely to reduce competition, promote coexistence and enhance ecosystem functioning (Maherali & Klironomos, 2007; Powell & Rillig, 2018).

2.3. Role of Arbuscular Mycorrhizal Fungi

Climate change and food security have now become major problems mainly in developing countries. Therefore, there is need to fulfill demand of growing population of developing countries by increasing food production through high-input agricultural practices and at the same time by minimizing negative environmental impact (Singh *et al.*, 2021). Different research studies conducted on AMF during the past two decades have highlighted their countless benefits on soil health and crop productivity. And it is widely believed that AMF could be considered as a replacement or lower down the use of chemical fertilizers up to 50% for best agricultural production in the near future, because mycorrhizal application can effectively reduce the quantitative use of chemical fertilizer input especially of phosphorus (Begum et al., 2019).

Arbuscular mycorrhizal fungi, often referred to as agroecosystem engineers, represent a key functional group of soil microbiota that are fundamental for soil fertility, crop productivity, yield quality and ecosystem resilience. They form a critical symbiotic relationship with most agricultural crops improving the nutritional status of their hosts, besides protecting them against several soil-borne plant pathogens and environmental stresses. In addition, they improve soil structure and quality mainly through the external hyphal network which creates a skeletal structure that enmeshes the soil particles, and by

production of glomalin related soil protein (GRSP) which binds soil particles together (Njeru, 2018).

2.3.1. Increase Soil Fertility (Nutrient Uptake)

Thousands of trials have proven that AMF can help plants overcome nutritional limitations by improving nutrient acquisition (Aggarwal *et al*., 2011). Experiments show that AMF absorb N, P, K, Ca, S, Cu, and Zn from the soil and transport them to nearby plant (Smith & Read, 2008). However, the most noticeable and consistent nutritional impact of AM fungus is enhanced immobile nutrient absorption, particularly P, Cu, and Zn (Nogueira & Cardoso, 2006). The rate of diffusion has a big role in the provision of immobile nutrients to roots. In soils with insufficient nutrients, plant uptake of nutrients considerably outpaces the rate at which nutrients seep into the root zone, resulting in a nutrient-depleted zone around the roots. Mycorrhizal fungi help to solve this problem by extending their exterior hyphae outside the depletion zone, allowing them to explore a larger region of the soil than the unaided root can (Habte, 2000).

The fungal mycelium scavenges for mineral nutrients as it spreads through the soil, and it is able to make contact with uninfected roots, sometimes of different host species. Extra-radical mycelium is smaller than roots, allowing penetration through crystalline minerals, aggregates, and organic substances with fewer pores than a root alone could (Aggarwal *et al*., 2011). Furthermore, AMF contributes significantly to plant growth and development by acquiring phosphorus (Smith & Read, 2008). Phosphorus is a necessary nutrient, but it can be scarce; a large portion of soil P is in organic forms, and some inorganic P is insoluble. Plants are unable to absorb both organic and insoluble inorganic phosphorus. Only a few plants can produce organic acids and phosphatases on their own to hydrolyze and release P. (Hakim *et al*., 2021). Increased soil exploration; increased phosphorus translocation into plants via arbuscules; modification of root environment; efficient utilization of P within plants; efficient transfer of P to plant roots; and increased storage of absorbed P are among the various mechanisms proposed to account for increased P uptake (Aggarwal *et al*., 2011). AM fungus may be able to increase the availability of accessible P and other immobile nutrients through biochemical means. Organic P sources can be mineralized by AMF phosphatases (Calabrese et al., 2019; Wipf et al., 2019). Because it is found within the fungal vacuoles where polyphosphate granules were found, alkaline phosphatase activity is linked to fungus phosphate metabolism. Enzymatic processes break

down polyphosphate granules in fine branches of arbuscules, releasing inorganic phosphorus into the cytoplasm (Judith Prieto Méndez, César A. González Ramírez, 2009). Although AMFs may not have as much of an impact on plant N nutrition as they do on P, they do provide their hosts with access to various types of N, improving plant N intake (Judith Prieto Méndez, César A. González Ramírez, 2009). An AMF's ability to breakdown organic matter and obtain N from an organic source has been established. They also discovered that AMF boosted the rate of N diffusion into its host. As a result, compared to non-mycorrhizal plants, mycorrhizal plants have more access to N sources. The most fascinating aspect of their findings is the possibility that AMF is saprophytic, especially when breakdown is required for nutrient uptake (Begum et al., 2019; Govindarajulu et al., 2005), discovered that AMF transmit a significant quantity of N from the soil to the root system when investigating N transfer by AMF. Extraradical hyphae of AMF take up inorganic N and transport it to intraradical hyphae as amino acids, according to the authors' proposed model (mainly arginine). Intraradical hyphae breakdown amino acids to obtain C, then transport the leftover N to the host plant as ammonium. These researchers are among a number of others who have proved AMF's significant contributions to plant N uptake (Adeleke, 2010).

AMF not only transfers nitrogen and phosphorus to the host plant; it has also been shown that it can deliver micronutrients (Smith & Read, 2008). Copper, zinc, magnesium, manganese, and cobalt are the elements in question. AM fungus aid the plant in two ways: first, they aid in the uptake of these elements that are considered rather immobile, and second, they take up and store these elements so that their concentrations do not reach hazardous levels. AM fungi could act as a sink for copper, cobalt and zinc (Lies *et al*., 2018).

2.3.2. Increase Resistance to Phytopathogen

There is also enough data to suggest that AMF protects plants from phytopathogens by reducing pathogen populations and activity (Diagne *et al*., 2020); competing with pathogens for colonization sites, nutrients, and photosynthates; and boosting plant disease resistance (Maherali & Klironomos, 2007). AMF accomplishes this by stimulating the release of pathogenesis-related (PR) proteins by the host plant and enhancing the nutritional uptake of the damaged plant to compensate for the pathogen's harm. Bio-control of AMF, on the other hand, is reliant on the degree of mycorrhization (Thygesen *et al*.,

2004). Thygesen *et al*., (2004) discovered that G. mosseae dramatically reduced the disease index of Aphanomyces outreaches at high percent colonization, regardless of the density of the pathogenic inoculum, whereas at low percent colonization, the AMF displayed little protection against the pathogen. Despite numerous observations of AMF on plants, research have revealed that the known favorable benefits of mycorrhizal symbioses on plant productivity are only an estimate, and other functional roles remain unknown (Diagne *et al*., 2020).

2.3.3. Increase Tolerance to Drought Stress

Drought stress has a variety of effects on plants; for example, a lack of water at the roots lowers transpiration and causes oxidative stress. Drought stress affects enzyme activity, ion uptake, and nutrient assimilation, all of which are detrimental to plant growth. The AMF relationship enhances root hydraulic conductivity and plant water uptake, or affects plant physiology in various ways to minimize the stress response to soil drought (Begum *et al*., 2019). In very dry conditions, mycorrhizal plants fare better than nonmycorrhizal plants. It demonstrates that in search of water and nutrients, the mycelial network spreads deeper and wider in the soil. Although better phosphate feeding and colonization by AMF can improve plant drought resistance, mycorrhizal colonization may affect the permeability of cell membrane to water (Lies *et al.*, 2018). AMF exert their influence during drought stress by increasing transpiration and lowering stomatal resistance, or by affecting the balance of plant hormones. Changes in leaf elasticity caused by AMF inoculation improve leaf water and turgor potential, as well as root length and depth, which may alter water relations and thus plant drought tolerance (Aggarwal *et al*., 2011).

2.3.4. Tolerance to Heavy Metals

Because of their capacity to strengthen the defense system of AMF-mediated plants to promote growth and development, AMF are commonly regarded to support plant establishment in soils contaminated with heavy metals. Heavy metals can build up in crops, fruits, vegetables, and soils, posing a variety of health risks. There are also numerous findings in the literature on the effects of AMF on the accumulation of metals in plants (Begum *et al*., 2019). AMF can help with heavy metal cleanup by hyphal "metal binding," which reduces the bioavailability of metals including Cu, Pb, Co, Cd, and Zn (Smith & Read, 2008). Inoculation with AMF, according to Diagne et al., (2020), produced the best outcomes in terms of percentage of seed germination, seedling sustainability, fresh weight,

and dry weight of plants. Root colonization of maize plants with Glomus isolates reduced heavy metal concentrations in shoots and roots and raised the amounts of critical elements like K, P, and Mg in roots in two distinct heavy metal-polluted soils. AMF inoculation significantly boosted maize growth and reduced Cd uptake in soil contaminated with Cd, implying that AMF can be utilized in conjunction with plants to mitigate heavy metals such as Cd in soils (Hildebrandt *et al*., 1999). Heavy metals can be immobilized in internal and exterior fungal hyphae, which have the ability to fix heavy metals in the cell wall and store them in the vacuole, or chelate with other substances in the cytoplasm, reducing metal toxicity in plants. The ability of AMF to increase morphological and physiological processes that increase plant biomass and, as a result, uptake of important immovable nutrients like Cu, Zn, and P, and thus reduced metal toxicity in the host plants, is responsible for the strong effects of AMF on plant development and growth under severe stressful conditions (Begum *et al*., 2019).

2.3.5. Increase Tolerance to Salinity

Soil salinization is well acknowledged to be a growing environmental concern that poses a serious danger to global food security. Plant growth is suppressed by salinity stress, which affects vegetative development and net assimilation rate, resulting in lower yield productivity (Begum *et al*., 2019). Natural occurrences of AMF have been documented in saline environments. It is well known that they helped promote the growth of various plant species under saline circumstances. This is mostly due to a confluence of biochemical, physiological, and dietary factors. The expansion of water absorption capacity and nutrient uptake, the buildup of osmoregulatory substances proline and carbohydrates, ionic homeostasis, and the reduction in Na+ and Cl uptake are all mechanisms involved in salinity tolerance in AMF infested plants (Aggarwal *et al*., 2011). Furthermore, AMF colonization has been shown to enhance stomatal conductance and minimize oxidative damage in plants exposed to salinity. For example, inoculating tomato plants watered with saline water with F. mosseae dramatically enhanced plant biomass, fruit fresh yield, and shoot P, K, Cu, Fe, and Zn contents (Diagne *et al*., 2020). Under stress conditions, Begum et al. (2019) found that mycorrhizal plants exhibited higher biomass output, increased proline synthesis, increased N absorption, and notable changes in ionic relations, especially reduced Na+ buildup, than nonmycorrhizal plants.

2.4. Methods in Arbuscular Myccorrhizal fungi on Inoculum Production

Mukhongo *et al*. (2016), reported that there are about 12 mycorrhizal inocula producers in the European Union, with the producers in the United Kingdom, Czech Republic, Germany, Switzerland, Spain, and France and more than 20 others worldwide. The un-culturability and obligate biotrophy of arbuscular mycorrhizal (AM) fungi have not allowed the development of large-scale inoculation programs (Saritha & Prasad Tollamadugu, 2018). The only feasible means for production of infective propagules is growing the inoculum in symbiosis with living host plants or in root organ cultures (Sherameti & Varma, 2010). Although such production systems provide an advantage by allowing a continuous monitoring of the infective capability of the inoculum, their major drawbacks include extensive production costs, slow turnover time, and difficulty excluding secondary root colonizers such as root pathogens (Nayak, 2008).

Large-scale multiplication of AMF aiming to produce mycorrhizal inoculant for field applications is generally carried out in substrate-based (nursery beds, pots, concrete tanks) (Habte, 2000), substrate-free (i.e., hydroponics and aeroponics boxes) (Saritha & Prasad Tollamadugu, 2018). Commercial inocula produced using these systems are available in several countries, especially in Asia and Europe (Mukhongo *et al*., 2016). AMF inoculum can be applied as spores, fragments of roots colonized by AMF, or a combination of the two and incorporated soil mycelium. AMF spores and hyphae can be isolated from the soil substrate and mixed with carrier substrate (Smith & Read, 2008). Commonly used carriers include pumice or clay, sand, perlite, vermiculite, soilrite, and soil or glass pellets. AMF taxa and strains may vary in their ability to colonize host plants depending on the source of inoculum (Habte, 2000). Spores may be the most reliable source of inoculum across various AM taxa, whereas fragments of colonized roots are effective for some taxa but not others. The entire substrate can also be used and homogenized into a crude soil carrier that includes plant roots and fungal spores as well as the soil mycelium (Govindarajulu *et al.,* 2005).

2.5. Soil trap cultures

The simplest kind of culture to produce; soil is collected from the area of interest and mixed with a disinfected substrate, which- could be soil, sand, or expanded montmorilonite clay. Field-collected AM spores are found to be low in numbers, parasitized with microbes

and fungi, lacking suitable information for taxonomic characters, and hindering a more accurate identification. Trapping is necessary to obtain healthy AM spores for identification as well for using as inoculum to establish monospecific culture (Rastegari *et* al., 2019). Spores collected directly from a field soil suffer from many problems: they appear healthy but are not viable (some persisting as dead husks for years or possibly decades); they lose or change appearance of their structural characters in response to root pigments, soil chemistry, temperature, moisture, and microbial activity; and they represent only those colonizing arbuscular fungi with enough activity and biomass to trigger sporulation. Trap cultures yield very different results, depending on all of the biotic and abiotic factors that go into growth, reproduction, and the partitioning of colonization and sporulation (INVAM, 2020).

2.6. Restoration of degraded land using AMF

In some extreme environments, some plant species cannot survive without AMF (Pennisi, 2004). Because of the positive effects of AMF on plant growth and water and nutrient uptake, AMF have been applied to reestablish degraded land (ecosystems). Hildebrandt *et al*. (1999), showed that AMF increased plant cover in a roadside prairie restoration site. Richter & Stutz, (2002), found that mycorrhizal inoculation benefited restoration efforts in abandoned agricultural fields in semiarid regions. Inoculation with mycorrhizae improved the development of grasses during the early stages in the restoration of mine waste (Ryszka & Turnau, 2007). Bingham & Biondini, (2011), reported that mycorrhizal hyphae should be taken into consideration in grassland restoration and management. Biotechnology that can improve the regeneration of plants in this area is important for ecosystem stability and the development of livestock farming.

As the fungal mycelium grows through soil, it scavenges for mineral nutrients and is able to make contact with uninfected roots, sometimes of different host species. The small extra-radical mycelium compared to roots which allows penetration into some crystalline minerals, aggregates and organic matter with smaller pores than could be exploited by root alone (Aggarwal *et al*., 2011).

The various mechanisms proposed to account for enhanced P uptake include, increased exploration of soil; increased translocation of phosphorus into plants through arbuscules; modification of root environment; efficient utilization of P within plants; efficient transfer

of P to plant roots; and increased storage of absorbed P (Aggarwal *et al*., 2011). AM fungi may have biochemical capabilities for increasing the supply of available P and other immobile nutrients. AMF phosphatases are able to mineralize organic P sources (Calabrese *et al*., 2019). Alkaline phosphatase activity is related to phosphate metabolism of fungus as it is present within the fungal vacuoles where polyphosphate granules were observed. The polyphosphate granules in fine branches of arbuscules are broken down by enzymatic activities releasing inorganic phosphorus in the cytoplasm (Judith Prieto Méndez, César A. González Ramírez, 2009). Although AMF influence on plant N nutrition is not as high as in the case of P, they give their host access to different forms of N, thereby increasing plant N uptake (Judith Prieto Méndez, César A. González Ramírez, 2009). Hodge *et al.*, (2001), demonstrated the ability of an AMF to decompose organic matter and acquire N from the organic source. They also showed that AMF increased the diffusion rate of N to its host. Hence, mycorrhizal plants have additional access to N sources compared to non-mycorrhizal plants. The most exciting part of their findings is that AMF could be saprophytic, especially when decomposition is required for nutrient uptake (Balliu *et al*., 2015). While exploring N transfer by AMF, Govindarajulu *et al*., (2005), showed that AMF transfer a substantial amount of N from the soil to the root system. Using a proposed model, the authors explained that extraradical hyphae of AMF take up inorganic N, and transfer it to intraradical hyphae as amino acids (mainly arginine). The intraradical hyphae decompose the amino acids to access the C, and then transfer the remaining N as ammonium to the host plant. These authors are among several that have demonstrated the remarkable contributions of AMF to N uptake by plants (Adeleke, 2010).

