EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON GROWTH, WATER STRESS, TOLERANCE TO PHYTOPHTHORA BLIGHT AND NUTRITIONAL QUALITY OF SELECTED PAPAYA HYBRIDS IN KENYA

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Effect of Arbuscular Mycorrhizal Fungi on Growth, Water Stress, Tolerance to Phytophthora Blight and Nutritional Quality of Selected Papaya Hybrids in Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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ACRONYMS AND ABBREVIATIONS

ABA	Abscisic Acid
AMF	Arbuscular Mycorrhizal Fungi
ANOVA	Analysis of Variance
C	Compost Manure
CHO	Carbohydrates
DNA	Deoxyribonucleic Acid
GA	Gibberellic Acid
GAE	Gallic Acid Equivalent
I	Inoculum Treatment
IAA	Indole Acetic Acid
IC	Inoculum and compost manure treatment
INVAM	International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi
JK	JKUAT Hybrid
JKUAT	Jomo Kenyatta University of Agriculture and Technology
Kshs	Kenya Shillings
M hybrid	Malkia F1 Hybrid

M	Molarity
PAL	Phenylalanine Ammonia-Lyase
PBi	Phytophthora Blight Infection
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
POD	Peroxidase
PPO	Polyphenol Oxidase
TCA	Trichloroacetic Acid

ABSTRACT

Papayas are major tropical fruits grown globally and locally due to their nutritional benefit and as a source of income from local and export markets. However, increased pest and disease pressure, low uptake of new technologies to improve growth and maturation has led to low production and poor quality of papaya fruits. Mycorrhizal fungi has potential application in plant water and nutrient uptake, growth and development and consequently enhancing plant's resilience to biotic and abiotic stress. This could be exploited for enhancing plant adaptation to stress and for improving overall crop performance. The aim of this study was to evaluate the effectiveness of indigenous arbuscular mycorrhizal fungi (AMF) on growth, water stress, tolerance to phytophthora blight and nutritional quality of JKUAT and Malkia F1 papaya hybrids. Soil samples were obtained from Mwea, Mitunguu and Juja areas from grass, banana and papaya plants' rhizosphere at a depth of 0-20 cm. AMF spores were isolated from the collected soil samples using sucrose method. Papaya seeds were sown on sterile coarse sand and transplanted to sterile soil media at 2 and 3 leaves seedling stage. The treatments (AMF inoculum, AMF inoculum and composted manure, composted manure and control) were introduced to the soil media at 4 weeks after transplanting at a ratio of 1:9 (treatments: soil media). Plant growth parameters were assessed every 4 weeks for a period of 20 weeks. Nutritional analysis of the fruits obtained included selected minerals, proximates, ascorbic acid, total polyphenols and total carotenoids. The collected data was subjected to analysis of variance (ANOVA) at $p \leq 0.05$ level of significant and significant means separated using Tukey's HSD test in Genstat statistical package 15th edition. The highest number of spores were isolated from the grass plants' rhizosphere. The results showed that combining manure and inoculum had highly significant effect ($p \leq 0.05$) on the performance of papaya plants compared to other treatments. Plant growth significantly differed ($p \leq 0.05$) between treatments with the highest root mass recorded in plants treated with a combination of AMF and manure (49.00g and 58.45g for JKUAT and Malkia F1 hybrids, respectively) and the least (16.11g and 18.24g, for JKUAT and Malkia F1 hybrids, respectively) recorded in sole compost manure treated plants at 20 weeks after transplanting. Root colonization differed significantly ($p \leq 0.05$) among the treatments and the hybrids at 20 weeks. The JKUAT hybrid treated with both AMF and manure had the highest root colonization of 78% while manure only treated plants had the least % colonization (41%). The stem girth of JKUAT hybrid with AMF and manure treatment and with pathogen infection increased from 0.8 cm to 3.9 cm while pathogen infected JKUAT hybrid with manure treatment only increased from 0.5 cm to 2.9 cm from 4 weeks to 20 weeks. The carotenoids contents of pathogen infected Malkia F1 hybrid with manure treatments only and a combination of AMF and manure treatments at 2 weeks after infection was 0.6mg/100g and 0.8mg/100g respectively while the non infected ones had 1.8 mg/100g and 2.4mg/100g respectively. At 20 weeks, Malkia F1 hybrid subjected to water stress and with inoculum treatment only had a stem girth of 3 cm while the control treatment had 0.32cm. Crude fibre content for Malkia F1 hybrid with both

AMF and manure treatments was 5.7% while manure treatment only had 2.0% and 1.41% for the controls. Increased root biomass and high root colonization in AMF treated plants led to vigorous growth in papaya plants and accumulation of more nutrients in the fruits.The grass rhizospheric soils had the most abundant spores and could be isolated and bulked to boost on soil media for papaya establishment. Treating papaya plants with AMF combined with manure from seedling stage is recommended for plants' tolerance to phytophthora blight disease, vigorous growth of papaya plants and accumulation of nutrients such as ascorbic acid in the fruits.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

