EVALUATION OF NATIVE ARBUSCULAR MYCORRHIZAL FUNGI FOR GROWTH AND MANAGEMENT OF RHIZOME ROT OF GINGER (*Zingiber officinale*)

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By

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(2020-11-104)

THESIS

Submitted in partial fulfillment of the requirement for the degree of

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2023

DECLARATION

I, Revathy S. (2020-11-104) hereby declare that the thesis entitled **"Evaluation of native arbuscular mycorrhizal fungi for growth and management of rhizome rot of ginger (*Zingiber officinale*)"** is a bonafide record of research done by me during the course of research and that it has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled **"Evaluation of native arbuscular mycorrhizal fungi for growth and management of rhizome rot of ginger (*Zingiber officinale*)"** is a record of research work done independently by **Revathy S. (2020-11-104)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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(REVATHY S.)

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LIST OF ABBREVIATIONS

Abbreviations	Expansion
µm	Micrometre
AMF	Arbuscular Mycorhhizal Fungi
CI	Challenge Inoculation
cm	Centimetre
COA	College of Agriculture
FYM	Farm Yard Manure
Hcl	Hydrochloric acid
hr	Hour
KOH	Potassium hydroxide
NaOH	Sodium Hydroxide
DAI	Days After Inoculation
DAP	Days After Planting
min	Minutes
mg	Milligram
g	Gram
N	Nitrogen
P	Phosphorus
K	Potassium
kg	Kilogram
ml	Millilitre
pH	Hydrogen ion concentration
et al.,	Co - workers
%	Percent
/	Per
LSD	Least Significant Difference
°C	Degree Celcius
⌶	Mean standard error

1. INTRODUCTION

1. Introduction

Biofertilizers are microorganism-containing substances that, when added to soil, increases fertility and promotes plant growth. They are environmental friendly and do not cause any harm to the crops like chemical fertilizer. Arbuscular mycorrhizal fungi (AMF) are ubiquitous in soil habitats and form beneficial association naturally with 80 % of the land species including agricultural crops which can be used as biofertilizer to benefit plants. They are endo-mycorrhizal fungi, which belongs to the phylum Glomeromycota and order Glomales and it is currently placed in the class Zygomycetes (Dodd *et al*., 1996). AMF, being an obligate symbiont depends totally on the host plant for their food and life cycle and in turn, mycorrhizal fungi benefit the host plant. Some important genera found in association with plants are *Glomus, Gigaspora*, *Acaulospora, Entrophospora and Scutellospora* of which *Glomus* is the most common fungal partner.

Arbuscular mycorrhizal fungi plays an important role in increasing plant growth and yield due to an increased supply of nutrient to the plant, especially phosphorous. AMF-colonized plant roots transport phosphorous four times higher than the non-colonized plants and also improves the uptake of other soil nutrients like N, K, Cu and Zn. AMF allow plants to uptake more nutrients and water from the soil due to increased surface area of fungal hyphae. AMF are ecologically and economically important organism that helps in alleviating environmental stresses like salinity, drought, temperature and heavy metals. Moreover, these fungi play a major role in soil aggregation process and stimulate microbial activity.

As an added advantage, AMF also have antagonistic effect against other pathogenic fungi like *Pythium*, *Phytophthora* and *Fusarium*. It confers the host with resistance and tolerance against many soil - borne and root - borne disease causing pathogens and phytoparasitic nematodes. For example the stem rust disease of wheat was alleviated after inoculation with AMF (El-Sharkawy *et al*., 2018) and Verticillium wilt resistance of cotton was also improved by AMF under field conditions (Zhang *et al*., 2018). AMF forms associations with most agricultural and horticultural crops including spice crops.

Ginger (*Zingiber officinale* Roscoe), a member of family Zingiberaceae, is one of the important aromatic spice crop grown worldwide. Ginger is commercially grown for its medicinal and flavouring properties. The use of ginger, both as fresh vegetable and dried spice helps to improve digestion and boost immunity. It is also used to treat arthritis, inflammations, indigestion, diabetes and other health problems. India is the largest producer and consumer of ginger in the world and holds the third position in ginger export (2023). About 43 % of global demand of ginger is met by India. According to the report given by APEDA, Kerala stands ninth in ginger production (2021-22) with production of 72.70 thousand tonnes. Wayanad is a major contributing district which stands first in ginger cultivation in Kerala in terms of area and production (Farm guide, 2022).

Ginger is a succulent crop and it is highly susceptible to biotic and abiotic stresses. Rhizome rot or soft rot disease (*Pythium myriotylum*) is the most devasting disease of ginger which ruins its yield. This disease is a major problem faced by ginger growing farmers in Wayanad. Since the cost of cultivation of ginger is high, farmers do not want to risk with crop failure, so they use large amount of chemicals such as pesticides, herbicides, fungicides, insecticides and fertilizers to prevent crop diseases and to increase yield. This indiscriminate use of chemicals has resulted in the loss of biodiversity and heavily fertilized soil. AMF is a potent biofertilizer, nutrient remedifier, eco-friendly organism which can be used as an alternative to chemical fertilizer and also as an antagonist against rhizome rot causing pathogen, *Pythium myriotylum*. There were reports that inoculating ginger with AM fungi increased plant growth and rhizome yield while suppressing *Pythium* population (Iyer and Sundararaju, 1993).

However, the potential for using AM fungi on a large scale in ginger cultivation is dependent on the development of efficient native AMF strains. As a result, extensive field studies are required to understand the diversity and abundance of native AM fungi inhabiting the crop's rhizosphere. Given the foregoing information, the current study was designed and carried out with the following objectives

- Isolation of native arbuscular mycorrhizal fungi from the rhizosphere soils of ginger from different locations in Wayanad district.
- Evaluating the effect of AMF on growth and rhizome rot management (*Pythium myriotylum*) in ginger under pot culture studies.

2. REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1. Introduction

Majority of terrestrial plant roots have symbiotic relationships with fungi. Mycorrhiza is a common symbiosis that serves as a channel for the transfer of energy and matter between plants and soil (Mohammadi *et al.*, 2011). The word "Mycorrhiza" is derived from the Greek words *mycos*, which means fungus, and *rhiza*, which means roots. Mycorrhizal associations are known to exist in more than 80% of angiosperms and almost all gymnosperms in nature (Barman *et al.*, 2016). AM fungi are strictly biotrophic, so they require living host root to grow and complete their life stages. To date, no synthetic medium has been developed that can support the full proliferation of AM fungi in the absence of living host (Kehri *et al*., 2018).

Most preferred plant species by mycorrhizal fungi are acacia, citrus, legumes, maize, onion, etc. Cruciferous plants are not preferred by fungi since they produce many plant extracts. AMF benefits the plant by improving nutrient uptake, water uptake, disease resistance, plant chemical defence, soil aggregation, and allelochemical transport and protection (Delavaux *et al.*, 2017). Ectomycorrhiza, Endomycorrhiza, and Ectendomycorrhiza are the three major groups of mycorrhiza. Ectomycorrhizal fungi and endomycorrhizal fungi are important in agriculture and forestry. Endomycorrhiza also known as vesicular arbuscular mycorrhiza (VAM) now called as arbuscular mycorrhizal fungi (AMF).

AMF plays a critical role in increasing plant growth and yield by increasing nutrient content, especially phosphorus supply to the host plant and increase plant resistance to various environmental stresses. These fungi can be found in the rhizosphere of a variety of vascular plants and play critical roles in agricultural ecosystem management and sustainability. The beneficial effect of indigenous AM fungi on agricultural plant nutrition is determined by the quantity and type of fungi present in the soil (Abbott and Robson, 1982).

According to Aguilera *et al*. (2014), AMF are important in ecological agriculture because they benefit the vast majority of crop plants and environment by acting as biofertilizers, bioprotectors, and biocontrol agents. From the tropics to the subtropics, the majority of plant families in various ecosystems are universally associated with AM fungi (Zhao *et al*., 2001 and Maffo *et al*., 2022). The fungi benefit their host plants in a variety of ways, including increased nutrient supply, protection against soil-borne plant pathogens, and drought tolerance (Harrier and Watson, 2004). AMF is distinguished by the presence of arbuscules and vesicles, which serve as reserve organs as well as a site for fungal multiplication (Supriya and Purshotam, 2011).

The soil was viewed as a vital element of the system, composed of interconnected physical, chemical, and biological factors, with the AMF serving as an integral part of a biological micro-ecosystem. Soil in disturbed areas are frequently deficient in available nutrients and lack nitrogen-fixing bacteria. Mycorrhizal fungi, which are typically associated with the root rhizosphere, aids in supplying the plant with essential nutrients like nitrogen, phosphorous, zinc etc. and also helps in the restoration of land degraded due to water scarcity by improving water uptake ability of plant.

Jayaprakash and Nagarajan conducted research on mycorrhizal biodiversity in medicinal plant species in the Wayanad district's Pookode Lake area. Mycorrhizal association was found in all plant species, according to the findings. AM fungal association surveyed 40 plant species from 20 families. The density of AM fungal spores per 100 g soil varied from 170 to 690 (2017).

2.2. Taxonomy of mycorrhiza

The taxonomic classification of AMF was created by grouping the fungal strains based on morphological similarities and differences. International Vesicular Arbuscular Mycorrhiza (INVAM) culture collection described approximately 160 AMF species using spore morphology (Goswani *et al*., 2018). Arbuscular mycorrhiza (AM) belongs to Glomales of class Zygomycetes and appeared nearly 400 million

years ago, according to both fossil discoveries and DNA sequences. These fungi are Glomeromycota members, which is a monophyletic phylum with 150-160 described species. Since they form intra-radical structures, AMF are also known as "endomycorrhizas"(Mohammadi *et al.*, 2011).

In 1809, German botanist Link classified these mycorrhizal association-forming species as *Endogone* and *Glomus* was placed in the Endogonaceae by Gerdemann and Trappe (1974), who also described two new genera, *Acaulospora* and *Gigaspora*. AMF includes one *Endogone* species, two *Sclerocystis* species, three *Entrophospora* species, twelve *Gigaspora* species, fourteen *Acaulospora* species, fifteen *Scutellospora* species, and sixty *Glomus* species. Two new families, Archaeosporaceae and Paraglomaceae, as well as two new genera, *Archaeospora* and *Paraglomus*, were added respectively by Morton and Redecker, 2001 and Kehri *et al.*, 2018).

2.3. Classification of Mycorrhiza

According to A.B Frank (1885), mycorrhiza are classified as Ectotrophic and Endotrophic. In ectotrophic mycorrhizae, fungus forms sheath on root surface and hyphae grow outward and between the outer cortical cells of the roots. While in endotrophic mycorrhizae, fungal hyphae enters the cortical cells of root and colonize the plant root enveloped by plasmalemmma of the host.

However, on the basis of strict morphological and anatomical features, mycorrhiza recently classified into Ectomycorrhizae and Endomycorrhizae.

2.3.1. Ectomycorrhizae

Ectomycorrhizal fungi are also known as sheathing fungi because, these fungi grow on the surface of plant root and forms mantle or sheath around them. Thickness of sheath varies from 20 to 40 mm and it constitutes about 20 – 40% of total dry weight of fungus which helps in better nutrient absorption and storage. The fungal hyphae penetrate the root only to a limited extent in cortical cells by growing between the cells intercellularly and forms Hartig net, which completely covers the plant roots

and produces metabolites to protect the plant roots against diseases (Peterson *et al*., 2004). Only 5% of vascular plant species are affected by these fungi. Most of the fungi involved belong to sub division Basidiomycotina and mainly found on plants families like Myrtaceae, Pinaceae, Fagaceae and Saliceae family of ornamental and forest species of trees (Himaya *et al.*, 2021). Fungi like *Amanita muscaria, Boletus edulis, Lactarius, Rhizopogon, Laccaria laccata, Inocybe, Scleroderma* are also seen associated in this mycorrhiza. These fungi can be cultured under *in vitro* conditions.

2.3.2. Endomycorrhizae (Vesicular-Arbuscular Mycorrhiza/ Arbuscular Mycorrhizae)

Endomycorrhiza is the most common mycorrhiza which colonizes majority of plants (nearly 90%) in both natural and agricultural conditions. It forms association with plants belonging to bryophytes, pteridophytes, gymnosperms, and angiosperms. Unlike ectomycorrhiza, these fungi grow inside the plant root and penetrate the root cell wall. The penetrating hyphae creates greater surface area between the fungal hyphae and host plant which facilitates greater transfer of nutrients between them. These fungi cannot be cultured under in vitro since they require living host for their growth and multiplication. Generally, the endomycorrhizal fungi are recognized as AM because of the development of arbuscules (Kaur *et al.*, 2014). These fungi are classified into four major groups. They are Arbuscular mycorrhizae, Ericoid mycorrhiza, Arbutoid mycorrhiza, Orchid mycorrhiza.

2.3.2.1. Arbuscular mycorrhizal fungi (AMF)

The term Vesicular Arbuscular Mycorrhiza (VAM) was commonly used before 1974, because it produces large swollen vesicles and intricately branched arbuscles within the plant cells. However, later it was noted that vesicles are not formed by some fungi like *Gigaspora and Scutellospora* (forms only arbuscles) and hence nowadays they are only called as Arbuscular Mycorrhizal fungi or AM fungi (Sharma and Mehta, 2018).

AM fungi belong to family Endogonaceae of sub division Zygomycotina which include important genera like *Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis, and Scutellospora*. These fungi have wide host range and plants found colonized naturally by this different AM fungal strains. AMF are used against many soil-borne pathogens as a biocontrol agent (St-Arnaud and Vujanovic, 2007).

Glomus

Glomus species has straight hyphae branched throughout the root cortex, often with 'H' branches. Vesicles formed are oval to elliptical when formed intercellularly. This group of fungi are common in temperate climates but apparently absent in the tropics. *Glomus* species often have an introverted wall thickening (Oehl *et al.* 2011).They have very thin hyphae (<1 µm), odd fan- like structures in the root cortex and small hyphal swellings which spread intra- and intercellularly (Morton, 1985).

Acaulospora

Acaulospora species have irregular or H-branched hyphae that spread in the outer root cortex with looped or coiled hyphae. Hyphae are thin-walled with variable staining intensity (stain weakly in trypan blue and acid fuchsin). Vesicles form intra- and intercellular patches and are more ovoid or irregularly lobed (Liu and Wang, 2003).

Gigaspora and Scutellospora

Gigaspora and Scutellospora species have thick, intraradical hyphae *Gigasporales* exhibit gigasporoid or scutellosporoid spore formation (Oehl *et al.* 2011), i.e. spores formed terminally on sporogenous cells and with either germ warts on the inner surface of the mono-walled spore wall (gigasporoid*)*, or a discrete germination shield on the innermost of 2–4 walls (scutellosporoid). Spore colour mostly range from very pale yellow or brown in colour for *Scutellospora* spp. and hyaline to pale coloured for *Gigaspora* spp. (Oehl *et al.,* 2017).

2.3.2.2. Arbutoid Mycorrhizae

Arbutoid association are found in the *pyrolaceae* family of order Ericales. Here, the host plants are mostly woody shrubs and trees. Roots are typically herorhizic, which are short roots being converted into mycorrhiza with a well-defined sheath and a hartig net. The intracellular coils, along with the mantle sheath and hartig net are the diagnostic features of arbutoid mycorhiza. They also form association with conifers. The fungi forming arbutoid mycorrhizae are mainly basidiomycetes which includes genera such as *Laccaria, Piloderma,* and *Rhizopogon,* and ascomycetes such as *Cenococcum*. (Sharma and Mehta, 2018).

2.3.2.3. Ericoid Mycorrhizae

Ericoid mycorrhiza forms symbiotic association with the plants belonging to the family Ericaceae. Many genera *like Epachis, Leucopogon, Monotoa, Rhodendron, Vaccinum* etc. develop ericoid mycorrhiza. Here, the plants belonging to Ericaceae are woody shrubs or small trees found in open or acid peat soil. They usually have fine roots on which the fungus establishes to the outermost layer of cortical cells forming dense intracellular cells. Ericoid fungi form symbiosis with several crop and ornamental plants like cranberries, blueberries and *Rhododendron* (Himaya *et al*., 2021).

2.3.2.4. Orchid Mycorrhizae

Orchid mycorrhiza forms association with roots of plant family *Orchidaceae*. These fungi are critically important during orchid germination because orchid seed has no energy reserve, they obtain their carbon from the fungal partner. Fungi infecting these orchids are mostly from the genus *Rhizoctonia* with the perfect stage *Ceratobasidium, Sebacina,* and *Tulasnella* occurring in the class Basidiomycetes and Ascomycetes (Sharma and Mehta, 2019).

Another group belonging to Basidiomycotina are *Armillaria, Corticium, Fomes, Marasmius,* and *Zerotus.* These fungi break down lignin and cellulose and

thus contribute to decaying organic matter. Mostly they form association with saprophytes and pathogenic fungi (Himaya *et al.*, 2021).

2.5. Host specificity of mycorrhiza

Arbuscular mycorrhizal fungi have been evaluated for their potential utility in the sustainable agriculture system. About 90 % of the land plants are known to form associations with soil fungi (Read, 1991). Mycorrhiza are reported to occur in 83% of dicotyledons and 79% of monocotyledonous families and all gymnosperms (Wilcox, 1991).

Wang and Qiu (2006) compiled data on the mycorrhizal status of 3,617 species from 263 plant families and discovered that 80% of the recorded species and 92% of the families were mycorrhizal. Members of the Chenopodiaceae and Cruciferae were previously reported to be non-mycorrhizal; however, some species in these families have recently been reported to have low or, in some cases, high levels of vesicular-arbuscular (VA) mycorrhizal infection. *Glomus fasciculatus* infection was found in four Chenopodiaceae and two Cruciferae species in our experiments, but only when a mycorrhizal companion plant, citrus or onion, was present (Brundrett, 2009).

2.6. Ecosystem services contributed by mycorrhiza

Studies have shown that AMF plays a major role in regulating plant species diversity and the ecosystem succession (Bauer *et al*., 2017). Therefore, exploring AMF diversity in different ecosystems has become inevictable (Hontoria *et al*., 2019).

AMF diversity has been reported in various ecosystems, such as grassland ecosystem (Goldmann *et al.*, 2020), agricultural ecosystem (Zhu *et al.*, 2020), forest ecosystem (Dos Passos *et al*., 2021 and Rozek *et al.*, 2020).

Mycorrhizal symbiosis has a significant impact on the health of plants as well as several ecosystem functions such as carbon, nitrogen, and phosphorous cycles,

plant diversity regulation, soil aggregation, and seedling survival (van der Heijden *et al.,* 2015) and restoration of degraded natural ecosystems (Neuenkamp *et al.,* 2019).

In the roots, AMF forms vesicles, arbuscules, and hyphae, as well as spores in the rhizosphere. The formation of a hyphal network by the AMF with plant roots significantly improves root access to a large soil surface area, resulting in improved plant growth by improving nutrient content and water uptake ability of plant (Bowles *et al*., 2016 and Begum *et al*., 2019).

Glomalin-related soil protein (GRSP) is secreted by mycorrhizal fungi in their mycorrhizosphere and regulates water translocation from soil to plants. GRSP was primarily composed of carbon, which protected the soil from desiccation and increases its water holding capacity (Sharma et al., 2017) and helps the plant establishment in water-stressed environments (Auge, 2004 and Fernandes *et al*., 2016).

AM associations can boost plant growth by increasing phosphorus uptake from soils with low to moderate phosphorus availability and also improves translocation (Begum *et al*., 2019) and soil quality by influencing its structure and texture, which in turn improves plant health (Zou *et al*., 2016 and Thirkell *et al*., 2017).

2.7. Isolation and identification of AMF

Gerdemann and Nicolson (1963) developed a technique for obtaining mycorrhizal spores from soil using wet sieving and decanting method, which has proven to be a valuable tool in mycorrhizal research. AM fungal spores are identified morphologically based on characteristics such as wall morphology, size, shape, colour, hyphal attachment, bulbous suspensor, surface ornamentation and response to staining compounds (Wright, 2005).

Giovannetti and Mosse (1980) investigated the methods for detecting VAM infection in roots, which proved to be a beneficial method for examining the percentage infection of AM fungi in roots, as well as the diversification and

distribution of AM fungi in different eco-systems. Vierheilig *et al*. (1998) developed a low-cost method for staining AM fungal colonisation in root tissues which is stable and reliable.

2.8. Mass multiplication of AMF

AMF propagation for large-scale application is still limited due to their obligate biotrophic nature, which makes them dependent on the host plant for survival. Various cultivation strategies, including soil and substrate-based techniques like pot cultures, as well as substrate-free methods like hydroponics and aeroponics, have been used for the mass production of AMF in the presence of roots (Gaur and Adholeya, 2000; Liu and Yang, 2008).

Because it is less artificial and more cost-effective, the substrate-based method is the most widely used. Previous research discovered that farmers were able to generate a large amount of AMF inoculum using substrate-based methods such as pot cultures and had a higher colonising efficiency in the host plants (Schlemper and Sturmer, 2014). Furthermore, substrate-based on-farm AMF production has gained popularity due to the use of indigenous soil, which effectively lowers inoculum costs while significantly improving plant growth (Douds *et al*., 2010 and Schlemper and Sturmer, 2014).

Fracchia *et al.* (2001) used a vermiculite-perlite mixture to develop a novel method for accurately establishing monosporic cultures of AMF with a high success rate. In the forest and agricultural systems, an inoculum should be effective for mass multiplication. Under production conditions, it must produce the desired growth response for the targeted plants (Mehrotra, 2005).

Oseni *et al*. (2010) concluded that seedlings inoculated with vermiculite containing AM fungi had better transplant results due to higher shoot, fresh weight, height, shoot and root ratio, and root biomass. He concluded that the fungi colonised 23.3 % of the roots in the vermiculite medium after AM inoculation.

2.8.1. Factors influencing mycorrhizal germination

Along with plant factors such as age, lifespan, and root density, edaphic factors also plays an important role in spore germination and colonisation (Abbott and Robson, 1991). The diversity and distribution of mycorrhiza varies widely due to various environmental conditions. These environmental factors that affect the distribution of AMF are soil structure, P, N nutrients in the soil, organic C content, water, pH, and soil temperature (Hartoyo *et al.,* 2021).

Soil properties such as pH, soil fertility and texture, available soil phosphorus, and geographical location, particularly altitude, have a strong influence on AMF communities (Jansa *et al.,* 2014). It has been discovered that pH has a significant impact on the distribution of AM fungi. AM fungi are abundant in acidic to natural soils. VAM host plant colonisation and spore production in soil also vary seasonally based on climate and host plant (Giovannetti, 1985).

According to Jamiokowska *et al.* (2018), important abiotic factors influencing the efficacy of arbuscular mycorrhiza consist of soil physicochemical properties, water and biogenic element availability, agricultural practices, and weather conditions.

2.9. Influence of AMF application on growth and yield attributes

2.9.1. Plant growth parameters

The potential benefits of mycorrhiza in the rhizosphere are the result of beneficial interactions between all rhizosphere microbes required for plant growth.

Borde *et al.,* 2010 reported that when compared to non-AM plants, growth parameters of garlic *(Allium sativum)* such as leaf area, plant fresh weight and dry weight and antioxidant enzyme activities were greater in AM plants due to increased antioxidant activity and proline content.

In comparison to non-AMF pea plants, Kristek *et al.* (2005) found higher mean biomass yield, dry matter, and number of pods, as well as higher nitrogen concentration in AMF inoculated pea plants. Alleh *et al.* (2012) also noticed that mycorrhizal inoculum increased onion yield and dry matter weight significantly.

Co-inoculating tomato with *Rhizophagus fasciculatus*, *Pseudomonas fluorescens,* and *Azotobacter chrococcum* significantly increased plant growth parameters, percentage root colonisation, spore number, and leaf number was reported by Kavatagi and Lakshman (2014).

Inoculation with *Rhizophagus irregularis* and *Funneliformis mosseae* increased the nutritional value of lettuce by increasing mineral nutrients (P, K, Mg, Cu, Fe, Ca), total soluble protein contents, tocopherol (vitamin E) and ascorbic acid (vitamin C) concentrations of leaves in mycorrhiza inoculated lettuce plants, according to Baslam *et al.* (2013).

Samanta and Verma (2006) noticed a significant rise in fruit protein content as well as an increase in plant height and dry weight as a result of AM fungi inoculation in *Capsicum annum*.

Pagano *et al.* (2010) and Cabello and Scotti (2010) investigated the effect of mycorrhizal inoculation on plant growth and found that inoculating native species of leguminous and forest plants with AM fungi resulted in increased height and diameter.

Boonlue *et al.* (2012) conducted experiment with fourteen AMF species isolated from the chilly organic fields. Experiment revealed that *Acaulospora appendicula* (HR0201), *Acaulospora denticulata* (RA2106), and *Glomus clarum* (RA0305) are the best chili growth promoting AMF species which are insensitive to P status, increased plant growth, flowering and fruiting in chili.

A study had found that AM symbiosis promoted the tomato seedling growth and Si absorption. It also improved the auxin response and IAA accumulation in

tomato, thereby promoted tomato seedling growth, including plant height, root length, and dry weight (Jentschel *et al.,* 2007 and Ju *et al.,* 2021).

2.9.2. Plant nutrition

Several researchers have thoroughly studied the effects of mycorrhizal fungi on nutrient uptake and plant growth. The importance of AM fungi in plant growth and nutrition acquisition has been demonstrated and it is largely acknowledged that plants associated with AMF will outperform the non-mycorrhizal plants.

AMF form symbiotic relationship with the plant roots to obtain vital nutrients from the host plant and, in turn, its supplies mineral nutrients such as N, P, K, Ca, Zn, as well as S to plants for nutritional support. AMF produce fungal structures like arbuscules, which helps in the exchange of inorganic minerals, carbon compounds and phosphorus to plants (Li *et al*., 2016 and Prasad *et al*., 2017).

Colla (2015) found that applying *Glomus intraradices* and *Glomus mosseae* with *Trichoderma atroviride* improved wheat grain productivity by 32.1 % and 8.3%, respectively. AM fungi altered the micronutrient availability and absorption of nitrogen, phosphorous and other micronutrients at grain filling stage, which resulted in high protein content and increased cereal crop quality and quantity.

According to Govindarajulu *et al.* (2005), AMF transfers a significant amount of nitrogen from the soil to the root system through extra- radical hyphae which taken up inorganic N and transferred it to the intra-radical hyphae as amino acids (mainly arginine). A study conducted by Garces-Ruiz *et al.* (2017) reported that phosphorous absorption rate was markedly increased in the AMF-colonized maize plants.

Similarly, inoculation of *Glomus etunicatum, Glomus clarum, Rhizophagus intraradices, and Glomus caledoniu* has increased the P and Zn absorption in pepper (*Capsicum annuum L.*), compensating for soil P and Zn deficiency (Ortas *et al*., 2011).

Glomus versiforme inoculation increased the plant growth parameters, photosynthesis, and Mg concentrations in plant tissues of citrus plants (*Poncirus trifoliate*) under low Mg conditions (Xiao *et al.*, 2014).

Rahman *et al*. (2020) AMF inoculation promoted the activity of iron chelate reductase and increased the content of Fe, Zn, S, and P; and relieved Fe deficiency in alfalfa (*Medicago sativa* L.).

Mycorrhizal symbiosis enhanced the concentrations of N, P, and Fe in *Pelargonium graveolens* L. under drought stress condition was reported by Amiri *et al*., 2017.

Tortora *et al.* (2007) found that AMF are involved in the N-fixation process through their interaction with microorganisms. They proposed that free living bacteria associated with AMF functioned as N-fixers for plants.

Tawaraya *et al*. (2006) illustrated that AMF in association with plants increased P availability by solubilizing the insoluble fraction of inorganic P, which increased phosphorous uptake by onion plants. AMF inoculated plants had higher P and Zn content than non-inoculated plants.

According to research conducted by Nzanna *et al.* (2011), AMF along with *Trichoderma harzianum* had the potential to improve seedling growth, development, and nutrient uptake of key elements such as N, P, S, Zn, and Mn in *Solanum lycopersicum*.

2.9.3. Physiological parameters

Ruiz-Lozano *et al*. (2015) reported that *Rhizophagus irregularis, Glomus intraradices inoculation* had increased the biomass production, efficiency of photosystem II, ABA accumulation, synthesis, and strigolactone production in lettuce and tomato under drought condition.

Glomus mosseae application in *Triticum aestivum* under drought condition had increased osmotic potential, chlorophyll content and antioxidant enzymes activity, ascorbic acid content, enzymes of N and P metabolism, and contents of N, P, and K (Rani, 2016).

Under salinity stress conditions, Xu *et al.* (2018) found that the AM fungus *Glomus tortuosum* improved physiological metabolisms by increasing chlorophyll content, light energy utilisation efficiency, gas exchange, and rubisco activity in maize.

Plant tolerance levels in saline soil were investigated by Chandrasekaran *et al.* (2019), who examined the physiological responses of C3 and C4 plants and discovered a positive response to AMF. In both plants, AMF plants had higher chlorophyll contents, gas exchange, water use efficiency, transpiration rate, and stomatal conductance, particularly in C3 plants.

Jabborova (2021) found that combination of bio char and AMF enhanced the fenugreek growth, total chlorophyll content, carotenoid content, relative water content, soil microbial biomass, and enzyme activity.

Sarathambal *et al.* (2022) investigated the effect of arbuscular mycorrhizal inoculation on black pepper cutting growth, mineral nutrient uptake, photosynthesis, and antioxidant activities and found that AM fungi were more prominent in improving root biomass and nutrient accumulations were higher in AM inoculated plants than in un-inoculated black pepper plants. The amount of acid phosphatase and dehydrogenase activity was significantly greater in AM-inoculated soil, and the net photosynthetic rate and stomatal conductance of AM-inoculated black pepper leaves were significantly higher than in un-inoculated plants.

2.9.4. Yield parameters

AMF have been used in large-scale field production of maize (Sabia *et al*., 2015), yam (Lu *et al.*, 2015), and potato (Hijri, 2016), proving that AMF have a high potential for crop yield enhancement.

Glomus versiforme has been linked to increased sugar, organic acid, vitamin C, flavonoids, and mineral content in citrus fruit, according to Zeng *et al*. (2014).

Sharma *et al.* (2011) documented that, AMF in wheat plots of both elevated and flat system showed significantly higher grain yield when compared to non AMF plots.

In tomato, inoculation with mixed mycorrhizal agents enriched Na, K, Mg, Ca, Cl, and citrate accumulation in soil which resulted in increased flowering, size, weight, improved color and shape, and enhanced nitrate, carotenoids, lycopene, and vitamin C content of tomato fruit (Bona *et al.,* 2016; Ramona *et al*., 2020; Ziane *et al*., 2021 and Chouyia *et al.,* 2022).

Field inoculation with mixed exotic AMF *viz., Diversispora versiformis, Funneliformis mosseae* and *Rhizophagus intraradices* significantly improved the fruit size, fruit weight, coloration value, quality, and soluble solids in *Citrus reticulate* (Cao *et al.,* 2021).

Inoculation with *S. constrictum* improved the stomatal conductance of tomato leaves, leaf water potential and relative water content, intensity of photosynthesis and tomato yield (Chitarra *et al*., 2016; Duc *et al.,* 2018 and Leventis *et al*., 2021).

2.10. Influence of AMF application on biotic and abiotic stress.

2.10.1. Abiotic stress

AMF plays an important role in abiotic stress tolerance. It improves host plant tolerance to salinity stress (Hashem *et al*., 2018), water stress (Pavithra and Yapa, 2018), heavy metal stress (Garg and Singh, 2017) and high temperature stress (Mathur *et al.,* 2016).

Pal and Pandey (2016) noted that AMF *Glomus mosseae, Glomus fasciculatum, Gigaspora decipiens* application in *Triticum aestivum* under drought condition has increased the plant growth parameters, and total chlorophyll pigments.

According to Liang *et al.* (2009), AMF can promote maize growth while decreasing the heavy metal uptake like Pb, Zn, and Cd and guarded the hosts from the toxicity of heavy metals in Pb, Zn, and Cd contaminated soils.

Hashem *et al*. (2018) reported that inoculating *Glomus etunicatum, Glomus intraradices, and Glomus mosseae* in *Cucumis sativus* L. increased biomass, photosynthetic pigment synthesis, and enhanced antioxidant enzymes under salinity stress.

Neumann and George (2009) reported that AMF symbiosis plays an important role in helping the leguminous plants to overcome drought stress under low phosphorous availability. Similarly, AMF treatment of tomato seedlings grown under salt condition was noticed to provide more seedling growth by avoiding the negative effects of salt was reported by Basak *et al.* (2011).

Mathur *et al.* (2016) studied the photosynthetic efficacy of maize *Zea mays* plants in the presence of arbuscular mycorrhizal fungi (AMF) under high temperature stress. *Rhizophagus intraradices, Funneliformis mosseae*, and *Funneliformis geosporum* application resulted in increased leaf length, plant height, leaf number, chlorophyll a, photosynthetic rate, stomatal conductance, and transpiration rate in maize plants.

Inoculation of *Funneliformis mosseae* alleviated the toxicity caused by heavy metal stress, such as Cu, Pb, and Zn, improved the tolerance of soybean (*Glycine max* L.) to heavy metal-contaminated soil and enhanced the soybean growth and P uptake (Adeyemi *et al.,* 2021).

Inoculation with *Funneliformis mosseae* and *Glomus gigantean*, improved the water absorption of carrot (*Daucus carota* L.) under drought stress, maintained the osmotic balance, increased photosynthetic efficiency, modified the content of phytohormones, and improved the quality and yield (Yadav *et al*., 2021)

2.10.2. Mycorrhiza and disease resistance

Pathogenic fungi cause extensive crop damage and yield loss. AMF has the capacity to control a variety of plant pathogenic fungi (Bodker *et al.,* 2002). The ability of AM fungi to restrict pathogen could be due to competition with pathogen for space, nutrition, and host photosynthesis. Mycorrhizal fungi attack pathogens and disease causing organisms that enter the root zone by secreting antibiotics and producing siderophores (iron chelating compounds) and observed that mycorrhizal plants produced more plant biomass and mobilised substantially more N and P uptake than non-mycorrhizal plants.