2.7. Green Legacy program of Ethiopia

Ethiopia has been carrying out national tree seedling planting campaigns since its millennium and planted billion of trees. The Government of Ethiopia envisions covering 22 million hectares of degraded lands with forest by 2030 by significantly enhancing the contribution of forestry to agriculture, water and energy. Ministry of Environment, Forest and Climate Change (MEFCC, 2018). The Ethiopian government has announced larger goal of planting 20 billion trees during a four-year period. All these development activities have been accomplished under the country's National Green Development programme, to combat climate change and environmental degradation.

The Green Legacy Initiative aimed to grow four billion trees to help curb the effects of deforestation and climate change (UNDP, 2019). The campaign was part of the effort to combat environmental degradation. Another aim was to raise the public's awareness about Ethiopia's fighting ecological degradation and educate communities on the importance of adopting green behavior, to become a green society. The general public in different regions participated widely in the campaign. The one-day campaign was reported as successful because the participants were able to plant the prepared seedlings for all parts of the regions in the country. With the green legacy day Ethiopia also broke the world record in tree planting having planted more than 200 million seed-lings in one day. It was reported in national media how international organizations supported the initiative for its decisive role in the fight against climate change.

Even if the country has been working to rehabilitate degraded lands and restore forests through national tree seedling planting campaigns since 2000, the survival rate of the seedling is low according to the reports of Adama Woreda Agriculture and Natural Resources Office (2020) and (2021), the survival seed lings is 40% and 50% respectively. The low level of seedlings survival and establishment in the field in Ethiopia has been a bottleneck to the realization of the national restoration target (Eba, 2017; Fisseha *et al.*, 2019). In fact, it causes loss of a huge amount of money allocated for this purpose. Poor field survival and establishment of trees in the country is caused by low moisture and nutrient content of soils at the planting site (Fisseha *et al.*, 2019; Mahari, 2014). In addition, selection of suitable tree species for a particular planting site, providing the necessary aftercare and utilization of relevant technologies that enhance tree seedlings moisture and nutrient relation are also important. One of such technology could be arbuscular mycorrhizal fungi (AMF) inoculation (Asmelash *et al.*, 2016).

Tree seedlings establishment, survival and growth in field are greatly impacted by water and soil nutrient availability. In addition, survival and adequate growth under field conditions depend on several factors including the nutritional status of seedlings before transplanting. AMFs are among soil microorganisms that greatly impact plant nutrition and growth under nursery and field conditions as they establish the AM association with their hosts. These fungi colonize the plant root cortex and spread their hyphae into the surrounding soil where they scavenge for low mobility nutrients like phosphorus, which they translocate back to the plant host; in turn, they receive from the plant carbon compounds to grow and complete their life cycle. They also confer to the plants resistance

against pathogens, and improvement in water relations, as well as impacting soil structure (Goetten *et al*., 2016). Considering all these benefits of the mycorrhizal association, inoculation of woody species' seedlings under nursery conditions is a strategy to reduce costs of chemical fertilizers and to produce seedlings with good vigor which would translate into high survival and growth at the field. Large scale inoculation in nurseries depends on the availability of AMF inoculant that can be either purchased commercially or produced by the nurseryman. Many studies have shown that nutrition and growth of native woody species are improved by the mycorrhizal association and that many species are moderate to very highly dependent on the association (Fisseha *et al*., 2019; Goetten *et al*., 2016).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Description of the Study Area

Two locations were chosen based on prior studies of arbuscular mycorrhizal fungi diversity and abundance associated with acacia trees from various land use systems in Ethiopia (Zerihun *et al*., 2013). The study was performed in selected areas of central Rift Valley and highland, regions of Ethiopia. The Central Rift Valley region is involving Batu and Bishoftu woody grassland where naturally different acacia tree species are dominating and the high land region is Sululta, the tertiary of Addis Ababa. Bishoftu and Batu are located in the East Showa zone, Oromia Regional State at the altitude of 1600 to 1960 meters above sea level. The Zone extends between $7^{o}33'$N-$9^{o}08'$N and from $38^{o}24'$E- $40^{o}05'$E, with a total area coverage of approximately 8,370.90 square kilometers (Figure: 9). Since a large part of the Zone is situated along with the Rift Valley system, rainfall ranges from 600 mm to 1000 mm with an average annual rainfall of 816 mm, with the temperature range from 10ºC in the uplands to over 30ºC in the depressions of the Rift Valley with a mean temperature of 20ºC (CSA, 2020).

Sululta is located in the central part of Ethiopia, Oromia Special Zone surrounding Addis Ababa. It is found on the road to Fiche (north Showa Zone, Oromia) 23 km from Addis Ababa to north. Geographically, the study area extends from $9^{o}30'00''$N to $9^{o}12'15''$N latitude and $38^{o}42'0''$E to $38^{o}46'45''$ E longitude with altitude of 2589 meters above sea level. The administrative area of the town is about 4471 hectares. Sululta has the similar climatological characteristics to Addis Ababa. Globally it is a part of a tropical humid climatic region, which is characterized by warm temperature and high rainfall (The maximum annual rain fall is 1447 mm with mean of 1140 mm and minimum of 834 mm). The soils of the area are derived from Mesozoic sedimentary and volcanic rocks. The major soil types of the Sululta area are Chromic Luvisols (Gelan, 2021).

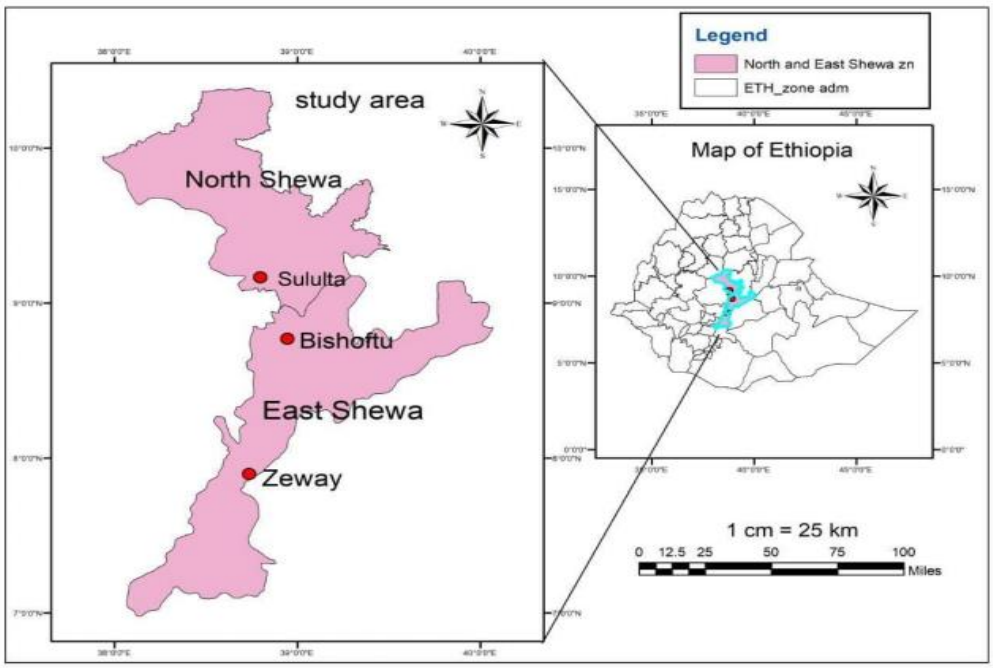


Figure 10: Map of study area, Oromia special zone and East Showa in Central Rift Valley of Oromia Regional State, Ethiopia (Developed from Google map).

3.2. Soil Sampling

Rhizosphere soil sampling was collected in February 2021. Triplicate samples were taken randomly from each plot within a 10m x 10m quadrant (Davison *et al*., 2012). From each plot, three samples of rhizosphere soil at depth of 0-30 cm were collected and pooled per quadrant to form a composite sample. From high land area *Acacia abyssinica* and from low land area *Acacia seyal* and *Acacia tortilis* were selected. The acacia tree species were characterized by relatively high AMF colonization and very high AMF abundance and diversity according the report of (Zerihun *et al*., 2013). Within each of these areas (approximately 100 m^2), three replicates of each acacia tree species were randomly selected and 3kg of rhizosphere soil were taken into a depth of 0-30 cm and that were pooled into a composite sample (9kg from each sample location for each species). Totally 45kg of soil samples were collected using sterilized plastic bags and stored at room temperature until further analysis is conducted.

3.3. Physicochemical analysis of soil

The physical and chemical properties of soils were analyzed at Horticoop Plc. Bishoftu Ethiopia and Awash Melkasa Agricultural Research Center (MRC). Soil pH was measured potentiometrically using a digital pH-meter in the supernatant suspension of (1:2.5 soil:

water), and soil texture was measured with the Boyacus hydrometer method (g/cm) using USDA textural triangle (Kim *et al*., 2006). The total organic carbon was determined by using the method of Meersmans *et al.,* (2009), and available phosphorus was determined by Olsen methods (De Silva *et al*., 2015). In the Olsen procedure, $NaHCO_3$ at nearly constant pH of 8.5 was used as extracting solution(De Silva *et a*l., 2015). The total N content was determined using the Kjeldahl method by oxidizing soil samples with sulfuric acid and converting the N compounds into NH_4^+ as ammonium sulfate (Yanagi *et al*., 2003).

3.4. Determination of AMF Spore Abundance of the Soil Sample

Before establishing trap culture, the abundance of AMF in the soil samples collected from sample locations was examined to determine the initial mycorrhizal status of the soil sample. Soil samples from the field were air-dried and sieved through a 2-mm sieve to remove coarse debris. AMF spores occurring in the soil samples were extracted by the wet sieving and decanting method (Gerdemann & Nicolson, 1963) followed by centrifugation in water and in a 60% sucrose solution (Shamini & Amutha, 2014). One hundred grams of soil was suspended in one liter of tap water in each batch and left to settle the heavier fractions to settle for two to three minutes. Then, the liquid fraction was decanted 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 some AMF sporocarps, and the third and fourth also to capture AMF spores and fine particles. The contents of the 180, 90, and 63 µm sieves were then, put into a centrifuge tube containing water and centrifuged at 2000 rpm for five minutes. After pouring off the supernatant, 60% sucrose was added to the pellet and mixed it carefully, then centrifuged for one min at 2000 rpm. 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 a plastic Petri dish for examination under the stereomicroscope. Enumeration of spore numbers per gram of dry soil was undertaken according to INVAM, http://invam.caf.wvu.edu.

3.5. Establishment of Trap Cultures (AMF Inoculum Production)

In order to obtain many healthy and infective AMF propagules for inoculation, a trap culture was established in the greenhouse at ASTU for four months (March to June 2021), according to INVAM (2020). The trap cultures in pots were set up in triplicates for all five

soil samples (*A. seyal* and *A. tortilis* each from Bishoftu and Batu and *A, abyssinica* from Sululta), A total of 15 pots was established. Each soil sample was thoroughly mixed with autoclaved sand (sterilized twice in autoclave at 121 ºC, 1 hour with intervals of 24 hrs.) (1:1; v/v), and about 3kg of mixed soil: sand sample was transferred to a plastic pot, and were irrigated for three days before sowing. Seeds of "Melkam" a sorghum cultivar and a mycotrophic crop from Melkasa Agricultural Research Center (MRC) were selected as an appropriate trap plant for its ability to induce high spore density, diversity, and species richness according to INVAM (2020). Surface sterilization of sorghum seeds was performed by soaking them for 15 minutes in a 0.5% sodium hypochlorite solution. Then, after washing the seeds with sterile water, they were sown at a two cm depth in each plastic pot and covered with sterilized sand. Pots were irrigated daily as needed. During the growing phase, no fertilizer was used. All seedlings were grown in the greenhouse under natural light and temperature conditions for four months. Finally, watering was reduced during the final weeks of the four months to maximize spore production. At the end of four month, the plants were cut near the base, and the cultures were air-dried and stored in zipped plastic bags at room temperature for 30 days before inoculation (INVAM, 2021).

3.6. Assessment of root Colonization and Spore Density of Trap Culture

The root samples of trap culture were washed several times in tap water cut into segments about 1–2 cm long. About 0.5 g of root segments was cleared in 10 % (w/v) KOH at 90^{o}C for 1 hour in water bath. And roots were further bleached with alkaline hydrogen peroxide (10% H_2O_2) for 3 minutes at room temperature. Thereafter, the roots were treated with 2% HCl (v/v) for 15-20 minutes at room temperature and finally stained in 0.05% w/v trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) at 90^{0}C for one 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. Finally, roots were mounted in PVLG mountant on 48 microscopic slides and covered with 24×24 mm coverslips (Wongchalee & Pukahute, 2012). AMF colonization was assessed from cleared and stained roots according to (Keymer *et al*., 2017). A total of 150 intersections were taken for each subsample to estimate percent AM 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. Hyphal colonization (HC) was calculated by dividing the proportion of nonnegative intersections to total number of intersections.

The assessment of spore density is performed by wet sieving and decanting method (Gerdemann & Nicolson, 1963), followed by centrifugation in water and in a 60% sucrose solution (Shamini & Amutha, 2014) as described above. Then, enumeration of spore numbers per gram of trap culture soil was undertaken according to INVAM, http://invam.caf.wvu.edu.

3.7. Experimental Design and Inoculation of AMF Inocula on to selected trees

Four plant seeds of *Azodichta indic*a (Neem*), Delonix regia, Sesbania grandflora* and *Cassia fistula* were obtained from Adama Woreda Agriculture and Natural Resources office. The experiment was arranged in a randomized complete block design with three replications in a greenhouse. The experimental treatments of pots (diameter of 15 cm and a depth of 20 cm and capacity of 3kg) were grouped into four treatments. i. T_1 [*A. abyssinica* of AMF (18.42 spores g^{-1} of soil)], ii. T_2 [*A. seyal* of AMF (32.4 spores g-1 of soil)], iii. T_3 [*A. tortilis* of AMF (22.4 spores g-1 of soil)], iv. T_4 [Control (Non Inoculum)].

Soil sample with low pH and low phosphorus levels was collected from Holeta for the experiment to evaluate the efficiency of the inoculants in poor fertile soil. The physical and chemical characteristics of the test soil were as follows :(P (6.44 Ppm), N (1.66), organic matter content (1.549 %), pH (4.75), and EC (0.059 ds/m) (Zerihun Belay & Fassil Assefa, 2011). AMF spore density of the test soil was 5.9 spores per gram. The unsterilized soil samples (2.7kg for each pot) were mixed thoroughly with 300g (10%) of the inoculum. Healthy selected seeds of (*Azodichta indic*a (Neem*) Delonix regia, Sesbania grandflora, and Cassia fistula* were surface disinfected by immersing in a 0.5% sodium hypochlorite solution for 15 min. The seeds were washed with sterilized water and allowed to germinate on a 0.75% (w/v) water agar for 48 hours at 25 ^{0}C before planting or sowing. Then, the germinated eight seeds were sown at 2 cm depth in each plastic pot. For each variety of plant seeds three pots were inoculated and one control (none inoculated). All pots were watered for four days per week grown in a greenhouse with natural light at ambient temperature during the growing period of four months.

3.8. Measurement of plant growth parameters and AMF spore density and percentage of root colonization.

Height of plant or Shoot height (SH) and root height (RH) was measured with a tapeline before harvest. Total leaves number (NL), root length (RL) fresh shoot mass (FSM) and fresh root mass (FRM) were measured and then dried at 70^{o}C in an oven for 48 h. Dry shoot mass (SDM) and dry root mass (RDM) were measured. The measurement of the plant growth parameters were conducted twice (at 60 and 120days) according to Kumar *et al.*, (2014).

Moreover, for each plant species, a Mycorrhizal Dependency (MD) value was calculated. It was determined by expressing the difference between the total dry of mycorrhizal plant (DWM) and the total dry weight of the non-mycorrhizal plant (DWNM) as a percentage of the dry weight of the mycorrhizal plant. The MD indexes calculated from these values, $\%\text{MD} = \frac{\text{DWM} - \text{DWNM}}{\text{DWM}} \times 100$, (Yaseen *et al.*, 2011)

AM root colonization was assessed on cleared and stained roots. Percent of AM root colonization was estimated using, a magnified intersection method McGonigle *et al.*, (1990), and a hairline graticule inserted in to eyepiece acted as the line of intersection with each root at 200x magnification under the compound microscope. A total of 150 intersections were taken for each subsample to estimate percent of AM root colonization under a compound microscope (FXACTA+OPTECH) at a magnification of 200X.