The agriculture sector is the pillar of Kenya's economy and it contributes 29.3% to the Gross Domestic Product. The sector also reports 80 % of national employment (Horticultural Crops Directorate, 2020). In the year 2020, value of horticultural production in Kenya amounted to Kshs 285.4 billion compared to Kshs 269.68 billion in the year 2019 and this was 5.8 % increase. However the area under cultivation decreased from 483,094 ha to 453,636 ha (6.7 %) (Horticultural Crops Directorate, 2020). The area under fruit increased by 6 % while value and production had increased by 9 and 14% respectively. This was ascribed to cultivation on new and young orchards as well as rains that were experienced in most parts of the country. In the year 2020, the main fruits grown in order of economic importance were; banana 32.73%, mango 17.34%, pineapple 13.56%, avocado 10.64 %, water melon 9.08 % and papaya 4.66 % while in the year 2019, banana 31.05 %, mango 19.24%, pineapple 13.85%, avocado 11.85%, water melon 9.18% and papaya 5.20% (Horticultural Crops Directorate, 2020). In the year 2020, *Carica papaya* contributed Ksh. 4.13 billion up from Ksh4.12 billion in the year 2019 (0.2 % increase). The production area increased by 954 Ha while papaya production increased by 1,025 tonnes in the year 2019 and this was due to increased production area. However the productivity dropped during the year 2020 due to mealy bugs and the papaya ring spot virus incidences mainly in Upper Eastern of Kenya (Horticultural Crops Directorate, 2020).

Papaya is a semi-woody fast-growing tropical herb and has a single, straight and hollow stem whose color ranges from light green to tan brown with noticeable leaf scars (Arvind *et al*., 2013). It exhibits an apical dominance which rarely branch lest the apical meristem is either injured or removed. Papaya cultivars are distinguished by leaf shape, type of stomata, wax structures on the leaf surface, color of the leaf petiole, the number of leaf

main veins and lobes at the leaf margins (Silva *et al*., 2007). Papaya fruit is rich in nutrients including carotenoids, vitamins A, B and C, dietary minerals, lycopene, different types of enzymes and dietary fibre. Papain is present in large amounts in unripe fruit and aids in digestion (*Yogiraj et al*., 2014).

Papaya is subtle to the growing environment and the changes affect the productivity as well as the quality of fruits. Extended moisture stress slows down the growth and more male or sterile flowers are produced, leading to poor fruit set (Jeyakumar *et al*., 2005). Fungi, viruses, nematodes and bacteria incur papaya production losses due to the various infections (Srivastava and Singh, 2022). Mycorrhiza is the mutualistic symbiotic association between soil-borne fungi and plants and are the most widespread among natural plant communities (Brundrett, 2002). The host plant receives soil water and nutrients from mycorrhizal fungus while the biotrophic fungus obtains photosynthates such as carbon compounds from the host (Augé 2001). Read (2002) categorized mycorrhiza in six major types; monotropoid, arbutoid, ecto, orchid, ericoid and arbuscular types in accordance to their morphology, structural and functional features, and plant taxonomic category forming the association.

The occurrence of mycorrhizal fungi enhances an increased volume of soils between fifteen centimeters and two hundred centimeters (15cm -200cm) for every centimeter of the colonized root and this depends on the predominant environmental conditions (Wang, 2017). The enlargement of the fungal hyphae improves the plant roots' capacity to not only absorb nutrients but also enhancing nutrient mobilization (Zhang *et al*., 2010). Mycorrhizae have been shown to be important in the mobilization of low mobile ions like phosphates, orthophosphate, zinc, copper, iron, manganese, organic nutrients and soluble inorganic nitrogen which include nitrate and ammonium (Smith *et al.*, 2003, Cruz *et al.*, 2004). This maximizes the primary production of the plants.

According to Tahat *et al*., (2010), an abundance of lateral root tips and developing meristems make highly branched root systems more susceptible for pathogen attack, resulting in an increasing demand for AMF to protect them. Fritz *et al*., (2006) compared

plants with inherently highly branched root systems and found that mycorrhizal plants had less necroses compared to non-mycorrhizal ones. Factors such as different root size and architecture, altered physiology and exudation patterns may contribute to microbial communal variations in the mycorrhizosphere, both quantitatively and qualitatively (Toljander *et al*., 2007; Finlay 2008). Pozo *et al*., (2009), also suggested that the processes of AM fungal establishment rather than resulting in the constitutive expression of defense, enhanced the plant's capability to stimulate defense mechanisms more effectively when attacked. Induced pathogen protection through this mechanism may either be through root exudation (Lioussanne *et al.*, 2008) or systemic within the plant (Guillon *et al*., 2002).