Dual inoculation of *Medicago sativa* with *Funnelliformis mosseae* and *Sinorhizobium medicae* reduced Fusarium wilt disease and resulted in a significant increase in lignin content (Wang *et al.* 2020).

Hu *et al.* (2020) reported that *Funneliformis caledonium* forms symbiosis with plant roots and increase P mobilization which helps in the suppression of *Phytophthora* blight of pepper than *Purpureocillium lilacinum* applied alone or in combination.

Application with *Rhizophagus irregularis* controlled the *Macrophomina phaseolina* infection in Soybean (Marquez *et al.,* 2018) and *Botrytis cinerea* in tomato (Sanmartin *et al*., 2020) under controlled condition.

Combined inoculation of *Funneliformis mosseae* and *Rhizophagus irregularis* in tomato found effective against *Fusarium oxysporum* (Singh *et al*., 2020). Similarly, *Rhizophagus irregularis* application reduced the *Magnaporthe oryzae* infection in Rice was reported by Campo *et al*. (2020).

According to Manian *et al.* (2006), *Glomus fasciculatum* improved tomato plant growth and reduced fusarium wilt and *Glomus claroideum* was found effective against *Fusarium oxysporum* in Cucumber (Ahammed *et al.,* 2020).

Nair *et al.* (2015) found that *Glomus fasciculatum* colonization in tomato plants suppressed the foliar damage during *Alternaria alternata* infection. Pre-inoculation of tomato plants with *F. mosseae* enhanced tomato resistance to early blight caused by *Alternaria.* Application of *F. mosseae* + *Glomus fasciculatum* found to be effective against *Fusarium* wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (Devi *et al*., 2022).

Iyer and Sundararaju (1993) observed that inoculating ginger with AM fungi increased plant growth and rhizome yield while suppressing *Meloidogyne* and *Pythium* populations. This appears to be the initial study on AM fungi, pathogenic fungi, and nematodes interactions.

The majority of studies used *Rhizophagus irregularis* and *F. mosseae* as biocontrol agent was found effective bio-protection for the vascular wilt disease (Boutaj *et al*., 2022). AMF fungus was noted for potential protective effect *against Xanthomonas translucens* in Wheat was recorded by Fiorilli *et al*. (2018).

Ozgonen and Erkilic (2007) reported that *Glomus mosseae*, *Glomus etunicatum*, *Glomus fasciculatum* and *Gigaspora margarita* increased the shoot height, fresh and dry weight of shoot and root. *Glomus mosseae* application significantly increased capsidiol level in pepper and suppressed the disease severity of *Phytophthora capsici* by 91.7%, 43.0% and 57.2% under pot, greenhouse and field conditions, respectively. *Glomus mosseae* enhanced the development of plants and reduced Phytophthora blight of pepper.

Bokhari *et al*. (2023) reported that using mycorrhizospheric fluorescent Pseudomonas (MRFP) combined with VAM fungi significantly suppressed root rot pathogens of sunflower in both pot and field experiments, and in some cases improved plant growth.

Glomus hoi, Clariodeoglomus clareoideum, and *Glomus mossae* have been reported to provide various defensive benefits to host populations against fungal pathogens such as Powdery mildew (Eck *et al*., 2022). Similarly, *Glomus* sp. has been

shown to improve resistance to *Rhizoctonia solani*-caused damping-off disease in Cucumber plants (Aljawasim *et al*., 2020). The AMF (*Rhizophagus irregularis*) has also been shown to improve plant resistance to the fungus *Fusarium oxysporum* in tomato plants (Wang *et al*., 2022).

Fujita *et al*. (2022) noticed that mycorrhizal application added effective disease resistance to avirulent and virulent bacterial and fungal pathogens in tomatoes. Kadam *et al*. (2020) stated in a review that mycorrhizal-induced disease resistance has enhanced physiological, phytohormonal, and metabolic changes in plants.

Raghavendra *et al*. (2018) studied wilt disease complex of pepper which is usually caused by the combined action of the bacterial pathogen *Ralstonia solanacearum*, the root-knot nematode *Meloidogyne incognita*, and the fungal pathogen *Phytophthora capsici*. *Glomus bagyarajii* was found to be the best AMF in enhancing plant height, stem girth and also showed lesser disease index (50.20%) than that of the control group (82.14%), indicating that AMF effectively controls the proliferation of all three pathogens.

Ralstonia solanacearum caused bacterial wilt is a globally significant soil-borne plant disease that has a significant impact on tomato production and causes significant economic losses. AMF (*Rhizophagus irregularis* MUCL 41833) alleviated *Ralstonia solanacearum* and Fusarium wilt symptoms in tomato (Chave *et al.*, 2017).

The work of various researchers revealed the studies on the isolation and identification of arbuscular mycorrhizal fungi using precision isolation techniques, taxonomic keys, and molecular methods. Arbuscular mycorrhizal fungi have been used by various workers in various agricultural crops to increase productivity through promoting plant growth characteristics and disease suppression. They provide an essential nutritional absorption system for mineral nutrients such as nitrogen, phosphorus, potassium, and trace elements such as zinc, manganese, and others to the plant, as well as making soluble phosphate available.

AMF also improves disease resistance, drought resistance, and host plant survival under stress conditions, and they are also employed to tolerate transplant shocks in micro-propagated plants. As a result, AMF inoculum can be used as an alternative to inorganic fertilisers. Though the occurrence of symbiotic colonisation of AM fungi has been well recorded by several workers in various parts of India, review of the literature reveals that not much work has been done in AMF role in Ginger plant.

2.11. Application of AMF on spice crop

Few studies have been conducted on the effects of commercial mycorrhizal inoculants on horticultural plant growth and development, as well as their impact on indigenous mycorrhizal populations (Antunes *et al*., 2009 and Mummey *et al*., 2009; and Cwala *et al*., 2010). Mycorrhiza has been shown to improve pepper fruit yield (Kaya *et al*., 2009), seedling quality, and compensate for P and zinc (Zn) deficiency in P and Zn deficient soils (Ortas *et al.*, 2011).

2.11.1. Ginger

Zingiber officinale Roscoe, or ginger, is a member of the Zingiberaceae family, which includes 47 genera and 1400 species, including turmeric (*Curcuma longa*) and cardamom. *Zingiber officinale* is the only species widely used for flavouring among the 150 species in the genus *Zingiber*. It is grown from April to December at an optimal elevation of 300 to 900 m (Nybe and Raj, 2016), preferring light shade and requiring a warm, humid climate.

Ginger is a very succulent herb, and its rhizomes are extremely susceptible to a variety of abiotic and biotic stresses. One of the most damaging diseases of ginger is rhizome rot, also known as soft rot (Dohroo, 2005 and Rai *et al*., 2018). The organisms associated with the rhizome rot disease vary depending on crop growth stage and geographical area. Correct disease diagnosis, as well as the intervention of one or more pathogens in a specific location, must be clearly understood so as to plan effective location-specific integrated management measures.

Ginger is grown in a wide range of soil types, but it is most commonly grown in black, red, and red loamy soils. The occurrence of rhizome rot disease was highest in crops grown in black soil (34.68%), and lowest in red loamy soils (22.62%). Ratankumar Singh (2018) noticed that the occurrence and advancement of rhizome rot complex disease in ginger was highest in July and August due to the coincidence of heavy rainfall and cool weather.

2.11.1.1. Impact of AMF application on ginger

According to the study conducted by Samanhudi *et al*. (2014) treatment with mycorrhizal fungi at different doses like 5, 10, and 15 g/plant increased the plant height, number of leaves, number of tillers, and fresh weight of ginger rhizome in comparison with non-mycorrhizal treatments.

Jabborova *et al.* (2022) investigated the effects of plant-growth-promoting *Bacillus endophyticus* IGPEB 33 and arbuscular mycorrhizal fungi (AMF) on plant growth of ginger (Zingiber officinale), physiological properties, and soil enzymatic activities. In comparison to the control, co-inoculation of *B. endophyticus* IGPEB 33 and AMF treatment increased plant height by 81%, leaf number by 70%, leaf length by 82%, and leaf width by 40%.

Joseph (1997) attempted to manage rhizome rot, the most destructive disease of ginger caused by *Pythium aphanidermatum*, using native arbuscular mycorrhizal fungi (AMF) and antagonists. Of the fourteen native AMF developed from ginger rhizosphere, Mi-l and Mi-4, identified as *Glomus* species, were the most efficient isolates for rhizome rot suppression, yield increase, and growth enhancement of ginger.

Jabborova (2022) investigated the effect of *Pseudomonas koreensis* IGPEB 17 and arbuscular mycorrhizal fungi (AMF) on plant growth and physiological properties of ginger (Zingiber officinale). The results showed that dual inoculation of *Pseudomonas koreensis* IGPEB 17 strain and AMF enhanced the plant growth and

physiological traits of ginger plants in comparison to *P. koreensis* IGPEB 17 and AMF alone.

Because of their diverse applications, mycorrhizal fungi are used as 'biofertilizers' due to their enhanced uptake of mineral nutrients which can be used in place of large amount of chemical fertilisers. Biofertilizers are emerging as a viable alternative to synthetic agrochemicals as a consequence of increased awareness about the negative environmental impact of these chemicals, as they promote crop growth and yield in an environmentally friendly manner (Basu *et al.,* 2021). However, little research has been conducted on the biodiversity of AM fungi in spices and their application in increasing *Zingiber officinale* yield. Given the foregoing, the goal of this study was to identify the most effective AM fungi for use as a biocontrol agent against rhizome rot caused by *Pythium myriotylum* of *Zingiber officinale*. From extensive literature review, it is clear that no work has been done to assess the AM fungal diversity of ginger crop in Wayanad region. The literature also clearly shows that the AM fungal species communicates differently in different environmental conditions. So the current study aims to investigate the diversity of AM fungi in this region as well as the efficacy of the selected AM fungi in the growth and yield of ginger as well as soft rot management.

3. MATERIALS & METHODS

3. Materials & methods

The research project titled “Evaluation of native arbuscular mycorrhizal fungi for the growth and management of rhizome rot of ginger (*Zingiber officinale*)” was conducted during 2020-2022. All the laboratory works were carried out in the department of Agricultural Microbiology, College of Agriculture, Vellanikkara and pot culture experiments were carried out at Regional Agricultural Research Station, Ambalavayal, Wayanad. The materials used and the methods followed are mentioned in this chapter.

3.1. Materials

3.1.1. Glassware

Glassware viz., Petri plate, volumetric flask, beakers, slides, coverslips, glass rod, funnel, pipette, test tube used for the laboratory work were of borosilicate.

3.1.2. Chemicals

All the chemicals used in the study were of either analytical reagent grade or molecular biology grade.

3.1.3. Sterilization

All the glass wares were sterilized in hot air oven at 160˚C for one hours and all the growth solutions were autoclaved at 121 ˚C and pressure 15 psi for 20 minutes.

3.1.4. Equipments and instruments

Equipments and instruments used in the study included autoclave, compound microscope, stereo microscope, laminar air flow chamber, pH meter, conductivity meter, weighing balance, sieves, and hot air oven.

3.2. Selection of site and collection of soil sample

For the soil sample collection, ten major ginger growing areas were identified in Wayanad district and the rhizospheric soil samples were collected from randomly selected ginger plants from farmer's field in selected location (Table 1).

Three ginger plants were selected randomly from a field. The plants were uprooted and soils adhering to the roots and adjacent to the rhizome were collected at the depth of 10 - 20 cm. The soil taken from three plants in the same field were pooled to get the representative soil sample. The shade dried samples were transferred to sterile polythene covers and stored under refrigerated conditions for further use.

Table 1: Location of soil sample collection and their GPS coordinates

Location	Sample code	GPS coordinates
Kalpetta	KAL	N 11˚63'2162" E 76˚08'3029"
Pachilakkad	PAC	N 11˚71'146" E 76˚4'46.332"
Edakkal	EDA	N 11˚37'28.98012" E 76˚13'39.53388"
Varadoor	VAR	N 11˚42'35.54568" E 76˚54'2.37944"
Anappara	ANA	N 11˚36'0.10476" E 76˚13'47.62308"
Vaduvanchal	VAD	N 11˚35'4.656" E 76˚13'16.56"
Ambalavayal	AMB	N 11˚37' 9.241" E 76˚12' 38.88072"
Malavayal	MAL	N 11˚63'0394" E 76˚24'7919"
Nenmeni	NEN	N 11˚37'31.0" E 76˚16'19.5"
Poomala	POO	N 11˚64'5986" E 76˚24'2075"

3.3. Chemical analysis of soil

3.3.1. Soil pH

Soil pH was determined by using pH meter (Jackson, 1982).

3.3.2. Electrical conductivity of the soil

The electrical conductivity of the soil was estimated using the instrument conductivity meter.

3.3.3. Organic carbon of soil

The organic carbon content of the soil was determined by colorimetric method (Walkey and black, 1934). One gram of the soil sample was taken in 100 ml conical flask and 10 ml of 1N $K_2Cr_2O_7$ solution and 10ml of Conc.H_2SO_4 were added to the conical flask and cooled. Then 10 ml of distilled water was added and stirred well and allowed to stand overnight. Finally, the reaction was read for green chromous in colorimeter.

3.3.4. Available nitrogen in soil

The soil's available nitrogen content was determined using the alkaline permanganate method described by Subbiah and Asija (1956). Twenty gram of soil was transferred to an 800 ml Kjeldahl digestion flask, which was then filled with 100 ml of 0.32% $KMnO_4$ solution, 100 ml of 2.5% NaOH solution, and 20 ml of water. The flask was attached to a standard Kjeldahl distillation unit, and 75 ml of the distillate was collected in a 25 ml boric acid indicator mixture. Bromocresol green and methyl red were used as indicators, and the absorbed ammonia was titrated with 0.05 N H_2SO_4 to determine the sample's available nitrogen content.

3.3.5. Available phosphorous of soil

The soil's available phosphorous content was colorimetrically determined using the ascorbic acid reduced molybdate blue colour method (Bray and Kutz, 1945;

Watanabe and Olsen, 1965). It was measured with a solution of 0.025N HCl and 0.03N NH_4F known as the Bray-1 extractant. Ten millilitres of extractant were mixed with one gram of soil and shaken for five minutes. The amount of phosphorus extracted was calculated by measuring the intensity of the blue colour produced in the filtrate after treatment with the molybadate-ascorbic acid reagent. A colorimeter at 880 nm is used to measure the colour.

3.3.6. Available potassium of soil

The soil's available potassium content was calculated using neutral ammonium acetate (Hanway and Heidel, 1952), and the concentration was measured using a flame photometer. Potassium was extracted from the soil by shaking for 5 minutes in 10 millilitres of 1 N ammonium acetate (pH 7) with 1 g of dried soil. The filtered extract was analysed on an atomic absorption at 776 nm to determine the available potassium.

3.4. Isolation and enumeration of AMF spores from rhizosphere soil

Arbuscular mycorrhizal fungi are obligate symbiont. Gerdemann (1995) for the first time used wet sieving and decanting technique to isolate mycorrhizal spores. Gerdemann and Nicolson's (1963) wet sieving and decanting method was used for the isolation of AMF spores.

The soil sample were finely powdered and passed through 2 mm sieve. Hundred gram sieved soil was mixed with 1000 ml of water. The mixture was stirred for about 15 minutes and allowed to settle for 30 minutes. The heavier particles settled at the bottom and the supernatant was passed carefully through series of sieves of different size *viz.,* 710 µm, 420 µm, 250 µm, 105 µm, and 45 µm. Residues that settled on the last two sieves were collected carefully and filtered through whatsman no. 1 filter paper. Then the spore containing filter paper was transferred to the Petri plate and observed under the stereo microscope.

3.5. Identification of AM fungal spores

The spores in the filter paper were observed under the stereo microscope and enumerated using grid intersect method given by Giovannetti and Mosse (1980). AMF were tentatively identified at genus level by observing and recording morphological characters of AMF spores like colour, size, shape, number of spore walls, surface ornamentation of spores, nature and size of subtending hyphae, and bulbous suspensor (INVAM, 2018).

3.6. Mass multiplication of spores

Isolated predominant spores were observed for abundance and morphological character of the each spores were recorded. Based on the abundance, five most predominant AMF spores were selected and approximately 10 AMF spores with exactly similar morphology of the selected spores were picked for mass multiplication using maize as host plant for further studies. The picked spores are surface sterilized with streptomycin sulphate 200 ppm for 15 minutes. Surface sterilized spores are transferred to funnel for multiplication. The funnels were filled with sand and soil (sterilized) at 1:1 ratio. The maize seeds are surface sterilized with mercuric chloride 0.1% for 3 minutes. The plants are kept in funnels (mother inoculum) for 20 days in shade net. After 20 days, mother inoculum is transferred to pot for mass multiplication (Kaushish et al., 2011).

Mass multiplication was carried out in clay pot containing potting mixture proposed by mixing vermiculite (7.5 kg) + perlite (2.5 kg) + soil (1 kg) + cow dung (0.5 kg). Pots were maintained in the shade net carefully and checked periodically for purity. After 60 days, total spore count and root staining were done to check the mycorrhizal infectivity.

3.7. Assessment of AM colonization

The root infectivity was assessed by using the procedure given by Phillips and Hayman (1970). The secondary and tertiary fine roots were collected from the maize plant and washed thoroughly in the tap water to remove soil particles. Then the roots

were soaked in 10 % KOH solution and kept in water bath at 90˚C for 1 hr. After 1 hr in water bath, KOH solution was discarded and the roots were washed in the tap water for 3 times. Further the roots were soaked in 2% HCl for 10 minutes for acidification and excess acid was drained and treated with trypan blue dye 0.005 % for 10 minutes. Excess dye was removed and roots were cut into 1 cm bits and placed in the glass slide, mashed softly and observed for the infectivity.

AMF colonization was assessed by the slide method given by Giovannetti and Mosse (1980). The stained root bits of 1 cm long were randomly selected and mounted on the microscopic slides. Approximately 10 roots bits were taken for each sample and checked for the infectivity. The percent root colonization was calculated by

$$\% \text{ AMF colonization} = \frac{\text{Number of root segments colonized}}{\text{Total number of root segments observed}} \times 100$$

3.8. Pot culture experiment

3.8.1. Experiment site

The pot culture experiment were conducted at Regional Agricultural Research Station, Ambalavayal, Wayanad district of Kerala. Geographically the site was located at an altitude of 974 m above MSL about 100 km, with latitude 11.6167˚ N, and longitude 76.21403˚ E.

3.8.2. Experimental soil

The initial nutrient status of the soils were analysed for the parameters like pH, EC, organic carbon, nitrogen, phosphorous, and potassium.

3.8.2.1. pH

The pH of the pot culture soil is determined by following the procedure as mentioned in 3.3.1.

3.8.2.2. Electrical conductivity

The electrical conductivity of the pot culture soil was determined by following the procedure as mentioned in 3.3.2.

3.8.2.3. Organic carbon content

The organic carbon content of the soil was determined by following the procedure as mentioned in 3.3.3.

3.8.2.4. Available nitrogen

The available nitrogen content of the soil was determined by following the procedure as mentioned in 3.3.4.

3.8.2.5. Available phosphorous

The available phosphorous content of the soil was determined by following the procedure as mentioned in 3.3.5.

3.8.2.6. Available potassium

The available potassium content of the soil was determined by following the procedure as mentioned in 3.3.6.

3.8.3. Ginger variety

The planting material *Zingiber officinale* variety "Rio de janeiro", which is widely cultivated by farmers in Wayanad district was selected for the experiment. It can be used as both raw ginger and dry ginger. It is a high dry ginger yielding and high oleoresin producing variety which is highly susceptible to rhizome rot. The planting material was collected from Regional Agricultural Research Station, Ambalavayal, Wayanad.

3.8.4. Season

The ginger rhizome was planted in May 2022 (2 rhizomes per pot) and harvested on December 2022.

3.8.5. Experimental Design

The experiment was carried out in completely randomized design (CRD) with eight treatments and five replications. Totally, about 80 pots were used for two experiments with 2 rhizomes in each pot.

3.8.6. Treatment details

Ginger cultivation was carried out in pot culture with the application of nitrogen and potassium as recommended in the package of practices of KAU (POP of KAU, 2016). The quantity of phosphate fertilizer applied was fixed based on soil testing data, so as to maintain lower P levels. The potting mixture is disinfected by solarisation. The solarisation was done by heaping the soil covered with polythene sheet in sun for 1 month.

In the present study, two experiments were conducted. Experiment 1 was conducted to assess the effect of AMF in growth and yield of ginger and Experiment 2 was conducted to evaluate the effectiveness of AMF spores in managing rhizome rot disease of ginger.

The quantity of phosphate fertilizer was reduced to one fourth of the recommended dose of KAU POP, 2016 for the treatment T_1 to T_5 in both the experiments.

3.8.6.1. Experiment 1 - Growth and yield promotion of ginger

T_1: PAC AMF 2 + POP of KAU

T_2: EDA AMF 1 + POP of KAU

T_3: ANA AMF 5 + POP of KAU

T_4: NEN AMF 2 + POP of KAU

T_5: POO AMF 3 + POP of KAU

T_6: Package of Practices Recommendation of KAU (2016) (Appendix II)

T_7: Organic Package (Ad hoc) of Practices Recommendation of KAU (2015) (Appendix III)

T_8: Absolute control

3.8.6.2. Experiment 2 - Soft rot management of ginger

T_1: PAC AMF 2 + CI+ POP of KAU

T_2: EDA AMF 1 + CI+ POP of KAU

T_3: ANA AMF 5 + CI + POP of KAU

T_4: NEN AMF 2 + CI + POP of KAU

T_5: POO AMF 3 + CI + POP of KAU

T_6: Package of Practices Recommendation of KAU (2016) + CI (Appendix II)

T_7: Organic Package (Ad hoc) of Practices Recommendation of KAU (2015) + CI (Appendix III)

T_8: Absolute control

The cultivation practice explained in Package of Practices Recommendation of KAU (2016) was followed for the treatments (T_1 to T_6) and Organic Package (Ad hoc) of Practices Recommendation of KAU (2015) was followed in T_7.

3.8.7. Layout of experiments

3.8.7.1. Experiment 1 – Effect of AMF on growth promotion of ginger

T_3R_1	T_8R_2	T_5R_3	T_8R_4	T_6R_5
T_7R_1	T_1R_2	T_4R_3	T_1R_4	T_7R_5
T_4R_1	T_7R_2	T_6R_3	T_7R_4	T_8R_5
T_8R_1	T_2R_2	T_3R_3	T_2R_4	T_5R_5
T_1R_1	T_6R_2	T_7R_3	T_6R_4	T_3R_5
T_5R_1	T_3R_2	T_2R_3	T_3R_4	T_4R_5
T_2R_1	T_5R_2	T_8R_3	T_5R_4	T_1R_5
T_6R_1	T_4R_2	T_1R_3	T_4R_4	T_2R_5

3.8.7.2. Experiment 2 – Effect of AMF on soft rot management of ginger

T_2R_1	T_8R_2	T_3R_3	T_6R_4	T_5R_5
T_7R_1	T_1R_2	T_4R_3	T_2R_4	T_3R_5
T_1R_1	T_5R_2	T_7R_3	T_3R_4	T_2R_5
T_3R_1	T_4R_2	T_2R_3	T_5R_4	T_8R_5
T_5R_1	T_6R_2	T_1R_3	T_4R_4	T_7R_5
T_6R_1	T_7R_2	T_5R_3	T_8R_4	T_4R_5
T_8R_1	T_2R_2	T_6R_3	T_7R_4	T_1R_5
T_4R_1	T_3R_2	T_8R_3	T_1R_4	T_6R_5

3.8.8. Potting mixture

The solarized soil was mixed with 20 t FYM + 2 t neem cake + 1 t ash + 4 t vermicompost/ha area. The initial nutrient status like pH, EC, nitrogen, phosphorous, potassium content of the soils were analysed.

3.8.9. Rhizome treatment

Healthy single bud rhizome weighing nearly (15g) were selected. Seed treatment was done as per the KAU package of practices (2016) for the treatments (T_1 to T_6) and ad hoc organic KAU package of practice (2015) for the treatment (T_7).

3.8.10. AMF inoculum

The selected and mass multiplied AMF inoculum was applied @ 15g / rhizome at the time of planting for T_1 to T_5 for both the experiment (KAU POP, 2016).

3.8.11. Challenge inoculation

The challenge inoculation was done in experiment 2 (disease management) with the pathogen *Pythium myriotylum* (causal organism of rhizome rot of ginger) to check the efficiency of AMF in managing rhizome rot of ginger.

The soft rot causing pathogen (*P. myriotylum*) was inoculated into each pot at 75 DAP. Ten gram of 5 day old *P. myriotylum* mycelia was blended in 100 mL sterile distilled water and made up to 1000 mL, and 50 mL of that mycelial suspension was inoculated into each pot (Dinesh *et al.*, 2015).

3.8.12. Fertilizer application

NPK was applied in the ratio of 75:50:50 Kg/ha/year for the first 6 treatments. Full dose of P_2O_5 and 50 % of K_2O was applied as basal. Half quantity of nitrogen was applied at 60 days after planting. The remaining quantity of N and K_2O applied at

120 days after planting. Treatment 7 was applied with *Azospirillum* (Ad hoc organic POP, 2015).

3.8.13. Intercultural operation

Hand weeding was done as and when required.

3.8.14. Harvesting

The crop was harvested during December, 2022. The fresh and dry weight of the rhizome was recorded and the final nutrient status of the soil was analysed.

3.9. Observations

Biometric observations of plants were taken in both the experiments at monthly intervals for the period of 6 months.

3.9.1. Height of the plant

The plant heights were measured from the base of the plant to growing tip at monthly interval. The plant height was expressed in cm.

3.9.2. Number of leaves

The number of fully opened leaves of selected shoot in the pot was recorded at monthly interval.

3.9.2. Number of tillers

The number of tillers developed per plant was recorded at monthly interval.

3.9.3. Leaf area

The length (l) and the width (w) of the leaf was recorded at the monthly interval. The leaf area was calculated by using the formula given by Montgomery (1911).

Leaf area = leaf length (L) × leaf width (w) × crop factor (k)

Where k =0.666 (Reddy and Reddy, 1995)

3.9.4. Percent disease incidence:

The percent disease incidence of the plants inoculated with the challenge inoculum *pythium myriotylum* was calculated in experiment 2 by using the formula,

$$\text{Percent disease incidence} = \frac{\text{Number of leaves of infected}}{\text{Total number of leaves observed}} \times 100$$

The disease intensity was assessed by using 0 to 5 rating scale (shahzad and bhat, 2005).

Category	**Numerical value**	**Leaf area infected %**
I	0	Disease free
II	1	0.1 – 10.0
III	2	10.1 – 25.0
IV	3	25.1 – 50.0
V	4	50.1 – 75.0
VI	5	>75

3.9.5. Fresh and dry weight of the rhizome

The fresh and dry weight of the rhizome was calculated after harvesting (180 DAP). The fresh weight was recorded immediately after harvest and dry weight was taken after drying the plant in hot air oven at 70˚C until completely dried.

3.9.6. Fresh and dry weight of the plant

The fresh and dry weight of the plant was calculated after harvesting (180 DAP). The fresh weight was recorded immediately after harvest and dry weight was taken after drying the plant in hot air oven at 60˚C until completely dried.

3.9.7. Oleoresin estimation

Oleoresin content was estimated by using Soxhlet extractor (Mbaeyi-Nwaoha *et al*., 2013). Acetone (99%) was used to extract 30 g of dried ginger rhizome powder. The extraction was carried out above the boiling points of the respective solvents and continued until the extraction was completed. After that, the recovered oleoresin was cooled and weighed. The oil extraction yield was calculated using the formula,

$$\text{Oil yield} = \frac{\text{Weight of oleoresin extracted (g)}}{\text{Weight of dried ginger rhizome powder (g)}} \times 100$$

3.9.8. Final nutrient status of pot cultured soil

3.9.8.1. pH

The pH of the pot culture soil is determined by following the procedure as mentioned in 3.3.1.

3.9.8.2. Electrical conductivity

The electrical conductivity of the pot culture soil was determined by following the procedure as mentioned in 3.3.2.

3.9.8.3. Organic carbon content

The organic carbon content of the soil was determined by following the procedure as mentioned in 3.3.3.

3.9.8.4. Available nitrogen

The available nitrogen content of the soil was determined by following the procedure as mentioned in 3.3.4.

3.9.8.5. Available phosphorous

The available phosphorous content of the soil was determined by following the procedure as mentioned in 3.3.5.

3.9.8.6. Available potassium

The available potassium content of the soil was determined by following the procedure as mentioned in 3.3.6.

3.9.9. Percent root colonization of ginger root

Percent root colonization of ginger root was determined by following the procedure as mentioned in 3.7.

3.9.10. Total spore count in pot culture soil

Total spore count in pot culture soil was determined by following the procedure as mentioned in 3.5.

3.9.11. Meteorological observation

The weather data like rainfall, relative humidity, maximum and minimum temperature, and sunshine hours were recorded during the cropping period May to November at meteorological observatory, Regional Agricultural Research Station, Ambalavayal and presented in Appendix I.

3.9.12. Statistical analysis

All the field data and the laboratory data recorded were analysed statistically by CRD using Grapes software package of KAU (Gopinath *et al*., 2020).

4. RESULTS

4. RESULT

The research work was carried out with an aim to study the diversity of native arbuscular mycorrhizal fungi from different rhizospheric soils of ginger in Wayanad district and to evaluate its effect on growth and soft rot management in ginger.

The rhizospheric soil samples were collected from ten different ginger growing areas of Wayanad district (Figure 1).

4.1. Location of soil samples collected from Wayanad district

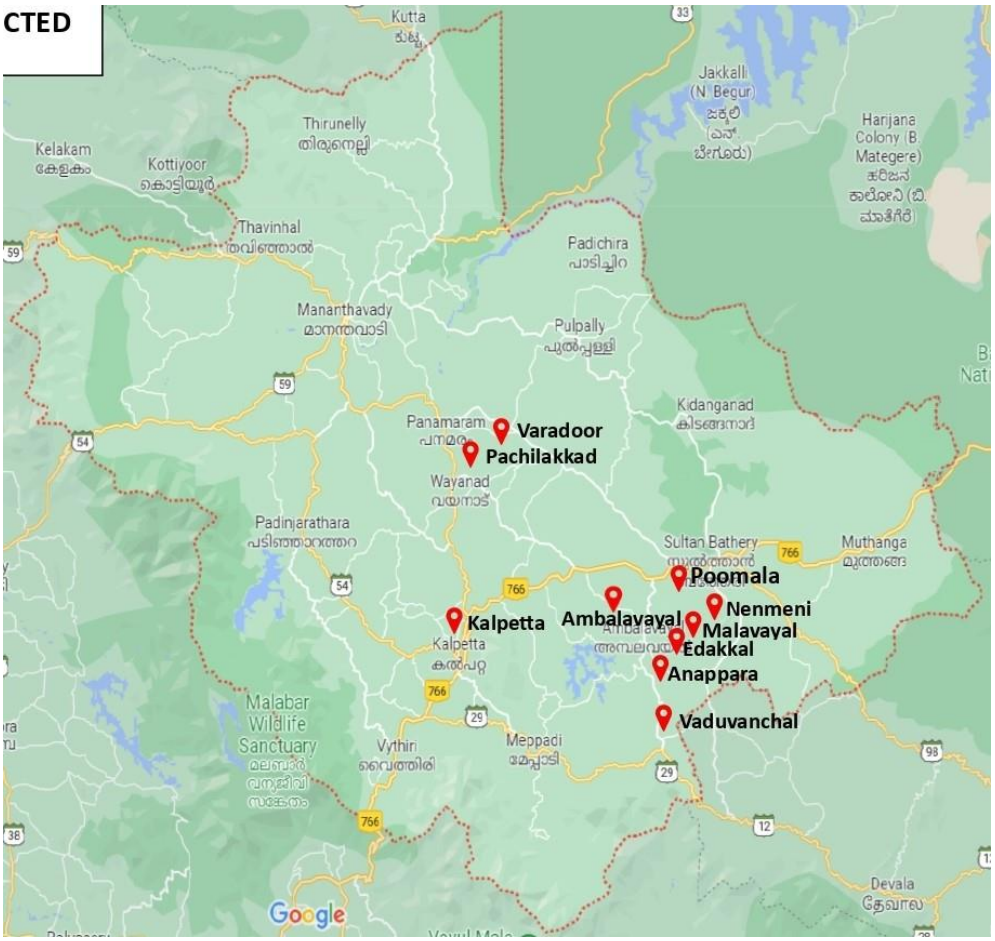


Figure 1: Location of soil samples collected from Wayanad district

4.2. Nutrient status of rhizospheric soil samples

The soil nutrient contents like pH, EC, organic carbon, nitrogen, phosphorous and potassium were analysed for the ten soil samples and presented in the Table 2.

The pH of the soil samples ranged between 4.51 and 6.14, which indicated that soil samples were acidic. The samples KAL, EDA, VAR, ANA, and NEN were more in acidic range between 4.51 and 4.91 than PAC, AMB, MAL, and POO whose pH ranged from 5.17 to 5.49 (slightly acidic). The sample VAD was less acidic with 6.14 which was near to neutral.

The EC value of the samples ranged from 0.05 to 0.23 ds/m which showed that the soils were non – saline. The organic carbon content of the soil ranged between 0.49 and 1.79 dS/m, which indicates except NEN all the other samples were high in organic carbon content. Similar to organic carbon content, nitrogen values ranged between 0.049 % and 0.179 % were higher in all the samples except NEN, which had medium nitrogen content.