AMF spores occurring in the soil rhizosphere after completing the growth of all the seedlings were examined by the wet sieving and decanting method (Gerdemann & Nicolson, 1963), followed by centrifugation in water and in a 60% sucrose solution (Walker *et al*., 1982), as described previously. Finally, the enumeration of spore numbers per gram of the soil was undertaken according to INVAM, http://invam.caf.wvu.edu.

3.9. Statistical analysis

The statistical analysis of the experiment was performed using the SPSS software package (version 26.0). AMF spore abundance and percentage of root colonization of the test pants and the growth responses (plant shoot height, root height, number of leaves, dry and fresh weight of root and shoot) data were subjected to one-way analysis of variance (ANOVA) using the treatments as factor. Tukey's honestly significant difference (HSD) post hoc test

was used for pair-wise multiple mean comparisons tests. Pearson's correlation coefficients among parameters were analyzed. All the tests of statistical significance were decided at $p < 0.05$. And data are reported as means ± SE, based on three replicates.

CHAPTER 4

4. RESULTS AND DISCUSSIONS

4.1. RESULT

4.1.1. Physicochemical analysis of soil

The soil sample used for the production of AMF inoculum in this experiment was dominated by the soil texture of the sandy loam in all the acacia species, while the *A. abyssinica* showed a clay loam fraction. The physico-chemical properties and AMF status of the field soil were shown in Table: 3. the soil samples were close to neutral, which ranged between pH values of 6.58 in *A. seyal* and 7.33 in *A. tortilis* from Batu. The soil organic carbon concentration of *A. abyssinica* from Sululta was 2.1%. Similarly, the soil total nitrogen concentration varied between 0.09 and 0.21% for Batu (*A. tortilis*) and Sululta (*A. abyssinica*), respectively. Additionally, the P content was observed to be the highest in Sululta (13.5 ppm) and the lowest in Batu with *A. seyal* (6.32ppm).

Regarding AMF analysis, it shows that the spore density of the rhizosphere soils sampled from all acacia species in highland and lowland showed numbers of AMF spores ranging from 10.47 to 18.89 spores g-1 soil. The highest average spore density of 18.89 spores g-1 soil was observed under *A. seyal* from Batu, and the lowest of 10.47 spores g-1 soil under *A. tortilis* from Bishoftu.

Table 2: AMF spore abundance and Physico-chemical parameters of soil samples from rhizosphere of acacia trees

Sampling area	Acacia sp.	pH	T.N (%)	Ave.P (ppm)	OC (%)	%Sand	%Clay	%Silt	Class of texture	SD (gm^{-1})
Sululta	*A. abyssinica*	6.84	0.21	13.5	1.08	32	36	32	clay loam (CL)	10.55±0.3d
Batu	*A. seyal*	6.58	0.15	6.32	2.1	62	4	34	sandy loam (SL)	18.89±0.07a
	A. tortilis	7.33	0.09	11.1	1.61	58	6	36	sandy loam (SL)	13.73±0.06c
Bishoftu	*A. seyal*	7.08	0.13	10.1	0.99	66	6	28	sandy loam (SL)	16.61±0.19b
	A. tortilis	7.13	0.14	9.58	1.66	50	14	36	sandy loam (L)	10.47±0.17d

Note- TN - total nitrogen, Ave.P - available phosphorus, OC - organic carbon, and SD – spore density.

4.1.2. Determination of AMF Spore Abundance of the Soil Sample and Establishment of Trap Cultures (AMF Inoculum Production)

The average number of AMF spore density enumerated in the trap culture ranged from 18.74 to 32.41 g^{-1}, (Table 4). There was a significant difference in AMF spore density among the areas of sampling and the acacia species. The highest spore densities were found associated with A. *seyal* from Batu. Sorghum that treated with highland *A. abyssinica rhizosphere*, recorded the lowest (10.55 g^{-1} soil*)* AMF spore density. All seedlings of sorghum in trap culture were colonized by AMF. The percentage of AMF root colonization significantly differs between tree species in the lowlands and highland ($p \leq 0.05$) (Table 4). Average hyphal colonization ranged between 56.96% and 94.9%. The highest colonization was found in *A. seyal* (94%) species from lowland Batu. The lowest colonization was in *A. abyssinica* species (56%)

Table 3: AMF spore abundance & percentage of root colonization in trap cultures of the inoculum.

sampling area	*Source of the soil inoculum*	SD (gm^{-1})	RC%		
			AC%	VC%	HC%
Sululta	*A. abyssinica*	18.74±0.47e	25.26±0.08e	14.6±0.15d	56.96±0.23e
Batu	*A. seyal*	32.41±0.21a	45.7±0.86a	25.6±0.45a	94.9±0.4a
	A. tortilis	22.43±0.06d	34.4±0.4c	19.9±0.45c	62.93±1d
Bishoftu	*A. seyal*	28.44±0.16b	41.76±0.48b	27.33±0.49a	79.1±1.17b
	A. tortilis	21.85±0.11d	28.46±0.4d	22.53±0.44b	61±0.56d

Note- SD- *spore density, RC- root colonization, AC- arbuscular colonization, VC – vascular colonization, and HC- hyphae colonization.* Different lowercase letters in the same column represent significant difference at 0.05 level; Mean values followed by the same letter are not significantly different at Pα0.05. Mean ± Standard error.

4.1.3. Growth Response of the Trees to AMF Inocula

4.1.3.1. A. indica (Neem)

In this study to identify the effects of the AMF on selected trees the growth parameters and mycorrhizal parameters was evaluated. Accordingly, results on the growth parameters of *A.indica* (Neem) after inoculating with AMF are presented in Table 5. It was observed that after 60 days the seedlings inoculated with AMF from *A. seyal* (T_2) showed significantly greater responses in all parameters over the non-inoculated or control (T_4) and those inoculated with AMF from *A. tortilis* (T_3). Henceforth, T_2 showed significantly greater responses in SH, FSM and DRM which were 14.5cm, 1.33g and 0.73g respectively than which inoculated with AMF from *A. abyssinica* (T_1) and the other parameters were equal in RH and showed lower in NL and FRM. After 120 days the seedlings inoculated with AMF from *A. seyal* (T_2) showed significantly greater responses at all parameters over the (T_1 and T_3) in addition to non-inoculated or control (T_4). Seedlings inoculated with AMF from *A. abyssinica* (T_1) and AMF from *A. tortilis* (T_3) showed equal responses in SH (29.33cm), NL (65.00), FRM (2.40g) and DRM (1.03g) parameters. However, T_1 showed greater responses in RH (21.50cm), FSM (3.90g) and DSM (1.83g).

Table 4: Effect of microbial inoculums on growth characteristics of *A.indica* (Neem)

	Parameters						
Treatment	SH (cm)	RH(cm)	NL	FSM (g)	FRM(g)	DSM(g)	DRM(g)
A.indica (Neem) after 60 days							
T_1	14.17±0.44a	7.67 ± 0.09b	34.67 ± 0.65a	1.20 ± 0.15b	2.03 ± 0.03a	0.53 ± 0.03b	0.97 ± 0.03a
T_2	14.5± 0.29a	7.83± 0.44a	29.00 ± 0.58b	1.33 ± 0.03a	1.73 ± 0.07b	0.73 ± 0.03a	0.78 ± 0.03b
T_3	11.30± 0.15b	6.57 ± 0.12c	22.33 ± 1.20c	1.03 ± 0.03c	1.00 ± 0.07c	0.47 ± 0.03c	0.33 ± 0.03c
$T_{4\ (control)}$	9.5± 0.00c	5.43 ± 0.12d	17.00 ± 0.58d	0.67 ± 0.07d	0.77 ± 0.03d	0.30 ± 0.00d	0.13 ± 0.03d
A.indica (Neem) after 120 days							
T_1	29.33 ± 0.29b	21.50 ± 0.29b	65.00 ± 1.76b	3.90 ± 0.17b	2.40 ± 0.09b	1.83 ± 0.03b	1.03 + 0.03b
T_2	32.37 ± 0.19a	22.83 ± 0.17a	77.00 ± 1.53a	4.63 ± 0.90a	4.10 ± 0.06a	2.07 ± 0.07a	1.73 + 0.03a
T_3	29.33 ± 0.44b	19.83 ± 0.17c	65.00 ± 1.73b	3.03 ± 0.03c	2.40 ± 012b	1.10 ± 0.00c	1.03 + 0.03 b
T_4	24.10 ± 0.21c	16.33 ± 0.17d	55.33 ± 0.88c	1.96 ± 0.03d	1.73 ± 0.07c	0.80 ± 0.06d	0.83 + 0.03c

Note: SH-Shoot Height, RH-Root Height, NL - Number of Leaves, FSM-Fresh Mass of Shoot, FRM-Fresh Mass of Root, DSM-Dry Mass of Shoot, DRM-Dry Mass of Root. T_1- *A. abyssinica* of AMF, T_2 - *A. seyal* AMF, T_3- *A. tortilis* and T_4 - Control (Non-Inoculum)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 ($P < 0.05$).

4.1.3.2. *Delonix regia*

Similarly, in this study growth response observed in *D.regia* is presented in Table 6. It was observed that after 60 days of growth the seedlings inoculated with T_2 (AMF from *A.seyal*) showed significant superiority in all parameters over other treatments (Table 6). Seedlings that inoculated with AMF from *A.abyssinica* (T1) showed greater responses over the seedlings that inoculated with AMF from *A.tortilis* (T3) in SH = 14.33cm, NL = 181.00, FRM = 1.33g, DSM = 1.07g and DRM = 0.70g. However, the parameter of FSM showed equal responses (T1 and T3 = 2.07g) and T3 showed greater response in RH = 13.20cm.

After 120 days seedlings inoculated with T2 (AMF from *A.seyal*) showed significantly greater responses at all growth parameters over the non-inoculated or control (T_4) and other inoculated treatments (T1 and T3) (SH = 23.53cm, RH = 20.50cm, NL = 295.67, FSM = 4.60g, FRM = 3.67g, DSM = 2.73g, and DRM = 2.20g). Secondly seedlings inoculated with AMF from *A.abyssinica* (T1) showed greater responses over seedlings that inoculated with AMF from *A.tortilis* (T3) in SH = 19.33cm, RH = 18.00cm, NL = 231.33, FRM = 3.07g, and DRM = 1.90g except FSM which was equal responses (2.07g).

Table 5: Effect of microbial inoculums on growth characteristics of *D.regia* (dire dawa zaf)

	Parameters						
Treatment	SH	RH	NL	FSM	FRM	DSM	DRM
D.regia after 60 days							
T_1	14.33 ± 0.17a	13.00 ± 0.29b	181.00 ± 5.57b	2.07 ± 0.09a	1.33 ± 0.09b	1.07 ± 0.03a	0.70 ± 0.06a
T_2	15.00 ± 0.29a	13.83 ± 0.17a	203.67 ± 1.96a	2.17 ± 0.03a	1.67 ± 0.03a	1.09 ± 0.03ab	0.72 ± 0.06a
T_3	14.00 ± 0.29a	13.20 ± 0.15ab	164.00 ± 7.02b	2.07 ± 0.13a	1.23 ± 0.03b	0.93 ± 0.03b	0.56 ± 0.01a
T_4	11.67 ± 0.17b	12.00 ± 0.00c	120.00 ± 1.15c	1.43 ± 0.03b	0.97 ± 0.03c	0.70 ± 0.06c	0.37 ± 0.01b
D.regia after 120 days							
T_1	19.33 ± 0.17b	18.00 ± 0.29b	242.33 ± 1.45b	3.50 ± 0.15b	3.07 ± 0.03b	2.10 ± 0.06b	1.90 ± 0.06b
T_2	23.53 ± 0.26a	20.50 ± 0.29a	295.67 ± 3.38a	4.60 ± 0.12a	3.67 ± 0.12a	2.73 ± 0.12a	2.20 ± 0.06a
T_3	17.83 ± 0.17c	16.13 ± 0.19c	231.33 ± 1.19c	3.90 ± 0.21b	3.00 ± 0.06b	2.20 ± 0.06b	1.80 ± 0.06b
T_4	15.27 ± 0.15d	12.53 ± 0.26d	187.33 ± 1.45d	2.53 ± 0.07c	2.13 ± 0.07c	1.67 ± 0.09c	1.10 ± 0.06c

Note: SH-Shoot Height, RH-Root Height, NL - Number of Leaves, FSM-Fresh Mass of Shoot, FRM-Fresh Mass of Root, DSM-Dry Mass of Shoot, DRM-Dry Mass of Root. T_1- *A. abyssinica* of AMF, T_2 - *A. seyal* AMF, T_3- *A. tortilis* and T_4 - Control (Non-Inoculum)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 ($P < 0.05$).

4.1.3.3. *Sesbania grandflora*

In this study, the result of *S.grandflora* is presented in Table: 7. After 60 days the seedlings inoculated with AMF from *A.seyal* (T_2) showed significantly greater response in all growth

parameters over the control (T_4). In addition, significant differences are observed between T1, T2 & T3 in the growth parameters such as SH, NL, FSM, DSM and DRM, (Table: 7) Growth responses in seedling inoculated with AMF from *A.tortilis* (T3) recorded 37.67cm, 15.33cm, 1.30g and 0.64g in SH, RH, FRM and DRM respectively. However, seedlings inoculated with AMF from *A.seyal* (T2) showed significantly greater growth responses in NL and FRM than T1 and T3 respectively. .

After 120 days the growth parameters showed variability among the treatments.. Accordingly the results in all growth parameters seedlings inoculated with AMF from *A.seyal* (T2) showed significantly greater responses (SH = 118.00cm, RH = 26.33cm, NL = 1024.33, FSM = 32.26g, FRM = 8.03g, DSM = 7.47g and DRM = 2.73g) over seedlings that were the control (T4) and other inoculated group (T1 and T3). Secondly, seedlings inoculated with AMF from *A.tortilis* (T3) and *A.abyssinica* (T1) also showed highly significantly greater than the control (T4) seedlings ($p<0.05$).

Table 6: Effect of microbial inoculums on growth characteristics of S.grandflora

	Parameters						
Treatment	SH	RH	NL	FSM	FRM	DSM	DRM
S.grandflora after 60 days							
T_1	37.33 ± 0.44a	14.23 ± 0.15b	243.33 ± 3.33a	4.13 ± 0.09b	1.10 ± 0.00b	2.93 ± 0.54ab	0.62 ± 0.02b
T_2	38.50 ± 0.29a	15.83 ± 0.33a	250.00 ± 10.00a	4.47 ± 0.03a	1.40 ± 0.00a	4.13 ± 0.17a	0.87 ± 0.03a
T_3	37.67 ± 0.44a	15.33 ± 0.17a	226.00 ± 4.00a	4.07 ± 0.03b	1.30 ± 0.10ab	2.40 ± 0.06bc	0.64 ± 0.02b
T_4	30.67 ± 0.67b	12.63 ± 0.09c	163.33 ± 8.81b	3.67 ± 0.07c	0.73 ± 0.03c	1.30 ± 0.12c	0.33 ± 0.03c
S.grandflora after 120 days							
T_1	99.50 ± 0.50b	20.83 ± 0.44b	946.00 ± 3.06b	27.33 ± 0.33b	6.73 ± 0.18b	6.33 ± 0.13b	2.10 ± 0.06b
T_2	118.00 ± 2.08a	26.33 ± 0.67a	1024.33 ± 9.24a	32.26 ± 0.64a	8.03 ± 0.03a	7.47 ± 0.26a	2.73 ± 0.09a
T_3	100.43 ± 0.35b	23.00 ± 0.58b	950.00 ± 1.15b	28.00 ± 0.58b	6.93 ± 0.07b	6.80 ± 0.06ab	2.27 ± 0.03b
T_4	85.00 ± 3.51c	17.90 ± 0.21c	733.33 ± 8.82c	23.00 ± 0.58c	5.23 ± 0.07c	4.57 ± 0.12c	1.67 ± 0.03c

Note: SH-Shoot Height, RH-Root Height, NL - Number of Leaves, FSM-Fresh Mass of Shoot, FRM-Fresh Mass of Root, DSM-Dry Mass of Shoot, DRM-Dry Mass of Root. T_1- *A. abyssinica* of AMF, T_2 - *A. seyal* AMF, T_3- *A. tortilis* and T_4 - Control (Non-Inoculum)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 ($P < 0.05$).