Arbuscular mycorrhizae are the commonest and extensively occurring of all the mycorrhizal relations. They have unlimited economic significance and they cannot be cultured on any laboratory media. Therefore, AMF subject is currently enticing great attention in forestry, horticultural and agricultural research (Bagyaraj 2014). In this study, isolated indigenous mycorrhizae fungi (IMF) spores were bulked in sorghum plants to obtain AMF inoculum which was inoculated on the soil media to determine its efficacy on the performance of the papaya plants, tolerance to phytophthora blight, water stress as well as nutritive value on the fruits.

1.2 Problem Statement

Soil borne diseases, physiological disorders, environmental stress, mechanical damage, or a combination of these factors are the leading causes of low harvest and post-harvest losses in horticultural production. In addition, soil erosion decreases the agricultural value of land through nutrient loss via runoff and sediment and this reduces soil fertility (Kurothe *et al*., 2014, Sahoo *et al*., 2015). Potassium, phosphorous and nitrogen are highly absorbed in papaya plants during its peak production whereas phosphorous nutrient is accumulated in the papaya fruits in greater amounts compared to other nutrients (Chandra 2014, Anjos *et al*., 2015). However, the growth of papaya plants is affected in areas with minerals deficiency such as phosphorous and zinc (Fallas-Corrales and van der Zee, 2020). The demand for phosphate fertilizers is very high and its supply is rapidly declining. The

global population is equally increasing and this is posing a threat to global food security (Igiehon and Babalola, 2017). On the other hand, over application of chemical fertilizers supersede AMF's capability to improve crop productivity, thus reducing the mycorrhizal activity and mask their effects on plants (Cruz *et al*., 2017). Moreover, there is an increased concern on the negative impacts of improper usage of these fertilizers such as environmental effects and health hazard (Alori *et al*., 2017).

Large scale production of AMF has been affected due to contamination of AMF communities by other microorganisms (Igiehon and Babalola, 2017). *Carica papaya* requires adequate moisture to avoid shedding of leaves, flowers and young fruits. However in very harsh environment, water becomes scarce and these symptoms are inevitable.

Diseases attack the plants at different stages of growth and most plants do not reach maturity and if the fruits are formed, they are shriveled and malformed and the plant ultimately dies. Plantlets ready for transplantation do not possess adequate resistance against soil microflora and the sudden exposure to microbial communities increases their mortality (Hao, *et al*., 2010). The quality of fruits is associated with the bio chemical, physical and nutritional attributes and ultimately, the consumer taste and preferences (Tarantino *et al*., 2018). However, this perceptive is highly changing due to the rising concern of consumers regarding the fruits' nutritional benefits (Di Vittori *et al*., 2018). Moreover, farmers search for ways to intensify fruit production in a sustainable way to guarantee fruit security and quality for the consumers. Plant bio stimulants, which generally includes microbial inoculants such as AMF can improve size, shape, appearance and sensory traits of the fruits. However, little has been achieved regarding enhancement of fruits' nutritional qualities using microbial inoculants (Calvo and Kloepper, 2014). The nutritional contents of fruits such as tomato have been increased where zinc, phosphorous and lycopene contents were enhanced through mycorrhizal inoculation (Giovannetti *et al*., 2012) and also mineral nutrients and fruit sugars were improved in citrus fruits through mycorrhizal inoculation (Zou *et al*., 2021).

Papaya is one of the fruit trees with mycorrhizal dependency status (Trindade *et al*., 2006) and inoculation of the soil with AMF can significantly affect the overall production and yield of the plant for both far and near markets.

1.3 Justification

Cultivation of fruit trees offers great potential for wealth generation if farmers are connected to markets such that input costs are reduced to favor the prices of their produce, as well as trained in farm management of existing fruit trees, cultivation of the improved, high value varieties and species, which are on demand on the present and future markets. Phosphorus element is an important mineral needed by plants and the growth and development of plants are diminished in its absence (Igiehon and Babalola, 2017). Arbuscular mycorrhizal fungi (AMF) are applied as biofertilizers and plants are able to effectively utilize mineral elements such as phosphorus, potassium and nitrogen from rhizospheric soils. Micro nutrients such as zinc and iron levels are enhanced in agricultural crops and biofortification through proper utilization of AMF. Boosting soil with microbial inoculants such as AMF also enhances phytoremediation, controls soil erosion as well as eliminating harmful organisms through the hyphae mycelia network (Igiehon and Babalola, 2017).