Among the ten samples, the highest phosphorous content was found in VAR with 356.44 Kg/ha, followed by POO with 282.99 Kg/ha of available phosphorous. The lowest phosphorous content was found in KAL (24.20 Kg /ha) and AMB sample (25.45 Kg /ha). Potassium content ranged from 115.69 to 702.91 Kg/ha. The samples KAL, VAR, NEN showed high potassium content while all the other samples were having medium potassium content.

4.3. Enumeration and characterization of AMF

Isolation and enumeration results were recorded and presented in Table 3**.**

Morphological characters of AMF spores such as colour, size, shape, Nature of hyphae, bulbous suspensor, wall structure, surface ornamentation, spore count were recorded (Table 4 to 12) and genus of the spores were tentatively identified (INVAM, 2018).

Table 2: Chemical properties of soils collected from Wayanad district

Sample	pH	EC (ds/m)	Organic carbon (%)	Nitrogen (%)	Phosphorous (Kg/ha)	Potassium (Kg/ha)
KAL	4.90 (acidic)	0.08 (low)	1.68 (High)	0.168 (High)	24.20 (High)	316.85 (High)
PAC	5.49 (acidic)	0.06 (low)	1.34 (High)	0.134 (High)	32.07 (High)	150.19 (Medium)
EDA	4.51 (acidic)	0.05 (low)	1.28 (High)	0.128 (High)	70.75 (High)	159.49 (Medium)
VAR	4.86 (acidic)	0.05 (low)	1.32 (High)	0.132 (High)	356.44 (High)	702.91 (High)
ANA	4.91 (acidic)	0.05 (low)	1.15 (High)	0.115 (High)	28.76 (High)	258.83 (Medium)
VAD	6.14 (slightly acidic)	0.23 (low)	1.60 (High)	0.160 (High)	74.47 (High)	214.82 (Medium)
AMB	5.17 (slightly acidic)	0.07 (low)	1.38 (High)	0.138 (High)	25.45 (High)	205.52 (Medium)
MAL	5.18 (slightly acidic)	0.07 (low)	1.11 (High)	0.111 (High)	129.92 (High)	184.13 (Medium)
NEN	4.58 (acidic)	0.07 (low)	0.49 (Medium)	0.049 (Medium)	79.03 (High)	554.74 (High)
POO	5.45 (slightly acidic)	0.13 (low)	1.79 High)	0.179 (High)	282.99 (High)	115.69 (Medium)

Table 3: Total spore count, number of morphotypes, and number of the most predominant spore obtained from soil samples collected from Wayanad district.

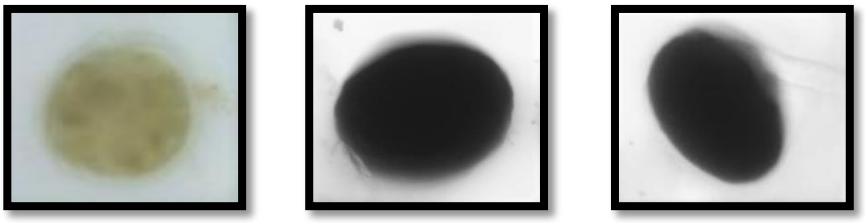
SI. No	Sample	Total spore count/ 100g of soil	Number of morphotypes	Count of predominant spore/ 100 g of soil
1	KAL	91	6	20
2	PAC	100	7	28
3	EDA	121	8	30
4	VAR	99	8	19
5	ANA	118	9	35
6	VAD	65	6	15
7	AMB	92	7	24
8	MAL	87	7	19
9	NEN	111	7	32
10	POO	119	9	27

Table 4: Morphological characters of AMF spores in rhizosphere soil sample collected from Kalpetta sample

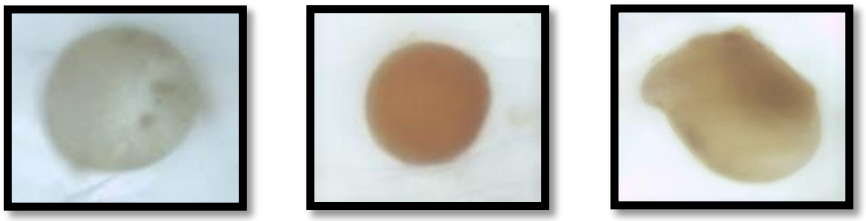
Isolates	Colour	Size (µm)	Shape	Number of spore walls	Nature of hyphae	Bulbous suspensor	Surface ornamentation	Number of spores/ 100g of soil	Tentative identification (Genus level)
KAL AMF 1	Light yellow	25.38	Globose	2	Straight hyphae	Absent	Smooth	20	*Glomus* sp.
KAL AMF 2	Black	60.49	Globose	3	Absent	Absent	Irregular	20	*Glomus* sp.
KAL AMF 3	Black	40.40	Oval	1	Bulbous	Present	Smooth	15	*Gigaspora* sp.
KAL AMF 4	White	32.57	Globose	2	Absent	Absent	Smooth	12	*Glomus* sp.
KAL AMF 5	Brownish orange	24.75	Sub globose	1	Subtending hyphae	Absent	Spiny	10	*Glomus* sp.
KAL AMF 6	Light brown	60.51	Sub globose	2	Bulbous hyphae	Present	Irregular	14	*Scutellospora* sp.

Table 5: Morphological characters of AMF spores in rhizosphere soil sample collected from Pachilakkad sample

Isolates	Colour	Size (µm)	Shape	Number of spore walls	Nature of hyphae	Bulbous suspensor	Surface ornamentation	Number of spores/ 100g of soil	Tentative identification (Genus level)
PAC AMF 1	Dark brown	47.77	Globose	1	Subtending hyphae	Absent	Smooth	15	*Glomus* sp.
PAC AMF 2	Brown	42.27	Sub globose	2	Absent	Absent	Regular	28	*Glomus* sp.
PAC AMF 3	Black	65.38	Globose	1	Absent	Absent	Irregular	15	*Glomus* sp.
PAC AMF 4	Brown	90.05	Ovoid	2	Bulbous	Present	Regular	13	*Scutellospora* sp.
PAC AMF 5	Pale yellow	23.59	Globose	2	Bulbous	Present	Spiny	11	*Gigaspora* sp.
PAC AMF 6	Brown	35.49	Sub globose	1	Bulbous	Present	Smooth	10	*Scutellospora* sp.
PAC AMF 7	Orange	55.53	Oval	2	Straight	Absent	Spiny	8	*Glomus* sp.

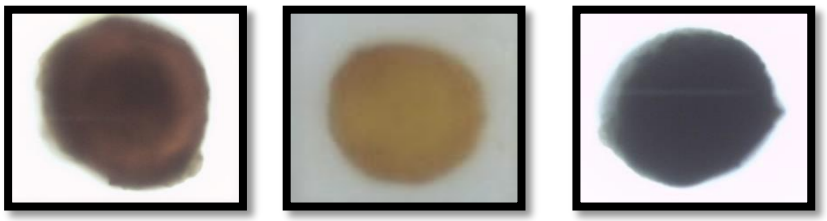


KAL AMF 1 KAL AMF 2 KAL AMF 3

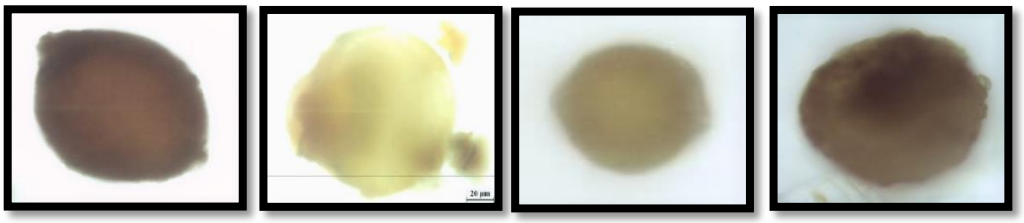


KAL AMF 4 KAL AMF 5 KAL AMF 6

Plate 1: AMF spores isolated from ginger rhizospheric soil of Kalpetta



PAC AMF 1 PAC AMF 2 PAC AMF3



PAC AMF 4 PAC AMF 5 PAC AMF 6 PAC AMF 7

Plate 2: AMF spores isolated from ginger rhizospheric soil Pachilakkad

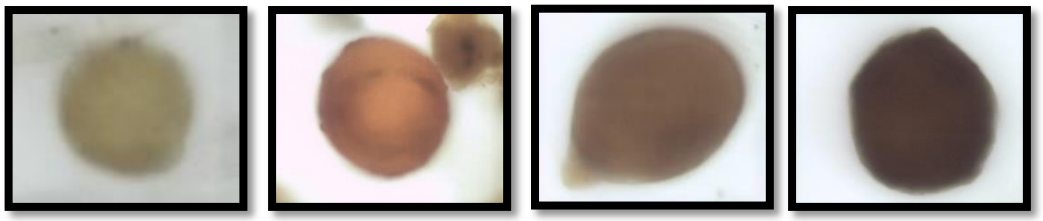
Table 6: Morphological characters of AMF spores in rhizosphere soil sample collected from Edakkal sample

Isolates	Colour	Size (µm)	Shape	Number of spore walls	Nature of hyphae	Bulbous suspensor	Surface ornamentation	Number of spores/100g of soil	Tentative identification (Genus level)
EDA AMF 1	Dark brown	75.62	Globose	2	Straight hyphae	Absent	Smooth	30	*Glomus* sp.
EDA AMF 2	Black	61.58	Globose	1	Bulbous	Present	Regular	18	*Scutellospora* sp.
EDA AMF 3	Dark brown	76.06	Ovoid	1	Bulbous	Present	Regular	16	*Scutellospora* sp.
EDA AMF 4	Yellow	27.79	Globose	2	Straight	Absent	Smooth	15	*Glomus* sp.
EDA AMF 5	Bright yellow	122.08	Sub globose	2	Subtending hyphae	Present	Smooth	12	*Gigaspora* sp.
EDA AMF 6	Orange	25.41	Ovoid	2	Bulbous hyphae	Present	Regular	10	*Scutellospora* sp.
EDA AMF 7	Brown	41.59	Globose	1	Straight hyphae	Absent	Smooth	10	*Glomus* sp.
EDA AMF 8	Dark brown	85.32	Ellipsoid	1	Absent	Absent	Irregular	10	*Glomus* sp.

Table 7: Morphological characters of AMF spores in rhizosphere soil sample collected from Varadoor sample

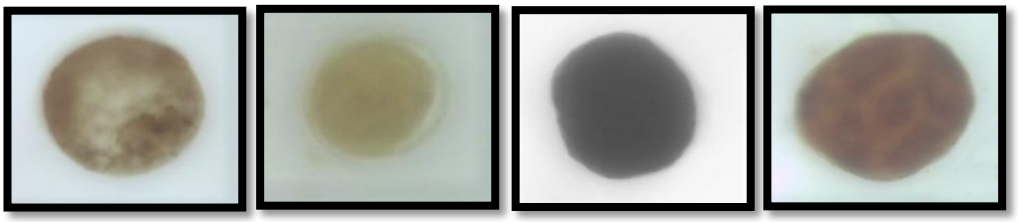
Isolates	Colour	Size (µm)	Shape	Number of spore walls	Nature of hyphae	Bulbous suspensor	Surface ornamentation	Number of spores/ 100g of soil	Tentative identification (Genus level)
VAR AMF 1	Brown	30.44	Globose	2	Bulbous	Present	Spiny	19	*Gigaspora* sp.
VAR AMF 2	Light yellow	49.08	Globose	1	Straight hyphae	Absent	Smooth	18	*Glomus* sp.
VAR AMF 3	Black	53.72	Sub globose	1	Absent	Absent	Smooth	15	*Glomus* sp.
VAR AMF 4	Brownish orange	36.11	ovoid	2	Bulbous hyphae	Present	Regular	12	*Scutellospora* sp.
VAR AMF 5	Dark brown	40.08	Ovoid	1	Straight	Absent	Regular	10	*Glomus* sp.
VAR AMF 6	Bright yellow	29.59	Ellipsoid	2	Subtending hyphae	Absent	Regular	10	*Gigaspora* sp.
VAR AMF 7	Yellow	22.22	Sub globose	2	Hyaline hyphae	Absent	Spiny	8	*Glomus* sp.
VAR AMF 8	Dark brown	28.17	Sub globose	2	Absent	Absent	Smooth	7	*Glomus* sp.

EDA AMF 1 EDA AMF 2 EDA AMF 3 EDA AMF 4

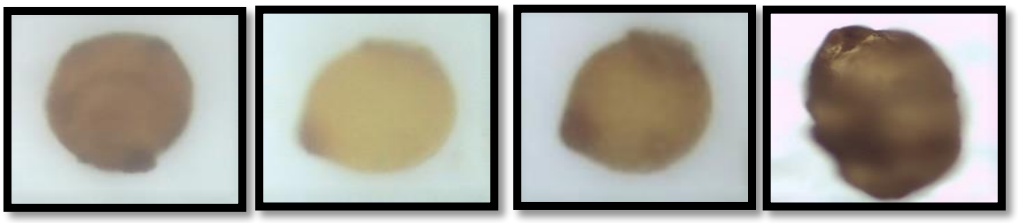


EDA AMF 5 EDA AMF 6 EDA AMF 7 EDA AMF 8

Plate 3: AMF spores isolated from ginger rhizospheric soil Edakkal



VAR AMF 1 VAR AMF 2 VAR AMF 3 VAR AMF 4



VAR AMF 5 VAR AMF 6 VAR AMF 7 VAR AMF 8

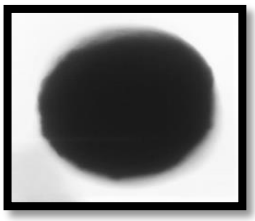
Plate 4: AMF spores isolated from ginger rhizospheric soil Varadoor

Table 8: Morphological characters of AMF spores in rhizosphere soil sample collected from Anappara sample

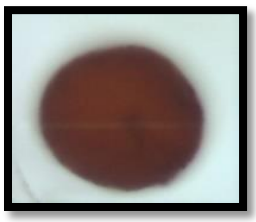
Isolates	Colour	Size (µm)	Shape	Number of spore walls	Nature of hyphae	Bulbous suspensor	Surface ornamentation	Number of spores/ 100g of soil	Tentative identification (Genus level)
ANA AMF 1	Black	22.53	Ovoid	1	Absent	Absent	Regular	10	*Glomus* sp.
ANA AMF 2	Brown	30.44	Globose	2	Absent	Absent	Irregular	14	*Glomus* sp.
ANA AMF 3	Light yellow	25.53	Round	2	Straight	Absent	Smooth	12	*Glomus* sp.
ANA AMF 4	White	45.93	Sub globose	2	Absent	Absent	Smooth	12	*Acaulospora* sp.
ANA AMF 5	Black	129.24	Globose	1	Straight hyphae	Absent	Irregular	35	*Glomus* sp.
ANA AMF 6	Dark brown	50.32	Ovoid	2	Bulbous hyphae	Present	Regular	10	*Scutellospora* sp.
ANA AMF 7	Bright yellow	23.28	Globose	1	Bulbous	Present	Smooth	9	*Scutellospora* sp.
ANA AMF 8	Light brown	38.54	Sub globose	2	Curved	Absent	Regular	8	*Glomus* sp.
ANA AMF 9	Light brown	53.69	Sub globose	2	Subtending hyphae	Absent	Irregular	8	*Glomus* sp.

Table 9: Morphological characters of AMF spores in rhizosphere soil sample collected from Vaduvanchal sample

Isolates	Colour	Size (µm)	Shape	Number of spore walls	Nature of hyphae	Bulbous suspensor	Surface ornamentation	Number of spores/ 100g of soil	Tentative identification (Genus level)
VAD AMF 1	Light brown	37.63	Globose	2	Straight hyphae	Absent	Regular	12	*Glomus* sp.
VAD AMF 2	Yellow	27.72	Sub globose	2	Absent	Absent	Irregular	15	*Glomus* sp.
VAD AMF 3	Black	60.63	Globose	1	Bulbous	Present	Smooth	10	*Gigaspora* sp.
VAD AMF 4	Dark brown	70.70	Globose	1	Absent	Absent	Spiny	12	*Glomus* sp.
VAD AMF 5	Reddish brown	35.78	Ovoid	1	Subtending hyphae	Absent	Smooth	8	*Glomus* sp.
VAD AMF 6	Light brown	67.46	Ovoid	1	Subtending hyphae	Absent	Regular	8	*Glomus* sp.



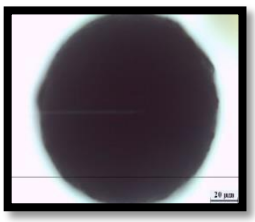
ANA AMF 1



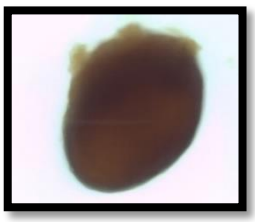
ANA AMF 2

ANA AMF 3

ANA AMF 4



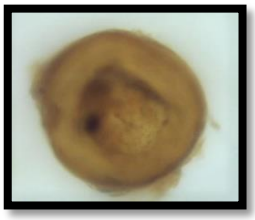
ANA AMF 5



ANA AMF 6

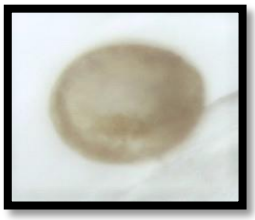
ANA AMF7

ANA AMF 8

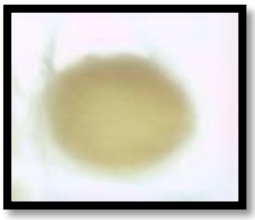


ANA AMF 9

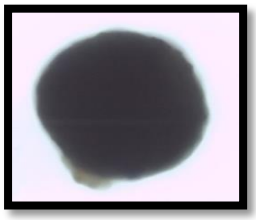
Plate 5: AMF spores isolated from ginger rhizospheric soil Anappara



VAD AMF 1



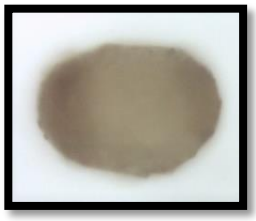
VAD AMF 2



VAD AMF 3

VAD AMF 4

VAD AMF 5



VAD AMF 6

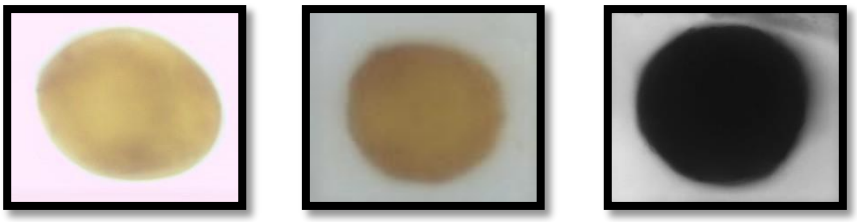
Plate 6: AMF spores isolated from ginger rhizospheric soil Vaduvanchal

Table 10: Morphological characters of AMF spores in rhizosphere soil sample collected from Ambalavayal sample

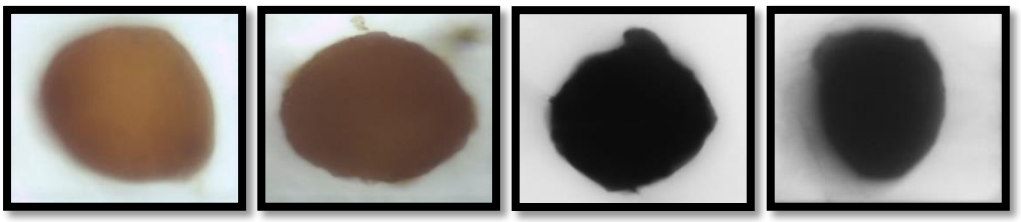
Isolates	Colour	Size (µm)	Shape	Number of spore walls	Nature of hyphae	Bulbous suspensor	Surface ornamentation	Number of spores/ 100g of soil	Tentative identification (Genus level)
AMB AMF 1	Yellow	65.78	Globose	1	Absent	Absent	Smooth	24	*Glomus* sp.
AMB AMF 2	Light brown	57.25	Ovoid	2	Subtending hyphae	Absent	Irregular	15	*Acaulospora* sp.
AMB AMF 3	Black	22.00	Sub globose	2	Bulbous	Present	Smooth	14	*Gigaspora* sp.
AMB AMF 4	Brown	43.49	Ovoid	1	Bulbous	Present	Regular	12	*Scutellospora* sp.
AMB AMF 5	Brown	66.42	Sub globose	1	Straight	Absent	Spiny	10	*Glomus* sp.
AMB AMF 6	Black	98.79	Ellipsoid	1	Straight	Present	Irregular	9	*Glomus* sp.
AMB AMF 7	Black	46.18	Ovoid	1	Absent	Absent	Irregular	8	*Glomus* sp.

Table 11: Morphological characters of AMF spores in rhizosphere soil sample collected from Malavayal sample

Isolates	Colour	Size (µm)	Shape	Number of spore walls	Nature of hyphae	Bulbous suspensor	Surface ornamentation	Number of spores/ 100g of soil	Tentative identification (Genus level)
MAL AMF 1	Black	71.28	Globose	1	Subtending hyphae	Absent	Spiny	19	*Glomus* sp.
MAL AMF 2	Black	36.86	Oval	1	Absent	Absent	Irregular	15	*Glomus* sp.
MAL AMF 3	Dark brown	43.57	Sub globose	2	Straight	Absent	Smooth	12	*Scutellospora* sp.
MAL AMF 4	Light brown	48.23	Globose	1	Absent	Absent	Irregular	11	*Glomus* sp.
MAL AMF 5	Brown	66.48	Oval	2	Bulbous	Present	Smooth	10	*Scutellospora* sp.
MAL AMF 6	White	57.44	Globose	2	Subtending hyphae	Absent	Smooth	10	*Acaulospora* sp.
MAL AMF 7	Brownish orange	28.98	Globose	2	Straight hyphae	Absent	Spiny	9	*Glomus* sp.

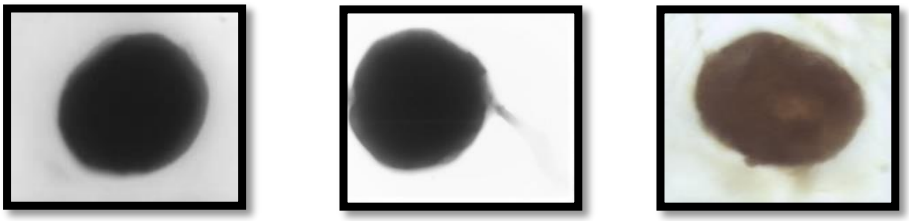


AMB AMF 1 **AMB AMF 2** **AMB AMF 3**

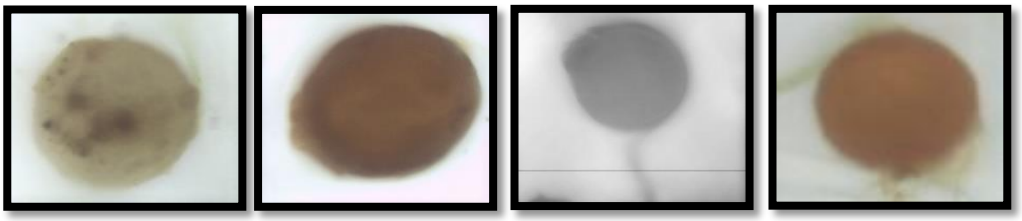


AMB AMF 4 **AMB AMF 5** **AMB AMF 6** **AMB AMF 7**

Plate 7: AMF spores isolated from ginger rhizospheric soil Ambalavayal



MAL AMF 1 **MAL AMF 2** **MAL AMF 3**



MAL AMF 4 **MAL AMF 5** **MAL AMF 6** **MAL AMF 7**

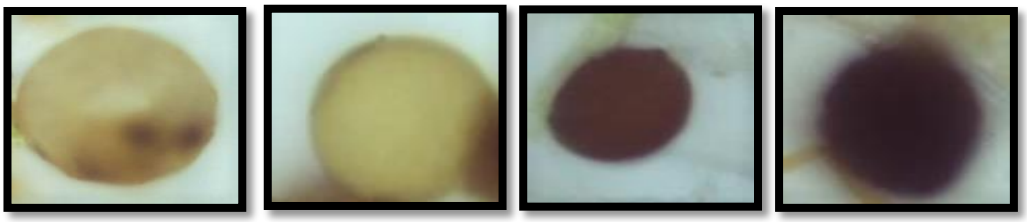
Plate 8: AMF spores isolated from ginger rhizospheric soil Malavayal location

Table 12: Morphological characters of AMF spores in rhizosphere soil sample collected from Nenmeni (sample)

Code	Colour	Size (µm)	Shape	Number of spore walls	Nature of hyphae	Bulbous suspensor	Surface ornamentation	Number of spores/ 100g of soil	Tentative identification (Genus level)
NEN AMF 1	Light brown	41.27	Globose	2	Absent	Absent	Spiny	18	*Glomus* sp.
NEN AMF 2	Light yellow	50.85	Globose	2	Subtending hyphae	Absent	Smooth	32	*Glomus* sp.
NEN AMF 3	Dark brown (small)	26.62	Sub globose	2	Subtending hyphae	Absent	Regular	16	*Scutellospora* sp.
NEN AMF 4	Black	28.17	Ellipsoid	2	Absent	Absent	Smooth	15	*Glomus* sp.
NEN AMF 5	Black	49.94	Ovoid	1	Straight	Absent	Irregular	12	*Glomus* sp.
NEN AMF 6	Dark brown	85.32	Ovoid	1	Bulbous	Present	Regular	10	*Scutellospora* sp.
NEN AMF 7	Brown	25.41	Ellipsoid	2	Bulbous	Present	Spiny	8	*Gigaspora* sp.

Table 13: Morphological characters of AMF spores in rhizosphere soil sample collected from Poomala sample

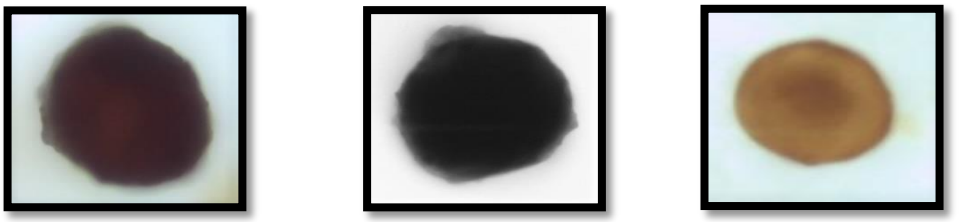
Code	Colour	Size (µm)	Shape	Number of spore walls	Nature of hyphae	Bulbous suspensor	Surface ornamentation	Number of spores/ 100g of soil	Tentative identification(Genus level)
POO AMF 1	Black	50.29	Globose	1	Straight	Absent	Spiny	15	*Glomus* sp.
POO AMF 2	Black	54.79	Sub globose	1	Absent	Absent	Irregular	20	*Glomus* sp.
POO AMF 3	Brownish Orange	38.54	Ovoid	2	Straight	Absent	Smooth	27	*Glomus* sp.
POO AMF 4	Light brown	52.89	Globose	2	Straight	Absent	Smooth	12	*Gigaspora* sp.
POO AMF 5	Light yellow (small)	28.19	Globose	1	Bulbous	Present	Irregular	12	*Scutellospora* sp.
POO AMF 6	White	47.93	Globose	1	Subtending hyphae	Absent	Regular	10	*Glomus* sp.
POO AMF 7	Black	67.24	Ovoid	2	Absent	Absent	Smooth	8	*Glomus* sp.
POO AMF 8	Brown	31.86	Sub globose	2	Subtending hyphae	Absent	Irregular	8	*Glomus* sp.
POO AMF 9	Yellowish orange	35.77	Globose	1	Absent	Absent	Spiny	7	*Glomus* sp.



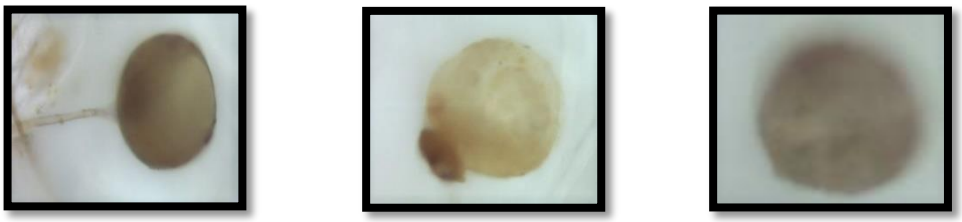
NEN AMF 1 NEN AMF 2 NEN AMF 3 NEN AMF 4

NEN AMF 5 NEN AMF 6 NEN AMF 7

Plate 9: AMF spores isolated from ginger rhizospheric soil Nenmeni



POO AMF 1 POO AMF 2 POO AMF 3



POO AMF 4 POO AMF 5 POO AMF 6

POO AMF 7 POO AMF 8 POO AMF 9

Plate 10: AMF spores isolated from ginger rhizospheric soil Poomala location

4.4. Selection of AMF for further study

By analysing the data from table 3, five most predominant spores were selected based on abundance for further studies are presented in the Table 14.

Table 14: Five AMF spores selected for the further studies

SI. No	Sample	Number of predominant spores/100g of soil	Spore Colour
1	PAC AMF 2	28	Brown spores
2	EDA AMF 1	30	Dark brown spores
3	ANA AMF 5	35	Black spores
4	NEN AMF 2	32	Light yellow spores
5	POO AMF 3	27	Brownish orange

The PAC AMF 2 sample had about 28 spores having same morphology in 100g of soil and 7 morphotypes. The colour of the selected spore was brown.

The EDA AMF 1 sample had about 30 number of spores of same type per 100g of soil and 8 different morphotypes of spores. The colour of the selected dominant spore was dark brown.

The ANA AMF 5 sample had 35 number of spores having exactly similar morphology per 100g of soil and the colour of that dominant spore was black. ANA has totally 8 different morphotypes of spores.

The NEN AMF 2 sample had 32 spores of similar morphology per 100g of soil and about 7 types of morphotypes. The colour of the dominant spore was light yellow.

The POO AMF 3 sample had 27 spores of same type in 100g of soil and totally about 9 morphotypes of spores were present. The colour of the dominant spore was brownish orange.

These five spores were selected based on abundance for further studies in ginger crop in Wayanad district for the growth and management of rhizome rot.

4.5. Mass multiplication of predominant AMF spores

The selected spores were mass multiplied for application in pot culture study. Mass multiplication was carried out in vermiculite and perlite medium with maize as host plant. The percent root colonization on maize roots were recorded (Table 15).

The brown spore selected from the place Pachilakkad has given name as PAC AMF 2, dark brown spore selected from Edakkal as EDA AMF 1, black spore from Anappara as ANA AMF 5, light yellow spore from Nenmeni as NEN AMF 2, and brownish orange spore from Poomala as POO AMF 3.

4.5.1. Percent root colonization of AMF spores using maize as a host

The maize roots were checked periodically for the AMF infection and results were recorded (Table 15).

Table 15: Percent root colonization of AMF on maize root

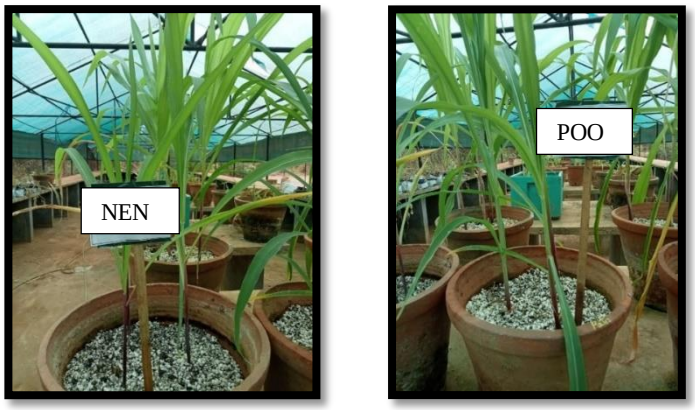
SI. No	Inoculum	40^{th} day	60^{th} day
1	PAC AMF 2	70 %	100 %
2	EDA AMF 1	50 %	90 %
3	ANA AMF 5	80 %	100 %
4	NEN AMF 2	60 %	90 %
5	POO AMF 3	50 %	80 %

The root staining done to check the infectivity which shows, on 40^{th} day the percent root infection was higher in ANA AMF 5 sample which was 80 % followed by 70 % by PAC AMF 2 sample. The infection was lowest in POO AMF 3 sample which was about 50 %.

PAC AMF 2 **EDA AMF 1** **ANA AMF 5** **NEN AMF 2** **POO AMF 3**

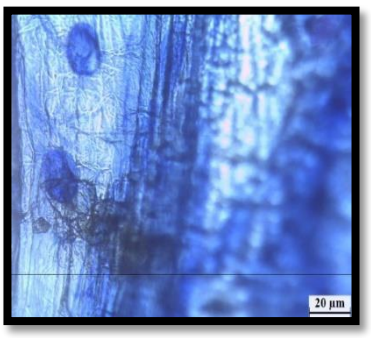
Plate 11: AMF multiplication by funnel technique

PAC AMF 2 **EDA AMF 1** **ANA AMF 5**

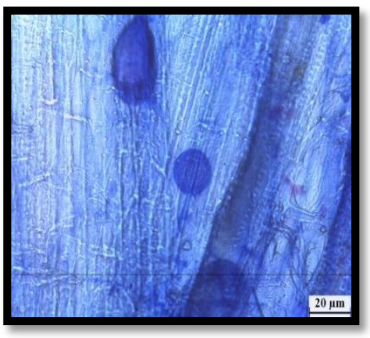


NEN AMF 2 **POO AMF 3**

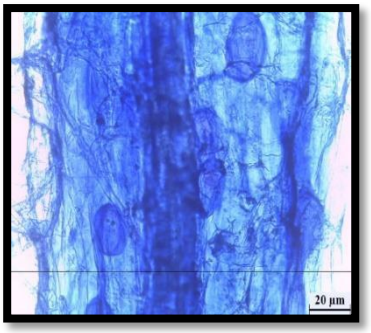
Plate 12: Mass multiplication of selected AMF spores using maize as host plant



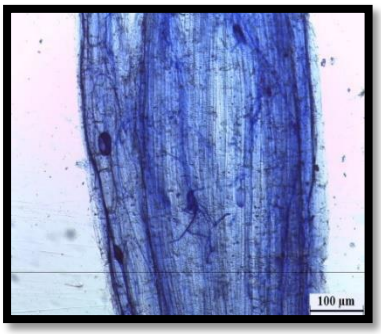
PAC AMF 2



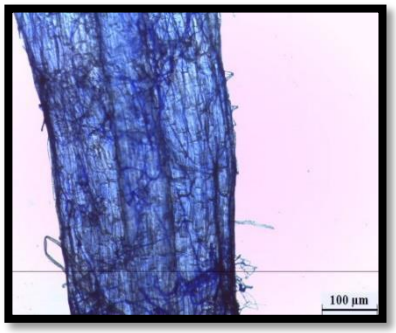
EDA AMF 1



ANA AMF 5



NEN AMF 2



POO AMF 3

Plate 13: Microscopic view of AMF infected root bits of maize

On 60th day of root staining, ANA AMF 5 and PAC AMF 2 sample showed highest percent root infection of 100 % and the lowest infection was recorded in POO AMF 3 sample.