4.1.3.4. *Cassia fistula*

In the same way, the results of this study showed that after 60 days of inoculation of the *Cassia fistula* with AMF growth parameters of SH ranged between 12.83cm and 20.30cm, RH ranged between 10.50cm and 13.33cm, NL ranged between 49.33 and 88.67, FSM

ranged between 1.37g and 2.83g, FRM ranged between 1.00g and 1.73g, DSM ranged between 0.60g and 1.33g and DRM ranged between 0.23g and 0.90g. The results of growth parameter of the seedlings inoculated with AMF from *A.seyal* (T2) showed significantly greater responses in SH = (42.3300cm), RH = (16.93cm), NL = (131.67), FSM = (13.23g), FRM = (4.50g), DSM = (5.40g) and DRM = (2.37g) over seedlings that were non-inoculated or control (T4) and other inoculated group (T1 and T3). Seedlings inoculated with AMF from *A.abyssinica* and *A.tortilis* were not showing significant differences in all growth parameters. However, T1 recorded significantly higher value of SH (18.77cm) than T3 (17.8cm) (Table: 8).Similarly, the growth results after 120 days showed SH ranged between 25.67cm and 42.33cm, RH ranged between 11.37cm and 16.93cm, NL ranged between 68.67 and 131.67, FSM ranged between 8.97g and 13.23g, FRM ranged between 2.17g and 4.50g, DSM ranged between 2.00g and 5.40g and DRM ranged between 0.90g and 2.37g (Table 8). Seedlings treated with T2 showed a significant difference with the control and other treatments. However, after 120 days of experiment no significant difference were recorded in SH, RH and NL between T1, T2, T3.

Table 7: Effect of microbial inoculums on growth characteristics of *C.fistula*

	Parameters						
Treatment	SH (cm)	RH (cm)	NL (no.)	FSM (g)	FRM (g)	DSM (g)	DRM (g)
C.fistula after 60 days							
T_1	18.77 ± 0.15b	12.33 ± 0.33b	82.67 ± 1.76b	2.43 ± 0.03b	1.57 ± 0.03a	1.10 ± 0.00b	0.67 ± 0.03ab
T_2	20.30 ± 0.20a	13.33 ± 0.17a	88.67 ± 2.03a	2.83 ± 0.09a	1.73 ± 0.13a	1.33 ± 0.03a	0.90 ± 0.06a
T_3	17.80 ± 0.15c	12.00 ± 0.29c	77.67 ± 1.45c	2.53 ± 0.09ab	1.47 ± 0.03a	1.17 ± 0.03ab	0.53 ± 0.09b
T_4	12.83 ± 0.17d	10.50 ± 0.29d	49.33 ± 0.67d	1.37 ± 0.07c	1.00 ± 0.00b	0.60 ± 0.06c	0.23 ± 0.03c
C.fistula after 120 days							
T_1	34.33 ± 1.20b	14.33 ± 0.33b	119.67 ± 6.06a	10.27 ± 0.15c	2.97 ± 0.09c	4.73 ± 0.12b	2.13 ±0.03b
T_2	42.33 ± 1.45a	16.93 ± 0.07a	131.67 ± 0.88a	13.23 ± 0.15a	4.50 ± 0.06a	5.40 ± 0.00a	2.37 ± 0.03a
T_3	39.00 ± 0.58ab	16.87 ± 0.59a	124.33 ± 2.33a	11.07 ± 0.07b	4.10 ± 0.06b	4.83 ± 0.17b	2.00 ± 0.00c
T_4	25.67 ± 0.89c	11.37 ± 0.19c	68.67 ± 1.33b	8.97 ± 0.03d	2.17 ± 0.09d	2.00 ± 0.00c	0.90 ± 0.00d

Note: SH-Shoot Height, RH-Root Height, NL - Number of Leaves, FSM-Fresh Mass of Shoot, FRM-Fresh Mass of Root, DSM-Dry Mass of Shoot, DRM-Dry Mass of Root. T_1- *A. abyssinica* of AMF, T_2 - *A. seyal* AMF, T_3- *A. tortilis* and T_4 - Control (Non-Inoculum)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 ($P < 0.05$).

4.1.4. Arbuscular Mycorrhizal Fungi Parameters

4.1.4.1. AMF Root Colonization and Spore Density

The seedlings of selected trees which were inoculated with AMF and the effect on their growth were evaluated. Accordingly the results on the root colonization parameters were measured twice, after 60 and 120 days. AMF root colonization observed in all tree seedlings inoculated. The AMF hyphal root colonization recorded (Figure: 10) after 60 days ranged between 18.52% and 33.92% for *A.indica (Neem),* between 25.74% and 35.17%, for *D.regia(dred awa zaf),* and between 20.29% and 56.12%, for *S.grandflora,* and between 45.25% and 49.80% for *C.fistula* (Table 9).

Similarly, after 120 days of inoculation the results of root colonization by AMF showed that *A.indica (Neem),* ranged between 30.80% and 43.90%, *D.regia,* ranged between 33.74% and 62.82%, *S.grandflora,* ranged between 45.26% and 71.05%, and *C.fistula* ranged between 53.43% and 61.90%, respectively (Table 9). When compared the AMF root colonization between 60 and 120 days it showed that the pattern of the root colonization was showed increasing with increasing of the height of the seedling. The percentage range of AMF root colonization shown in *A.indica* increased from 30.83 – 37.46 in (T1), from 33.92 – 43.90 in (T2) and from 18.52 – 30.80 in T3). In the same way, *D.regia* showed changes from 25.74 – 57.69 (T1), from 35.17 – 62.82 (T2) and T3 26.94 – 3374. Similarly in *S.grandflora* T1 increased from 42.12 – 71.03, T2 from 56.12 – 71.03 and T3 increased from 20.29 – 45.26. In the same way changes were observed in *C.fistula,* T1 from 45.25 – 53.43, T2 from 49.80 – 61.90 and T3 from 45.94 – 58.86.

AMF spore density after 120 days of inoculation in T1 showed 35.37, 35.33, 56.10 and 29.73 (soil gm^{-1}) of the inoculum for *A.indica, D.regia, S.grandflora* and *C.fistula,* respectively. Results showed in T2 were 39.83, 40.23, 45.43, and 33.23 for *A.indica, D.regia, S.grandflora* and *C.fistula,* respectively. Accordingly, trees inoculated with T3 were 36.37, 32.60, 35.27 and 29.43 for *A.indica, D.regia, S.grandflora* and *C.fistula,* respectively.

Table 8: Effect of AMF inoculum inoculation on AMF root colonization (RC) and spore density (SD), after 60 and 120 days in *A.indica, D.regia, S.grandflora*, and *C.fistula*. Colonization of the roots and spore density

Treatment		%RC after 60	%RC after 120	SD (soil gm^{-1})
Sesbania grandflora	T1	42.12 ± 3.48b	71.03 ± 1.12a	56.10 ± 0.36a
	T2	56.12 ± 0.67a	71.05 ± 1.12a	45.43 ± 0.15b
	T3	20.29 ± 3.62c	45.26 ± 3.98b	35.27 ± 0.29c
Azodchta indica (Neem)	T1	30.83 ± 0.00a	37.46 ± 1.14b	35.37 ± 0.39a
	T2	33.92 ± 1.27 a	43.9 ± 0.77a	39.83 ± 0.23a
	T3	18.52 ± 1.85 b	30.80 ±1.00c	36.37 ± 1.79a
Delonix regia	T1	25.74 ± 0.05b	57.69 ± 0.60b	35.33 ± 0.26b
	T2	35.17 ± 1.98a	62.82 ± 0.00a	40.23 ± 0.15a
	T3	26.94 ± 0.75b	33.74 ± 0.21c	32.60 ± 0.23c
Cassia fistula	T1	45.25 ± 1.05a	53.43 ± 0.43c	29.73 ± 0.23b
	T2	49.80 ± 2.24a	61.90 ± 0.29a	33.23 ± 0.35a
	T3	45.94 ± 3.18a	58.86 ± 0.70b	29.43 ± 0.33b

Note: %RC- AMF root colonization, SD- spore density. Different lowercase letters in the same rows represent significant difference at 0.05 level; Mean values followed by the same letter are not significantly different at $P \leq 0.05$. Mean ± Standard error.

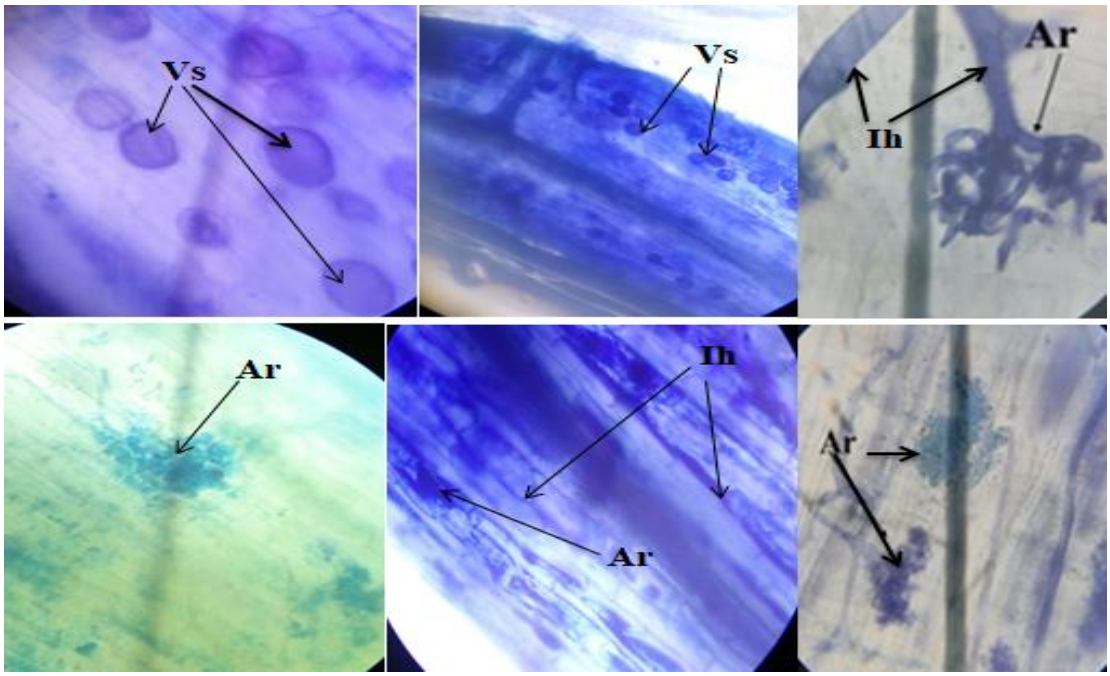


Figure 11: Microscopic picture of root colonization Intraradical hyphae (Ih), Arbuscule (Ar), and vesicles (Vs).

4.1.5. Comparison in Mycorrhizal dependency among treatment and plant species after 60 days and after 120 days

Comparing of mycorrhizal dependency among the treatments and the trees after 60 days and 120 days were subjected to two-way ANOVA (Table 10). MD after 60 days showed significant differences within treatments, which ranged between 27.92% and 43.75%. The highest mycorrhizal dependency was recorded in T2 and the mycorrhizal dependencies of the three treatments were 27.92%, 35.98% and 43.75% for T3, T1, and T2, respectively. In the same way mycorrhizal dependencies among the tree seedlings ranged between 27.76% and 46.99% and the highest mycorrhizal dependency was recorded in *C.fistula,* (46.99%) and lowest for *A.indica* (27.76%).

In the same pattern the mycorrhizal dependency among the treatments and the trees after 120 days were showed significantly differences among each other. Among treatments T1, T2, and T3 the result showed that 42.17%, 54.90% and 59.91% respectively. In the same ways mycorrhizal dependency among the plant species showed significantly differences between them, (37.39%, 51.42%, 58.06%, and 62.44% for *A.indica, D.regia, C.fistula* and *S.grandflora* respectively).

Table 9: Comparison MD among treatment and Tested plant species after 60 days and after 120 days

	MD After 60 days	MD After 120 days
Treatment		
T_1	35.99b	54.90b
T_2	43.75a	59.99a
T_3	27.92c	42.17c
Tested plant species		
S.grandflora	39.51b	62.44a
A.indica (Neem)	27.76c	37.39d
D.regia	29.28c	51.42c
C.fistula	46.99a	58.06b

Note: MD- mycorrhizal dependency, T_1- *A. abyssinica* of AMF, T_2 - *A. seyal*, T_3-*A. tortilis*. Different lowercase letters in the same column represent significant difference at 0.05 level; Mean values followed by the same letter are not significantly different at $P \leq 0.05$.

4.1.6. Correlation between the Parameters

To determine the potential of inoculums and seedling development response, a Pearson correlation analysis was performed between the parameters in each seedling (p□0.05). As a

consequence, Pearson's correlation results revealed that the plant growth parameters in each seedling have been strong positive and significant correlated. Furthermore, Pearson's correlation analyses revealed a high positive association between root colonization and plant height in all plant varieties of seedlings (Table: 11).

Moreover, Pearson's correlation results indicated also strong positive correlation between root colonization and plant root height. In the same trend, very strong positive correlations were found between shoot height and root height with spore density except in *A.indica* which showed only positive correlations in SD and FRM; SD and DRM as well as RC and DRM (Table 11).

Table 10: Pearson correlation coefficients between the parameters in each selected trees.

A.indica (Neem)

	SH(cm)	RH(cm)	NB(no.)	FSM(g)	FRM(g)	DSM(g)	DRM(g)	RC	SD
SH	1								
RH	.935**	1							
NL	.916**	.874**	1						
FSM	.925**	.964**	.911**	1					
FRM	.881**	.807**	.922**	.862**	1				
DSM	.831**	.936**	.814**	.966**	.794**	1			
DRM	.833**	.780**	.910**	.842**	.987**	.790**	1		
RC	.877**	.870**	.707*	.778**	.582*	.686*	0.498	1	
SD	.752**	.896**	.629*	.854**	0.509	.865**	0.469	.840**	1

D.regia

	SH(cm)	RH(cm)	NB(no.)	FSM(g)	FRM(g)	DSM(g)	DRM(g)	RC	SD
SH	1	.							
RH	.961**	1							
NL	.991**	.973**	1						
FSM	.876**	.887**	.905**	1					
FRM	.944**	.967**	.963**	.956**	1				
DSM	.930**	.897**	.941**	.951**	.958**	1			
DRM	.894**	.936**	.925**	.871**	.942**	.852**	1		
RC	.707*	.823**	.772**	.809**	.852**	.730**	.911**	1	.
SD	.795**	.894**	.850**	.889**	.923**	.830**	.941**	.963**	1

S.grandflora

	SH(cm)	RH(cm)	NB(no.)	FSM(g)	FRM(g)	DSM(g)	DRM(g)	RC	SD
SH	1								
RH	.928**	1							
NL	.897**	.881**	1	.					
FSM	.934**	.947**	.912**	1					
FRM	.951**	.940**	.961**	.952**	1				
DSM	.917**	.923**	.955**	.942**	.960**	1	.		
DRM	.953**	.968**	.916**	.962**	.971**	.933**	1		
RC	.743**	.742**	.941**	.806**	.852**	.882**	.765**	1	
SD	.889**	.852**	.988**	.912**	.952**	.944**	.890**	.954**	1

C.fistula

	SH(cm)	RH(cm)	NB(no.)	FSM(g)	FRM(g)	DSM(g)	DRM(g)	RC	SD
SH	1								
RH	.926**	1							
NL	.930**	.925**	1		.				
FSM	.908**	.849**	.814**	1					
FRM	.943**	.939**	.849**	.925**	1				
DSM	.915**	.879**	.960**	.821**	.867**	1			
DRM	.899**	.865**	.973**	.818**	.817**	.983**	1	.	
RC	.878**	.863**	.977**	.735**	.781**	.972**	.983**	1	
SD	.920**	.885**	.978**	.834**	.854**	.994**	.993**	.979**	1

Note: SH-shoot height, RH-Root height, NL - Number of Leaves, FSM Fresh Shoot Mass, FRM Fresh Root Mass, DSM- Dry Shoot Mass, DRM- Dry Root Mass, RC- Hyphal Root Colonization, and SD – Spore Density. **. Correlation is significant at the 0.01 level (2-tailed) and *. Correlation is significant at the 0.05 level (2-tailed)

4.2. DISCUSSION

The current study focused on evaluating the effects of AMF inoculation isolated from highland and lowland areas to improve the growth and survival of the seedlings of the selected tree seedlings (*A.indica, D.regia, S.grandflora and C.fistula*) of GLP of Ethiopia under low fertility of soil.

In this study, rhizosphere of selected acacia species was used as source of AMF inoculum. The amount of AMF in soil samples collected from sample locations was evaluated before trap culture was established to identify the initial mycorrhizal status of the soil sample. *A. seyal* rhizosphere soil had the highest average spore density of AMF (18.89 spores g^{-1} soil), followed by *A.tortilis* (13.73 spores g^{-1} soil) from lowland (Batu) and *A. abyssinica* (10.55 g^{-1} soil) from highland (Sululta). Subsequently, this finding agrees with Zerihun *et al.*, (2013), who found that *A. seyal* from Batu has showed the highest average spore density and root colonization. Similarly, in this study after trap culture *A. seyal* considerably had the greatest average AMF spore density and root colonization (32.41 spores g^{-1} soil and 94%) and *A.tortilis* (13.73 spores g^{-1} soil and 62.93%) from lowland (Batu) and *A. abyssinica* (10.55 g^{-1} soil and 56.96%) from highland (Sululta). As a result, the highest source of AMF spores g^{-1} soil inoculum was the rhizosphere soil of *A. seyal* from lowland (Batu) and the lowest in *A. abyssinica* from highland (Sululta).

The results obtained from *A.indica, D.regia, S.grandflora and C.fistula* seedlings after inoculation of with AMF for 60 and 120 days showed significantly greater responses in all parameters over the control (T_4). This is similar with the previous report by Chen *et al.*,

(2020), who reported that AMF inoculation of *Catalpa bungei* in drought stress conditions significantly increased the growth parameters of the tree.

Although the growth rate of the trees (*A.indica, D.regia, S. grandflora* and *C. fistula*) varied they showed better growth in all parameters when treated by treatment T_2, followed by treatment T_1. In contrast, the control (T_4) showed the lowest growth rate in all parameters of the trees. This indicates the effects of AM fungi on plant growth parameters, which clearly showed positive responses in all the inoculated trees compared to the control. Aggarwal *et al*., (2011) reported that this may be due to the fact that AMF facilitate the soil structure which helps the plant roots in the acquisition of Phosphorus (P) and other mineral nutrients from the soil. In addition, their function ranges from stress alleviation to bioremediation in soils polluted with heavy metals. They may also enhance the protection of plants against pathogens and increase the plant diversity.

From the treatments, AMF from *A. seyal* (T_2) showed significantly greater responses in all plant growth parameters and mycorrhizal parameters over the (T_1 and T_3) in addition to control (T_4). This may be due to high mycorrhizal presence in the soil improving the plant growth. Similarly, (Michelsen, 1993), documented results that showed AMF inoculation in unsterile soil definitely boosted the growth of the seedlings, where he grew *Acacia abyssinica and Acacia sieberiana* for three and four months, respectively, in different soils; soil inoculated with a tropical or with a temperate isolate of the AM fungus Glomus *mosseae*. In comparison to seedlings in inoculation with the tropical *G. mosseae* isolate raised the shoot dry weight of *A. sieberiana*. Likewise inoculation with the tropical *G. mosseae* also increased the shoot dry weight of *A. abyssinica*.

The shoots and roots of the seedlings treated with AMF increased not only in height and mass but also showed significantly higher colonization by AMF and spore density than control. This indicated that trees inoculated by AMF improved in all aspects of the growth parameters directly by the support of the AMF. This result is in agreement with a report by Kumar *et al*., (2014), who stated that AMF (*Glomus fasciculatum*) inoculum improved directly plant height, plant spread, and number of branches; reduced disease incidence; and increased tuber dry mass of *Coleus forskohlii*. Likewise in the current study, after 60 days of inoculation the results of root colonization by AMF showed in *Sesbania grandflora* ranged between 20.29% and 56.12% which is the highest in trees inoculated with T_2. Similarly the results of root colonization by AMF after 120 days ranged between 45.26 %

and 71.05 % here also T_2 is the highest. Likewise spore density ranged between 35.27 and 56.10 g^{-1} soil which is the highest in trees inoculated by T_1 which is the highest among others (Table 9).

Accordingly after 120 days of inoculation the results of root colonization by AMF showed that *A.indica (Neem),* ranged between 30.83 % and 43.9 % which showed significantly higher root colonization over control. This meant that ultimate coherence was observed between AMF root colonization and plant growth parameters. Additionally, very strong positive correlations ($p \leq 0.05$) in AMF root colonization with AMF spore density and mycorrhizal dependency of the trees were found. Which is similar with previous work reported by Nanjundappa *et al*., (2019).

Mycorrhizal dependency is related in part to the morphology of the plant root system. Plants having less extensive root systems are often dependent on mycorrhiza than plants with extensive fibrous roots (Zerihun *et al*., 2020), Such plants are often obligate mycorrhizal crops that are unable to complete their life cycle in the absence of AMF because of insufficient P uptake and hence insufficient growth (Shuab *et al*., 2014). The mycorrhizal dependency percentages observed in all plants (*A.indica, D.regia, S.grandflora,* and *C.fistula*) after 60 and 120 days increased, although not equally. The mycorrhizal dependency percentages increased from ranges of 37.39% to 62.44%. This is probably due to the shortage (lack) of nutrient in the soil and to overcome these constraints the plant increase their symbiosis relationship with AMF. Previously Njeru, (2018), reported that AMF facilitate soil structure and quality mainly through the external hyphal network which creates a skeletal structure that enmeshes the soil particles, and by production of glomalin related soil protein (GRSP) which binds soil particles together.

In regards to evaluating the differences in AMF response (mycorrhizal dependency), root colonization, and spore density among the selected trees, the trees showed high growth response, in root colonization, and spore density. The highest RC values were recorded in *S.grandflora* grown under T_2 and followed by T_1 and T_3 inoculations, respectively (Table 9). Likewise the value of RC showed higher response in *D.regia,* followed by *C.fistula* and, *A.indica* respectively. Moreover, the highest SD values were recorded in *C.fistula* and then, next to *C.fistula,* followed by *S.grandflora, D.regia* and *A.indica,* respectively. This result may be due to the fact that legumes require more P than non-leguminous plants because maintaining the biological N_2 fixation process in root nodules is highly dependent

on P. In this case, *S.grandflora, D.regia* and *C.fistula* are legume plants for this reason these plants had more associations with AMF that may have positively influenced the plant P nutrition and, indirectly, the biological nitrogen fixation process (Bini *et al*., 2018).

Additionally, in this study all parameters were positively correlated with each other. And colonization by AMF had positive effects on the growth parameters of the selected trees seedlings and on spore density and mycorrhizal dependency. In addition, strong positive correlations ($p \leq 0.05$) in AMF root colonization with AMF spore density and mycorrhizal dependency of the trees were found. This was also in agreement with previous report of Bhale, (2017) who studied relation between root colonization percentage and spore density in the rhizosphere soil, and found strong positive correlation in Chilli ($r = 0.799$) and Brinjal ($r = 0.899$).

Comparable to other studies, this study also found that AMF colonization with total dry biomass, number leaves, root height, shoot height, fresh shoot mass, fresh root mass, dry shoot mass and dry root mass were positively correlated. This indicated that likely this growth benefit is due to the large extracellular hyphae AMF network, which transports water and nutrients to roots, increasing their absorption range and helping plants in the low nutrient soil (Chen *et al.*, 2020). These findings were quite in agreement with the results reported by Pei *et al*. (2020) who showed positive correlation of AMF colonization with Flavonoids and plant biomass in Chinese tallow tree. They reported that the plant biomass and AMF colonization rate were positively correlated ($r = +0.15$, $P = 0.0346$).

Furthermore, in this investigation, all parameters were directly correlated. AMF colonization had a favorable impact on the selected tree seedlings' growth parameters, as well as spore density and mycorrhizal dependence. Other studies had previously revealed similar results (Bhale, 2017; Chen et al., 2020 and Pei *et al*., 2020). AMF root colonization was also found to have very high positive relationships ($p \leq 0.05$) with AMF spore density and tree mycorrhizal density. This was also in line with a previous study by (Bhale, 2017), who found a substantial positive association between root colonization percentage and spore density in the rhizosphere soil in Chilli ($r = 0.799$) and Brinjal ($r = 0.899$). In general all growth parameters had strong positive and significant correlation with each other and with mycorrhizal parameters. Except in *A. indica* SD and FRM; SD and DRM as well as RC and DRM only showed positive correlation regarding to the trees association with AMF, legume plants (*D.regia, S.grandflora* and *C.fistula*) showed greater response

compared that of non-legume plant (*A.indica*) (Table 11). Likewise AMF from lowland, *A. seyal* showed the greatest in AMF.

5. CONCLUSION AND RECOMMENDATION

5.1. CONCLUSIONS

In the present study, AMF inoculants from rhizosphere soil of Acacia trees found in lowlands (*A. seyal* and *A. tortilis*) and highlands (*A. abyssinica*) were collected and spore density was checked prior to the preparation of trap culture. Application of AMF inoculants which were isolated from Acacia trees of *A. seyal* and *A. abyssinica* rhizosphere showed better influence on the growth of the plants (*A. indica, D. regia, S. grandflora, and C. fistula)* respectively.

It is evident from the result that the application of AMF has greatly improved seedling growth and biomass of the seedlings. This could be important to development of such a sustainable bio fertilizer technology for maximum growth and survival seedlings in Green Legacy program of Ethiopia. Therefore, from the present research, it could be concluded that application of the AMF Inocula used in this experiment demonstrate the possibility of increasing the survival and growth of seedlings.

In this regard, plants with inoculation of AMF had a significant increase in many parameters including height, total fresh and dry mass. In addition, plants with inoculation AMF also had a significant increase in root colonization and spore density. Generally, it can be concluded that among the treatments, AMF from *A.seyal* from lowland showed positive responses than the rest treatment and followed by *A.abyssinica* from highland.

The leguminous plants *S.grandflora, D.regia,* and *C.fistula* had more associations with AMF than that of the non-leguminous plant *A.indica,* which may have positively influenced their P nutrition and, indirectly, the biological nitrogen fixation process, which in turn translated into their growth parameters.

5.2. RECOMMENDATIONS

During this study, the potential of survival growth of the plant seedlings on field and tolerance to environmental stress were not evaluated. Therefore, it is necessary in the future to evaluate their effectiveness under different environmental conditions and against different field trials. Moreover, the application of the inoculums showed increases in all growth parameters, however during this experiment no investigation was carried out regarding the effect of these inoculums in nutrient acquisition. Therefore, it should be determined what the potential of this inoculum on these parameters. However, the application of present findings required to be confirmed by further studies under field conditions.

6. REFERENCE

Adamu, A., Assefa, F., Mariam, A. H., & Bekele, E. (2001). Studies of rhizobium inoculation and fertilizer treatment on growth and production of faba bean (*Vicia faba*) in some "yield-depleted" and "yield sustained" regions of Semien Shewa. In *SINET: Ethiopian Journal of Science* (Vol. 24, Issue 2). https://doi.org/10.4314/sinet.v24i2.18186

Adeleke, A. B. (2010). Effect of Arbuscular Mycorrhizal Fungi and Plant Growth-Promoting Rhizobacteria on Glomalin Production. *Production*, 166.

Adhikari, K., Bhandari, S., Niraula, D., & Shrestha, J. (2020). Use of neem (Azadirachta indica A. Juss) as a biopesticide in agriculture: A review. *Journal of Agriculture and Applied Biology*, *1*(2), 100–117. https://doi.org/10.11594/jaab.01.02.08

Aggarwal, A., Kadian, N., Tanwar, A., Yadav, A., & Gupta, K. K. (2011). Role of arbuscular mycorrhizal fungi (AMF) in global sustainable development. *Journal of Applied and Natural Science*, *3*(2), 340–351. https://doi.org/10.31018/jans.v3i2.211

Ahmed, S., Bamofleh, S., & Munshi, M. (1989). Cultivation of Neem (Azadirachta indica, Meliaceae) in Saudi Arabia. *Economic Botany*, *43*(1), 35–38. https://doi.org/10.1007/BF02859323

Akiyama, H., Yan, X., & Yagi, K. (2010). Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N2O and NO emissions from agricultural soils: Meta-analysis. *Global Change Biology*, *16*(6), 1837–1846. https://doi.org/10.1111/j.1365-2486.2009.02031.x

Akiyama, K., Matsuzaki, K. I., & Hayashi, H. (2005). Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature*, *435*(7043), 824–827. https://doi.org/10.1038/nature03608

Alemayehu, N. (2020). East African Journal of Sciences (2020) The Use of Biofertilizer by Smallholder Farmers and its Impact on Productivity of Pulse- Cereal Cropping System in Arsi Zone , Oromia Regional State , Southeastern Ethiopia. *East African Journal of Sciences*, *14*, 1–12.

Anon. (1992). *Annual Report 1991, & International Centre for Research in Agroforestry*

ICRAF. Nairobi. (1992). In: Ndungu J. N. and Boland D. J. (1994). Sesbania seed collections in Southern Africa. Developing a model for collaboration between a CGIAR Centre and NARS.

Argaw, A. (2013). Evaluation of symbiotic effectiveness and size of resident Rhizobium leguminosarum var. viciae nodulating lentil (Lens culinaris medic) in some Ethiopian soils. *Archives of Agronomy and Soil Science*, *59*(7), 929–945. https://doi.org/10.1080/03650340.2012.690144

Asif, M., Nawaz, H., Nadeem, R., Mahmood, K., & Asif, A. (2007). Cassia fistula (Golden Shower): A Multipurpose Ornamental Tree. *Floriculture and Ornamental Biotechnology*, *1*(January), 20–26. http://www.globalsciencebooks.info/Online/GSBOnline/images/0706/FOB_1(1)/FOB_1(1)20-26o.pdf

Asmelash, F., Bekele, T., & Birhane, E. (2016). The potential role of arbuscular mycorrhizal fungi in the restoration of degraded lands. *Frontiers in Microbiology*, *7*(JUL), 1–15. https://doi.org/10.3389/fmicb.2016.01095

Asmelash, F., Bekele, T., Kebede, F., & Belay, Z. (2021). The arbuscular mycorrhizal fungi status of selected tree nurseries in the Ethiopian highlands. *Journal of Forestry Research*, *32*(3), 1189–1201. https://doi.org/10.1007/s11676-020-01169-9

Azani, N., Babineau, M., Bailey, C. D., Banks, H., Barbosa, A. R., Pinto, R. B., Boatwright, J. S., Borges, L. M., Brown, G. K., Bruneau, A., Candido, E., Cardoso, D., Chung, K. F., Clark, R. P., Conceição, A. D. S., Crisp, M., Cubas, P., Delgado-Salinas, A., Dexter, K. G., … Zimmerman, E. (2017). A new subfamily classification of the leguminosae based on a taxonomically comprehensive phylogeny. *Taxon*, *66*(1), 44–77. https://doi.org/10.12705/661.3

Bagyaraj, D. J. (2014). Mycorrhizal fungi. *Proceedings of the Indian National Science Academy*, *80*(2), 415–428. https://doi.org/10.16943/ptinsa/2014/v80i2/55118

Bahiru, Z., 1991. A History of Modern Ethiopia. Addis Ababa University Press, Addis Ababa.

Bakhoum, N., Fall, D., Fall, F., Diouf, F., Hirsch, A. M., Balachandar, D., & Diouf, D. (2018). Senegalia senegal (synonym: Acacia senegal), its importance to sub-Saharan

Africa, and its relationship with a wide range of symbiotic soil microorganisms. *South African Journal of Botany*, *119*, 362–368. https://doi.org/10.1016/j.sajb.2018.10.007

Balliu, A., Sallaku, G., & Rewald, B. (2015). AMF inoculation enhances growth and improves the nutrient uptake rates of transplanted, Salt-stressed tomato seedlings. *Sustainability (Switzerland)*, *7*(12), 15967–15981. https://doi.org/10.3390/su71215799

Barrow, C. J. (2012). Biochar: Potential for countering land degradation and for improving agriculture. *Applied Geography*, *34*, 21–28. https://doi.org/10.1016/j.apgeog.2011.09.008

Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., Ahmed, N., & Zhang, L. (2019). Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance. *Frontiers in Plant Science*, *10*(September), 1–15. https://doi.org/10.3389/fpls.2019.01068

Belay, Z., & Assefa, F. (2011). Symbiotic and phenotypic diversity of Rhizobium leguminosarum bv. viciae from Northern Gondar, Ethiopia. *African Journal of Biotechnology*, *10*(21), 4372–4379. https://doi.org/10.4314/ajb.v10i21.