4.5.2. AMF spore count after mass multiplication

After mass multiplication for two months (60 DAP), spore count of the soils were recorded (Table 16).

Table 16: AMF spore count in the mass multiplied inoculum

SI. No	Inoculum	Tentatively identified genus	Total spore count/10g of soil
1	PAC AMF 2	*Glomus* sp.	15
2	EDA AMF 1	*Glomus* sp.	18
3	ANA AMF 5	*Glomus* sp.	22
4	NEN AMF 2	*Glomus* sp.	12
5	POO AMF 3	*Glomus* sp.	10

The ANA AMF 5 sample showed highest spore count of about 22 spores/ 10 g of soil followed by EDA AMF 1 sample, which recorded about 18 spores/ 10 g of soil. The lowest spore count was recorded in POO AMF 3 sample of about 10 spores/ 10 g of soil

4.6. Pot culture experiments

The mass multiplied samples were used as inoculum in the pot culture study. The pot culture experiment was carried out in Regional Agricultural Research station, Ambalavayal in Wayanad district.

4.6.1. Initial nutrient status of the potting mixture

The initial nutrient status of pot culture soil was analysed and presented in the Table 17.

Table 17: Nutrient status of potting mixture

SI. No	Potting mixture	Nutrient status
1	pH	5.93 (Acidic)
2	EC (dSm^{-1})	1.14 (Non-saline)
3	Organic carbon (%)	1.60 (High)
4	Available Nitrogen (Kg/ha)	332.42 (Medium)
5	Available Phosphorous (Kg/ha)	105.38 (High)
6	Available potassium (Kg/ha)	523.94 (High)

The pH of final pot culture soil was 5.93 which was acidic. The EC content of the soil was low (1.14). The organic carbon content was high in the soil of about 1.60 % and the nitrogen content was in medium range 332.42 Kg / ha (medium). The phosphorous and potassium content in the soil was high of about 105.38 Kg/ ha and 523.94 Kg/ ha respectively.

4.7.1. Experiment 1– Effect of AMF on growth and yield promotion of ginger

4.7.1.1 Plant height

The result pertaining to the effect of AMF on plant height by different treatments at monthly interval are presented in the Table 18.

The plant height was affected by different AMF treatments. At 60 DAP, the plant height ranged from 18.3 to 48.76 cm. The treatment T_5 (POO AMF 3) and T_3

(ANA AMF 5) had highest plant height with 48.76 cm and 48.7 cm respectively. The shortest plants were recorded in the T_8 (Absolute control) followed by T_7 (KAU, 2015) with 18.3 cm and 24.44 cm respectively.

At 90 DAP, the highest plant height was recorded in the T_5 (POO AMF 3 - 53.9 cm) which was followed by T_3 (ANA AMF 5 - 53.8 cm). Plants of absolute controls pots were the shortest (23.7 cm) followed by T_7 (KAU, 2015).

T_1 (PAC AMF 2) had the highest plant height of about 77.9 cm at 120 DAP which was followed by T_3 (ANA AMF 5) and T_5 (POO AMF 3). Likewise, T_1 (PAC AMF 2) showed highest plant height at 150 DAP and 180 DAP with 95.70 cm and 103.70 cm respectively, followed by T_5. Treatment T_8 (Absolute control) and T_7 (KAU, 2015) treatments were significantly lower throughout the growing season.

4.7.1.2. Number of leaves

The effect of AMF on number of leaves were recorded at monthly interval (Table 19).

At 60 DAP, the effect of AMF on number of leaves ranged from 7 to 3.4. Treatment T_6 (KAU, 2016) had more number of leaves (6.8) which was followed by the treatment T_3 (ANA AMF 5) and the less number of leaves were observed in T_8 (absolute control).

The T_6 (KAU, 2016) had the maximum number of leaves after 90 days of planting (9.4) which was followed by T_3 (ANA AMF 5) and T_1 (PAC AMF 1) with 9.2 and 9 respectively. The absolute control showed the lesser number of leaves.

At 120 DAP and 150 DAP, the treatment T_1 (PAC AMF 2) had the maximum number of leaves of about 18.6 and 21.8 respectively, which was followed by the treatment T_5 (POO AMF 1). Treatment T_8 (absolute control) had lesser number of leaves.

Table 18: Effect of AMF on plant height of ginger at monthly interval

TREATMENT	60 DAP (cm)	90 DAP (cm)	120 DAP (cm)	150 DAP (cm)	180 DAP (cm)
T_1	42.66^{ab}	50.80^{a}	77.90^{a}	95.70^{a}	103.70^{a}
T_2	41.42^{ab}	48.50^{a}	72.50^{ab}	79.20^{ab}	82.56^{ab}
T_3	48.70^{a}	53.80^{a}	76.96^{a}	81.50^{ab}	84.50^{ab}
T_4	37.78^{b}	43.10^{ab}	64.44^{ab}	68.64^{bc}	72.60^{bc}
T_5	48.76^{a}	53.90^{a}	75.74^{a}	90.90^{ab}	95.20^{ab}
T_6	44.64^{ab}	50.10^{a}	72.60^{ab}	76.90^{abc}	79.60^{abc}
T_7	24.44^{c}	31.60^{bc}	50.34^{bc}	53.50^{cd}	55.44^{cd}
T_8	18.30^{d}	23.70^{c}	28.34^{c}	31.60^{d}	33.50^{d}
LSD (0.05)	12.13	15.97	23.11	25.02	26.81

(T_1 – PAC AMF 2 + POP of KAU, T_2 – EDA AMF 1 + POP of KAU, T_3 – ANA AMF 5 + POP of KAU, T_4 – NEN AMF 2 + POP of KAU, T_5 – POO AMF 3 + POP of KAU, T_6 – KAU, 2016, T_7 – KAU, 2015, T_8 – Absolute control, DAP – Days after planting).

Table 19: Effect of AMF on number of leaves in ginger at monthly interval

TREATMENT	60 DAP	90 DAP	120 DAP	150 DAP	180 DAP
T_1	6.00^{ab}	9.00^{a}	18.60^{a}	21.80^{a}	22.60^{a}
T_2	6.00^{ab}	8.80^{a}	16.40^{a}	15.60^{abc}	15.40^{bc}
T_3	6.80^{a}	9.20^{a}	17.00^{a}	16.80^{ab}	15.00^{bc}
T_4	5.40^{bc}	8.00^{ab}	14.00^{ab}	14.00^{bcd}	14.20^{bc}
T_5	6.00^{ab}	8.60^{ab}	17.80^{a}	17.40^{ab}	16.60^{ab}
T_6	7.00^{a}	9.40^{a}	17.20^{a}	16.00^{abc}	14.80^{bc}
T_7	4.20^{cd}	6.80^{bc}	9.60^{bc}	10.00^{cd}	10.40^{cd}
T_8	3.40^{d}	5.80^{c}	6.80^{c}	7.40^{d}	6.60^{d}
LSD (0.05)	1.47	1.92	6.15	6.64	6.09

(T_1 – PAC AMF 2 + POP of KAU, T_2 – EDA AMF 1 + POP of KAU, T_3 – ANA AMF 5 + POP of KAU, T_4 – NEN AMF 2 + POP of KAU, T_5 – POO AMF 3 + POP of KAU, T_6 – KAU, 2016, T_7 – KAU, 2015, T_8 – Absolute control, DAP – Days after planting).

At 180 DAP, T_1 (PAC AMF 2) recorded more number of leaves (22.6) followed by the treatment T_5 (POO AMF 1 - 16.6). The number of leaves recorded at 180 DAP was less than the number of leaves at 150 DAP in all treatments except T_7 (KAU, 2015) and T_1 (PAC AMF 2).

4.7.1.3. Number of tillers

The results of effect of AMF on number of tillers in ginger plant was recorded (Table 20).

At 60 DAP, the T_5 (POO AMF 3) treatment showed more number of tillers (4.8) which was followed by the treatment T_1 (PAC AMF 2) and the less number of leaves were recorded in T_8 (Absolute control) which was followed by T_7 (KAU, 2015).

At 90 DAP and 120 DAP, the treatment T_1 (PAC AMF 2) showed higher number of tillers of about 6 and 9.4 respectively, which was followed by the treatment T_5 (POO AMF 3).

At 150 DAP and 180 DAP, the treatment T_5 (POO AMF 3) recorded with more number of tillers with 12.4 and 14.4 respectively, which was followed by the treatment T_1 (PAC AMF 2). The treatment T_8 (Absolute control) had the lesser number of tillers throughout the cropping season.

4.7.1.4. Leaf area

The result obtained on monthly basis regarding the effect of AMF on leaf area of ginger plant is presented in the Table 21.

The leaf area of the treatments are not significantly different at 60 and 90 DAP, comparatively treatment T_1 (PAC AMF 2) showed higher leaf area of 30.17 and 34.89 respectively.

At 120, 150, and 180 DAP, the different AMF treatment had affected the ginger leaf area. T_5 (POO AMF 3) showed higher leaf area of 38.41, 40.83, and 39.01

respectively, which was followed by T_1 (PAC AMF 2). The treatment T_8 (Absolute control) showed lower leaf area throughout the cropping period.

The treatment T_5 (POO AMF 3 - 40.83) at 150 DAP had higher leaf area, which was on par with all the other treatment, except T_8 (Absolute control). The treatment T_5 (POO AMF 3- 39.01) had maximum leaf area at 180 DAP followed by T_1 (PAC AMF 2 – 38.41). Leaf area of the treatments at 180 DAP are lesser than the leaf area observed at 150 DAP.

4.7.1.5. Fresh and dry weight of ginger rhizome

The results of fresh weight and dry weight of ginger per rhizome was recorded and presented in the Table 22.

The fresh weight of ginger rhizome was higher in the treatment T_5 (POO AMF 3- 160 g) which was on par with the treatment T_1 (PAC AMF 2- 152.5 g). The lowest fresh weight was recorded in the treatment T_8 (30 g) which was an absolute control. T_6 (KAU, 2016) was on par with T_2 (EDA AMF 1), T_3 (ANA AMF 5), T_4 (NEN AMF 2).

The dry weight of ginger rhizome was higher in the treatment T_5 (POO AMF 3- 65 g), which was on par with the treatment T_1 (PAC AMF 2 – 58 g). The lowest dry weight was recorded in the treatment T_8 (20 g) which was an absolute control.

4.7.1.6. Fresh and dry weight of ginger plant shoot

The results of fresh weight and dry weight of plant shoot per rhizome was recorded and presented in the Table 23.

The fresh weight of plant shoot was higher in the treatment T_5 (POO AMF 3 – 67.5 g), followed by the treatment T_1 (PAC AMF 2 – 64 g). The lowest fresh weight was recorded in the treatment T_8 (17.5 g) which was an absolute control.

Table 20: Effect of AMF on number of tillers of ginger at monthly interval

TREATMENT	60 DAP	90 DAP	120 DAP	150 DAP	180 DAP
T_1	4.00^{ab}	6.00^{a}	9.40^{a}	10.00^{ab}	13.00^{a}
T_2	3.00^{bc}	5.00^{ab}	7.00^{b}	7.40^{bc}	7.80^{bc}
T_3	3.60^{ab}	4.20^{bc}	6.60^{b}	8.60^{bc}	8.80^{b}
T_4	3.00^{bc}	3.40^{cd}	6.00^{b}	7.20^{bc}	7.40^{bc}
T_5	4.80^{a}	5.00^{ab}	8.00^{ab}	12.40^{a}	15.00^{a}
T_6	3.40^{bc}	4.20^{bc}	6.40^{b}	9.80^{ab}	10.00^{b}
T_7	2.80^{bc}	3.20^{cd}	3.40^{c}	5.40^{cd}	5.40^{cd}
T_8	2.20^{c}	2.20^{d}	2.60^{c}	3.60^{d}	3.40^{d}
LSD (0.05)	1.30	1.52	2.23	3.47	2.84

(T_1 – PAC AMF 2 + POP of KAU, T_2 – EDA AMF 1 + POP of KAU, T_3 – ANA AMF 5 + POP of KAU, T_4 – NEN AMF 2 + POP of KAU, T_5 – POO AMF 3 + POP of KAU, T_6 – KAU, 2016, T_7 – KAU, 2015, T_8 – Absolute control, DAP – Days after planting).

Table 21: Effect of AMF on leaf area of ginger at monthly interval

TREATMENT	60 DAP	90 DAP	120 DAP	150 DAP	180 DAP
T_1	30.17	34.89	37.08^{ab}	40.68^{a}	38.41^{a}
T_2	25.18	29.27	32.89^{ab}	35.37^{a}	34.67^{ab}
T_3	28.79	30.14	34.19^{ab}	38.91^{a}	36.17^{ab}
T_4	26.15	28.45	31.02^{ab}	34.07^{a}	37.18^{a}
T_5	29.63	34.67	38.41^{a}	40.83^{a}	39.01^{a}
T_6	26.08	30.78	33.96^{ab}	37.14^{a}	35.83^{ab}
T_7	24.10	27.67	25.63^{bc}	27.73^{ab}	24.43^{bc}
T_8	22.82	24.58	17.76^{c}	17.06^{c}	13.63^{c}
LSD (0.05)	NS	NS	12.19	14.89	12.47

(T_1 – PAC AMF 2 + POP of KAU, T_2 – EDA AMF 1 + POP of KAU, T_3 – ANA AMF 5 + POP of KAU, T_4 – NEN AMF 2 + POP of KAU, T_5 – POO AMF 3 + POP of KAU, T_6 – KAU, 2016, T_7 – KAU, 2015, T_8 – Absolute control, DAP – Days after planting).

Table 22: Effect of AMF on fresh weight and dry weight of ginger rhizome

Treatment	Fresh weight g/plant	Dry weight g/plant
T_1	152.50[a]	58.00[a]
T_2	104.50[b]	40.50[bc]
T_3	118.00[b]	43.00[b]
T_4	109.00[b]	39.00[bc]
T_5	160.00[a]	65.00[a]
T_6	126.00[b]	44.50[b]
T_7	71.50[c]	26.50[cd]
T_8	30.50[d]	20.00[d]
LSD (0.05)	23.93	12.36

Table 23: Effect of AMF on fresh weight and dry weight of ginger plant shoot

Treatment	Fresh weight of plant shoot (g)	Dry weight of plant shoot (g)
T_1	64.00[ab]	15.00[a]
T_2	48.00[bcd]	14.00[a]
T_3	48.50[bcd]	12.50[ab]
T_4	46.00[cd]	12.50[ab]
T_5	67.50[a]	16.50[a]
T_6	51.50[abc]	13.50[a]
T_7	35.00[d]	9.00[b]
T_8	17.50[e]	4.50[c]
LSD (0.05)	16.32	4.25

(T_1 – PAC AMF 2 + POP of KAU, T_2 – EDA AMF 1 + POP of KAU, T_3 – ANA AMF 5 + POP of KAU, T_4 – NEN AMF 2 + POP of KAU, T_5 – POO AMF 3 + POP of KAU, T_6 – KAU, 2016, T_7 – KAU, 2015, T_8 – Absolute control, DAP – Days after planting).

The dry weight of plant shoot was higher in the treatment T_5 (POO AMF 3 – 16.5 g), which was followed by the T_1 (PAC AMF 2 – 15 g), T_2 (EDA AMF 1 - 14 g) and T_6 (PAC AMF 2 – 13.5 g). The lowest dry weight was recorded in the treatment T_8 (4.5 g) which was an absolute control.

4.7.1.7. Percent disease incidence of rhizome rot of ginger

The effect of AMF on disease incidence was recorded on monthly interval is presented in Table 24 and the disease scoring was done according to the formula given by Shahzad and Bhat, 2005.

Table 24: Disease scoring of naturally occurred ginger rhizome rot

Treatment	Rhizome rot disease incidence (%)	Disease severity
T_1	9.00^c	1 (Highly resistant)
T_2	8.33^c	1 (Highly resistant)
T_3	9.00^c	1 (Highly resistant)
T_4	11.66^c	2 (Resistant)
T_5	8.00^c	1 (Highly resistant)
T_6	11.66^c	2 (Resistant)
T_7	29.00^b	3 (Tolerant)
T_8	70.00^a	4 (Susceptible)
LSD (0.05)	6.56	

The percent disease incidence was higher in the control T_8 (70 %) and lower in T_5 (POO AMF 3 - 8 %) and followed by treatment T_2 (EDA AMF 1 - 8.33 %). All the treatments were on par, except T_8 and T_7 (KAU, 2015).

Treatments like T_1, T_2, T_3, and T_5 were highly resistant to rhizome rot, T_4 and T_6 (KAU, 2016) were resistant, T_7 (KAU, 2015) was tolerant to rhizome rot and T_8 (control) was susceptible to rhizome rot.

4.7.1.8. Oleoresin content

The effect of AMF on the oleoresin content of the ginger is presented in the Table 25.

Table 25: Effect of AMF on oleoresin content in ginger

Treatment	Oleoresin content (%)
T_1	10.03^{c}
T_2	12.50^{a}
T_3	11.25^{b}
T_4	8.32^{ef}
T_5	8.65^{def}
T_6	9.45^{cd}
T_7	9.10^{cde}
T_8	7.58^{f}
LSD (0.05)	1.113

The oleoresin content was higher in the treatment T_2 (EDA AMF 1) of about 12.5%, followed by Treatment T_3 (ANA AMF 5 - 11.25 %). The lowest oleoresin content was recorded in the treatment T_8 of about (Control - 7.58 %).

4.7.1.9. Per cent root colonisation and final spore count by AMF

The result of percent root colonisation and final spore count is presented in the Table 26.

Per cent root colonization was higher in the treatment T_1 (PAC AMF 2 - 90%), followed by the treatment T_5 (POO AMF 3 - 80%). The treatment T_3 (ANA AMF 5) and T_4 (NEN AMF 2) showed 70% of root colonisation and 60 % by T_2 (EDA AMF 1).

Spore count ranged from 67- 51 per 10 g of soil. The highest spore count was found in the treatment T_5 (POO AMF 3 - 67) and lowest spore count was reported in

Experiment 1 - Pot culture study for growth promotion of ginger

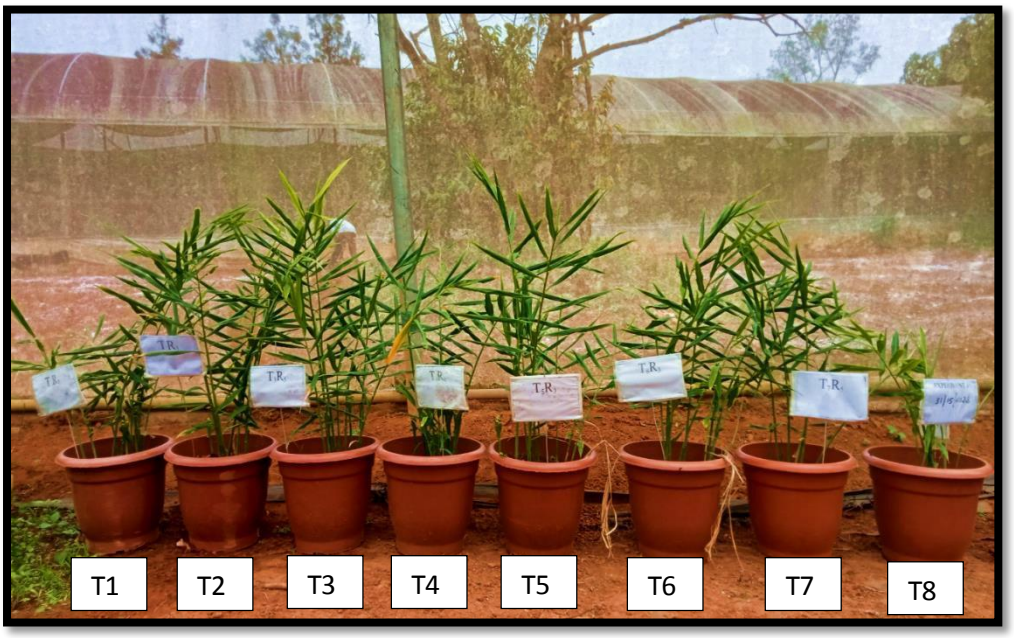


Plate 14: Ginger plants in pot culture plants at 150 DAP

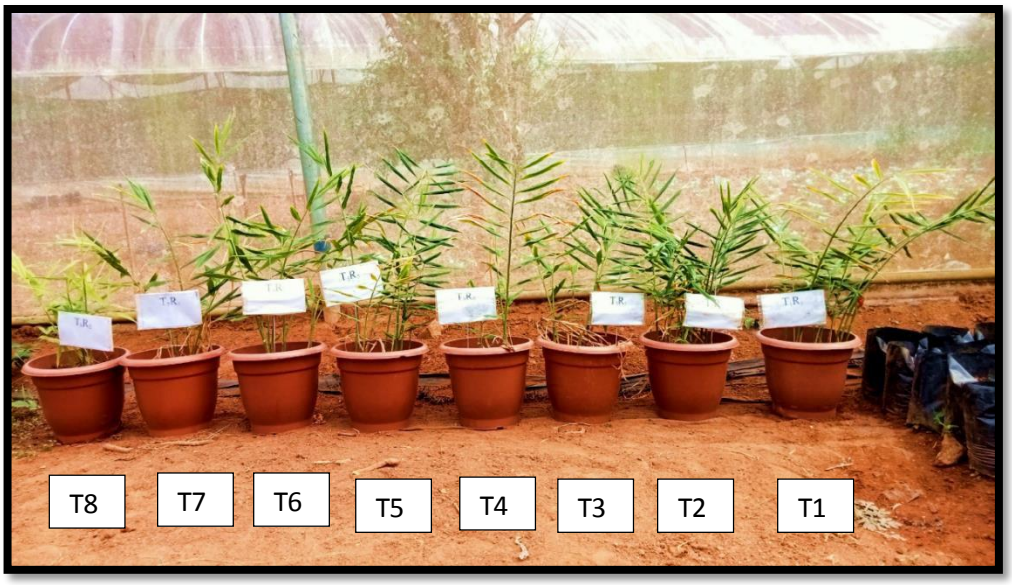


Plate 15: Ginger plants at pot culture plants at 180 DAP

(T_1 – PAC AMF 2, T_2 – EDA AMF 1, T_3 – ANA AMF 5, T_4 – NEN AMF 2, T_5 – POO AMF 3, T_6 –POP of KAU, 2016, T_7– Organic POP of KAU, 2015, T_8 – Absolute control).

Plate 16: Rhizome yield of ginger at harvest (180 DAP)

T_4 (NEN AMF 2 - 51). Treatment T_1 had 64, T_2 had 58, and T_3 had 60 number of spores.

Table 26: Final spore count and per cent root colonization of Ginger

Treatment	Final spore count per 10 g of soil	Per cent root colonisation
T_1 (PAC AMF 2)	64	90%
T_2 (EDA AMF 1)	58	60%
T_3 (ANA AMF 5)	60	70%
T_4 (NEN AMF 2)	51	70%
T_5 (POO AMF 3)	67	80%

4.7.2. Experiment 2 – Effect of AMF on soft rot management (*Pythium myriotylum)*

4.7.2.1. Plant height

The results of effect of AMF on plant height on monthly interval was recorded in the Table 27.

At 60 and 90 DAP, the treatment T_2 (EDA AMF 1) showed the increased plant height of about 44.16 cm and 49.2 cm respectively, followed by the treatment T_1 (PAC AMF 2). Control plants showed lesser plant height in both months.

At 120 DAP and 150 DAP, the treatment T_1 (PAC AMF 2) recorded highest plant height with 70.5 cm, and 85.80 cm respectively, followed by the treatment T_2 (EDA AMF 1). Treatment T_8 (control) showed the lowest plant height.

At 180 DAP, the treatment T_1 (PAC AMF 2) recorded highest plant height with 90 cm and treatment T_8 (21 cm) showed lowest plant height. T_1 (PAC AMF 2) was on par with all the treatments except T_7 (KAU, 2015) and T_8 (control).

4.7.2.2. Number of leaves

The results pertaining to the effect of AMF on number of leaves in ginger crop is presented in the Table 28.

At 60 DAP, the treatment T_3 (ANA AMF 5) exhibited more number of leaves (6.4) which was on par with all the treatment except T_7 (KAU, 2015) and T_8 (control) and at 90 DAP, there was no significant different in number of tillers among all the treatment.

At 120 DAP, treatment T_1 (PAC AMF 2) showed maximum number of leaves (16.2) followed by T_2 (EDA AMF 1) and minimum number of leaves were recorded in T_8 (control – 4.0). The treatment T_1 (PAC AMF 2) recorded higher number of leaves at 150 DAP (19.6) which was on par with all the treatment except T_8 (control).

At 180 DAP, more number of leaves were recorded in the treatment T_2 (PAC AMF 2 - 17) which was less than the number of leaves at 150 DAP. The minimum number of leaves (5.8) were observed in the treatment T_8 (Absolute control) throughout the cropping season.

4.7.2.3. Number of tillers

The observation on the effect of AMF on number of tillers is presented in the Table 29.

At 60 DAP, the effect of AMF on number of tillers in ginger crop was not significantly different and the treatment T_2 (EDA AMF 1) exhibited more number of tillers at 90 DAP, which is on par with all the treatments.

The treatment T_1 (PAC AMF 2) showed maximum number of tillers at 120 DAP (8.6) which was on par with the all the treatment, except T_7 (KAU, 2015) and T_8 (control).

The treatments were not significantly different on number of tillers at 150 DAP and the treatment T_1 (PAC AMF 2) recorded significantly higher number

Table 27: Effect of AMF on plant height of ginger at monthly interval

TREATMENT	60 DAP (cm)	90 DAP (cm)	120 DAP (cm)	150 DAP (cm)	180 DAP (cm)
T_1	42.14[a]	48.50[a]	70.50[a]	85.80[a]	90.00[a]
T_2	44.16[a]	49.20[a]	69.10[a]	77.20[a]	79.90[ab]
T_3	39.76[a]	42.40[a]	63.50[a]	73.80[a]	75.10[ab]
T_4	38.86[a]	45.00[a]	58.74[a]	70.00[a]	73.50[ab]
T_5	37.30[a]	39.90[ab]	61.88[a]	69.46[ab]	72.10[ab]
T_6	32.92[ab]	33.30[abc]	57.86[a]	64.00[ab]	66.74[ab]
T_7	22.20[bc]	23.70[bc]	39.50[b]	45.00[bc]	41.20[c]
T_8	18.50[c]	19.50[c]	18.94[c]	27.90[c]	21.00[d]
LSD (0.05)	13.76	16.38	17.69	24.72	29.98

(T_1 – PAC AMF 2 + CI + POP of KAU, T_2 – EDA AMF 1 + CI + POP of KAU, T_3 – ANA AMF 5 + CI + POP of KAU, T_4 – NEN AMF 2 + CI + POP of KAU, T_5 – POO AMF 3 + CI + POP of KAU, T_6 – KAU, 2016 + CI , T_7 – KAU, 2015 + CI , T_8 – Absolute control, DAP – Days after planting).

Table 28: Effect of AMF on number of leaves in ginger at monthly interval

Treatment	60 DAP	90 DAP	120 DAP	150 DAP	180 DAP
T_1	6.20[a]	8.20	16.20[a]	19.60[a]	16.00[a]
T_2	7.00[a]	8.00	15.20[a]	18.40[a]	17.00[a]
T_3	6.40[a]	7.00	12.00[ab]	16.00[ab]	13.00[ab]
T_4	5.80[a]	7.80	13.00[ab]	16.00[ab]	12.60[ab]
T_5	6.00[a]	7.40	13.40[a]	17.20[ab]	13.40[ab]
T_6	5.80[a]	6.60	12.60[ab]	15.00[ab]	13.00[ab]
T_7	3.80[b]	5.40	9.00[b]	12.00[b]	9.00[bc]
T_8	2.80[b]	4.80	4.00[c]	6.00[c]	5.80[c]
LSD (0.05)	1.49	NS	4.00	5.43	5.64

(T_1 – PAC AMF 2 + CI + POP of KAU, T_2 – EDA AMF 1 + CI + POP of KAU, T_3 – ANA AMF 5 + CI + POP of KAU, T_4 – NEN AMF 2 + CI + POP of KAU, T_5 – POO AMF 3 + CI + POP of KAU, T_6 – KAU, 2016 + CI , T_7 – KAU, 2015 + CI , T_8 – Absolute control, DAP – Days after planting).

of tillers (8.4) at 180 DAP, followed by T_2 (EDA AMF 1) and T_5 (POO AMF 3) and the lowest was recorded in T_8 (2.2).

4.7.2.4. Leaf area

The results obtained on the effect of AMF on leaf area of the ginger crop was recorded (Table 30).

The treatments were not significantly different at 60, 90 and 120 DAP. Treatment T_1 (PAC AMF 2) showed higher leaf area during these days and T_8 (control) showed lesser leaf area among all the treatments.

At 150 DAP, treatments T_1 (PAC AMF 2) showed the highest leaf area of about 36.12, which was on par with all the treatments and T_8 (control) showed the lowest leaf area 14.41.

AT 180 DAP, treatment T_1 (PAC AMF 2) showed higher leaf area of 37.02, which was followed by T_5 (POO AMF 3 – 36.36). Plants in T_8 pots recorded the lowest leaf area 13.17.

4.7.2.5. Percent disease incidence of above ground symptoms of rhizome rot of ginger

The results obtained on the effect of AMF on disease resistance against *Pythium myriotylum* is presented in the Table 31.

The effect of AMF on disease resistance was significantly different among treatments. Throughout the cropping season the disease incidence was low in the treatment T_1 (23.13% - 90 DAP, 49.46% - 120 DAP, 65.87% - 150 DAP, and 73.84% – 180 DAP) followed by T_5 (29.87% - 90 DAP, 51.17% - 120 DAP, 69.54 % - 150 DAP, and 75.20% – 180 DAP) and higher in the treatment T_8 (46.33% - 90 DAP, 85.33% - 120 DAP, 92.62% - 150 DAP, and 100 % – 180 DAP) followed by T_7 (44.28% - 90 DAP, 66.152% - 120 DAP, 84.18% - 150 DAP, and 88 % – 180 DAP).

At later stage of growth, all the treatments were highly susceptible to rhizome rot, except T_1 (PAC AMF 2) and T_5 (POO AMF 3).

4.7.2.6. Fresh weight and dry weight of ginger rhizome

The results of fresh weight and dry weight of ginger per rhizome was recorded and presented in the Table 32.

The fresh weight of ginger rhizome was significantly higher in the treatment T_1 (PAC AMF 2 – 120 g), followed by the treatment T_5 (POO AMF 3 - 94 g). The lowest fresh weight was recorded in the treatment T_8 (control - 27 g).

The dry weight of ginger rhizome was significantly higher in the treatment T_1 (PAC AMF 2 - 51 g), followed by the treatment T_5 (POO AMF 3 - 40 g). The lowest dry weight was recorded in the treatment T_8 (control – 12.5 g).

4.7.2.7. Fresh weight and dry weight of ginger shoot

The results of fresh weight and dry weight of plant shoot was recorded and presented in the Table 33.

The fresh weight of plant shoot was significantly higher in the treatment T_1 (PAC AMF 2 - 58 g), followed by the treatment T_2 (EDA AMF 1 - 42 g). The lowest fresh weight was recorded in the treatment T_8 (control - 13 g).

The dry weight of plant shoot was higher in the Treatment T_1 (PAC AMF 2 - 13 g) and T_5 (POO AMF 3 - 13 g), followed by the treatment T_2 (EDA AMF 1 - 12 g). The lowest dry weight was recorded in the treatment T_8 (control - 4.5 g).

Table 29: Effect of AMF on number of tillers in ginger at monthly interval

TREATMENT	60 DAP	90 DAP	120 DAP	150 DAP	180 DAP
T_1	4.00	5.00^{a}	8.60^{a}	7.00	8.40^{a}
T_2	4.20	4.80^{a}	6.60^{a}	6.60	5.80^{b}
T_3	4.20	3.20^{abc}	7.00^{a}	5.80	5.20^{bc}
T_4	3.80	3.20^{abc}	7.40^{a}	5.20	4.60^{bc}
T_5	3.00	4.00^{ab}	7.00^{a}	6.20	5.80^{b}
T_6	3.20	4.00^{ab}	7.60^{a}	6.40	5.40^{bc}
T_7	3.00	2.60^{bc}	3.80^{b}	4.80	3.40^{cd}
T_8	2.60	2.00^{c}	2.40^{b}	2.40	2.20^{d}
LSD (0.05)	NS	1.90	2.30	NS	2.20

(T_1 – PAC AMF 2 + CI + POP of KAU, T_2 – EDA AMF 1 + CI + POP of KAU, T_3 – ANA AMF 5 + CI + POP of KAU, T_4 – NEN AMF 2 + CI + POP of KAU, T_5 – POO AMF 3 + CI + POP of KAU, T_6 – KAU, 2016 + CI , T_7 – KAU, 2015 + CI , T_8 – Absolute control, DAP – Days after planting).