Besserer, A., Puech-Pagès, V., Kiefer, P., Gomez-Roldan, V., Jauneau, A., Roy, S., Portais, J. C., Roux, C., Bécard, G., & Séjalon-Delmas, N. (2006). Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. *PLoS Biology*, *4*(7), 1239–1247. https://doi.org/10.1371/journal.pbio.0040226

Bhale, U. (2017). *Mycorrhizal association of Solanaceous*. *April 2016*.

Bindraban, P. S., van der Velde, M., Ye, L., van den Berg, M., Materechera, S., Kiba, D. I., Tamene, L., Ragnarsdóttir, K. V., Jongschaap, R., Hoogmoed, M., Hoogmoed, W., van Beek, C., & van Lynden, G. (2012). Assessing the impact of soil degradation on food production. *Current Opinion in Environmental Sustainability*, *4*(5), 478–488. https://doi.org/10.1016/j.cosust.2012.09.015

Bingham, M. A., & Biondini, M. (2011). Nitrate leaching as a function of plant community richness and composition, and the scaling of soil nutrients, in a restored temperate grassland. *Plant Ecology*, *212*(3), 413–422. https://doi.org/10.1007/s11258-010-9832-8

Bini, D., Santos, C. A. dos, Silva, M. C. P. da, Bonfim, J. A., Cardoso, E. J. B. N., & Andreote, F. D. (2018). Intercropping Acacia mangium stimulates AMF colonization and soil. *Sci. Agric. V*, *75*(2), 102–110. http://dx.doi.org/10.1590/1678-992X-2016-0337

Bonfante, P., & Genre, A. (2010). Mechanisms underlying beneficial plant - Fungus interactions in mycorrhizal symbiosis. *Nature Communications*, *1*(4), 1–11. https://doi.org/10.1038/ncomms1046

Branzini, A., González, R. S., & Zubillaga, M. (2012). Absorption and translocation of copper, zinc and chromium by Sesbania virgata. *Journal of Environmental Management*, *102*, 50–54. https://doi.org/10.1016/j.jenvman.2012.01.033

Brundrett, M., Bougher, N., Dell, B., Grove, T., & Malajczuk, N. (eds. . (2004). *Working with Mycorrhizas in Forestry and Agriculture* (Issue January 1995).

Calabrese, S., Cusant, L., Sarazin, A., Niehl, A., Erban, A., Brulé, D., Recorbet, G., Wipf, D., Roux, C., Kopka, J., Boller, T., & Courty, P. E. (2019). Imbalanced Regulation of Fungal Nutrient Transports According to Phosphate Availability in a Symbiocosm Formed by Poplar, Sorghum, and Rhizophagus irregularis. *Frontiers in Plant Science*, *10*. https://doi.org/10.3389/fpls.2019.01617

Chen, W., Meng, P., Feng, H., & Wang, C. (2020). Effects of arbuscular mycorrhizal fungi on growth and physiological performance of catalpa bungei C.A.Mey. under drought stress. *Forests*, *11*(10), 1–29. https://doi.org/10.3390/f11101117

Davison, H. K., Maraist, C. C., Hamilton, R. H., & Bing, M. N. (2012). To Screen or Not to Screen? Using the Internet for Selection Decisions. *Employee Responsibilities and Rights Journal*, *24*(1), 1–21. https://doi.org/10.1007/s10672-011-9178-y

De Silva, C. S., Koralage, I. S. A., Weerasinghe, P., & Silva, N. R. N. (2015). The Determination of Available Phosphorus in Soil: A Quick and Simple Method. *OUSL Journal*, *8*(0), 1. https://doi.org/10.4038/ouslj.v8i0.7315

Dessalegn, R., 1994. The unquiet countryside: the collapse of “Socialism” and the rural agitation 1990 and 1991. In: Zegeye, A., Pausewang, S. (Eds.), Ethiopia in Change: Peasantry, Nationalism and Democracy. British Academic Press, London.

Dessalegn, R., 2004. Searching for Tenure Security? The Land System and New Policy Initiatives in Ethiopia. Forum for Social Studies, Discussion Paper 12.

De Vries, F. T., & Bardgett, R. D. (2015). *Biodiversity Climate change Impacts Report Card Technical paper 16 Climate change effects on soil biota in the UK*. http://www.manchester.ac.uk/research

Diagne, N., Ngom, M., Djighaly, P. I., Fall, D., Hocher, V., & Svistoonoff, S. (2020). Roles of arbuscular mycorrhizal fungi on plant growth and performance: importance in biotic and abiotic stressed regulation. *Diversity*, *12*(10), 1–25. https://doi.org/10.3390/d12100370

dos Santos, R. S., Ferreira, J. S., & Scoriza, R. N. (2017). Inoculum production of arbuscular mycorrhizal fungi native to soils under different forest covers. *Revista Ceres*, *64*(2), 197–204. https://doi.org/10.1590/0034-737X201764020013

Eba, M. S. (2017). Growth and survival rate of endemic trees of Ethiopia: Olea africana and Hagenia abysinicca in Lake Haramaya Watershed, Eastern Ethiopia. *Journal of Horticulture and Forestry*, *9*(5), 33–39. https://doi.org/10.5897/jhf2017.0486

Editor, R. A. (2013). *Symbiotic Endophytes*. *37*, journ. https://doi.org/10.1007/978-3-642-39317-4

Evans, D. O. (1990). *What is Sesbania? Botany, taxonomy, plant geography, and natural history of the perennial members of the genus.* (B. Macklin & D. O. Evans P (eds.); In: Mackli). Nitrogen Fixing Tree Association.

Evans, D. O., & Rotar, P. p. (1987). *Sesbania in Agriculture* (L. P. Donald (ed.); Westview T). Westview Press / Boulder and London.

Finlay, L. (2008). A dance between the reduction and reflexivity: Explicating the "phenomenological psychological attitude." *Journal of Phenomenological Psychology*, *39*(1), 1–32. https://doi.org/10.1163/156916208X311601

Fisseha, A., Tamrat, B., & Zerihun, B. (2019). Comparative field survival and growth of selected Ethiopian native tree species and the effect of whole soil arbuscular mycorrhizal fungi inoculation. *Journal of Horticulture and Forestry*, *11*(2), 19–31. https://doi.org/10.5897/jhf2018.0573

Gajalakshmi, S., & Abbasi, S. A. (2004). Neem leaves as a source of fertilizer-cum-pesticide vermicompost. *Bioresource Technology*, *92*(3), 291–296. https://doi.org/10.1016/j.biortech.2003.09.012

Gelan, E. (2021). Municipal Solid Waste Management Practices for Achieving Green Architecture Concepts in Addis Ababa, Ethiopia. *Technologies*, *9*(3), 48. https://doi.org/10.3390/technologies9030048

Gerdemann, J. W., & Nicolson, T. H. (1963). Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society*, *46*(2), 235–244. https://doi.org/10.1016/s0007-1536(63)80079-0

Giovannini, L., Palla, M., Agnolucci, M., Avio, L., Sbrana, C., Turrini, A., & Giovannetti, M. (2020). Arbuscular mycorrhizal fungi and associated microbiota as plant biostimulants: Research strategies for the selection of the best performing inocula. *Agronomy*, *10*(1). https://doi.org/10.3390/agronomy10010108

Girma, N., Mekibib, F., Fikre, A., Keneni, G., Rao, G., & Ojiewo, C. (2019). Estimate of Heritability and Correlation Analysis for Nitrogen Fixation, Yield and Associated Traits in Chickpea (. *Int. J. Eng. Res.*, *10*(5), 1266–1280.

Goetten, L. C., Moretto, G., & Stürmer, S. L. (2016). Influence of arbuscular mycorrhizal fungi inoculum produced on-farm and phosphorus on growth and nutrition of native woody plant species from Brazil. *Acta Botanica Brasilica*, *30*(1), 9–16. https://doi.org/10.1590/0102-33062015abb0175

Gomes, M. G. M., King, J. G., Nunes, A., Colegrave, N., & Hoffmann, A. A. (2019). The effects of individual nonheritable variation on fitness estimation and coexistence. *Ecology and Evolution*, *9*(16), 8995–9004. https://doi.org/10.1002/ece3.5437

Gomez-Roldan, V., Fermas, S., Brewer, P. B., Puech-Pagès, V., Dun, E. A., Pillot, J. P., Letisse, F., Matusova, R., Danoun, S., Portais, J. C., Bouwmeester, H., Bécard, G., Beveridge, C. A., Rameau, C., & Rochange, S. F. (2008). Strigolactone inhibition of shoot branching. *Nature*, *455*(7210), 189–194. https://doi.org/10.1038/nature07271

Govindarajulu, M., Pfeffer, P. E., Jin, H., Abubaker, J., Douds, D. D., Allen, J. W., Bücking, H., Lammers, P. J., & Shachar-Hill, Y. (2005). Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature*, *435*(7043), 819–823.

https://doi.org/10.1038/nature03610

Habte, M. (2000). Mycorrhizal Fungi and Plant Nutrition. *Plant Nutrient Management in Hawaii's Soils, Approaches for Tropical and Subtropical Agriculture*, 127–131.

Hakim, S., Naqqash, T., Nawaz, M. S., Laraib, I., Siddique, M. J., Zia, R., Mirza, M. S., & Imran, A. (2021). Rhizosphere Engineering With Plant Growth-Promoting Microorganisms for Agriculture and Ecological Sustainability. *Frontiers in Sustainable Food Systems*, *5*(July). https://doi.org/10.3389/fsufs.2021.617157

Hanif, U., Syed, S. H., Ahmad, R., & Malik, K. A. (2010). Economic impact of climate change on the agricultural sector of Punjab. *Pakistan Development Review*, *49*(4), 771–796. https://doi.org/10.30541/v49i4iipp.771-798

Hildebrandt, U., Kaldorf, M., & Bothe, H. (1999). The zinc violet and its colonization by arbuscular mycorrhizal fungi. *Journal of Plant Physiology*, *154*(5–6), 709–717. https://doi.org/10.1016/S0176-1617(99)80249-1

Hodge, A., Campbell[2], C. D., & Fitter, A. H. (2001). An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material Arbuscular mycorrhizal fungi (order Glomales), which form mycorrhizal symbioses with two out of three of all plant species. *NATURE Www.Nature.Com*, *413*(20), 297–299.

Hoffmann, J. H., & Moran, V. C. (1991). Biological control of Sesbania punicea (Fabaceae) in South Africa. *Agriculture, Ecosystems and Environment*, *37*(1–3), 157–173. https://doi.org/10.1016/0167-8809(91)90144-M

IJdo, M., Cranenbrouck, S., & Declerck, S. (2011). Methods for large-scale production of AM fungi: Past, present, and future. *Mycorrhiza*, *21*(1), 1–16. https://doi.org/10.1007/s00572-010-0337-z

Judith Prieto Méndez, César A. González Ramírez, A. D. R. G. and F. P. G. (2009). Tropical and Subtropical Agroecosystems. *Tropical and Subtropical Agroecosystems*, *10*(1870–0462), 29–44. http://www.redalyc.org/pdf/939/93911243003.pdf

Kassa, H. (2018). Reshaping the Terrain Landscape Restoration in Ethiopia. *Global Landscape Forum*, *August*, 1–4.

Keller, E., 2002. Ethnic federalism, fiscal reform development and democracy in Ethiopia. African Journal of Political Scene 7, 1.

Keymer, A., Pimprikar, P., Wewer, V., Huber, C., Brands, M., Bucerius, S. L., Delaux, P. M., Klingl, V., von Röpenack-Lahaye, E., Wang, T. L., Eisenreich, W., Dörmann, P., Parniske, M., & Gutjahr, C. (2017). Lipid transfer from plants to arbuscular mycorrhiza fungi. *ELife*, *6*, 1–33. https://doi.org/10.7554/eLife.29107

Kim, L., Han, K., Cho, H., Oh, D., & Beach, H. (2006). *A Precision Test of Hydrometer Method for Determining Soil Texture*. *39*(5), 315–320.

Koide, R. T., & Kabir, Z. (2000). Extraradical hyphae of the mycorrhizal fungus Glomus intraradices can hydrolyse organic phosphate. *New Phytologist*, *148*(3), 511–517. https://doi.org/10.1046/j.1469-8137.2000.00776.x

Kumar, S., Bauddh, K., Barman, S. C., & Singh, R. P. (2014). Organic matrix entrapped bio-fertilizers increase growth, productivity, and yield of Triticum aestivum L. and transport of NO3-, NO2-, NH4+ and PO4-3 from soil to plant leaves. *Journal of Agricultural Science and Technology*, *16*(2), 315–329.

Kumar, V. S., & Navaratnam, V. (2013). Neem (Azadirachta indica): Prehistory to contemporary medicinal uses to humankind. *Asian Pacific Journal of Tropical Biomedicine*, *3*(7), 505–514. https://doi.org/10.1016/S2221-1691(13)60105-7

Kwesiga, F. R., Franzel, S., Place, F., Phiri, D., & Simwanza, C. P. (1999). Sesbania sesban improved fallows in eastern Zambia: Their inception, development and farmer enthusiasm. *Agroforestry Systems*, *47*(1–3), 49–66. https://doi.org/10.1023/a:1006256323647

Lies, A., Delteil, A., Prin, Y., & Duponnois, R. (2018). Role of Rhizospheric Microbes in Soil. *Role of Rhizospheric Microbes in Soil*, 277–298. https://doi.org/10.1007/978-981-10-8402-7

Lin, S. M., Liang, H. Y., & Hommersand, M. H. (2008). Two types of auxiliary cell ampullae in Grateloupia (Halymeniaceae, Rhodophyta), including G. taiwanensis sp. nov. and G. orientalis sp. nov. from Taiwan based on rbcL gene sequence analysis and cystocarp development. *Journal of Phycology*, *44*(1), 196–214. https://doi.org/10.1111/j.1529-8817.2007.00443.x

Lugenja, M. M. S. (1988). *Forage germplasm collection in Tanzania. Paper presented at the First TALIRO Annual Scientific Conference, Arusha, February 1988*.

Mahamat, O. B., Younes, S., Otchom, B. B., Franzel, S., Mahamat, A.-D. O., Fadoua, A., & Soumaya, E. ismaili. (2021). Ecology, Morphology, Distribution, and Use of Sesbania tchadica (Sesbania Sesban) from the Republic of Chad: A Review. *Research Square*. https://doi.org/10.21203/rs.3.rs-543115/v1

Mahari, A. (2014). Factors Affecting Survival of Tree Seedlings in the Drylands of Northern Ethiopia. *Journal of Natural Sciences Research*, *4*(16), 26–29.

Maherali, H., & Klironomos, J. N. (2007). Influence of phylogeny on fungal community assembly and ecosystem functioning. *Science*, *316*(5832), 1746–1748. https://doi.org/10.1126/science.1143082

Marx, D., Marrs, L., & Cordell, C. (2002). Practical use of the mycorrhizal fungal technology in forestry, reclamation, arboriculture, agriculture, and horticulture. *Dendrobiology*, *47*, 27–40.