Table 30: Effect of AMF on leaf area of ginger at monthly interval

TREATMENT	60 DAP	90 DAP	120 DAP	150 DAP	180 DAP
T_1	31.02	33.96	35.83	36.11[a]	37.02[a]
T_2	24.47	26.81	28.45	31.02[a]	30.15[ab]
T_3	28.11	29.38	32.49	34.07[a]	32.32[ab]
T_4	24.88	26.38	27.95	28.98[a]	27.67[ab]
T_5	29.37	32.03	33.96	35.37[a]	32.36[a]
T_6	27.75	28.98	29.27	30.78[a]	29.71[ab]
T_7	24.58	25.60	22.35	24.79[ab]	21.09[bc]
T_8	22.52	23.00	15.37	12.89[b]	13.17[c]
LSD (0.05)	NS	NS	NS	12.54	13.00

(T_1 – PAC AMF 2 + CI + POP of KAU, T_2 – EDA AMF 1 + CI + POP of KAU, T_3 – ANA AMF 5 + CI + POP of KAU, T_4 – NEN AMF 2 + CI + POP of KAU, T_5 – POO AMF 3 + CI + POP of KAU, T_6 – KAU, 2016 + CI , T_7 – KAU, 2015 + CI , T_8 – Absolute control, DAP – Days after planting).

Table 31: Percent disease incidence (PDI) of above ground symptoms of rhizome rot

Treatment	90 DAP PDI (%)	Disease scoring	120 DAP PDI (%)	Disease scoring	150 DAP PDI (%)	Disease scoring	180 DAP PDI (%)	Disease scoring
T_1	23.13^{c}	2 (Resistant)	49.46^{d}	3 (Tolerant)	65.87^{d}	4 (Susceptible)	73.85^{d}	4 (Susceptible)
T_2	28.96^{bc}	3 (Tolerant)	61.69^{bcd}	4 (Susceptible)	76.72^{bc}	5 (Highly susceptible)	89.25^{b}	5 (Highly susceptible)
T_3	30.74^{abc}	3 (Tolerant)	67.38^{b}	4 (Susceptible)	77.79^{bc}	5 (Highly susceptible)	84.48^{bc}	5 (Highly susceptible)
T_4	29.87^{bc}	3 (Tolerant)	65.21^{bcd}	4 (Susceptible)	75.49^{bcd}	5 (Highly susceptible)	84.82^{bc}	5 (Highly susceptible)
T_5	26.82^{c}	3 (Tolerant)	51.17^{cd}	4 (Susceptible)	69.54^{cdd}	4 (Susceptible)	75.20^{d}	4 (Susceptible)
T_6	23.44^{c}	2 (Resistant)	56.89^{bcd}	4 (Susceptible)	72.81^{cd}	4 (Susceptible)	79.78^{bc}	5 (Highly susceptible)
T_7	44.28^{ab}	3 (Tolerant)	66.15^{bc}	4 (Susceptible)	84.18^{ab}	5 (Highly susceptible)	88^{bc}	5 (Highly susceptible)
T_8	46.33^{a}	3 (Tolerant)	85.33^{a}	5 (Highly susceptible)	92.62^{a}	5 (Highly susceptible)	100^{a}	5 (Highly susceptible)
LSD (0.05)	**16.42**		**15.93**		**9.79**		**8.82**	

(T_1 – PAC AMF 2 + CI + POP of KAU, T_2 – EDA AMF 1 + CI + POP of KAU, T_3 – ANA AMF 5 + CI + POP of KAU, T_4 – NEN AMF 2 + CI + POP of KAU, T_5 – POO AMF 3 + CI + POP of KAU, T_6 – KAU, 2016 + CI , T_7 – KAU, 2015 + CI , T_8 – Absolute control, DAP – Days after planting).

Table 32: Effect of AMF on fresh weight and dry weight of ginger rhizome

Treatment	Fresh weight g/plant	Dry weight g/plant
T_1	120.00[a]	51.00[a]
T_2	95.50[b]	39.00[b]
T_3	69.00[bc]	34.50[bc]
T_4	85.00[b]	36.50[b]
T_5	94.00[b]	40.00[b]
T_6	89.50[b]	39.50[b]
T_7	54.00[c]	26.00[c]
T_8	27.00[d]	12.50[d]
LSD (0.05)	25.36	10.45

Table 33: Effect of AMF on fresh weight and dry weight of plant shoot

Treatment	Fresh weight of plant shoot (g)	Dry weight of plant shoot (g)
T_1	58.00[a]	13.00[a]
T_2	42.00[b]	12.00[a]
T_3	31.00[bc]	10.50[ab]
T_4	34.50[bc]	11.50[a]
T_5	38.50[b]	13.00[a]
T_6	36.50[b]	11.50[a]
T_7	22.50[cd]	6.50[bc]
T_8	13.00[d]	4.50[c]
LSD (0.05)	12.94	4.47

(T_1 – PAC AMF 2 + CI + POP of KAU, T_2 – EDA AMF 1 + CI + POP of KAU, T_3 – ANA AMF 5 + CI + POP of KAU, T_4 – NEN AMF 2 + CI + POP of KAU, T_5 – POO AMF 3 + CI + POP of KAU, T_6 – KAU, 2016 + CI , T_7 – KAU, 2015 + CI , T_8 – Absolute control, DAP – Days after planting).

Experiment 2 – Pot culture study for rhizome rot management of ginger

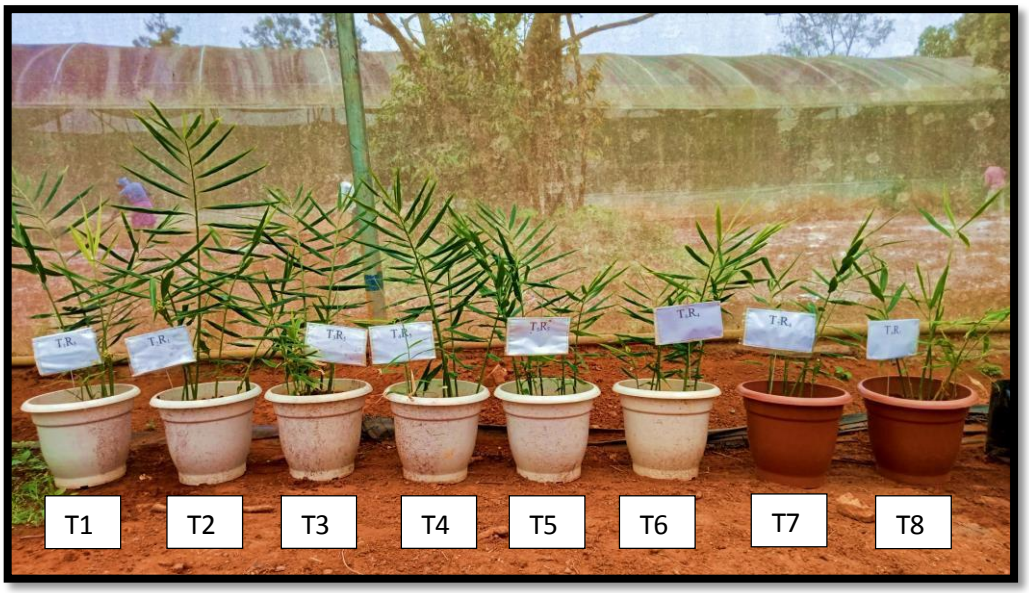


Plate 17: Ginger plants in pot culture plants at 150 DAP

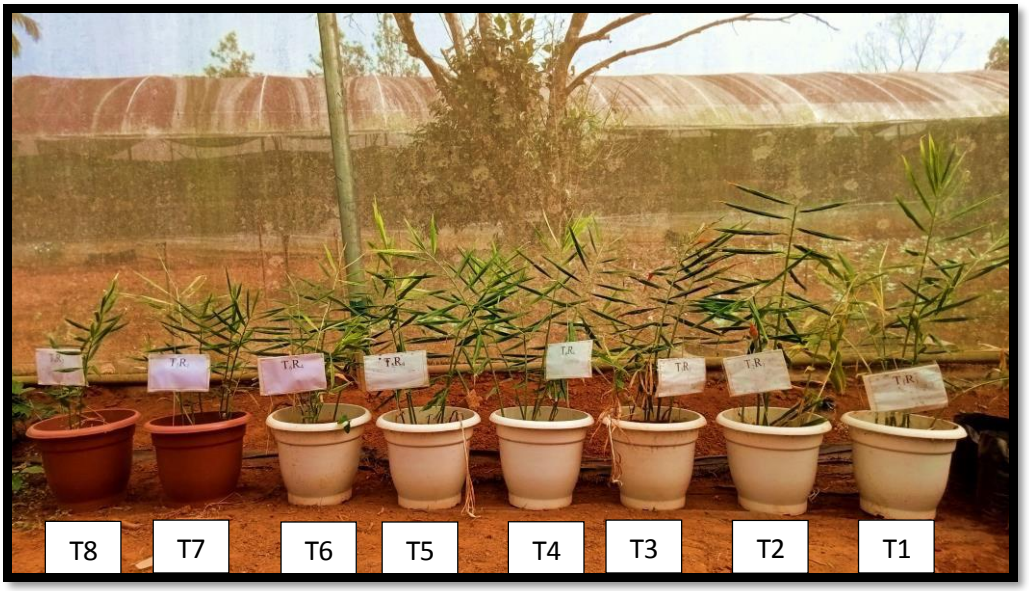
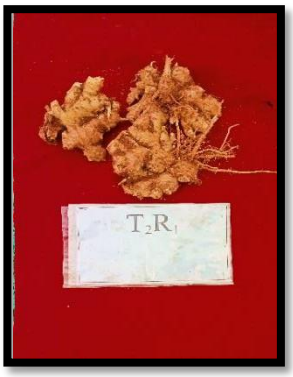


Plate 18: Ginger plants in pot culture plants at 180 DAP

(T_1 – PAC AMF 2 + CI, T_2 – EDA AMF 1 + CI, T_3 – ANA AMF 5 + CI, T_4 – NEN AMF 2 + CI, T_5 – POO AMF 3 + CI, T_6 –POP of KAU, 2016 +CI, T_7– Organic POP of KAU +CI, 2015, T_8 – Absolute control).

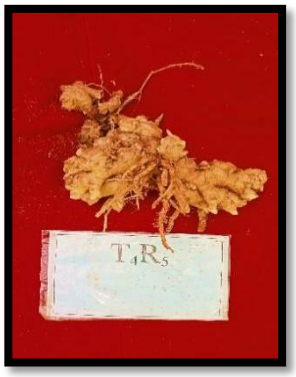
Plate 19: Above and below ground symptoms of rhizome rot

T1 (PAC AMF 2)

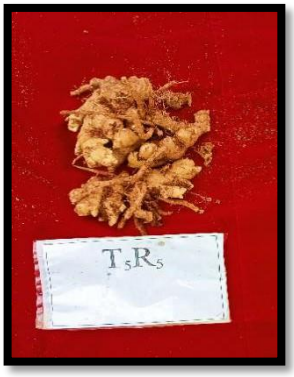


T2 (EDA AMF 1)

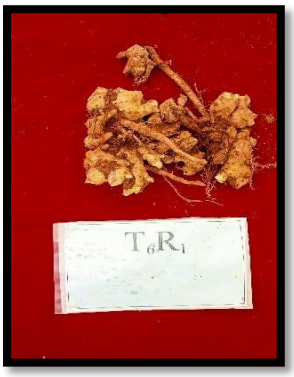
T3 (ANA AMF 5)



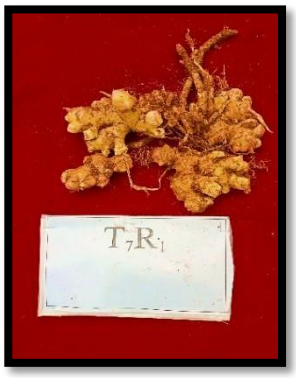
T4 (MAL AMF 2)



T5 (POO AMF 3)



T6 (POP of KAU, 2016)



T7 (POP of KAU, 2015)

T8 (Control)

Plate 20: Rhizome yield of ginger at harvest (180 DAP)

4.7.2.8. Percent disease incidence of rhizome rot of ginger

The role of AMF on rhizome rot disease incidence is presented in the Table 34.

Table 34: Percent disease incidence of below ground symptoms of rhizome rot

Treatment	Rhizome rot disease incidence (%)	Disease severity
T_1	26.57[c]	**3** (Tolerant)
T_2	32.66[c]	**3** (Tolerant)
T_3	31.57[c]	**3** (Tolerant)
T_4	41.99[c]	**3** (Tolerant)
T_5	26.57[c]	**3** (Tolerant)
T_6	31.57[c]	**3** (Tolerant)
T_7	68.66[b]	**4 (**Susceptible)
T_8	92[a]	**5** (Highly susceptible)
LSD (0.05)	20.43	

The disease incidence was higher in the treatment T_8 (Absolute control - 92 %) followed by T_7 (Organic POP, KAU). The rhizome rot incidence was recorded lowest in the treatment T_1 (PAC AMF 2) and T_5 (POO AMF 3) of about 26.57 %. All the treatments are on par with each other, except T_7 (KAU, 2015) and T_8 (control).

4.7.2.9. Per cent root colonisation and final spore count by AMF

The result of percent root colonisation is presented in the Table 35.

Per cent root colonization was higher in the treatment T_1 (PAC AMF 2 - 80%), followed by the treatment T_2 and T_3 (70%). The treatment T_4 and T_5 showed 60% of root colonisation.

Spore count ranged from 55- 37 per 10 g of soil. The highest spore count was found in the treatment T_1 (PAC AMF 2 - 55) and lowest spore count was reported in

T_4 (NEN AMF 2 - 37). Treatment T_2 had 48, T_3 had 40, and T_5 had 45 number of spores.

Table 35: Final spore count and percent root colonisation of Ginger

Treatment	Final spore count per 10 g of soil	Per cent root colonisation
T_1 (PAC AMF 2)	55	80%
T_2 (EDA AMF 1)	48	70%
T_3 (ANA AMF 5)	40	70%
T_4 (NEN AMF 2)	37	60%
T_5 (POO AMF 3)	45	60%

4.7.5. Final nutrient status of pot culture soil

The final nutrient status of pot culture soil was analysed and presented in the Table 36.

Table 36: Final nutrient status of Pot culture soil

SI. No	Parameters	Final Nutrient content
1	pH	6.59 (near to neutral)
2	EC (dSm^{-1})	0.46 (non - saline)
3	Organic carbon (%)	2.45 (high)
4	Available Nitrogen (Kg/ha)	294.78 (medium)
5	Available Phosphorous (Kg/ha)	82.59 (high)
6	Available potassium (Kg/ha)	316.85 (high)

The pH of final pot culture soil was 6.59 which was near to neutral. The EC content of the soil was very low (0.46). The organic carbon content was high in the soil of about 2.45 % and the nitrogen content was in medium range 294.78 (medium). The phosphorous and potassium content in the soil was high of about 82.59 Kg/ ha and 316.85 Kg/ ha respectively.

In the experiment for growth promotion of ginger, T_1 (PAC AMF 2) performed best in plant height, number of leaves and T_5 (POO AMF 3) was significantly higher in rhizome yield (on par with T_1) and performed better in growth parameters like number of tillers and leaf area which is followed by T_1 (PAC AMF 2). In the experiment for rhizome rot management, T_1 (PAC AMF 2) showed better results in plant height, number of tillers, leaf area and significantly higher in rhizome yield. T_1 (PAC AMF 2) also showed lesser rhizome rot incidence. Finally, T_1 (PAC AMF 2) was identified as best performing AMF isolate for growth promotion and rhizome rot management of ginger.

5. DISCUSSION

5. DISCUSSION

Arbuscular mycorrhizal fungi (AMF) are soil fungi belonging to the phylum Glomeromycota that forms symbiotic relationship with the roots of higher plants. Being an obligate symbiont, AMF is dependent on the host plant for their fixed carbon. Over 80% of arbuscular mycorrhizal fungi form symbiotic relationships with vascular plants (Smith and Read, 2008). Mycorrhizal associations help the host plants to thrive in adverse soil conditions and drought situations by increasing the root surface area and mineral uptake efficiency. The abundance of AM fungal spores has been noted to be determined more by host plant species and environmental characteristics than by AM fungal species. AM symbiosis has shown to boost host resistance to a variety of fungal and bacterial pathogens. Since AMF lives in soil and infects plant roots, they have the greatest impact on soil-borne diseases.

Ginger (*Zingiber officinale*) is an important horticultural crop grown primarily for its aromatic rhizomes, which is used as spice, condiment, flavouring agent, and for their potential medicinal properties since time immemorial, and widely distributed in tropical and subtropical regions of Asia. The present study was conducted to evaluate the impact of native arbuscular mycorrhizal fungi on the growth of ginger and also to evaluate its effectiveness in managing the soil borne pathogen *Pythium myriotylum*, the causal organism of rhizome rot of ginger. Numerous studies have shown that arbuscular mycorrhizal fungi improve plant resistance to a variety of pathogens (Harrier and Watson, 2004; Pozo *et al.*, 2007 and Bi *et al*., 2007).

Hoeksema *et al.* (2010) reported that plant responses to AM fungi are highly variable, with host physiology, genotype, edaphic factors, environmental conditions, and root excretions. Effective mycorrhizal symbiosis is critical for successful crop growth. As a result, screening for efficient AMF for a specific crop suitable for a specific agro-climatic region is required.

In the present study, arbuscular mycorrhizal fungal spores were isolated from the native soils of ten ginger growing fields of Wayanad district. Analysing the initial nutrient status of the soil is necessary to know the mycorrhizal spore colonization. The soil samples were analysed for chemical parameters like pH, EC, organic carbon,

nitrogen, phosphorous, and potassium. Total spore count and spore diversity was also recorded for all the samples.

5.1. AMF spore count and diversity

In an experiment conducted by Gopal *et al.* (2005) where diversity of arbuscular mycorrhizal fungi from rhizosphere soils of solanaceous crops in bacterial wilt areas of Kerala was studied and documented that the total AMF spores in the samples ranged from 26 - 1012 spores per 10 g of soil. The maximum number of AMF spores (1012 per 10 g of soil) was recorded in the case of brinjal from Eruthyampathy area of Kerala. In a study conducted by Aiswarya *et al*. (2017) on the AMF diversity of selected medicinal plant species from Kodikuthimala, Malappuram district of Kerala, 25 plant species belonging to 15 families were analyzed for arbuscular mycorrhizal association. Maximum spore population was observed in *Gloriosa superba* (574/100g of soil) and minimum in *Euphorbia hirta* (143/10g of soil).

In the present work, average spore count of the sample was low when compared to spore count observed in above mentioned studies conducted in Kerala. Spore count ranged from 65 spores per 100g of soil to 121 spores per 100g of soil. Bagyaraj (1991) reported that soil ecological and environmental factors like physical (Humidity, temperature, and light), chemical (pH, soil N, soil P, and organic matter), and biological factors (host and soil microorganism) influence the natural occurrence of VA mycorrhizal fungi in soils.

The soil samples were acidic with the pH ranging between 4.51 and 6.14 (pH of Wayanad soil ranges between 5.3 and 6.3 and is slightly acidic in nature). In soil, effects of pH are difficult to evaluate since many chemical properties of soil vary with changes in pH. Kumar *et al*. (2008) reported that AMF colonization was higher in acidic soil compared to neutral and alkaline soil in an experiment conducted in Karnataka. He documented that root colonization of acidic soil was 68% in kolar (pH-5.43), 52 % in Bangalore (pH - 6.60), 50.40 % in Mandya (pH- 6.35) and 49.80 Hassan (pH- 6.22) and the least colonization was observed in soil with alkaline pH – 7.02 (Tumkur). In contrary, study conducted by Patale (2018) has reported that

alkaline conditions (8 to 8.95) favoured mycorrhizal spores and root colonization. From the above mentioned studies, it is clear that pH alone is not a factor that influences the spore colonization. Other factors like nitrogen, potassium, phosphorous content, climatic conditions and habitat in which they grow also affects the spore population.

The role of mycorrhiza in plant growth is also determined by the amount of phosphorus and nitrogen status in the soil, and the balance of these two macronutrients in the soil. Baath *et al.* (1989) reported that low levels of soil P and intermediate N levels increased mycorrhizal infection whereas infection was reduced at high P and N content. In addition, Azcon *et al*. (2003) noted that mycorrhizal plants with highest N and P levels had decreased amount of nutrients absorbed per unit of root mass of lettuce.

All the samples collected for the study had high levels of phosphorous content ranging between 24.20 Kg/ha and 356.44 Kg/ha. The high level of phosphorous content in the soil could be one of the reasons for the low spore number in the sample. Bonneau *et al*. (2013) reported that AM symbiosis can be disrupted by environmental factors such as high P availability, which can inhibit symbiotic interaction. Balzergue *et al.* (2011) also demonstrated that, plants do not produce the necessary signals for the establishment of mycorrhizal symbiosis when P availability is more. These findings are in agreement with the result of the present study where high phosphorous content has negatively affected the spore count.

The nitrogen content was also high in the collected samples. It ranged between 0.049 % and 0.179 %. High nitrogen content has adversely affected the spore population. Hayman (1975) found that N fertilizers (188 Kg N/ ha) had a negative influence on the mycorrhizal population. Zhang *et al*. (2016) also reported that, nitrogen addition reduced spore population diversity and richness of AMF and suppressed the spore density and the hyphal length density. These results indicate that nitrogen content of soils could greatly influence the distribution and abundance of AM fungi.

From these studies, it is understood that mycorrhizal spore count is affected by many factors like pH, phosphorous and nitrogen content in the soil. Increased nitrogen and phosphorous content in the soil could be due to increased fertilizer application in the ginger field by farmers to get bumper yield and heavy pesticide application to reduce rhizome rot incidence also affects AMF growth in the soil. Similar results were observed by Verzeaux *et al.* (2017) that addition of phosphatic and nitrate fertilizers reduced AMF spore count and hyphal colonization by inducing deleterious effect in the AMF life cycle. Bonneau *et al*. (2013) rightly pointed out that high concentrations of N and P can limit mycorrhizal colonization. As reported by Rajesh kumar, 2002 the reason for the low spore number in the field soil could also be due to variations in host plant and soil fertility.

Morphological characterization of the 74 spores isolated from ten locations of Wayanad district was carried out for the characteristics like colour, size, shape, nature of hyphae, bulbous suspensor, number of spore wall, and surface ornamentation. Based on morphological characters, the isolates were tentatively identified at genus level. All the characterized AMF spores belonged to one of the four genera, *Glomus, Gigaspora, Scutellospora,* and *Acaulospora.* Among the 74 isolates, 47 isolates showed similar character with *Glomus,* 15 with *Scutellospora,* 9 with *Gigaspora,* and 3 with *Acaulospora*. The genus *Glomus* was found most dominant in the soils of Wayanad. Similar results were recorded by Khade and Rodrigues (2002) in their experiment conducted to assess the AM fungal association in commonly occurring pteridophytes from two sites located in the Western Ghats region of Goa. They recorded a total of 18 AM fungi belonging to 5 genera *viz., Acaulospora, Gigaspora, Glomus*, *Sclerocystis* and *Scutellospora.* The studies indicated that the genus *Glomus* is ubiquitous in various ecosystems in India, indicating greater adaptability of *Glomus* to various soil conditions. The dominancy of *Glomus* in the present study is in accordance with the findings of many other workers (Mridha and Dhar, 2007; Burni *et al.,* 2009 and Sharma *et al.,* 2009). The predominance of *Glomus* spp. under varying soil conditions might be due to their wide adaptability to varied soil conditions and their ability to survive in acidic as well as in alkaline soils (Pande and Tarafdar, 2004).

5.2. Mass multiplication of selected AMF spores

Due to obligatory biotrophism, AMF multiplication occurs in axenic in vitro cultivation using transformed roots or in trap pots cultivated with plants (Selvakumar *et al*. 2018). *In vitro* cultivation allows for the rapid multiplication of viable, pure, and decontaminated propagules, making it useful for AMF multiplication, which establishes symbiosis in 3 to 4 days (Dalpe and Seguin, 2010). Because it is less artificial, more cost-effective, and capable of producing large amounts of highly efficient inoculants in the mycorrhizal system, the AMF multiplication method that uses cultivation trap pots with substrate or soil. AMF and host plant compatibility has been demonstrated, resulting in differences in spore multiplication, mycorrhizal colonisation, extra-radicular hyphae growth, and production of glomalin-related soil protein (Parniske, 2008 and Smith and Read, 2008).

Mass multiplication of AMF spores was done for its application in the pot culture experiment. The mass multiplied inoculum had lesser spore count *viz*., PAC AMF 2 (28 per 10 g of soil), EDA AMF 1 (30 per 10 g of soil), ANA AMF 5 (35 per 10 g of soil), NEN AMF 2 (32 per 10 g of soil) and POO AMF 3 (27 per 10 g of soil). One reason for the low spore count could be varied environmental and climatic conditions, as the mass multiplication was carried out in Thrissur, where the average temperature was 27°C during the period and 23°C in Wayanad district (humid). This difference in climatic condition might affected the AMF sporulation. Also, mass multiplication was carried out in maize plant which is not the natural host of spores collected from Wayanad district which could also be the reason for low spore count. The substrate used for mass multiplication contained vermiculite and perlite in addition to soil. Even though, spore count was less in mass multiplied sample, it is higher when compared to the spore count of the sample collected from Wayanad district. This may be due to usage of substrate suited for AMF sporulation. Oseni *et al*. (2010) reported that seedlings inoculated with vermiculite containing AM fungi colonised 23.3 % of the roots in the vermiculite medium after AM inoculation.

All these findings are in agreement with the result of Muthukumar and Udaiyan (2000) and Zubek *et al*. (2010) who reported that the colonisation by arbuscular mycorrhizal fungi varied greatly depending on the host plants and the

habitats in which they grow. They also insisted that the type of substrate in which the plants grow had a significant impact on the intensity of mycorrhizal colonisation. Any plant species can be infected by any AM fungal species, but the extent and severity of infection vary depending on the host endophyte combinations.

Per cent root colonization of mass multiplied roots was analysed to check its infectivity. Root colonization of the samples were PAC AMF 2 (100 %), EDA AMF 1 (90 %), ANA AMF 5 (100 %), NEN AMF 2 (90%) and POO AMF 3 (80 %). Even though the spore count was less, root colonization was satisfactory. The root colonization is positively correlated with the spore count in the sample. The sample with high root colonization had higher number of spores and those with less root colonization had lesser number of spores. Similar findings were noticed by Gunwal *et al.* (2021), who discovered a favourable relationship between mycorrhizal infection within the roots and soil spore counts. The number of spores found in the rhizosphere soil was found to be directly proportional to the degree of colonisation in most cases.

5.3. Influence of AMF on growth and yield parameters

Pot culture experiment was conducted in Regional Agricultural Research Station, Ambalavayal, Wayanad district. The initial nutrient status of the potting mixture was analysed (Table 17). The pH of the potting mixture was acidic (5.93), EC (1.14 dS/m), organic carbon content was high (1.60 %), available nitrogen was medium (332.42 Kg/ha), phosphorous and potassium content was very high with 105.38 Kg/ha and 523.94 Kg/ha respectively. A study carried out by Balzergue *et al.* in 2011 with pea plants, revealed that AM symbiosis was arrested almost completely by a high P supply at a very early stage. So, the phosphorous fertilizer application is reduced to one fourth of the recommended dose.

Two experiments were conducted simultaneously in pot culture study. The first experiment focussed on the effect of arbuscular mycorrhizal fungal application on growth and yield promotion of ginger and the second experiment was designed to assess the efficacy of AMF in managing soft rot disease of ginger. For this, in the second experiment, the soil was artificially inoculated with the fungus *Pyhtium*

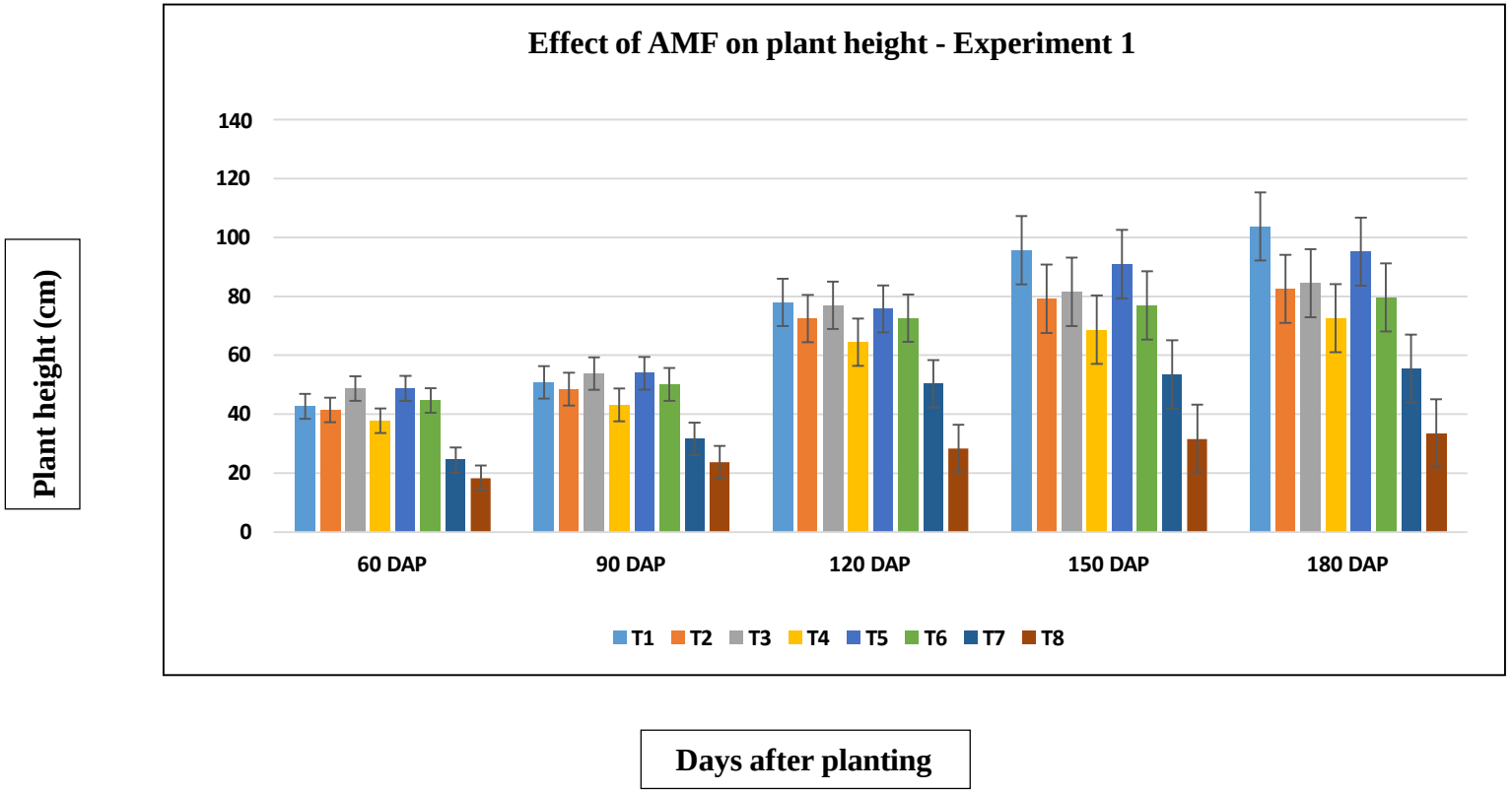


Fig 2: Effect of AMF on plant height of ginger at monthly interval

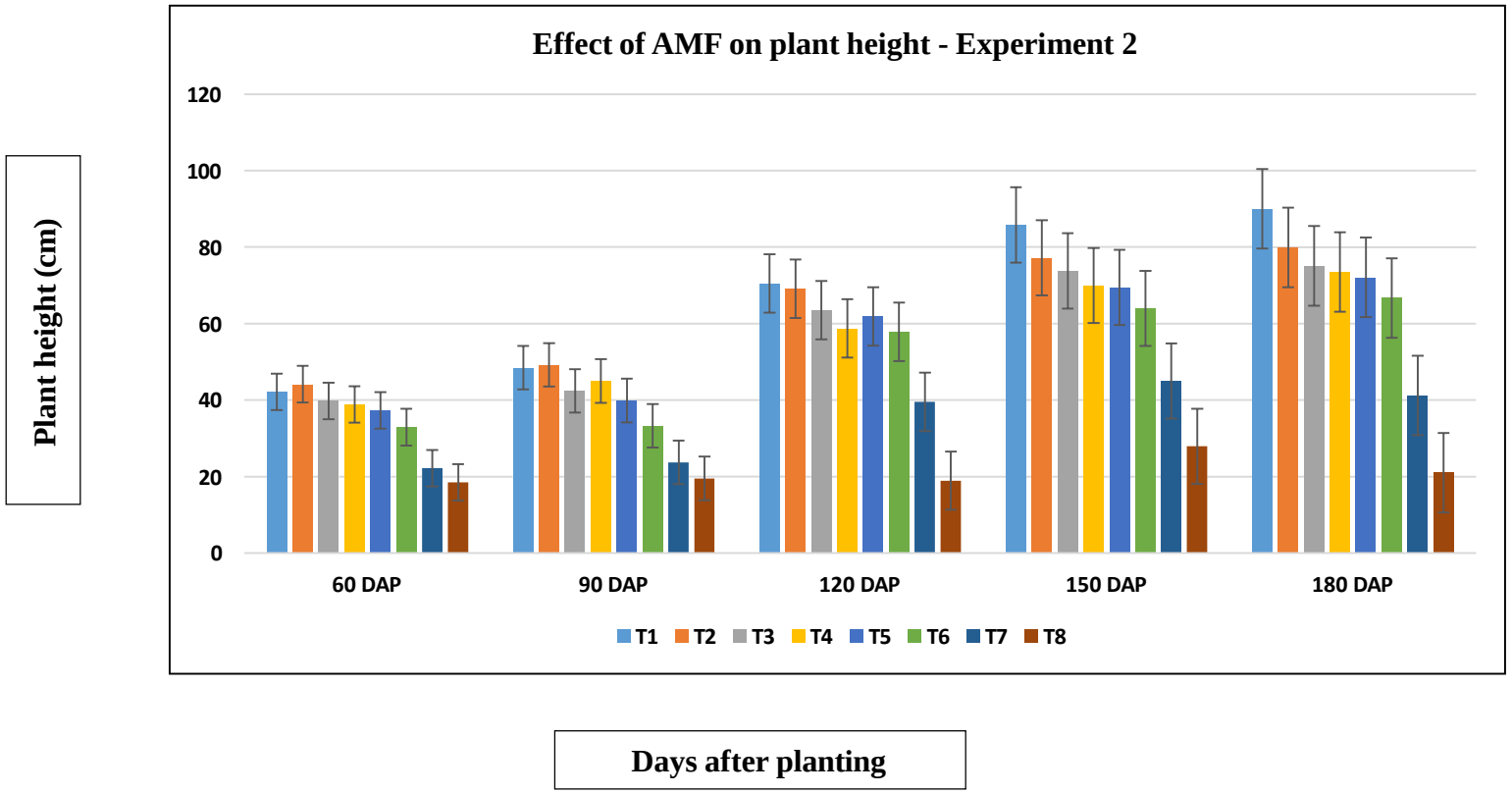


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Fig 3: Effect of AMF on plant height of ginger at monthly interval

myriotylum, the causal agent of rhizome rot. Treatments T_1 to T_5 was applied with selected AMF spores.

In the first experiment (Growth promotion), plant height was higher in all the AMF inoculated treatments throughout the cropping period and at 180 DAP the height ranged between 72.60 cm and 103.70 cm when compared to control plants which showed lowest height 33.50 cm (Figure 2). T_1 (PAC AMF 2) showed maximum plant height of 103.70 cm and minimum plant height was seen in T_8 (Control - 33.50 cm). Similar results were obtained by Samanhudi *et al.* (2014) who observed that arbuscular mycorrhizal fungi (AMF) application in ginger plant increased the plant height when compared to non-mycorrhizal plants. Powell (1984) also reported that mycorrhizal plants grew faster and produced more yield than non-mycorrhizal plants. Corresponding to the first experiment, plant growth parameters of ginger plants in second experiment also showed better performance in AMF inoculated pots than the control pots. The plant height at 180 DAP was higher in AMF inoculated pots T_1 (PAC AMF 2 - 90 cm) but control plants showed lesser height ranged 21 cm (Figure 3). Similar results were observed by Iyer and Sundararaju (1993) in their experiment on interaction of mycorrhiza with *Meloidogyne incognita* and *Pythium aphanidermatum* on ginger (*Zingiber officinale* Rosc). The plants inoculated with AMF fungi showed better growth than the plants that were devoid of AMF. In our study, AMF applied pots showed better growth even after challenge inoculation with *Pythium*.

At 180 DAP, all growth parameters of AMF inoculated treatments performed better than the control plants. T_1 (PAC AMF 2) showed significantly higher number of leaves (22.6) and control plants T_8 (control) showed minimum number of leaves (6.6) (Figure 4). This is in agreement with the results of Siddiqui and Sayeed (2009) who reported that, the number of leaves is significantly increased by AMF treatment as compared to the plants that are not applied with AMF. Similarly, AMF greatly influenced the number of tiller production in ginger. The treatment T_1 (PAC AMF 2) showed maximum number of tillers (15.00) and control plants showed minimum number of tillers T_8 (3.4) (Figure 6). The maximum leaf area was recorded in AMF treated plot T_5 (POO AMF 3 - 39.01) and minimum leaf area was recorded in T_8

(control) plants (13.63) of ginger crop (Figure 8). The leaf area and leaf area index have a direct relationship with the number of leaves produced. Increased leaf number increases leaf area, allowing the plant to increase photosynthetic rate and produce better growth. According to Samanhudi et al. (2014), mycorrhizal treatment at different doses (5, 10, and 15 g/plant) increased plant height, number of leaves, number of tillers, and fresh weight of ginger rhizome, which is consistent with the current study's findings. Number of tillers and number of leaves at 180 DAP was lesser than 150 DAP. This could be due to senescence of leaves and drying up of plants at later growth stages.

In the second experiment pertaining to soft rot management, all growth parameters like number of leaves were higher in T_2 (EDA AMF 1 - 17) which was followed by T_1 (PAC AMF 2 – 16.00) and lower in T_8 (control – 5.8), number of tillers were higher in T_1 (PAC AMF 2 – 8.40) and lower in T_8 (control – 2.2) and leaf area was maximum inT_1 (PAC AMF 2 – 37.02) and minimum in T_8 (control – 13.17) (Figure 5, 7, 9). Similar results were also reported by Rosendahl and Rosendahl (1990) found that AM colonisation reduced damping-off caused by *Pythium ultimum* in pea (*Pisum sativum* L.) seedlings. Jung *et al*. (2012) also found that AM-colonized tomato plants had lesser root infection by *Phytophthora parasitica*, and they proposed that the reduction in root pathogens is due to direct competition for root space and resources. According to Jacott (2017), AM symbiosis has been shown to increase host resistance to a variety of fungal and bacterial pathogens, particularly root pathogens.

The fresh weight and dry weight of rhizomes in first experiment were significantly higher in AMF inoculated treatments when compared to control pots (Figure 10, 11). Fresh weight was higher in T_5 (POO AMF 3 – 160 g/plant) which was on par with T_1 (PAC AMF 2) and lower in T_8 (control - 30 g/plant). Dry weight of rhizome was higher in T_5 (POO AMF 3 - 65 g/plant) was on par with T_1 (PAC AMF 2) and lower in T_8 (control - 20 g/plant). These findings agreed with those of Trisilawati *et al.* in 2019, who found that AMF application increased *Centella* yield (fresh and dry weight of stolon, root, and biomass), asiaticoside content (with an increase of 0.1% to 0.6%), and P and K nutrient uptake. Similarly, the fresh weight and dry weight of rhizomes of second experiment was also significantly higher in AMF