McGonigle, T. P., MILLERS, S. M. H., EVANS, D. G., SWAN, J. A., & FAIRCHILD, G. L. (1990). A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol*, *115*, 495–503. https://doi.org/doi:10.1111/j.1469-8137.1990.tb00476.x

Meersmans, J., Van Wesemael, B., & Van Molle, M. (2009). Determining soil organic carbon for agricultural soils: A comparison between the Walkley & Black and the dry combustion methods (north Belgium). *Soil Use and Management*, *25*(4), 346–353. https://doi.org/10.1111/j.1475-2743.2009.00242.x

MEFCC. (2018). *National Forest Sector Development Program*. *I*, 151. https://www.et.undp.org/content/dam/ethiopia/docs/2018/National Forest Sector Development Programme Volume III Synthesis report.pdf

Mekonnen, Z., & Environment, E. (2019). *Perceived and Observed Changes in Climate and Environment , Vulnerability and Adaptation in Central Rift Valley , Ethiopia*. *June 2018*. https://doi.org/10.13140/RG.2.2.10115.04644

Michelsen, A. (1993). Growth improvement of Ethiopian acacias by addition of vesicular-

arbuscular mycorrhizal fungi or roots of native plants to non-sterile nursery soil. *Forest Ecology and Management*, *59*(3–4), 193–206. https://doi.org/10.1016/0378-1127(93)90002-5

Millar, N. S., & Bennett, A. E. (2016). Stressed out symbiotes: hypotheses for the influence of abiotic stress on arbuscular mycorrhizal fungi. *Oecologia*, *182*(3), 625–641. https://doi.org/10.1007/s00442-016-3673-7

Mukhongo, R. W., Tumuhairwe, J. B., Ebanyat, P., AbdelGadir, A. H., Thuita, M., & Masso, C. (2016). Production and use of arbuscular mycorrhizal fungi inoculum in sub-Saharan Africa: Challenges and ways of improving. *International Journal of Soil Science*, *11*(3), 108–122. https://doi.org/10.3923/ijss.2016.108.122

Mulissa, J. M., Carolin, R. L., Ruth, A. S., & Fassil, A. (2016). Phosphate solubilization and multiple plant growth promoting properties of rhizobacteria isolated from chickpea (Cicer aeritinum L.) producing areas of Ethiopia. *African Journal of Biotechnology*, *15*(35), 1899–1912. https://doi.org/10.5897/ajb2015.15172

Nanjundappa, A., Bagyaraj, D. J., Saxena, A. K., Kumar, M., & Chakdar, H. (2019). Interaction between arbuscular mycorrhizal fungi and Bacillus spp. In soil enhancing growth of crop plants. *Fungal Biology and Biotechnology*, *6*(1), 1–10. https://doi.org/10.1186/s40694-019-0086-5

Narayanasamy, P. (2001). Indigenous Pest Suppression. *Biocontrol Potential and Its Exploitation in Sustainable Agriculture*, 87–111. https://doi.org/10.1007/978-1-4615-1377-3_7

Nayak, A. (2008). On the way to theory: A processual approach. *Organization Studies*, *29*(2), 173–190. https://doi.org/10.1177/0170840607082227

Ndungu, J. N., & Boland, D. J. (1994). Sesbania seed collections in Southern Africa - Developing a model for collaboration between a CGIAR Centre and NARS. *Agroforestry Systems*, *27*(2), 129–143. https://doi.org/10.1007/BF00705470

Njeru, E. M. (2018). Exploiting diversity to promote arbuscular mycorrhizal symbiosis and crop productivity in organic farming systems. *AIMS Agriculture and Food*, *3*(3), 280–294. https://doi.org/10.3934/AGRFOOD.2018.3.280

Nogueira, M. A., & Cardoso, E. J. B. N. (2006). Plant growth and phosphorus uptake in mycorrhizal rangpur lime seedlings under different levels of phosphorus. *Pesquisa Agropecuaria Brasileira*, *41*(1), 93–99. https://doi.org/10.1590/S0100-204X2006000100013

Ortega, U., Duñabeitia, M., Menendez, S., Gonzalez-Murua, C., & Majada, J. (2004). Effectiveness of mycorrhizal inoculation in the nursery on growth and water relations of Pinus radiata in different water regimes. *Tree Physiology*, *24*(1), 65–73. https://doi.org/10.1093/treephys/24.1.65

Otieno, K. (1987). *Sesbania germplasm collection in Tanzania. A report submitted to IDRC, Regional Office, Nairobi, Kenya*.

Palenzuela, J., Azcón-Aguilar, C., Barea, J.-M., da Silva, G. A., & Oehl, F. (2013). Septoglomus altomontanum, a new arbuscular mycorrhizal fungus from mountainous and alpine areas in Andalucía (southern Spain). *IMA Fungus*, *4*(2), 243–249. https://doi.org/10.5598/imafungus.2013.04.02.09

Parniske, M. (2008). Arbuscular mycorrhiza: The mother of plant root endosymbioses. *Nature Reviews Microbiology*, *6*(10), 763–775. https://doi.org/10.1038/nrmicro1987

Pei, Y., Siemann, E., Tian, B., & Ding, J. (2020). Root flavonoids are related to enhanced AMF colonization of an invasive tree. AoB PLANTS, 12(1), 1–7.

Peng, S., Guo, T., & Liu, G. (2013). The effects of arbuscular mycorrhizal hyphal networks on soil aggregations of purple soil in southwest China. *Soil Biology and Biochemistry*, *57*, 411–417. https://doi.org/10.1016/j.soilbio.2012.10.026

Pennisi, E. (2004). The birth of the nucleus. *Science*, *305*(5685), 766–768. https://doi.org/10.1126/science.305.5685.766

Powell, J. R., & Rillig, M. C. (2018). Biodiversity of arbuscular mycorrhizal fungi and ecosystem function. *New Phytologist*, *220*(4), 1059–1075. https://doi.org/10.1111/nph.15119

Raf, H., Service, U. F., & Family, F. B. (1994). *Delonix regia*. 433–435.

Rao, M. R., Nair, P. K. R., & Ong, C. K. (1997). Biophysical interactions in tropical agroforestry systems. *Agroforestry Systems*, *38*(1–3), 3–50.

https://doi.org/10.1023/a:1005971525590

Rastegari, A. A., Yadav, A. nath, & Gupta, A. (2019). Prospects of Renewable Bioprocessing in Future Energy Systems. In *Biofuels and Biorefineries* (Vol. 10, Issue August). https://doi.org/10.1007/978-3-030-14463-0_13

Richter, B. S., & Stutz, J. C. (2002). Mycorrhizal inoculation of big sacaton: Implications for grassland restoration of abandoned agricultural fields. *Restoration Ecology*, *10*(4), 607–616. https://doi.org/10.1046/j.1526-100X.2002.01041.x

Rillig, M. C., & Mummey, D. L. (2006). Mycorrhizas and soil structure. *New Phytologist*, *171*(1), 41–53. https://doi.org/10.1111/j.1469-8137.2006.01750.x

Rybczyński, J. J., Davey, M. R., & Mikuła, A. (2014). The gentianaceae - Volume 1: Characterization and ecology. *The Gentianaceae - Volume 1: Characterization and Ecology*, *1*, 1–329. https://doi.org/10.1007/978-3-642-54010-3

Ryszka, P., & Turnau, K. (2007). Arbuscular mycorrhiza of introduced and native grasses colonizing zinc wastes: Implications for restoration practices. *Plant and Soil*, *298*(1–2), 219–229. https://doi.org/10.1007/s11104-007-9356-8

Sanders, I. R. (2002). Ecology and evolution of multigenomic arbuscular mycorrhizal fungi. *American Naturalist*, *160*(4 SUPPL.). https://doi.org/10.1086/342085

Saritha, M., & Prasad Tollamadugu, N. V. K. V. (2018). The status of research and application of biofertilizers and biopesticides: Global scenario. In *Recent Developments in Applied Microbiology and Biochemistry*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-816328-3.00015-5

Schnepf, A., Leitner, D., Schweiger, P. F., Scholl, P., & Jansa, J. (2016). L-system model for the growth of arbuscular mycorrhizal fungi, both within and outside of their host roots. *Journal of the Royal Society Interface*, *13*(117). https://doi.org/10.1098/rsif.2016.0129

Schussler, A., & Walker, C. (2010). The Glomeromycota. *The Royal Botanic Garden Kew, December 2010*. http://www.arbuscular-mycorrhiza.net/species_infos/higher_taxa/funneliformis_claroideoglomus_rhizophagus_redeckera.pdf

Sedrez dos Reis, M., Ladio, A., & Peroni, N. (2014). Landscapes with Araucaria in South America: Evidence for a cultural dimension. *Ecology and Society*, *19*(2). https://doi.org/10.5751/ES-06163-190243

Shamini, S., & Amutha, K. (2014). Techniques For Extraction of Arbuscular Mycorrhizal Fungi Spores. *International Journal of Frontiers in Science and Technology*, *2*(2), 1–6.

Sherameti, I., & Varma, A. (2010). *Soil Biology Series Editor Ajit Varma , Amity Institute of Microbial Sciences ,. 19*, 492.

Shuab, R., Lone, R., Naidu, J., Sharma, V., Imtiyaz, S., & Koul, K. K. (2014). Benefits of Inoculation of Arbuscular Mycorrhizal Fungi on Growth and Development of Onion (Allium cepa) Plant. *& Environ. Sci*, *14*(6), 527–535. https://doi.org/10.5829/idosi.aejaes.2014.14.06.12347

Sileshi, G. W., Debusho, L. K., & Akinnifesi, F. K. (2012). Can integration of legume trees increase yield stability in Rainfed Maize cropping systems in Southern Africa? *Agronomy Journal*, *104*(5), 1392–1398. https://doi.org/10.2134/agronj2012.0063

Singh, A., Kumari, R., & Yadav, A. N. (2021). *Fungal Secondary Metabolites for Bioremediation of Hazardous Heavy Metals* (Issue April). https://doi.org/10.1007/978-3-030-68260-6_4

Smith, S. E., & Read, D. J. (2008). Mycorrhizal Symbiosis. In *Soil Science* (Vol. 137, Issue 3). https://doi.org/10.1097/00010694-198403000-00011

Soka, G., & Ritchie, M. (2014). Arbuscular mycorrhizal symbiosis and ecosystem processes: Prospects for future research in tropical soils. *Open Journal of Ecology*, *04*(01), 11–22. https://doi.org/10.4236/oje.2014.41002

Souza, T. (2015). Handbook of arbuscular mycorrhizal fungi. *Handbook of Arbuscular Mycorrhizal Fungi*, 1–153. https://doi.org/10.1007/978-3-319-24850-9

Syamsiyah, J., Herawati, A., & Mujiyo. (2018). The potential of arbuscular mycorrhizal fungi application on aggregrate stability in alfisol soil. *IOP Conference Series: Earth and Environmental Science*, *142*(1). https://doi.org/10.1088/1755-1315/142/1/012045

Systems, G. I. (2006). Management of the Urban Environment Using Remote Sensing and

Geographical Information Systems. *Journal of Human Ecology*, *20*(4), 269–277. https://doi.org/10.1080/09709274.2006.11905938

Teshale, T., 1995. The Making of Modern Ethiopia: 1896–1974. The Red Sea Press, Lawrenceville, NJ

Tessema, S. (1988). Improving ruminant livestock production on small farm systems under semi-arid farming conditions of Eastern Kenya. *UNDP/FAO Dryland Farming Research Development Project*, 13–25.

Thygesen, K., Larsen, J., & Bødker, L. (2004). Arbuscular mycorrhizal fungi reduce development of pea root-rot caused by Aphanomyces euteiches using oospores as pathogen inoculum. *European Journal of Plant Pathology*, *110*(4), 411–419. https://doi.org/10.1023/B:EJPP.0000021070.61574.8b

Trappe, J. M. (2005). A.B. Frank and mycorrhizae: The challenge to evolutionary and ecologic theory. *Mycorrhiza*, *15*(4), 277–281. https://doi.org/10.1007/s00572-004-0330-5

UNDP. (2019). *the Green Legacy Campaign in Ethiopia Setting a New World Record of Tree Seedlings Planted*.

Van Der Heijden, M. G. A., Scheublin, T. R., & Brader, A. (2004). Taxonomic and functional diversity in arbuscular mycorrhizal fungi - Is there any relationship? *New Phytologist*, *164*(2), 201–204. https://doi.org/10.1111/j.1469-8137.2004.01205.x

Vaughan, S., 2003. Ethnicity and power in Ethiopia. Ph.D. Dissertation, School of Social and Political Studies, University of Edinburgh.

Walker, C., Mize, C. W., & McNabb, H. S. (1982). Populations of endogonaceous fungi at two locations in central Iowa. *Canadian Journal of Botany*, *60*(12), 2518–2529. https://doi.org/10.1139/b82-305

Wang, W., Shi, J., Xie, Q., Jiang, Y., Yu, N., & Wang, E. (2017). Nutrient Exchange and Regulation in Arbuscular Mycorrhizal Symbiosis. *Molecular Plant*, *10*(9), 1147–1158. https://doi.org/10.1016/j.molp.2017.07.012

Wipf, D., Krajinski, F., van Tuinen, D., Recorbet, G., & Courty, P. E. (2019). Trading on the arbuscular mycorrhiza market: from arbuscules to common mycorrhizal networks. *New Phytologist*, *223*(3), 1127–1142. https://doi.org/10.1111/nph.15775

Wongchalee, P., & Pukahute, C. (2012). Diversity of mushrooms in Dry Dipterocarp forest at Phuphan National Park, Sakon Nakhon Province. *Natural Science*, *04*(12), 1153–1160. https://doi.org/10.4236/ns.2012.412a140

Yanagi, Y., Hamaguchi, S., Tamaki, H., Suzuki, T., Otsuka, H., & Fujitake, N. (2003). Relation of chemical properties of soil humic acids to decolorization by white rot fungus–Coriolus consors. *Soil Science and Plant Nutrition*, *49*(2), 201–206. https://doi.org/10.1080/00380768.2003.10409998

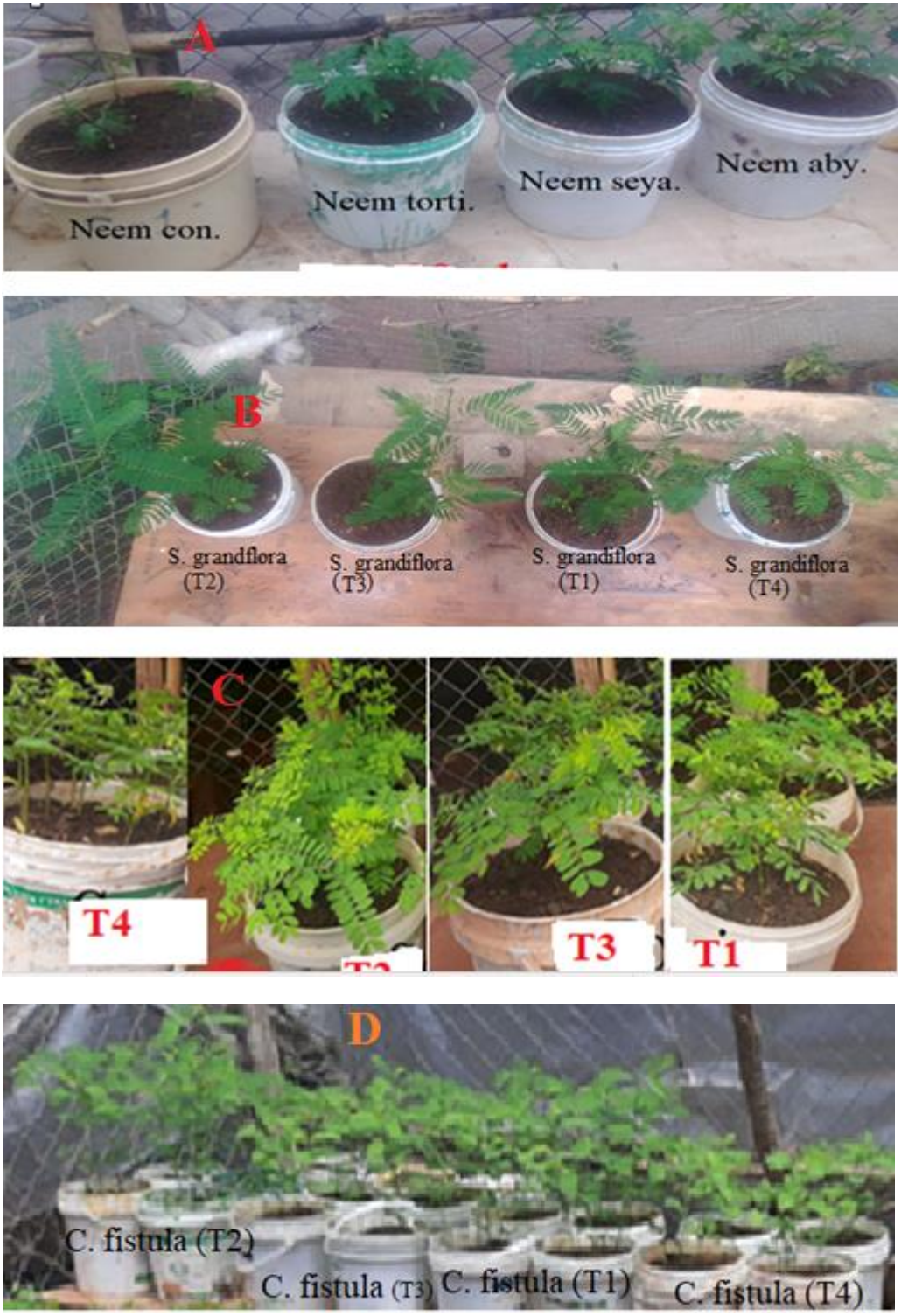
Yaseen, T., Burni, T., & Hussain, F. (2011). Effect of arbuscular mycorrhizal inoculation on nutrient uptake, growth and productivity of cowpea (Vigna unguiculata) varieties. *African Journal of Biotechnology*, *10*(43), 8593–8598. https://doi.org/10.5897/ajb10.1494