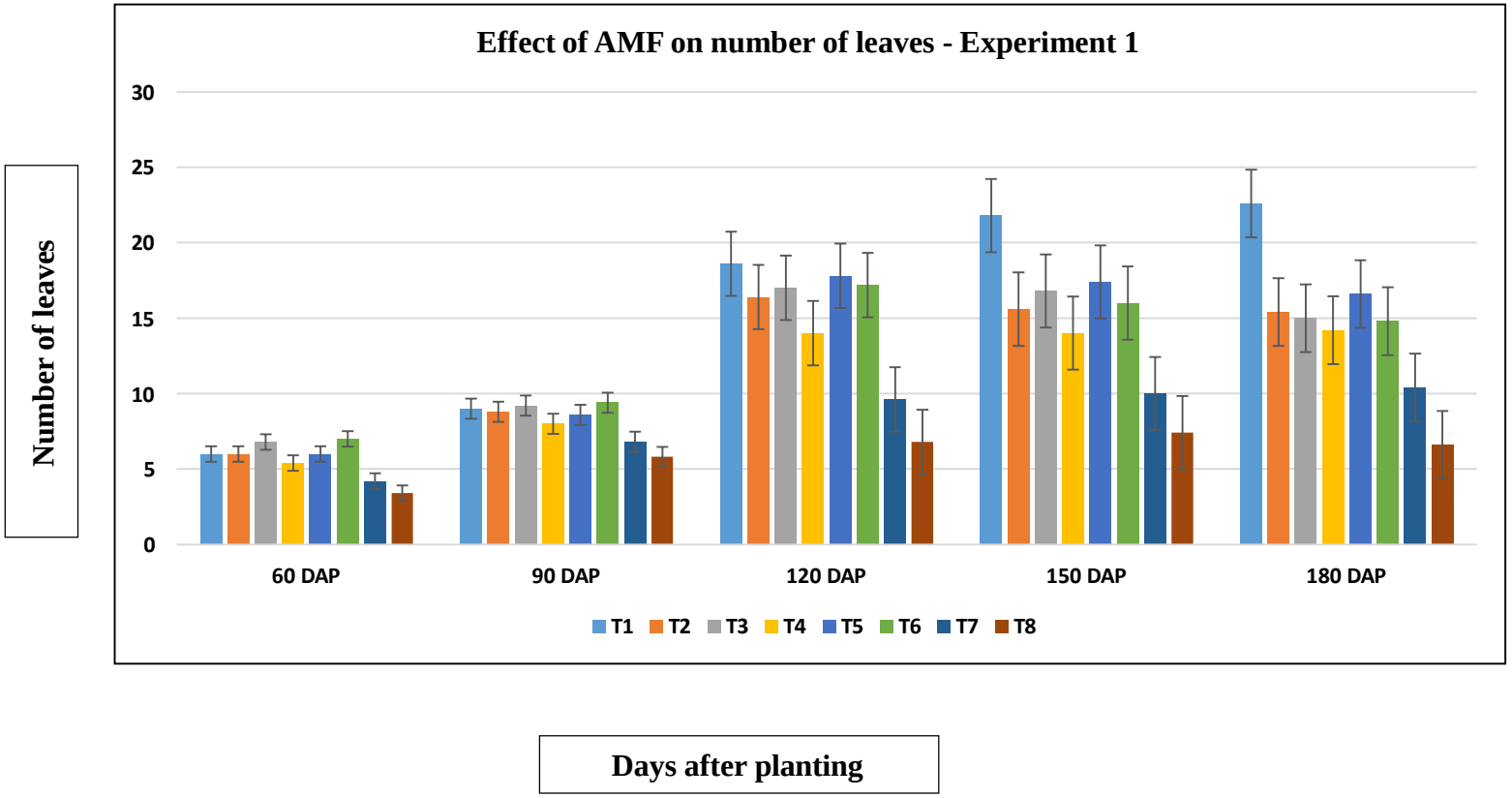


Fig 4: Effect of AMF on number of leaves of ginger at monthly interval

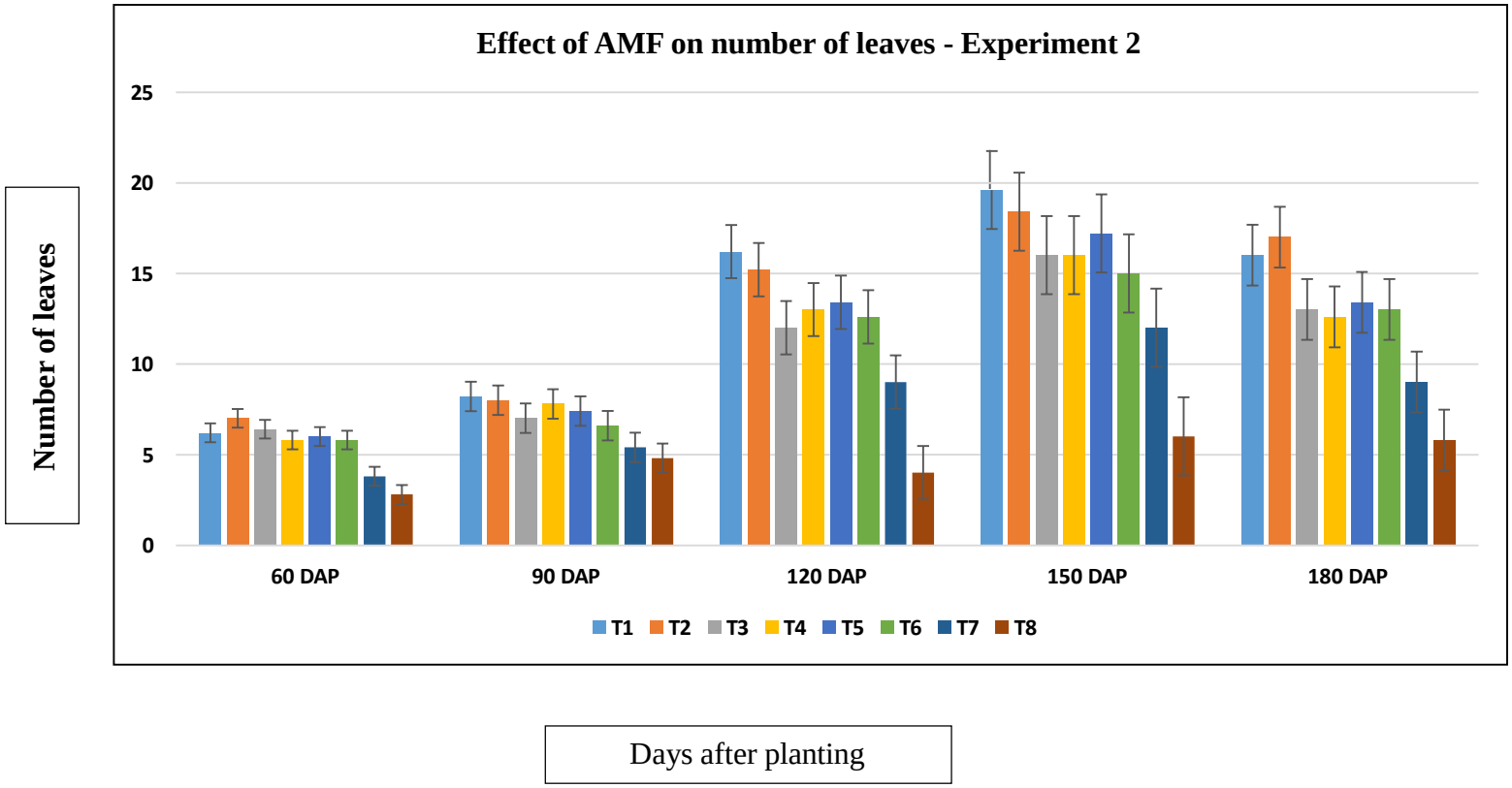


Fig 5: Effect of AMF on number of leaves of ginger at monthly interval

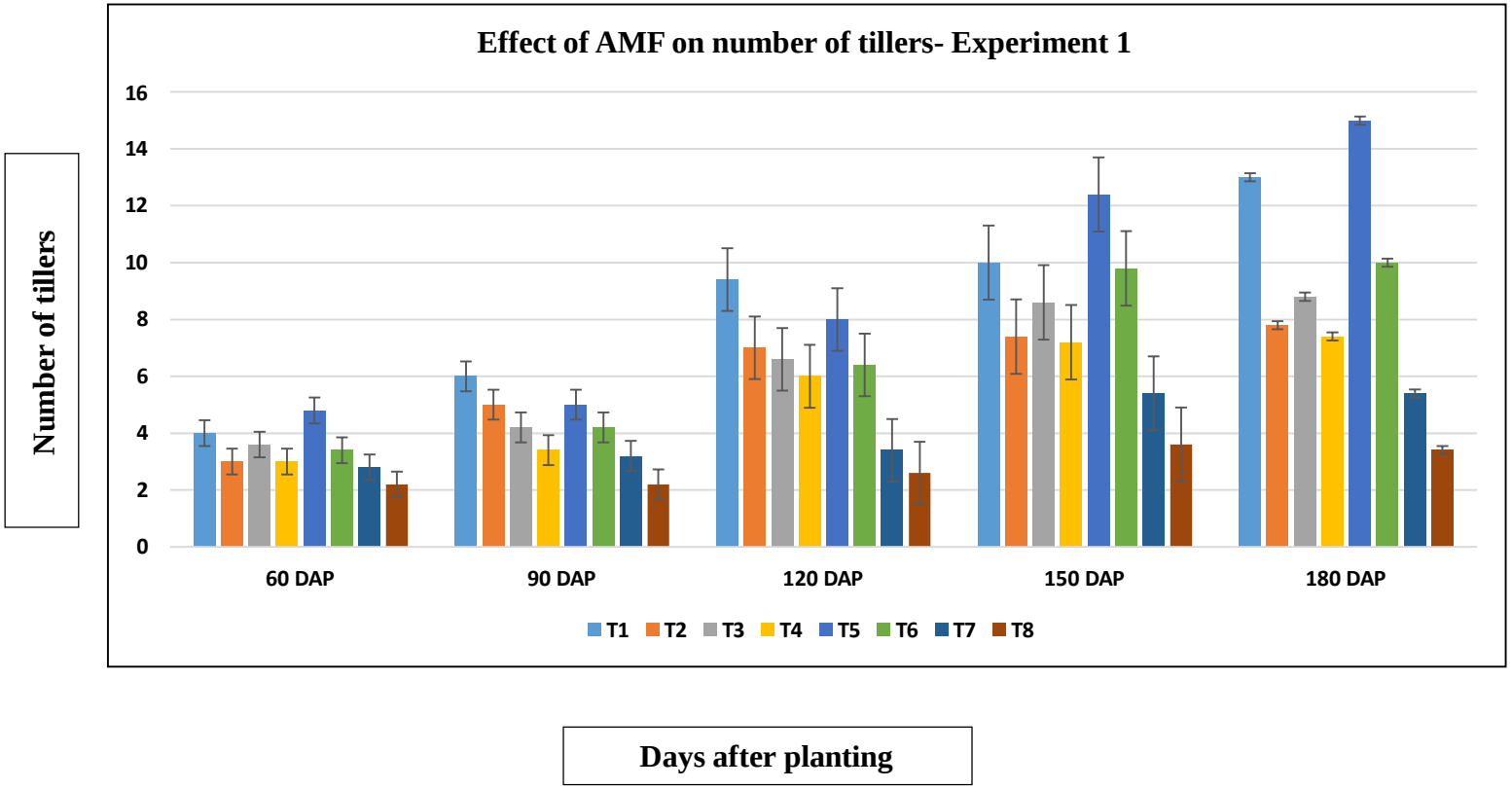


Fig 6: Effect of AMF on Number of tillers of ginger at monthly interval

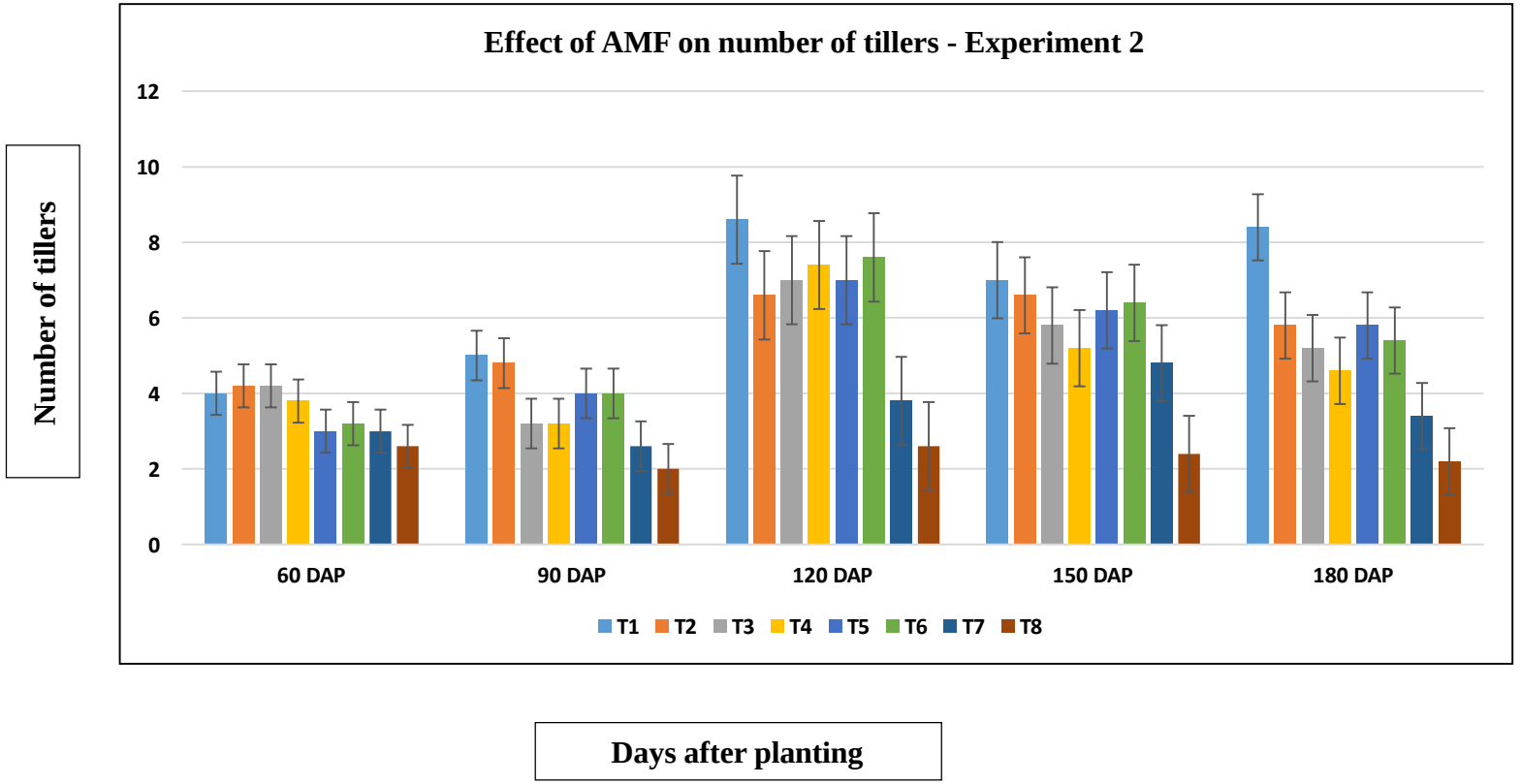


Fig 7: Effect of AMF on number of tillers of ginger at monthly interval

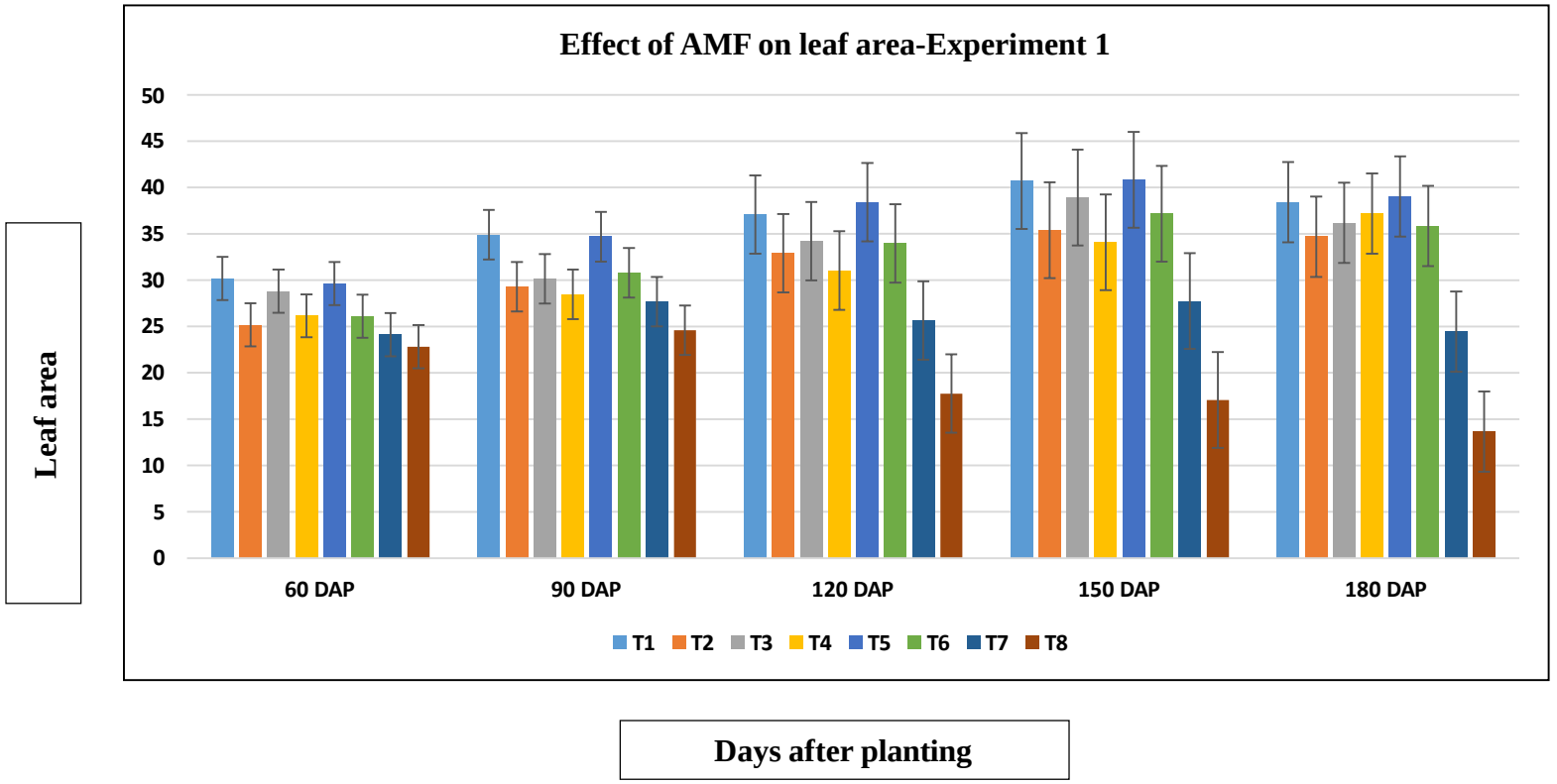


Fig 8: Effect of AMF on leaf area of ginger at monthly interval

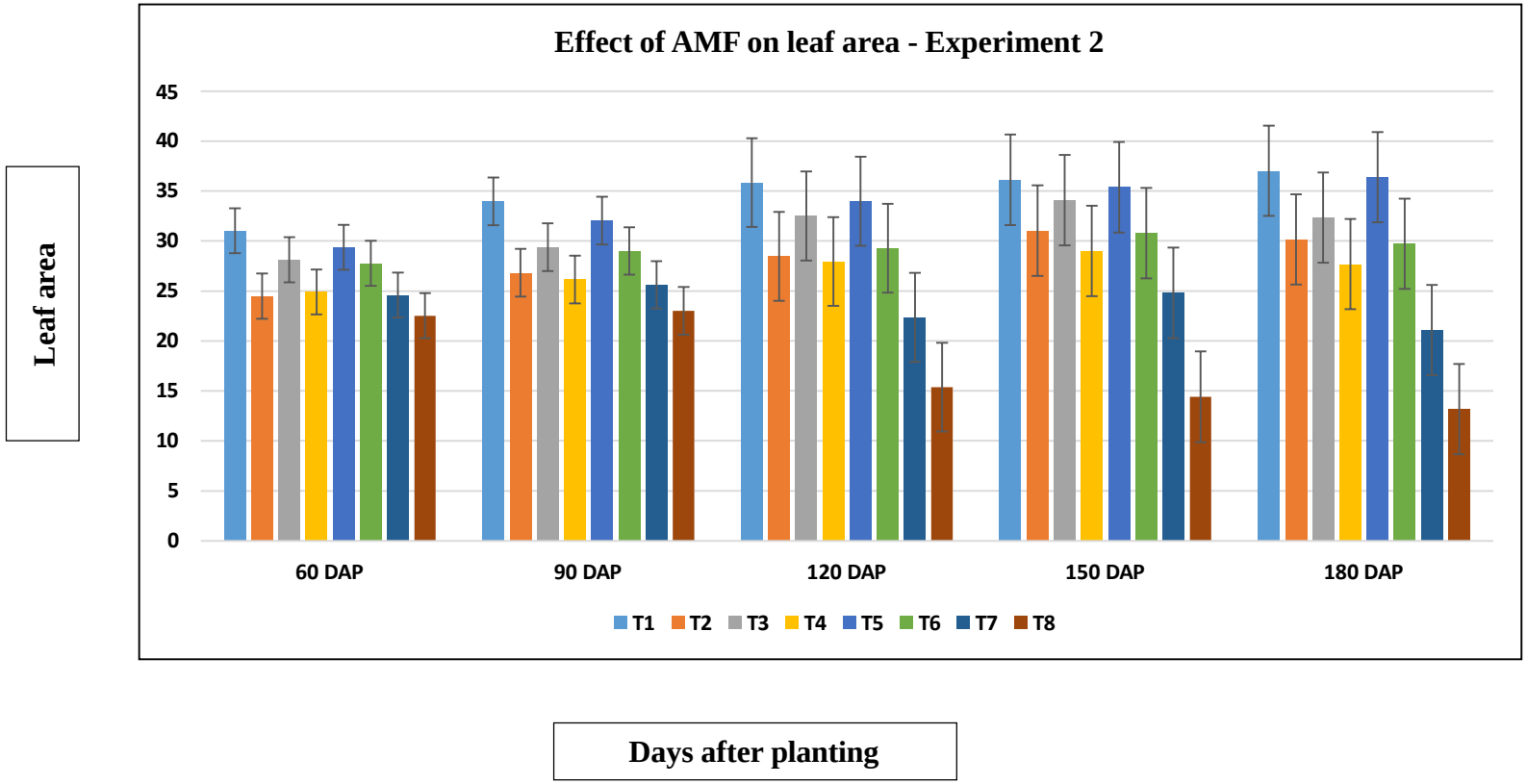


Fig 9: Effect of AMF on leaf area of ginger at monthly interval

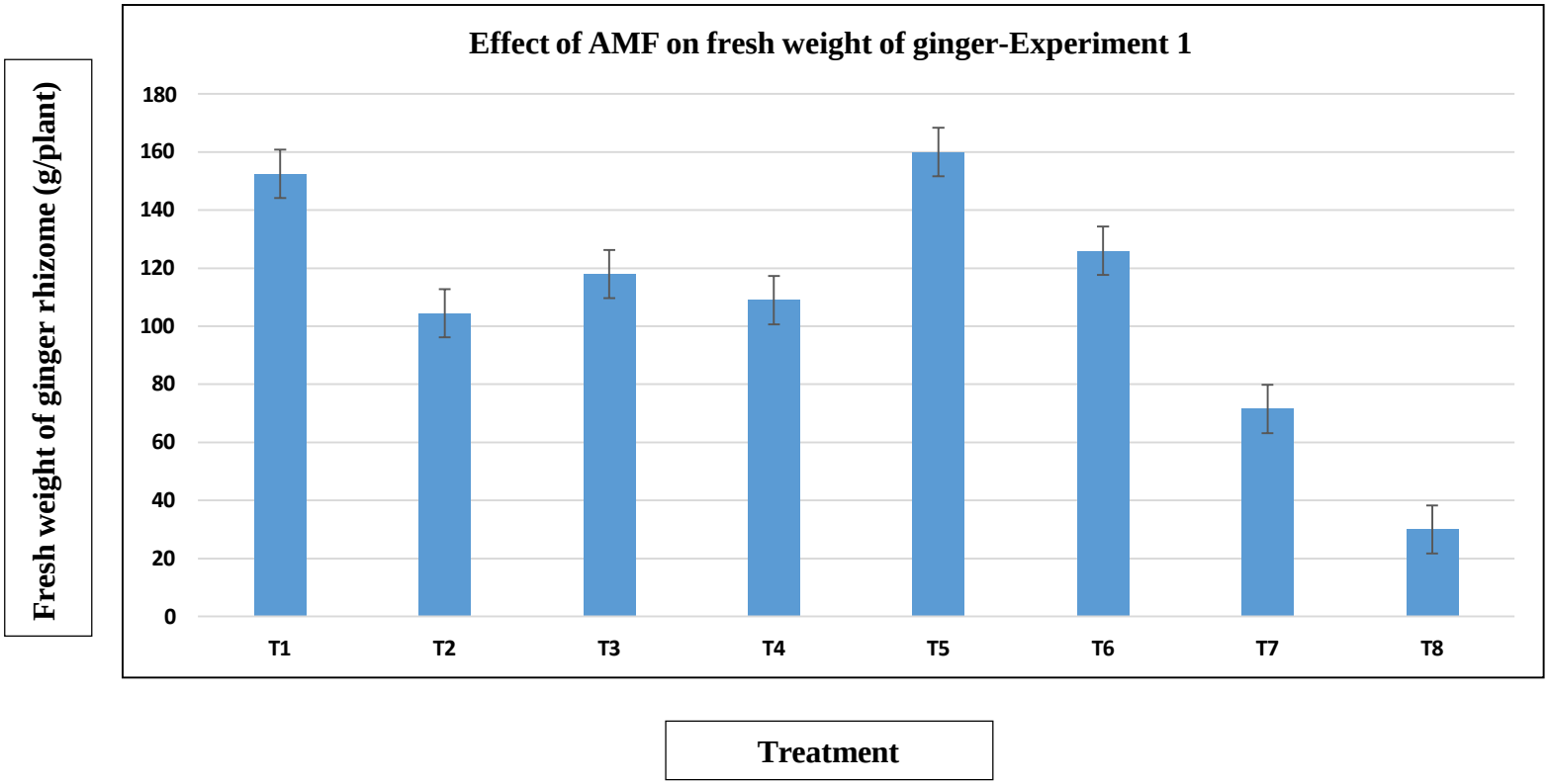


Fig 10: Effect of AMF on fresh weight of ginger rhizome

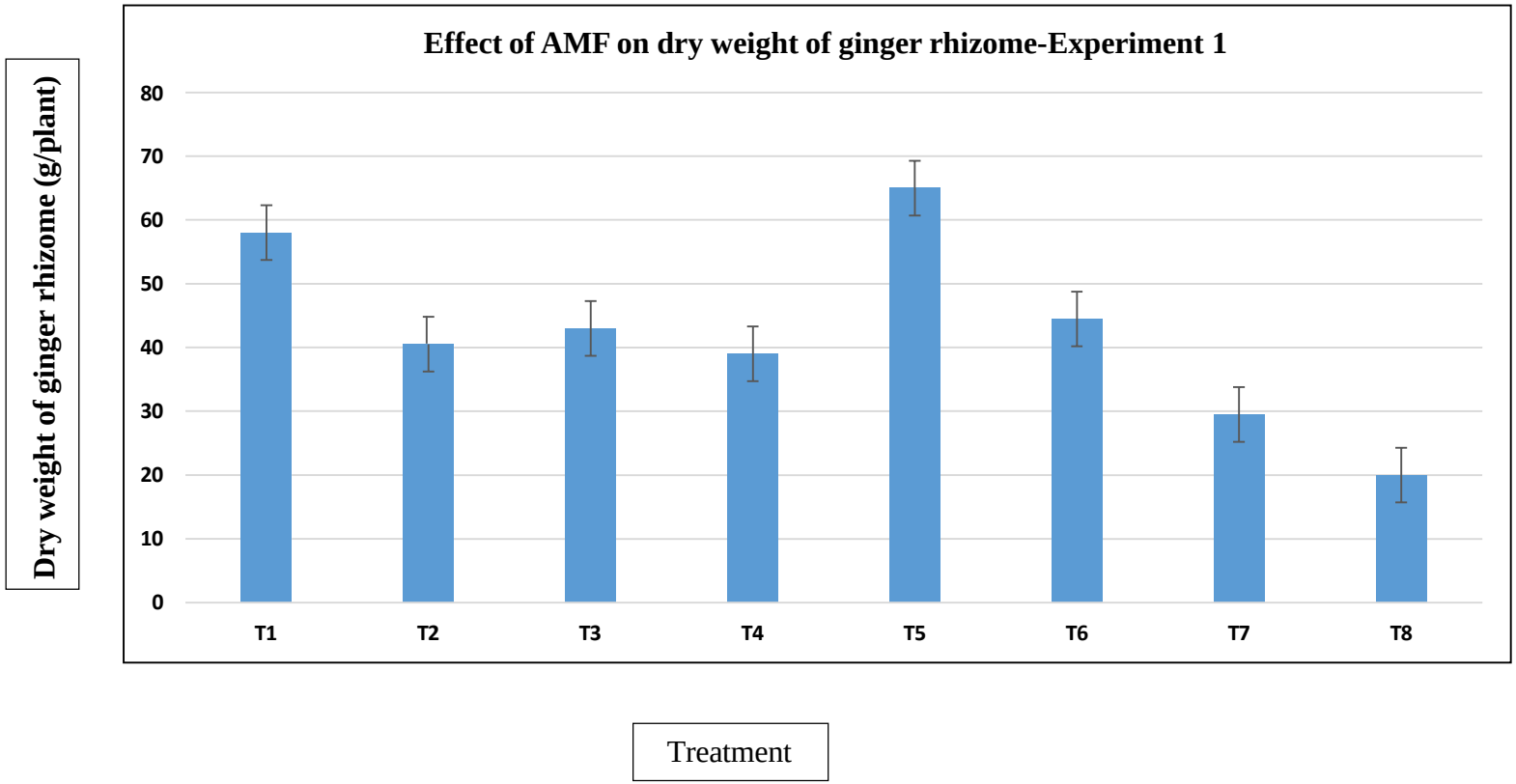


Fig 11: Effect of AMF on dry weight of ginger rhizome

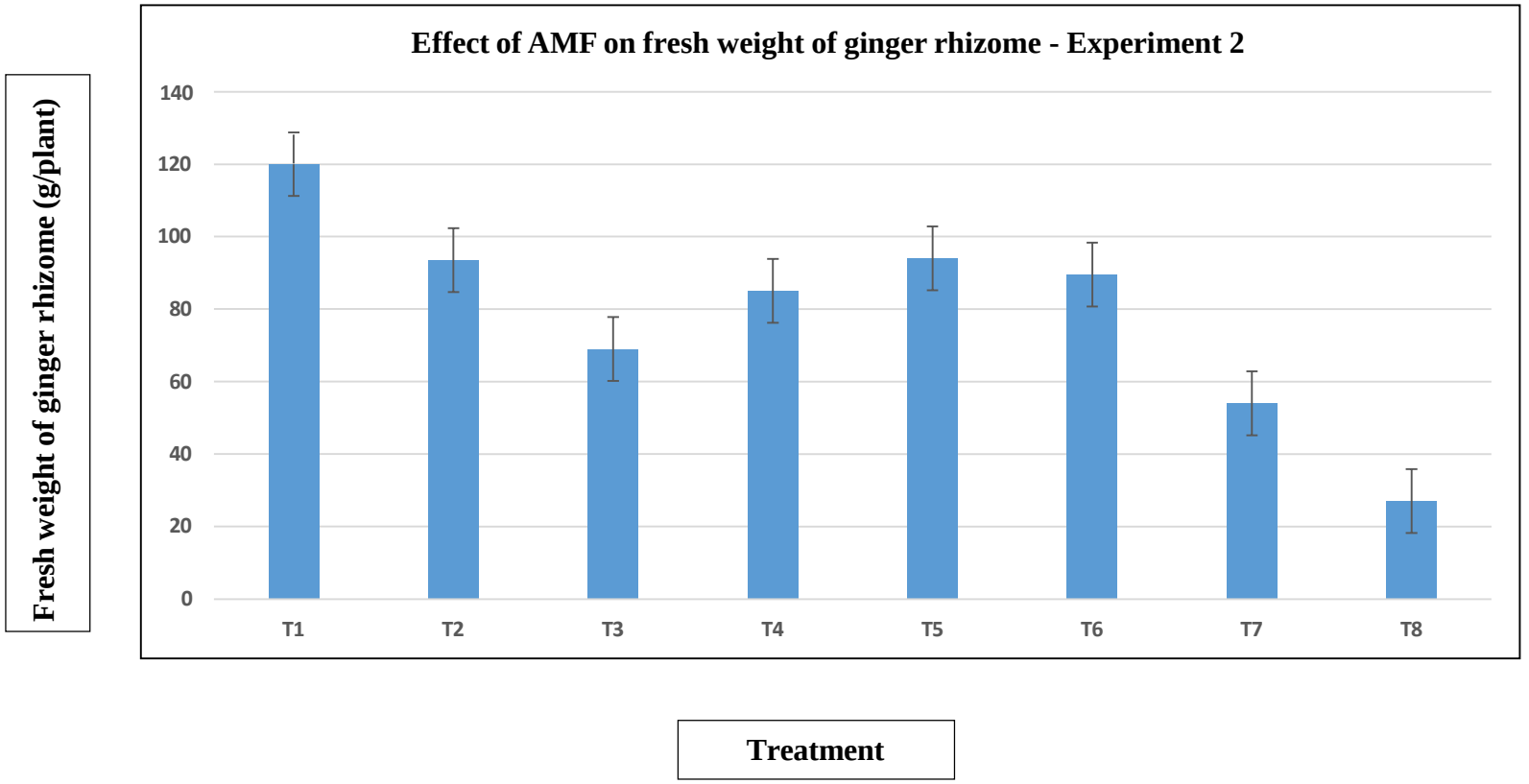


Fig 12: Effect of AMF on fresh weight of ginger rhizome

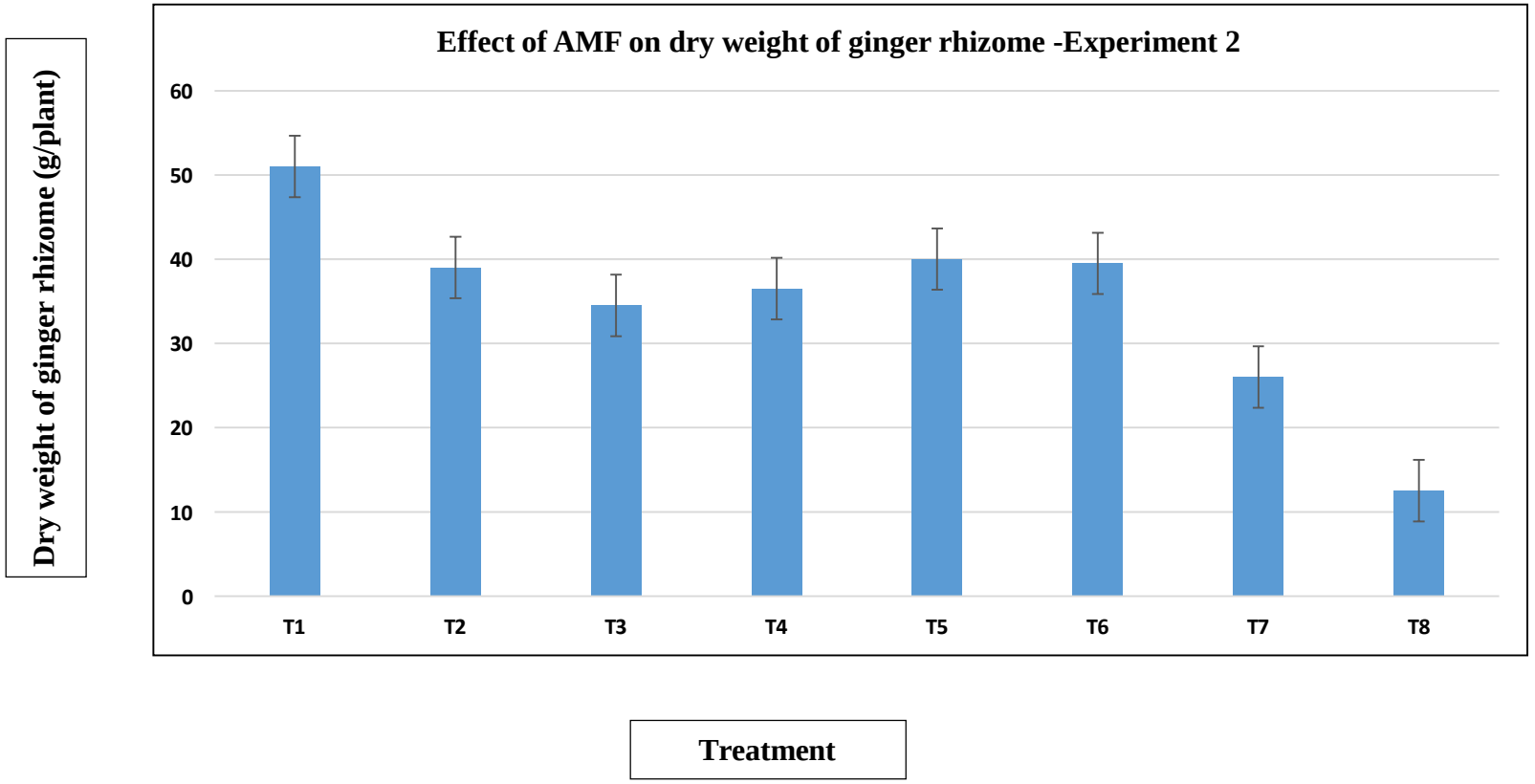


Fig 13: Effect of AMF on dry weight of ginger rhizome

inoculated treatments T_1 (PAC AMF 2) with 120 g/plant and 51 g/plant respectively (Figure 12, 13). Kavitha and Nelson (2014) conducted a study on the effect of AMF on the growth and yield of sunflower and found that fresh and dry weight of roots of AMF inoculated plants were increased which is in agreement with the present study. The plants inoculated with *Glomus mosseae* showed highest response followed by *Glomus fasiculatum* and *Acaulospora scrobiculata.*

Oleoresin content was higher in the AMF inoculated plants than control plants. Silva *et al*. (2008) found that inoculation with *S. herogama* and *G. decipiens* resulted in higher oleoresin yields, corresponding to 3.48% and 1.58% of ginger rhizome fresh biomass, respectively. The results indicate that screening and inoculation of arbuscular mycorrhizal fungi in ginger plants is a feasible procedure for increasing *Z. officinale* oleoresin production and, as a result, the aggregate value of ginger rhizome production.

5.4. Management of soft rot by AMF

Ginger farmers in Wayanad, Kerala, have reported rhizome rot as a major productivity issue in the growing season (2019–2020). Before COVID pandemic in early 2020, ginger farmers in Wayanad were confronted with a disease that caused excessive loss of ginger seeds, which were often stored belowground until sale. The loss of such seed rhizomes was a major source of concern for ginger farmers in Wayanad district and other parts of Karnataka State (Mao and Yan, 2014; Rosangkima *et al*., 2018 and Harsha *et al*., 2021).

Rhizome rot (also known as soft rot) is one of the most devastating diseases of ginger worldwide, causing losses of 50-90% in major production areas such as India's tropical regions (Dohroo, 2005). Rhizome rot completely destroyed ginger crops in the Indian states of Kerala and Tamil Nadu, according to Dohroo (2005). A survey was conducted in January 2020 among farmers cultivating ginger on farms of 1-2 acres in Wayanad and approximately 80 acres in Karnataka's Shimoga and Hassan districts. In the survey, soft rot disease was identified as a major issue for ginger farmers. Ginger rhizome rot complex disease has the potential to cause yield loss of

up to 90% as reported by Nirmal *et al*. (1992). The second experiment was thus carried out to identify the effective AMF isolate in managing soft rot disease.

In second experiment (artificially applied with *Pythium*), AMF inoculated plants showed lower disease incidence *viz.,* T_1 (PAC AMF 2) – 26.57 %, T_5 (POO AMF 3) – 26.57 %, T_3 (ANA AMF 5) – 31.57 %, T_2 (EDA AMF 1) – 32.66 %, T_4 (NEN AMF 2) – 41.99 % than the control plants which showed 92 % infectivity. Eventually, at the later stage of growth, some plants in control pots died, while the AMF treated plants survived throughout the experiment (Figure 15). Bagyaraj (2018) studied the interaction of AMF, Rhizobium, and two pathogenic fungi, *Pythium ultimum* and *Phytophthora megasper*, and discovered that the presence of mycorrhizal fungi reduced the number of plant deaths caused by *P. megasper*, which is consistent with the findings of the current study. Aguk *et al*. (2018) also reported that when compared to the control, the disease index and incidence of *Ralstonia solanacearum* inoculated with *G. rhizogenes* and *G. mosseae* were reduced by 9.7% and 49.8%, respectively.

Natural disease incidence was noticed in first experiment, AMF inoculated plants showed less rhizome rot incidence *viz.,* T_5 (POO AMF 3) - 8 %, T_2 (EDA AMF 1) - 8.33 %, T_1 (PAC AMF 2) - 9 %, T_3 (ANA AMF 5) - 9 %, T_4 (NEN AMF 2) - 11.66 % than control plants (70 %) (Figure 14). The natural occurrence of disease might be due to the use of Rio-de-janeiro variety of ginger which is highly susceptible to rhizome rot. According to survey conducted in 2016, maximum mean rhizome rot incidence was observed in Reo-de-Janeiro (28.33%) in Karnataka district and AMF application reduced disease incidence of ginger crop. Song *et al.* (2015) found that inoculating tomato plants with the AMF, *F. mosseae*, resulted in a substantial decrease in disease incidence and severity of early blight compared to control plants that did not receive mycorrhizal inoculation.

5.5. Final root colonization and nutrient status at harvest stage

Root colonization of AMF infected plants was assessed at the final stage of experiment. In experiment 1, maximum root colonization was observed in T_1 (PAC AMF 2 - 90 %), followed by T_5 (POO AMF 3 - 80 %), T_3 (ANA AMF 5 - 70 %) and

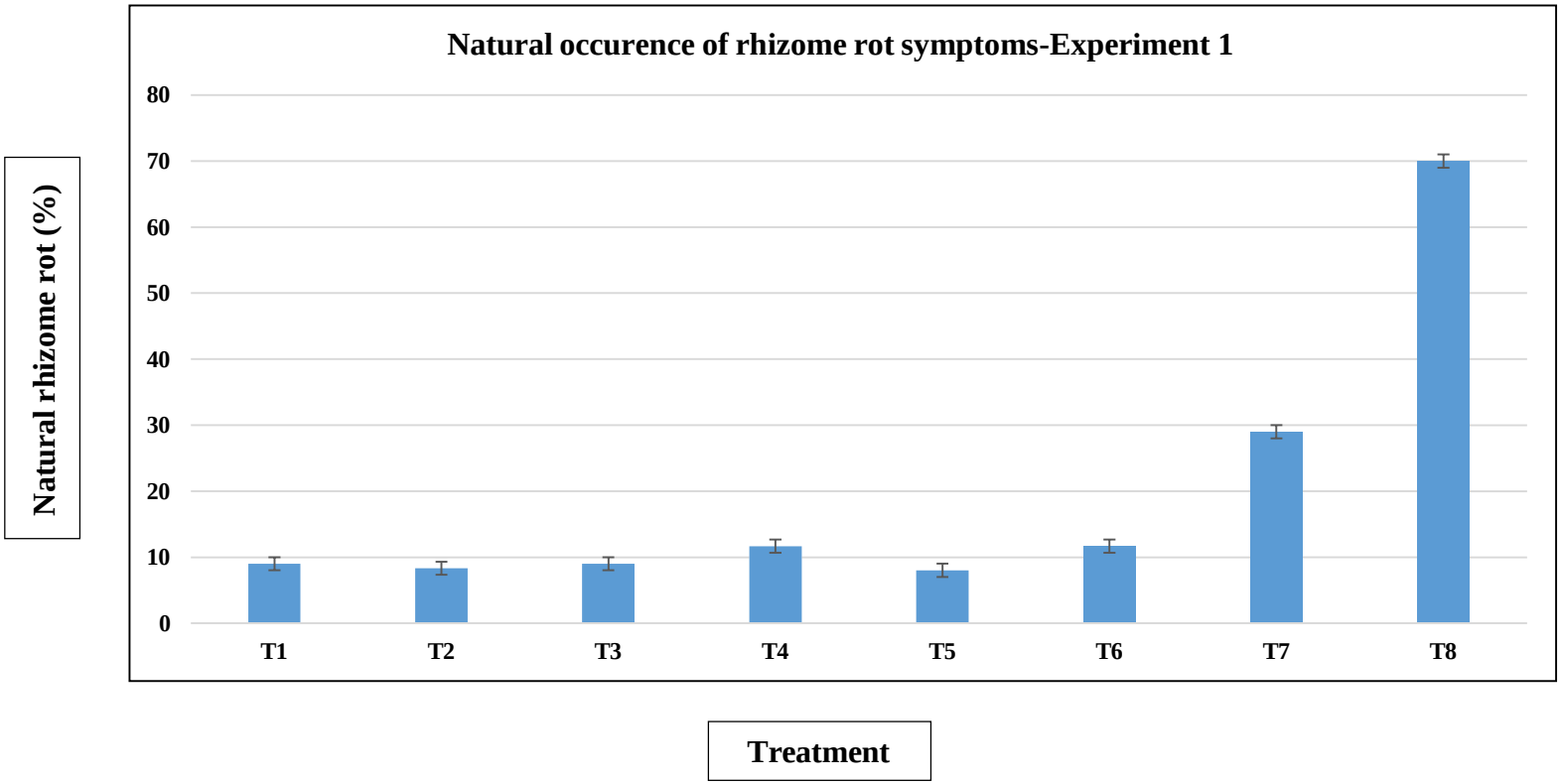


Fig 14: Natural occurrence of rhizome rot symptoms (%)

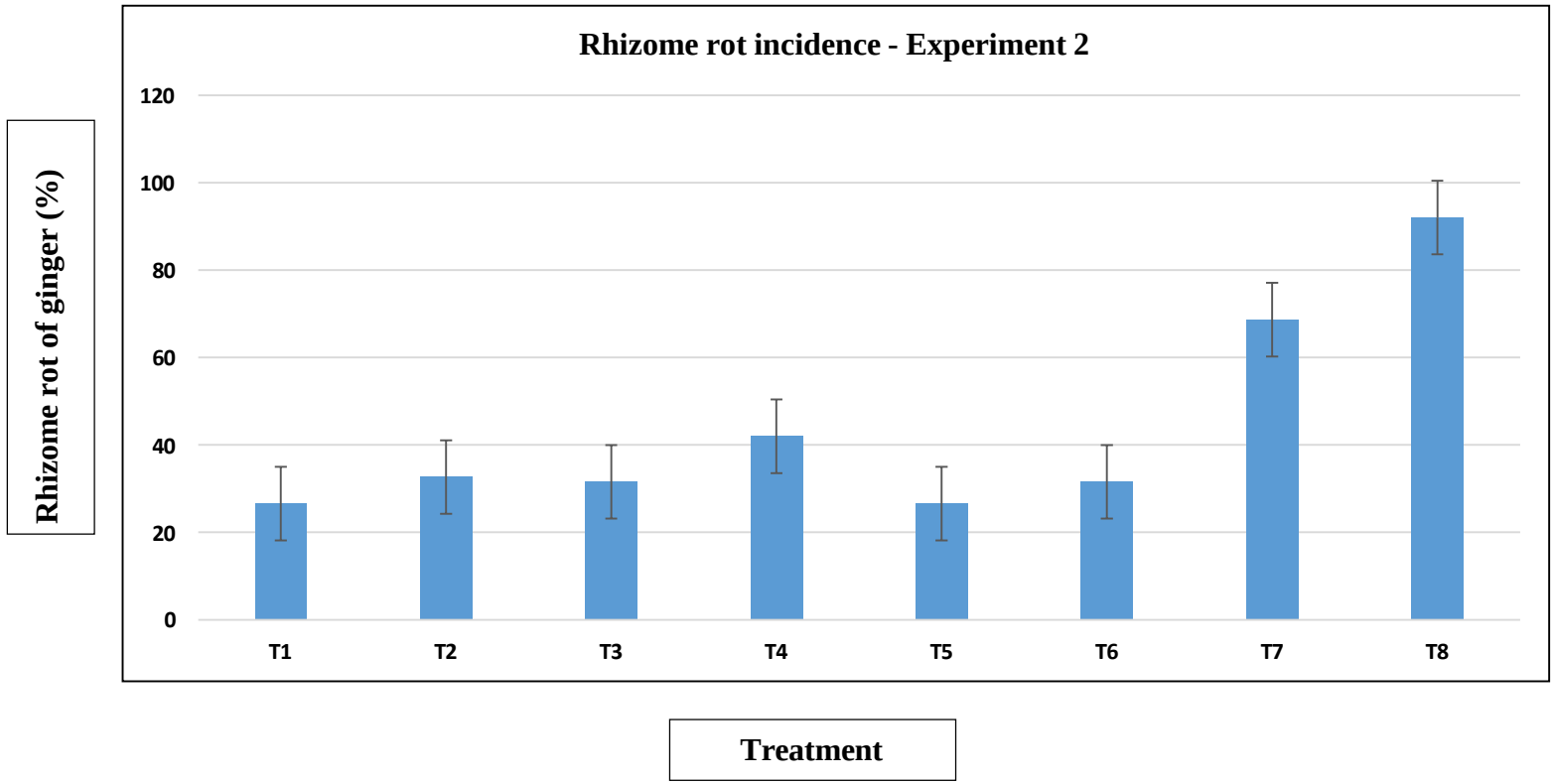


Fig 15: Effect of AMF on rhizome rot incidence (%)

T_4 (NEN AMF 2 - 70 %) and minimum was recorded in T_2 (EDA AMF 1 - 60 %). In experiment 2 increased root colonization was reported in T_1 (PAC AMF 2 - 80 %), followed by T_2 (EDA AMF 1 - 70 %) and T_3 (ANA AMF 5 - 70 %) and lowest was recorded in T_4 (NEN AMF 2 - 60 %) and T_5 (POO AMF 3 - 60 %). One common interpretation is that a higher concentration of AMF in plant roots benefited the host plants most. Treatment T_1 (PAC GRS-AMF 1) outperformed in all parameter than other treatment, this may be due to higher root colonization. As reported by Smith and Smith (2011) better performance of the AMF inoculated plants could be due to AMF hyphal network, which allows access to a large soil surface area in the mycorrhizal root, it was generally assumed that the mycorrhiza-induced stimulation of plant growth was the result of improved nutrient uptake, particularly phosphorus. Higher root colonized plants (T_1 - PAC AMF 2) performed better than the other treatments. As reported by Purin and Rillig (2008) some AMF are better at colonising plant hosts than others and the host may also influence root colonisation patterns.

The final nutrient status of the soil was analysed. The pH of the soil was acidic (6.59), EC value was 0.46 ds/m, organic carbon content was high (2.45 %), available nitrogen was medium (294.78 Kg/ha), phosphorous and potassium content was high with 82.59 Kg/ha and 316.85 Kg/ha respectively (Table 36). The organic carbon content of the soil had increased due to the activity of AMF. Phosphorous and potassium content was reduced as compared to initial nutrient status, because of the reduction of phosphorous fertilizer to one-fourth and potassium fertilizer to one-half. Felfoldi *et al.* (2022) reported that addition of arbuscular mycorrhiza, without fertilization, can have a beneficial effect on tomato crops. Fertilizers application decreased the interaction of tomato roots with beneficial fungi. The plants inoculated with AMF produced more vigorous plants with better developed root system, bigger fruits, and a higher level of production compared with non-inoculated plants.

In first experiment, T_5 (POO AMF 3) gave higher rhizome yield (T_5 on par with T_1) and in growth parameters like number of tillers and leaf area and T_1 (PAC AMF 2) performed better in growth parameters like plant height, number of leaves. In second experiment (Soft rot management), T_1 (PAC AMF 2) performed better in plant height, number of tillers, leaf area and significantly higher yield of ginger. T_1 also

recorded lesser disease incidence of rhizome rot. Final spore count and per cent root colonization was also recorded highest in the T_1. Though T_5 performed better in first experiment, it is on par with T_1 in parameter like rhizome yield in experiment 1. Treatment T_1 outperformed in all parameters in both the experiment. Finally, the treatment T_1 (PAC AMF 2) was identified as best performing treatment for improving the growth, yield and for managing the rhizome rot disease. The current study revealed that the native AM fungi, significantly increased the plant growth parameters, fresh and dry weight of rhizome, and disease resistance. Isolation, identification and development of such indigenous biofertilizers would benefit the farmers of Wayanad district to increase the rhizome yield in ginger without heavy dependence on expensive fertilizers and pesticides.

Future line of work,

- Identification of the isolated AMF through molecular techniques.
- Field evaluation of best performing AMF isolates under different agro-climatic zone and under different seasons.
- Development of effective AMF inoculum for supply among farmers.

6. SUMMARY

6. SUMMARY

The research work entitled “Evaluation of native arbuscular mycorrhizal fungi for the growth and management of rhizome rot of ginger (*Zingiber officinale*)” was carried out during the academic year 2020-2022. All the laboratory works were carried out at Department of Agricultural Microbiology, College of Agriculture, Vellanikkara and pot culture experiments were carried out at Regional Agricultural Research Station, Ambalavayal, Wayanad district. The objective of the study was to isolate the indigenous Arbuscular Mycorrhizal Fungi (AMF) from the rhizosphere soils of ginger in Wayanad district and to evaluate the selected AMF strain for the growth promotion characters and rhizome rot management in ginger. The important findings are summarized in this chapter.

1. The rhizospheric soil samples were collected from the ten different ginger growing areas of Wayanad district namely, Kalpetta (KAL), Pachilakkad (PAC), Edakkal (EDA), Varadoor (VAR), Anappara (ANA), Vaduvanchal (VAD), Ambalavayal (AMB), Malavayal (MAL), Nenmeni (NEN), and Poomala (POO).
2. The chemical properties of the soils like pH, EC, organic carbon, nitrogen, phosphorous and potassium contents were analysed. The pH of the soils were acidic ranged from 4.51 to 6.14. EC value of the samples ranged from 0.05 to 0.23 (non-saline). The organic carbon content and nitrogen content of all the samples were high except NEN, which had medium range.
3. Phosphorous content of all the samples were high ranged from 24.20 Kg/ha to 356.44 Kg/ha. The samples KAL, VAR, NEN had high potassium content while all the other samples were in medium range. The high phosphorous and nitrogen had affected the spore population in the soil.
4. From the ten samples, totally 74 AMF spores having similar morphology were identified and observed for morphological characters like size, shape, colour, nature of hyphae, bulbous suspensor, number of spore wall and surface ornamentation.

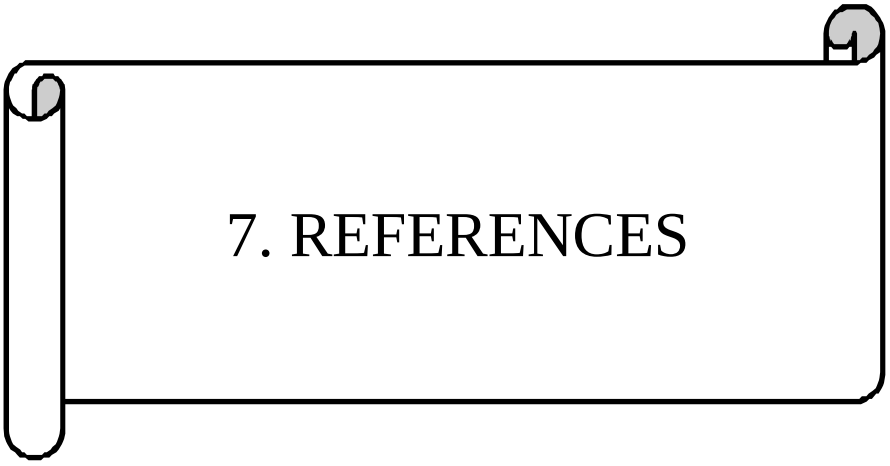
5. Using morphological data, AMF spores were identified at genus level. Among 74 isolates, 47 isolates shared similar characters with *Glomus*, 15 with *Scutellospora*, 9 with *Gigaspora* and 3 with *Acaulospora*.
6. Based on abundance, five predominant AMF isolates were selected for the mass multiplication and further study. They included brown spore from PAC AMF 2 sample (28 spores /100 g of soil), dark brown spores from EDA AMF 1 sample (30 spores /100 g of soil), black spore from ANA - AMF 5 sample (35 spores /100 g of soil), yellow spores from NEN AMF 2 sample (32 spores /100 g of soil), and brownish orange spores from POO AMF 3 sample (27 spores/100 g of soil).
7. Percent root colonization of the maize roots after mass multiplication ranged from 80 % to 100 %. Sample PAC AMF 2 and ANA AMF 5 showed higher root colonization of 100 %, EDA AMF 1 and NEN AMF 2 showed 90 % root colonization and POO AMF 3 showed lesser root colonization of 80 %.
8. The spore count of the mass multiplied inoculum ranged from 12 to 22 spores/10g of soil. ANA AMF 5 sample had higher spore count of 22 spore/10 g of soil, EDA AMF 1 had 18 spore/10 g of soil, PAC AMF 2 had 15 spore/10 g of soil, NEN AMF 2 had 12 spore/10 g of soil and POO AMF 3 had lower spore count of 10 spore/10 g of soil.
9. *Glomus* sp. (Black spore) selected from ANA AMF 5 sample showed highest root colonization and maximum number of spores in mass multiplied inoculum. The host plant maize and potting mixture used for multiplication supported the AMF root colonization.
10. In first experiment (growth and yield promotion), plant height was affected by different AMF treatment which ranged between 33.50 cm and 103.70 cm. T_1 (PAC AMF 2) had the maximum plant height with 103.70 cm and number of leaves at 180 DAP were higher in T_1 (22.60). Parameters like number of tillers and leaf area were higher in T_5 with 15.00 (followed by T1) and 39.01 (on par with T_1) respectively.
11. The fresh weight and dry weight of the ginger rhizome was recorded significantly greater in T_5 (POO AMF 3) with 160 g/plant and 65 g/plant

respectively, followed by T_1 (PAC AMF 2). Fresh and dry weight of plant shoot was observed maximum in T_5 (POO AMF 3) with 67.50 g and 16.50 g.

12. The natural disease incidence was observed in the first experiment. T_5 (POO AMF 3) showed lesser percent disease incidence of 8% which was on par with all the treatment except T_8 (Absolute control). The final spore count (64) and root colonization (90 %) of ginger were higher in T_1 (PAC AMF 2).
13. In second experiment (soft rot management), plant height ranged between 21 cm and 90 cm at 180 DAP. T_1 (PAC AMF 2) had the maximum plant height with 90 cm and number of leaves at 180 DAP were maximum in T_2 (EDA AMF 1 - 17). Parameters like number of tillers and leaf area were higher in T_1 (PAC AMF 2) with 8.4 and 37.02 respectively.
14. The fresh weight and dry weight of the ginger rhizome was recorded significantly greater in T_1 (PAC AMF 2) with 120 g/plant and 51 g/plant respectively. Fresh and dry weight of ginger plant shoot was observed maximum in T_1 (PAC AMF 2) with 58 g and 13 g respectively.
15. Development of above ground soft rot symptoms were lesser in T_1 (73.85 %) which is on par with T_5 (75.20%) whereas, POP recommendations of KAU (2016) showed 79.78 %, Organic POP of KAU (2015) showed 88 % and absolute control showed 100 % disease infectivity. Disease scoring revealed that, except T_1 (PAC AMF 2) and T_5 (POO AMF 3) all the other treated plants were highly susceptible to soft rot.
16. T_1 (PAC AMF 2) and T_5 (POO AMF 3) showed lesser soft rot of 26.57 % whereas POP recommendations of KAU (2016) showed 31.57 %, Organic POP of KAU (2015) showed 68.66 % and absolute control showed 92 % disease infectivity. Disease scoring revealed that, AMF treated plants and T_6 (POP recommendations of KAU, 2016) were tolerant to rhizome rot. T_7 (Organic POP of KAU, 2015) plants were susceptible and T_8 (absolute control) plants were highly susceptible to soft rot. The final spore count (55) and root colonization (80 %) of ginger were higher in T_1 (PAC AMF 2).

The present study indicated that AMF treated plants performed better than other treatments in all aspects. Finally, based on the growth parameters like plant

height, number of leaves, number of tillers, leaf area, yield parameters like fresh and dry weight of rhizome and disease incidence, treatment T_1 which was inoculated with PAC AMF 2 (*Glomus* sp.) was identified as the best treatment which performed better in both growth promotion and disease management. However, extensive field trial at different season and different agro ecological zone is necessary to confirm it. Molecular work is necessary to identify the AMF isolate at species level.