Zerihun, B., Mauritz, V., & Fassil, A. (2013). Diversity and abundance of arbuscular mycorrhizal fungi associated with acacia trees from different land use systems in Ethiopia. *African Journal of Microbiology Research*, *7*(48), 5503–5515. https://doi.org/10.5897/ajmr2013.6115

Zerihun, B., Negash, M., Kaseva, J., Vestberg, M., & Kahiluoto, H. (2020). Native forests but not agroforestry systems preserve arbuscular mycorrhizal fungal species richness in southern Ethiopia. *Mycorrhiza*, *30*(6), 749–759. https://doi.org/10.1007/s00572-020-00984-6

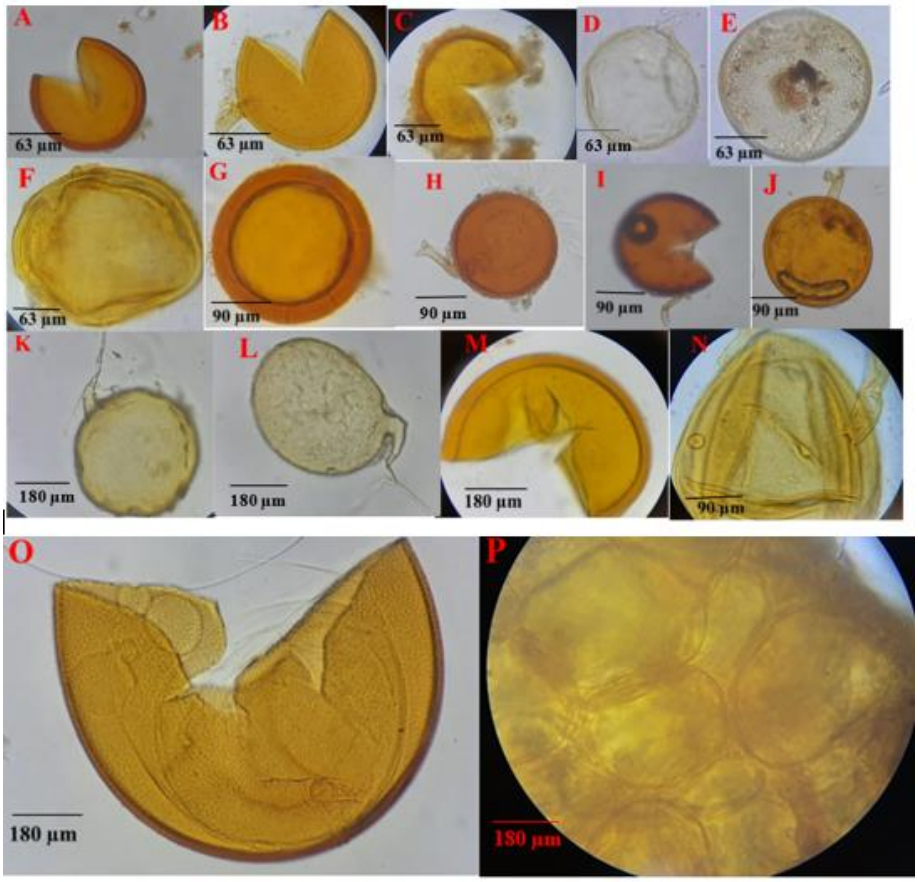
7. LIST OF APPENDICES

Appendices 1: Representative pictures to showing the growth response of *A. indica*, *S. grandflora*, *D. regia* and *C. fistula* to the different inoculums.



A- *Azodichta indic*a, B- *Sesbania grandflora* C- *Delonix regia* D- *Cassia fistula.* A- *Azodichta indic*a, B- *Sesbania grandflora* C- *Delonix regia* D- *Cassia fistula.* T_1 - *A. abyssinica* of AMF, T2 *A. seyal* of AMF, T3 *A. tortilis* of AMF T4 Control (Non Inoculum)

Appendices 2: Microscopic photos of some morphotypes extracted and identified spores from the AMF inoculum. All photos are from slides made in PVLG



A (C*laroideoglomus etunicatum*), **B** and **C** (***Claroideoglomus claroideum*), D** (*paraglomus occultum*), **E** (*Acaulospora sp*.), **F** (*Rhizophagus aggregatus*), **G** (*Glomus sp*. (Sporcarpic thick wall)), **H** and **I** (*Septoglomus constrictum*), **J** (*Funneliformis geosporum*), **K** and **L** *(Funneliformis mosseae*), **M** (*Gigaspora sp*.,) **N** (*Rhizophagus clarus*), **O** (*Acaulospora scrobiculata*/ *tuberculate*), **P** (*Sclerocystis sinuosum* (sporocarps, A dense layer of tightly interwoven hyphae, thick, covering all spores to keep them tightly packed)).

Appendices 3: Representative pictures to give further illustration of all practical works beginning from trap culturing to the end of laboratory session.

A (Trap culturing), **B** (Intation of sporylation), **C** (AMF inoculum storing), **D** (Pot setup before planting the vegetables), **E** (Growing of the vegetables), **F and G** (wet sieving & decanting), **H** (Centrifugation), **I** (Enumeration and extraction of AMF spore under stereomicroscope), and **J** (Estimation percent AM root colonization under a compound microscope).

Appendices 4: One-way ANOVA

Result analysis output of the plant growth parameters in each vegetable from SPSS software. *A. indica* (Neem), *S. grandflora*., *D. regia* and *C. fistula*, respectively.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Shoot height Neem 120	Between Groups	105.287	3	35.096	131.608	.000
	Within Groups	2.133	8	.267		
	Total	107.420	11			
Root height Neem 120	Between Groups	71.063	3	23.688	189.500	.000
	Within Groups	1.000	8	.125		
	Total	72.063	11			
Number of leaves Neem 120	Between Groups	709.667	3	236.556	34.201	.000
	Within Groups	55.333	8	6.917		
	Total	765.000	11			
Fresh shoot mass Neem 120	Between Groups	11.877	3	3.959	131.963	.000
	Within Groups	.240	8	.030		
	Total	12.117	11			
Fresh root mass Neem 120	Between Groups	9.257	3	3.086	142.410	.000
	Within Groups	.173	8	.022		
	Total	9.430	11			
Dry shoot mass Neem 120	Between Groups	3.217	3	1.072	160.833	.000
	Within Groups	.053	8	.007		
	Total	3.270	11			
Dry root mass Neem 120	Between Groups	1.430	3	.477	190.667	.000
	Within Groups	.020	8	.003		
	Total	1.450	11			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Shoot height Ses 120	Between Groups	1641.860	3	547.287	42.829	.000
	Within Groups	102.227	8	12.778		
	Total	1744.087	11			
Root height Ses 120	Between Groups	113.843	3	37.948	49.822	.000
	Within Groups	6.093	8	.762		
	Total	119.937	11			
Number of leaves Ses 120	Between Groups	141397.583	3	47132.528	361.400	.000
	Within Groups	1043.333	8	130.417		
	Total	142440.917	11			
Fresh shoot mass Ses 120	Between Groups	129.477	3	43.159	48.267	.000
	Within Groups	7.153	8	.894		
	Total	136.630	11			
Fresh root mass Ses 120	Between Groups	11.940	3	3.980	129.081	.000
	Within Groups	.247	8	.031		
	Total	12.187	11			
Dry shoot mass Ses 120	Between Groups	13.849	3	4.616	59.566	.000
	Within Groups	.620	8	.077		
	Total	14.469	11			
Dry root mass Ses 120	Between Groups	1.749	3	.583	58.306	.000
	Within Groups	.080	8	.010		
	Total	1.829	11			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Shoot height DD 120	Between Groups	107.882	3	35.961	331.946	.000
	Within Groups	.867	8	.108		
	Total	108.749	11			
Root height DD 120	Between Groups	101.336	3	33.779	167.497	.000
	Within Groups	1.613	8	.202		
	Total	102.949	11			
Number of leaves DD 120	Between Groups	17851.000	3	5950.333	415.140	.000
	Within Groups	114.667	8	14.333		
	Total	17965.667	11			
Fresh shoot mass DD 120	Between Groups	6.700	3	2.233	35.263	.000
	Within Groups	.507	8	.063		
	Total	7.207	11			
Fresh root mass DD 120	Between Groups	3.587	3	1.196	68.317	.000
	Within Groups	.140	8	.018		
	Total	3.727	11			
Dry shoot mass DD 120	Between Groups	1.729	3	.576	26.603	.000
	Within Groups	.173	8	.022		
	Total	1.902	11			
Dry root mass DD 120	Between Groups	1.950	3	.650	65.000	.000
	Within Groups	.080	8	.010		
	Total	2.030	11			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Shoot height Cas 120	Between Groups	470.667	3	156.889	44.825	.000
	Within Groups	28.000	8	3.500		
	Total	498.667	11			
Root height Cas 120	Between Groups	62.416	3	20.805	55.358	.000
	Within Groups	3.007	8	.376		
	Total	65.423	11			
Number of leaves Cas 120	Between Groups	7416.250	3	2472.083	73.610	.000
	Within Groups	268.667	8	33.583		
	Total	7684.917	11			
Fresh shoot mass Cas 120	Between Groups	28.830	3	9.610	268.186	.000
	Within Groups	.287	8	.036		
	Total	29.117	11			
Fresh root mass Cas 120	Between Groups	10.213	3	3.404	204.267	.000
	Within Groups	.133	8	.017		
	Total	10.347	11			
Dry shoot mass Cas 120	Between Groups	20.876	3	6.959	219.746	.000
	Within Groups	.253	8	.032		
	Total	21.129	11			
Dry root mass Cas 120	Between Groups	3.817	3	1.272	763.333	.000
	Within Groups	.013	8	.002		
	Total	3.830	11			

Appendices 5: Two-way ANOVA

result analysis output of the mycorrhizal dependency, hyphal root colonization, and spore density using crop and treatment as factors in order to test for interactions

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Variety	(J) Variety	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Mycorrhizal Dependency after 60	Cae	DDZ	19.2389*	1.69010	.000	14.5766	23.9012
		Neem	7.4856*	1.69010	.001	2.8232	12.1479
		Ses	17.7111*	1.69010	.000	13.0488	22.3734
	DDZ	Cae	-19.2389*	1.69010	.000	-23.9012	-14.5766
		Neem	-11.7533*	1.69010	.000	-16.4157	-7.0910
		Ses	-1.5278	1.69010	.803	-6.1901	3.1346
	Neem	Cae	-7.4856*	1.69010	.001	-12.1479	-2.8232
		DDZ	11.7533*	1.69010	.000	7.0910	16.4157
		Ses	10.2256*	1.69010	.000	5.5632	14.8879
	Ses	Cae	-17.7111*	1.69010	.000	-22.3734	-13.0488
		DDZ	1.5278	1.69010	.803	-3.1346	6.1901
		Neem	-10.2256*	1.69010	.000	-14.8879	-5.5632
Mycorrhizal Dependency after 120	Cae	DDZ	20.6733*	1.11507	.000	17.5973	23.7494
		Neem	-4.3756*	1.11507	.003	-7.4516	-1.2995
		Ses	6.6444*	1.11507	.000	3.5684	9.7205
	DDZ	Cae	-20.6733*	1.11507	.000	-23.7494	-17.5973
		Neem	-25.0489*	1.11507	.000	-28.1249	-21.9728
		Ses	-14.0289*	1.11507	.000	-17.1049	-10.9528
	Neem	Cae	4.3756*	1.11507	.003	1.2995	7.4516
		DDZ	25.0489*	1.11507	.000	21.9728	28.1249
		Ses	11.0200*	1.11507	.000	7.9439	14.0961
	Ses	Cae	-6.6444*	1.11507	.000	-9.7205	-3.5684
		DDZ	14.0289*	1.11507	.000	10.9528	17.1049
		Neem	-11.0200*	1.11507	.000	-14.0961	-7.9439

Based on observed means.
The error term is Mean Square(Error) = 5.595.

*. The mean difference is significant at the .05 level.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Variety	(J) Variety	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Root Arbuscul colonization	Cae	DDZ	-1.9992*	.70841	.039	-3.9185	-.0798
		Neem	5.1125*	.70841	.000	3.1932	7.0318
		Ses	5.1675*	.70841	.000	3.2482	7.0868
	DDZ	Cae	1.9992*	.70841	.039	.0798	3.9185
		Neem	7.1117*	.70841	.000	5.1923	9.0310
		Ses	7.1667*	.70841	.000	5.2473	9.0860
	Neem	Cae	-5.1125*	.70841	.000	-7.0318	-3.1932
		DDZ	-7.1117*	.70841	.000	-9.0310	-5.1923
		Ses	.0550	.70841	1.000	-1.8643	1.9743
	Ses	Cae	-5.1675*	.70841	.000	-7.0868	-3.2482
		DDZ	-7.1667*	.70841	.000	-9.0860	-5.2473
		Neem	-.0550	.70841	1.000	-1.9743	1.8643
Root Vesicle Colonization	Cae	DDZ	5.2783*	.95817	.000	2.6823	7.8744
		Neem	-.7225	.95817	.874	-3.3185	1.8735
		Ses	3.3342*	.95817	.008	.7381	5.9302
	DDZ	Cae	-5.2783*	.95817	.000	-7.8744	-2.6823
		Neem	-6.0008*	.95817	.000	-8.5969	-3.4048
		Ses	-1.9442	.95817	.199	-4.5402	.6519
	Neem	Cae	.7225	.95817	.874	-1.8735	3.3185
		DDZ	6.0008*	.95817	.000	3.4048	8.5969
		Ses	4.0567*	.95817	.001	1.4606	6.6527
	Ses	Cae	-3.3342*	.95817	.008	-5.9302	-.7381
		DDZ	1.9442	.95817	.199	-.6519	4.5402
		Neem	-4.0567*	.95817	.001	-6.6527	-1.4606
Root Hyphal Colonization	Cae	DDZ	1.1658	1.32534	.815	-2.4250	4.7567
		Neem	5.0550*	1.32534	.003	1.4642	8.6458
		Ses	11.9992*	1.32534	.000	8.4083	15.5900
	DDZ	Cae	-1.1658	1.32534	.815	-4.7567	2.4250
		Neem	3.8892*	1.32534	.030	.2983	7.4800
		Ses	10.8333*	1.32534	.000	7.2425	14.4242
	Neem	Cae	-5.0550*	1.32534	.003	-8.6458	-1.4642
		DDZ	-3.8892*	1.32534	.030	-7.4800	-.2983
		Ses	6.9442*	1.32534	.000	3.3533	10.5350
	Ses	Cae	-11.9992*	1.32534	.000	-15.5900	-8.4083
		DDZ	-10.8333*	1.32534	.000	-14.4242	-7.2425
		Neem	-6.9442*	1.32534	.000	-10.5350	-3.3533
Spore Dencity	Cae	DDZ	-.2083	.36118	.938	-1.1869	.7702
		Neem	-6.7075*	.36118	.000	-7.6861	-5.7289
		Ses	4.4250*	.36118	.000	3.4464	5.4036
	DDZ	Cae	.2083	.36118	.938	-.7702	1.1869
		Neem	-6.4992*	.36118	.000	-7.4777	-5.5206
		Ses	4.6333*	.36118	.000	3.6548	5.6119
	Neem	Cae	6.7075*	.36118	.000	5.7289	7.6861
		DDZ	6.4992*	.36118	.000	5.5206	7.4777
		Ses	11.1325*	.36118	.000	10.1539	12.1111
	Ses	Cae	-4.4250*	.36118	.000	-5.4036	-3.4464
		DDZ	-4.6333*	.36118	.000	-5.6119	-3.6548
		Neem	-11.1325*	.36118	.000	-12.1111	-10.1539

Based on observed means.
The error term is Mean Square(Error) = .783.

*. The mean difference is significant at the .05 level.