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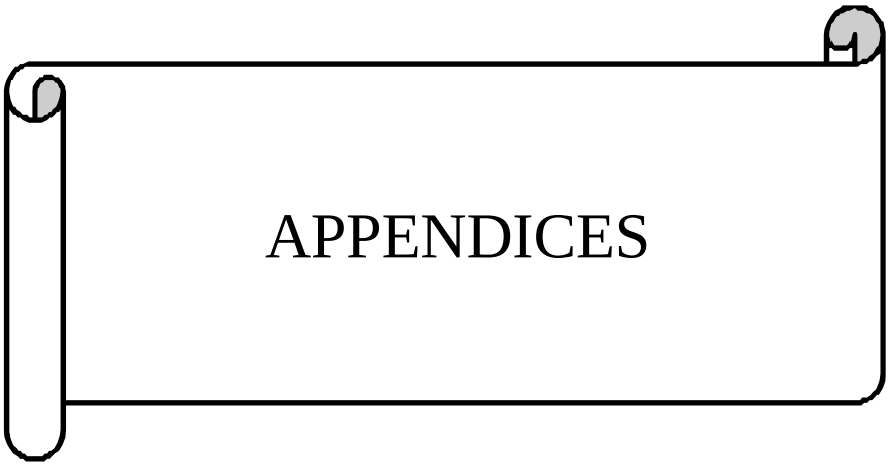

APPENDICES

APPENDIX I

Daily weather data during the experiment at Regional Agricultural Research Station, Ambalavayal, Wayanad

Date	Min Temp	Max Temp	RH 1	RH 2	Rain fall	Sunshine hours	Soil temperature		
31.05.22	20.2	27.3	93	67	0	6	22.4	23.6	25.2
01.06.22	20.3	27.3	93	86	5	2.6	21.2	22.9	24.1
02.06.22	19.2	26.9	95	77	0	4.8	21.3	22.9	24.2
03.06.22	19.6	27.2	96	73	0	6.3	21.9	22	24.3
04.06.22	19.3	27.3	91	76	0	8.2	22.2	23.2	24.2
05.06.22	20.2	28	95	73	0	8.8	23.4	24.8	25.4
06.06.22	18.9	27.1	89	72	6.8	5.3	22.8	23.9	24.8
07.06.22	20.1	27.3	96	80	30.4	5.2	23.2	24	26.1
08.06.22	19.4	25.8	91	76	1	1.3	22.8	26.8	24.1
09.06.22	18.9	26.9	80	77	0	7.7	22.9	23.9	24.2
10.06.22	19.2	27.7	96	77	0	6.8	22.8	23.7	24.9
11.06.22	19.8	26.9	93	79	0	6.0	22.5	23.8	24.1
12.06.22	19.8	25.2	96	81	0	4.3	21.3	23.8	24.2
13.06.22	19.2	25.3	95	63	11.2	3.7	21.3	23.9	24.4
14.06.22	19.9	28.3	88	78	0	7.3	22.8	23.9	24.3
15.06.22	20.6	28.2	96	70	1.8	7.3	23.2	24.2	25.6
16.06.22	19.9	27	95	63	0	7.0	22.6	23.8	24.8
17.06.22	19.7	28.2	96	81	2	5.9	22.7	23.9	24.9
18.06.22	19.2	28.7	93	74	12.6	6.0	23.1	23.9	25.1
19.06.22	19.1	27	96	76	3.2	3.5	22.8	23.6	24.9
20.06.22	19.1	26.2	91	79	4.9	2.9	22.9	23.9	28.0
21.06.22	19.9	25.8	95	75	5.2	3.8	22.9	23.9	25.1
22.06.22	19.6	25.2	96	88	12.9	0.4	22.9	23.0	24.9
23.06.22	19.1	25.2	95	95	11	0.9	22.9	23.9	25.1
24.06.22	20.2	24.8	96	92	7.6	3.2	21.8	22.9	23.9
25.06.22	19.8	24.9	98	96	26	0.5	22.9	23.6	24.2
26.06.22	20.1	22.1	98	77	7.5	0.0	21.2	22.4	22.9
27.06.22	19.1	25.9	88	69	8.3	6.5	21.2	22.9	23.9
28.06.22	19.1	25.3	98	86	14	2.4	21.2	22.8	23.6
29.06.22	19.6	25.2	96	91	17.4	1.5	20.9	21.9	22.8
30.06.22	19.6	25.2	98	96	15.9	0.6	20.2	21.2	22.8
01.07.22	19.1	22.6	98	82	39.9	0.0	20.3	21.9	22.9
02.07.22	20.1	23.9	98	68	14.2	0.0	21.2	22.9	23.6
03.07.22	19.1	25.1	98	91	52	1.3	21.2	22.8	23.5
04.07.22	18.1	23.1	98	96	18.4	0.0	21.2	22.4	23.6

05.07.22	18.9	21.2	98	95	49.4	0.0	21.1	22.8	23.2
06.07.22	19.4	21.2	93	94	48.4	0.0	21.1	22.8	23.1
07.07.22	19.4	21.3	98	94	29.1	0.0	21.2	22.8	23.6
08.07.22	18.4	21.1	89	89	33.1	0.0	21.1	22.3	22.9
09.07.22	18.3	22.8	98	96	35.4	0.0	21.2	22.3	22.9
10.07.22	19.4	21.3	98	96	70.4	0.0	21.2	22.1	22.8
11.07.22	19.2	21.3	96	93	50.5	0.0	20.1	20.6	21.9
12.07.22	19.2	21.3	98	98	31.1	0.0	20.3	21.1	22.5
13.07.22	19.2	21.6	98	95	53	0.0	20.2	20.9	21.9
14.07.22	19.2	22.6	96	93	53.9	0.0	19.8	20.1	21.2
15.07.22	19.1	22.7	96	96	24.2	0.0	19.7	20.1	20.9
16.07.22	19.1	22.8	98	96	52.1	0.0	19.3	20.1	21.4
17.07.22	19.8	22.2	96	96	40.1	0.0	19.2	20.2	21.3
18.07.22	19.2	23.2	96	95	29.4	0.0	19.1	20.3	21.2
19.07.22	19.4	22.4	98	80	18.4	0.0	19.8	20.1	21.5
20.07.22	19.9	25.9	93	83	0	3.2	21.2	22.2	23.2
21.07.22	20.1	26.7	93	75	1	1.8	21.9	22.6	23.5
22.07.22	19.9	27.4	93	95	0.8	6.5	21.3	22.5	23.6
23.07.22	20.1	26.8	93	86	1.5	2.2	21.2	22.8	23.6
24.07.22	19.9	24.8	96	85	6.9	1.1	21.1	22.7	23.3
25.07.22	18.1	25.1	88	73	0	2.2	21.2	22.5	22.6
26.07.22	19.8	26.8	93	77	0	6.5	21.9	22.9	23.6
27.07.22	19.1	25.6	94	76	3	1.4	22.1	22.9	23.1
28.07.22	19.1	26.8	95	73	1	3.3	21.3	22.9	23.7
29.07.22	20.2	27.3	93	71	21.8	5.7	22.2	23.1	24.2
30.07.22	20	28.2	96	67	7.2	6.5	22.2	23.2	24.2
31.08.22	19.9	28	93	80	24.6	5.8	21.2	22.8	23.9
01.08.22	20.1	26.9	96	83	8.2	5.3	23.2	24.2	24.9
02.08.22	19.9	26.4	96	98	147.2	2.2	22.2	22.4	23.6
03.08.22	20.1	26.3	96	87	4.3	2.4	22.2	22.9	23.6
04.08.22	20.1	25.2	98	96	3.8	0.0	22.2	23.1	23.9
05.08.22	19.3	25.2	98	93	60.4	0.0	20.2	30.9	23.1
06.08.22	18.9	20.9	98	96	25.8	0.0	20.1	20.9	22.3
07.08.22	19.6	21.3	98	91	31.7	0.0	20.1	21.9	22.1
08.08.22	19.9	21.6	91	95	42.9	0.0	20.1	21.1	22.2
09.08.22	19.6	22.6	98	96	31.7	0.0	20.6	22.3	22.9
10.08.22	19.3	25.5	98	91	10.7	0.4	20.0	22.2	22.9
11.08.22	19.6	23.6	95	93	9.2	4.5	20.1	21.6	22.9
12.08.22	19.2	22.8	98	75	26	0.6	21.0	21.6	22.1
13.08.22	18.9	25.2	81	84	1.2	6.6	21.1	21.9	22.9
14.08.22	17.4	25.2	96	84	0	6.2	21.2	22.2	23.7
15.08.22	19.1	25.6	86	70	0	0.5	21.3	22.4	23.9

16.08.22	18.4	25.8	88	76	0	7.2	21.3	21.9	23.8
17.08.22	19.3	28.2	89	61	0	10.0	22.2	23.1	23.9
18.08.22	19.2	29.4	83	63	0	8.5	22.9	23.9	24.7
19.08.22	20.2	28	96	76	0	7.3	21.6	22.9	23.9
20.08.22	18.8	26.8	93	73	0	3.2	22.2	23.7	24.2
21.08.22	19.8	26.9	90	77	0	4.7	22.4	23.9	24.6
22.08.22	20.2	26.6	96	91	1.2	3.9	21.2	22.9	24.3
23.08.22	19.8	22.2	98	77	36.3	0.0	20.1	21.9	22.9
24.08.22	19.6	25.2	98	81	8	0.8	20.8	21.2	22.8
25.08.22	18.3	25.4	98	86	33.6	0.4	20.3	21.4	22.9
26.08.22	19.1	25.4	95	72	27.7	4.9	20.9	21.7	23.2
27.08.22	19.2	25.4	98	72	13.8	4.0	20.9	21.7	23.2
28.08.22	18.9	26.1	91	68	9.5	2.6	20.8	21.3	20.0
29.08.22	19.4	26.9	96	72	36.2	4.7	20.4	21.3	22.7
30.08.22	19.8	25.7	96	74	4.4	0.4	20.0	21.2	22.4
31.08.22	19.2	28.2	98	67	20.3	3.7	22.2	23.3	24.1
01.09.22	19.1	26.3	98	98	24.2	0.1	21.2	22.8	23.9
02.09.22	19.5	25.5	98	77	7.7	1.2	22.2	23.4	24.1
03.09.22	19.6	25.3	93	77	0	6.7	22.6	23.7	24.6
04.09.22	20.2	26.4	93	75	0	8.2	22.4	23.9	24.5
05.09.22	19.1	26.9	96	72	15.8	5.0	21.2	22.9	23.9
06.09.22	19.4	28.2	95	96	3.2	5.7	21.4	22.8	23.8
07.09.22	19.4	25	98	93	28.4	1.0	21.6	23.4	23.1
08.09.22	18.4	23.4	96	86	14.2	0.0	20.9	21.3	22.9
09.09.22	18.3	23.8	98	86	7.9	0.0	20.8	21.3	22.4
10.09.22	19.6	23.8	95	87	13.3	0.0	20.7	21.2	22.5
11.09.22	19.4	23.4	91	87	11	0.0	20.4	21.1	22.6
12.09.22	19.7	24	95	93	1.3	2.8	20.5	21.2	22.9
13.09.22	18.2	23.8	91	76	1	2.5	20.9	21.4	22.9
14.09.22	18.9	24.9	96	91	3	4.6	21.1	22.2	23.1
15.09.22	17.6	25.6	84	85	0.3	1.9	21.3	28.4	23.9
16.09.22	17.1	25.8	81	70	0	5.9	21.2	22.8	23.9
17.09.22	17.4	27.2	84	74	0	6.1	21.4	22.6	23.4
18.09.22	18.2	26.4	85	64	0	6.2	21.3	22.9	23.9
19.09.22	18.1	27.2	98	74	0	7.1	21.0	22.9	23.9
20.09.22	17.2	25.9	86	61	0	3.7	21.3	22.9	24.1
21.09.22	17.3	26.2	82	56	0	4.5	21.4	22.6	27.9
22.09.22	17.2	28.7	79	53	0	8.6	20.4	22.9	23.9
23.09.22	17.4	27.7	82	71	0	8.7	22.4	23.9	24.5
24.09.22	18.3	26.3	89	84	0	8.7	21.9	22.6	28.8
25.09.22	17.3	25.8	81	60	0	3.6	21.9	22.6	23.9
26.09.22	17.3	28.2	89	61	1	6.6	22.2	23.4	24.1

27.09.22	18.6	28.9	95	50	0	8.3	21.3	22.9	24.8
28.09.22	18.7	28.4	96	64	5	8.3	21.8	22.9	24.1
29.09.22	16.2	28	68	55	0	6.8	21.0	22.9	24.3
30.09.22	16.3	27.4	89	86	0	5.7	22.1	23.1	24.6
01.10.22	16.4	27.4	96	63	0.3	1.7	21.3	22.9	23.9
02.10.22	16.7	25.8	86	79	0	0.6	21.8	22.9	23.7
03.10.22	18.3	28.2	98	70	28.4	5.4	21.6	22.5	23.4
04.10.22	18.2	27.2	96	70	0	2.1	21.6	22.8	23.8
05.10.22	18.6	27.3	95	58	0.3	2.9	21.1	22.8	23.1
06.10.22	19.5	28.2	96	65	6	4.5	21.2	22.4	23.6
07.10.22	15.4	24.5	87	67	1.9	0.0	21.0	22.1	23.1
08.10.22	15.7	24.6	98	70	11.2	5.4	21.2	22.5	23.1
09.10.22	17.3	26.2	98	52	0	6.7	21.3	22.9	23.9
10.10.22	19.4	27.6	98	61	48.8	1.3	21.2	21.9	22.9
11.10.22	19.4	25.8	96	69	0	0.1	21.0	21.8	22.7
12.10.22	18.7	26.4	96	64	0	1.8	21.2	22.5	22.7
13.10.22	17.3	27.5	80	67	0	4.1	21.4	22.7	23.6
14.10.22	17.3	27.4	98	70	19.8	4.7	21.0	21.4	22.9
15.10.22	17.2	26.2	96	62	13.7	2.3	21.0	22.1	22.9
16.10.22	17.8	28.2	98	74	53.2	0.0	21.0	22.2	23.2
17.10.22	19.7	26.4	98	75	4.3	0.0	21.9	22.4	22.1
18.10.22	19.4	26.4	96	78	6.3	0.0	22.2	23.6	24.1
19.10.22	18.4	25.8	98	76	2	0.0	22.4	23.7	24.3
20.10.22	18.4	25	98	75	1.2	0.0	20.9	21.3	22.9
21.10.22	18.2	25.8	98	83	2.2	0.0	21.9	22.9	24.1
22.10.22	18.1	26.3	98	67	1	0.0	21.8	22.9	23.9
23.10.22	19.1	26.3	98	62	0	0.0	22.2	23.9	24.9
24.10.22	19.1	28.1	96	66	0	9.2	22.7	23.9	24.9
25.10.22	19.4	28.3	96	65	0	5.7	23.1	24.5	25.1
26.10.22	16.4	28.3	87	64	0	8.9	23.4	24.6	25.3
27.10.22	16.9	28.6	86	76	0	8.4	21.2	22.4	24.9
28.10.22	16.5	27.5	81	83	0	8.1	22.0	22.9	24.8
29.10.22	17	26.3	89	75	0	7.8	22.9	23.8	24.8
30.10.22	16.8	25.4	94	74	0	5.3	22.1	22.9	23.9
31.10.22	19.3	26.2	93	84	0	3.2	22.2	23.2	24.6
01.11.22	16.9	26.3	93	74	0	6.9	22.4	23.9	24.6
02.11.22	17.4	26.9	84	77	0	2.5	21.0	22.7	24.2
03.11.22	17.3	26.3	98	77	26.8	4.5	21.8	22.6	24.1
04.11.22	19.1	26.2	77	73	3.6	4.4	22.2	23.4	24.1
05.11.22	18.6	26.8	83	77	0	6.5	21.8	22.8	23.9
06.11.22	18.7	26.2	93	74	0	0.0	21.9	22.9	23.9
07.11.22	17.8	26.9	98	80	0	8.4	21.9	23.1	24.7

08.11.22	16.1	26.6	96	79	0	7.1	23.2	22.7	24.2
09.11.22	17.3	26.7	98	72	0	6.5	22.2	23.1	24.4
10.11.22	17.1	27.3	98	72	0	8.8	22.8	23.4	24.8
11.11.22	18.2	27.3	84	73	0	8.6	22.4	23.2	24.1
12.11.22	17.1	21.9	96	93	18.2	8.7	19.8	20.8	21.9
13.11.22	17.1	20.9	96	77	4	8.7	19.7	20.9	21.9
14.11.22	18.6	26.2	91	80	0	3.6	21.6	20.9	24.1
15.11.22	19.1	27.3	96	84	8.4	6.6	19.8	21.2	22.7
16.11.22	19.1	26.8	96	78	0	8.3	20.8	21.4	23.7
17.11.22	18.1	27.2	93	57	0	8.3	20.2	22.2	23.8
18.11.22	15.2	26.8	84	60	0	6.8	21.2	22.8	23.5
19.11.22	13.8	25.8	74	75	0	5.7	21.3	22.9	23.9
20.11.22	13.9	25.8	83	78	0	1.7	21.4	22.9	23.7
21.11.22	15	26.2	98	74	0	3.2	21.9	22.9	24.4
22.11.22	14.9	26.2	91	81	0	1.8	22.9	23.7	24.6
23.11.22	15.9	25.9	96	69	0	6.5	22.9	23.9	24.8
24.11.22	17.2	24.8	98	78	0	2.2	21.8	22.8	23.4
25.11.22	17.4	25.6	98	60	0	1.1	21.0	22.9	28.7
26.11.22	16	21.7	96	78	0	2.2	21.8	22.9	23.9
27.11.22	17.2	25	95	69	0	6.5	21.7	22.6	23.4
28.11.22	16.9	25	98	72	0	2.2	22.4	23.7	24.3
29.11.22	17.4	25.2	98	69	0	1.1	20.9	21.3	22.9
30.11.22	17.2	25.4	96	80	0	2.7	21.9	22.9	24.1
01.12.22	14.4	26	96	68	0	6.7	21.8	22.9	23.9
02.12.22	15.7	28	94	76	0	8.1	22.2	23.9	24.9
03.12.22	17.3	26.2	96	62	0	3.7	22.7	23.9	24.9
04.12.22	17.1	28.2	96	63	0	7.5	23.1	24.5	25.1
05.12.22	17.4	26.7	80	62	0	6.0	23.4	24.6	25.3
06.12.22	15.1	25.2	94	63	0	3.9	21.2	22.4	24.9

APPENDIX II

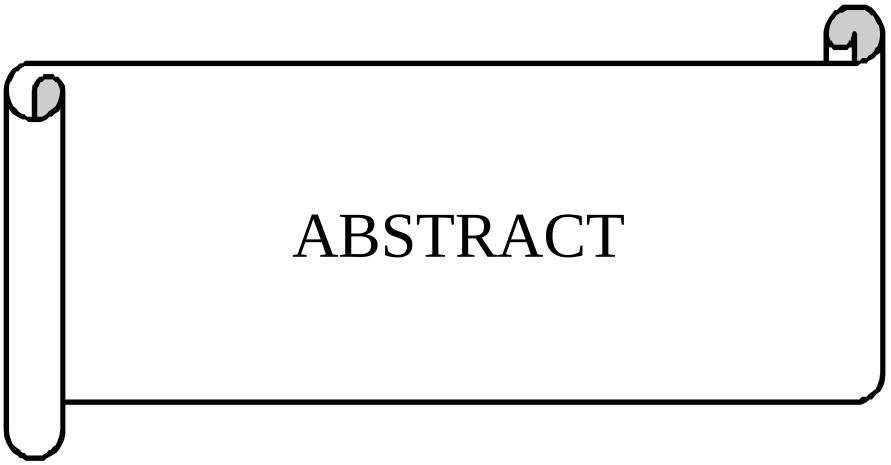
Package of Practices Recommendation of KAU (2016)

- Seed rhizome treatment: Mancozeb (0.3 %) and Quinalphos (0.075%) for 30 min.
- Apply 20 t FYM + 2 t Neem cake + 1 t Ash + 4 t vermicompost/ha.
- N: P_2O_5: K_2O (75:50:50 Kg/ha) full dose of P_2O_5 and 50 per cent of K2Oapplied as basal. Half the quantity of N applied at 60 DAP. The remaining quantity of N and K2O applied at 120 DAP.
- Based on soil testing, P_2O_5 was reduced to one fourth of the recommended dose.
- AMF was applied @ 15 g / plant at the time of planting

APPENDIX III

Organic Package (Ad hoc) of Practices Recommendation of KAU (2015)

- Seed rhizome treatment: *Pseudomonas* @ 20g/litre for 30 minutes.
- Compost @ 25 tonnes as basal and 3t/ha each at 60DAP and 120DAP.
- FYM, *Trichoderma*, neem cake mixture @ 100 g / planting pit at the time of planting.
- *Azospirillum* @ 2.5 kg /ha as basal and 120 DAP.


ABSTRACT

EVALUATION OF NATIVE ARBUSCULAR MYCORRHIZAL FUNGI FOR GROWTH AND MANAGEMENT OF RHIZOME ROT OF GINGER (*Zingiber officinale*)

By

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ABSTRACT OF THE THESIS

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Abstract

Mycorrhizae are mutualistic symbiotic associations based on bidirectional nutrient transfer between soil fungi and the roots of vascular plants. Over 80% of terrestrial plants can form symbiotic relationship with AMF in the natural environment. AMF being an obligate symbiont, it is completely dependent on the host plant for their life cycle and their relationship always been linked with improved plant performance. Colonisation of plant roots with mycorrhizal fungi greatly increases the root surface area which helps in water and nutrient uptake by plants. The mycorrhizal fungi not only provides the host plant with nutrients, such as phosphate and nitrogen, but also contributes to abiotic (drought, salinity, heavy metals) and biotic (root pathogens) stress tolerance of the host.

The present study was undertaken to assess the effect of native arbuscular mycorrhizal fungi for the growth promotion and management of rhizome rot in ginger (*Zingiber officinale*). The rhizospheric soil samples were collected from ten different ginger growing areas of Wayanad district namely Kalpetta (KAL), Pachilakkad (PAC), Edakkal (EDA), Varadoor (VAR), Anappara (ANA), Vaduvanchal (VAD), Ambalavayal (AMB), Malavayal (MAL), Nenmeni (NEN), and Poomala (POO).

Isolation of AMF spores, identification through morphological character and mass multiplication of selected spores were carried out at the Department of Agricultural Microbiology, College of Agriculture, Vellanikkara. Totally 74 types of AMF spores were isolated from the ten samples and tentatively identified at genus level based on morphological characters like colour, size, shape, nature of hyphae, bulbous suspensor, number of spore walls, surface ornamentation. Among 74 isolates, 47 isolates showed characteristics similar to *Glomus,* 15 to *Scutellospora,* 9 to *Gigaspora,* and 3 to *Acaulospora*.

From among these isolates, five most predominant AMF species were selected based on abundance. They included brown spore from PAC AMF 2 sample (28 spores /100 g of soil), dark brown spores from EDA AMF 1 sample (30 spores /100 g of soil), black spore from ANA AMF 5 sample (35 spores /100 g of soil), yellow spores from NEN AMF 2 sample (32 spores /100 g of soil), and brownish orange spores from POO AMF 3 sample (27 spores/100 g of soil).

Mass multiplication of selected spores were carried out with maize as host plant in vermiculite – perlite medium. Percent root colonization of maize roots ranged between 80 % to 100 % and spore count of the inoculum ranged from 10 spores / 10 g of inoculum to 22 spores / 10 g of inoculum. *Glomus* sp. (Black spore) selected from ANA AMF 5 sample showed highest root colonization and maximum number of spores in mass multiplied inoculum.

The pot culture experiment was conducted in a completely randomized block design (CRD) with two experiments with eight treatment and five replication at Regional Agricultural Research Station, Ambalavayal of Wayanad district. First experiment was conducted to assess the effect of AMF on the growth and yield promotion of ginger and the treatment consisted of T_1 (PAC AMF 2), T_2 (EDA AMF 1), T_3 (ANA AMF 5), T_4 (NEN AMF 2), T_5 (POO AMF 3), T_6 (POP of KAU, 2016), T_7 (Organic POP of KAU, 2015), T_8 (Absolute control). The second experiment was conducted to assess the efficacy of AMF in the management of soft rot of ginger. For this, the above treatments were repeated along with the challenge inoculation with *Pythium myriotylum*, the causal organism of rhizome rot.

In the experiment for growth and yield promotion of ginger, T_5 (POO AMF 3 - *Glomus* sp.) significantly higher in ginger yield (T_5 on par with T_1) and performed better in growth parameters like number of tillers and leaf area (T_5 followed by T_1) and T_1 performed better in growth parameters like plant height, number of leaves. In second experiment (rhizome rot management), T_1 performed better in plant height, number of tillers, leaf area and yield of ginger. T_1 also recorded lesser disease incidence of soft rot. Final spore count and per cent root colonization was also recorded highest in the T_1. Finally T_1 (*Glomus* sp. selected from PAC GRS - AMF 1) was identified as the best AMF isolate which performed better in growth promotion as well as in disease suppression. However, extensive field studies are needed under different season and agro ecological zone, in order to develop an efficient AMF isolate for ginger in high range zones of Wayanad district.

സംഗ്രഹം

മണ്ണിലെ കുമിളുകളും വാസ്കുലർ സസ്യങ്ങളുടെ വേരുകളും തമ്മിലുള്ള ദ്വിദിശ പോഷക കൈമാറ്റത്തെ അടിസ്ഥാനമാക്കിയുള്ള പരസ്പര സഹവർത്തിത്വ കൂട്ടുകെട്ടുകളാണ് മൈക്കോറൈസ. എൺപത് ശതമാനം ഭൗമ സസ്യങ്ങൾക്കും സ്വാഭാവിക പരിതസ്ഥിതിയിൽ മൈക്കോറൈസ കുമിളുകളുമായി സഹജീവി ബന്ധം സ്ഥാപിക്കുവാൻ കഴിയും. മൈക്കോറൈസ ഒരു നിർബന്ധിത സിംബയോൺട് ആയതിനാൽ, അവരുടെ ജീവിത ചക്രം പൂർണ്ണമായും ആതിഥേയ സസ്യങ്ങളെ ആശ്രയിച്ചിരിക്കുന്നു. മൈകോറൈസൽ കുമിളുകൾ ഉപയോഗിച്ചുള്ള വേരുകളുടെ സംക്രമണം, ജലവും പോഷകങ്ങളും ആഗിരണം ചെയ്യുവാനുള്ള സസ്യങ്ങളുടെ കഴിവിനെ മെച്ചപ്പെടുത്തുകയും, ഫോസ്ഫേറ്റ്, നൈടജൻ തുടങ്ങിയ പോഷകങ്ങൾ ആഗിരണം ചെയ്തു നൽകുകയും ചെയ്യുന്നു. ഇതിനു പുറമെ മൈക്കോറൈസ സസ്യങ്ങളുടെ അബയോട്ടിക് (വരൾച്ച, ലവണാംശം, ഹെവി ലോഹങ്ങൾ), ബയോട്ടിക് (രോഗകീട ബാധ) സമ്മർദങ്ങളെ അതിജീവിക്കുവാനുള്ള കഴിവിനെ പരിപോഷിപ്പിക്കുന്നു.

വയനാട് ജില്ലയിലെ കാർഷിക പരിസ്ഥിതി മേഖലയിൽ കൃഷി ചെയ്യുന്ന ഇഞ്ചിയിയുടെ വളർച്ച മെച്ചപ്പെടുത്തുന്നതിനും, ഇഞ്ചിയിൽ വ്യാപകമായി കണ്ടുവരുന്ന നാശകാരിയായ മൂടുചീയൽ രോഗത്തെ ഒരു പരിധി വരെ നിയന്ത്രിക്കുവാൻ കഴിവുള്ളതുമായ തദ്ദേശീയമായ മൈക്കോറൈസ കുമിളുകളെ കണ്ടെത്തുവാനും, അവയുടെ പ്രായോഗികത വിലയിരുത്തുവാനും ഉദ്ദേശിച്ചുകൊണ്ടാണ് ഈ ഗവേഷണപഠനം നടത്തിയത്.

ഇതിനായി വയനാട് ജില്ലയിൽ ഇഞ്ചി കൃഷി ചെയ്തുവരുന്ന പത്തു പ്രദേശങ്ങൾ; കൽപ്പറ്റ (കെഎഎൽ), പച്ചിലക്കാട് (പിഎസി), എടക്കൽ (ഇഡിഎ), വരദൂർ (വിഎആർ), ആനപ്പാറ (എഎൻഎ), വടുവൻചാൽ (വിഎഡി), അമ്പലവയൽ (എഎംബി) മലവയൽ (എംഎഎൽ), നെന്മേനി (എൻഇഎൻ), പൂമാല (പിഒഒ).ൽ നിന്നും റൈസോസ്ഫിയർ മണ്ണിന്റെ സാമ്പിളുകൾ ശേഖരിച്ചു. ഈ മണ്ണിൽ നിന്നും മൈക്കോറൈസ സ്പോറുകളെ വേർതിരിച്ചെടുത്ത്, അവയുടെ നിറം, വലിപ്പം, ആകൃതി, ഹൈഫയുടെ സ്വഭാവം, ബൾബസ് സസ്പെൻസർ, സ്പോർ ആവരണങ്ങളുടെ എണ്ണം, തുടങ്ങിയ രൂപശാസ്ത്രപരമായ പ്രതീകങ്ങളെ അടിസ്ഥാനമാക്കി ജനുസ് തലത്തിൽ പ്രാഥമികമായി തിരിച്ചറിഞ്ഞു. ആകെ വേർതിരിച്ച 74 ഐസൊലേറ്റുകളിൽ, 47 എണ്ണം ഗ്ലോമസ്, 15 എണ്ണം സ്കൂടെല്ലോസ്പോറ, 9 എണ്ണം ഗിഗാസ്പോറ, 3 എണ്ണം അകോളോസ്പോറ എന്നീ ജനുസ്സുകളുടെ സ്വഭാവസവിശേഷതകൾ കാണിച്ചു. ഇവയിൽ നിന്ന് സ്പോറുകളുടെ ആധിക്യത്തിന്റെ അടിസ്ഥാനത്തിൽ ഏറ്റവും കൂടുതലായി കാണപ്പെട്ട PAC AMF 2 (തവിട്ടു നിറം; 28 സ്പോറുകൾ /100 ഗ്രാം മണ്ണ്), EDA AMF 1 (കടുംതവിട്ടു നിറം; 30 സ്പോറുകൾ /100 ഗ്രാം മണ്ണ്), ANA AMF 5 (കറുത്ത നിറം; 35 സ്പോറുകൾ /100

ഗ്രാം), NEN AMF 2 (മഞ്ഞ നിറം ; 32 സ്പോറുകൾ /100 ഗ്രാം മണ്ണ്), POO AMF 3 (ഓറഞ്ചു നിറം; 27 സ്പോറുകൾ /100 ഗ്രാം മണ്ണ്), എന്നീ അഞ്ച് എഎംഎഫ് ഇനങ്ങളെ തിരഞ്ഞെടുത്തു. വെർമിക്കുലൈറ്റ് - പെർലൈറ്റ് മീഡിയത്തിൽ ആതിഥേയ സസ്യമായി ചോളം ഉപയോഗിച്ച് തിരഞ്ഞെടുത്ത സ്പോറുകളുടെ പ്രവർദ്ധനം നടത്തി. പ്രവർദ്ധനം നടത്തിയ മൈകോറൈസ സ്പോറുകൾക്ക് ഇഞ്ചിയുടെ വളർച്ചയിലും രോഗനിയന്ത്രണതിനുമുള്ള കഴിവ് വിലയിരുത്തുന്നതിനായി വയനാട് ജില്ലയിലെ അമ്പലവയൽ പ്രാദേശിക കാർഷിക ഗവേഷണ കേന്ദ്രത്തിൽ, എട്ട് പ്രയോഗരീതികളും അഞ്ച് ആവർത്തനങ്ങളുമുള്ള പൂർണ്ണമായും ക്രമരഹിതമായ ബ്ലോക്ക് ഡിസൈനിൽ (സിആർഡി), ചട്ടിയിൽ വളർത്തിയ ഇഞ്ചികളിൽ പരീക്ഷണം പരീക്ഷണങ്ങൾ നടത്തി.

ഇഞ്ചിയുടെ വളർച്ചയിലും വിളവ് മെച്ചപ്പെടുത്തുന്നതിലും, ഗ്ലോമസ് ജനസ്സിൽ പെട്ട T5 (POO AMF 3) മികച്ചതാണെന്നു കണ്ടെത്തി. മൂടുചീയൽ രോഗത്തെ നിയന്ത്രിക്കുവാനുള്ള മൈകോറൈസായുടെ ശേഷി നിർണ്ണയിക്കാനായുള്ള രണ്ടാമത്തെ പരീക്ഷണത്തിൽ, T1 (PAC GRS - AMF 1) മികച്ചതാണെന്നു കണ്ടെത്തി. രണ്ടു പരീക്ഷണങ്ങളുടെയും വിശദമായ അപഗ്രഥനത്തിൻ്റെ അടിസ്ഥാനത്തിൽ T1 (PAC GRS - AMF 1) നെ ഏറ്റവും മികച്ച AMF ഐസൊലേറ്റായി തിരിച്ചറിഞ്ഞു. വയനാട് ജില്ലയിലെ ഇഞ്ചി കൃഷിക്ക് അനുയോജ്യമായി കണ്ടെത്തിയ ഈ എഎംഎഫ് ഐസൊലേറ്റ് വികസിപ്പിക്കുന്നതിന് വിവിധ സീസണുകളിലും കാർഷിക പരിസ്ഥിതി മേഖലയിലും വിപുലമായ ഫീൽഡ് പഠനങ്ങൾ ആവശ്യമാണ്.