Plant roots can naturally establish associations with pathogenic, beneficial and neutral groups of soil organisms. Beneficial organisms like arbuscular mycorrhizal fungi may influence the structure, diversity and productivity of papaya. Mycorrhizal association helps plants to survive well under stressed environments such as high temperature, water stress, high salt levels and some mineral deficiency (Kapoor *et al*., 2013). Arbuscular mycorrhizal fungi has filaments which endorse drought resistance in the host plant by improving water holding capacity of the soil (Christopher, 2015).

Mycorrhizal colonization plays a role in alleviating the transplanting shock brought about by unfavorable environmental and nutritional conditions (Sharmila *et al*., 2000). At any given time, the overall performance of plants with mycorrhizae will be superior to that of

non- mycorrhizae plants (Sharmila *et al*., 2000). The yield of the papaya fruits could be enhanced by lesser invasion of soil borne pathogens and ability to withstand environmental stress. Root pathogens and AMF interactions decreases disease severity and reduces the symptoms for fungal pathogens such as *Phytophthora cactorum*, *Phytophthora vignae* and *Phytophthora parasitica* (Lioussanne *et al*., 2009). Combining these AMF potentials under agricultural situation will help to boost food security and sustain agriculture globally (Igiehon and Babalola, 2017). There is an increased awareness about the microbial association between the host plants and rhizospheric microbes (Hou and Oluranti 2013).

The final quality of the fruit depends on environmental conditions, agronomic practices and post-harvest intervention. Majority of the plant bio stimulants increase seed germination, enhances the growth and yield of plants as well as improves plants' tolerance against biotic and abiotic stresses (Ruiz and Egea, 2008). Microbial inoculants such as AMF has not been investigated on JKUAT and Malkia F1 papaya hybrids nutritional quality and hence has not yet been considered as plant biostimulants (Soppelsa *et al*., 2018). Adoption of new agricultural inputs and techniques is critical to meet the high consumer demand for improved fruits' nutritional quality (Du Jardin, 2015). Maximum benefit can only be obtained from inoculation with arbuscular mycorrhizal fungi isolates, in the greenhouse or field, indigenous or commercially obtained and with the selection of compatible host-fungus-substrate combinations (Carpio, 2002). Such studies have not been conducted on papaya production thus prompting this research work.

1.4 Objectives

1.4.1 Overall Objective

To determine the effects of amending soil with arbuscular mycorrhizal fungi (AMF) on growth, biotic and abiotic stresses tolerance and nutritional quality of selected papaya hybrids for improved yield and fruit quality

1.4.2 Specific Objectives

1. To isolate and characterize rhizospheric indigenous arbuscular mycorrhizal fungal (AMF) species from Mwea, Mitunguu and Juja sub-counties
2. To determine the efficacy of amending soil with arbuscular mycorrhizal fungi (AMF) on the management of phytophthora blight and water stress on selected papaya hybrids.
3. To determine the effects of amending soil with arbuscular mycorrhizal fungi (AMF) on growth and development of selected papaya hybrids
4. To determine the effect of amending soil with arbuscular mycorrhizal fungi (AMF)on the fruit nutrient content of selected papaya hybrids

1.5 Hypotheses

1. The isolated rhizospheric indigenous arbuscular mycorrhizal fungal (AMF) species from Mwea, Mitunguu and Juja sub-counties have similar characteristics
2. Soil amendment with arbuscular mycorrhizal fungal (AMF) has no effect on the management of phytophthora blight and water stress on selected papaya hybrids.
3. Soil amendments with arbuscular mycorrhizal fungal (AMF) has no effect on growth and development of selected papaya hybrids.
4. Soil amendments with arbuscular mycorrhizal fungal (AMF) has no effect on the fruit nutrient contents of selected papaya hybrids.

CHAPTER TWO

LITERATURE REVIEW

2.1 Importance of Papaya Fruit

Papaya is a fruit known for its exceptional nutritional value, as phytonutrients exist in both ripe and unripe fruits (Karunamoorthi *et al*., 2014). The papaya fruit contains nutrients including riboflavin, folate, thiamine, niacin, fiber content, vitamins A, B and C (Alara *et al*., 2020). The fruit contains sugars; sucrose, glucose and fructose at 48.3g/10g, 29.8 g/100 g and 21.9 g/100 g respectively and essential mineral elements such as phosphorous, iron and potassium in the ripened fruit It also has carotenoids contents, such as β-cryptoxanthin and β-carotene as well as major pigments such as lycopene (Koul *et al*., 2022). It is low in calories since it does not contain protein and fat contents (Koul *et al*., 2022).

The papaya fruit can be utilized to generate value-added products such as candy, pickles and papaya-mix juices (Samreen, et al., 2015). Inside the fruit is filled with multiple small, black seeds. These seeds are sometimes used as a substitute for black pepper due to their spicy and savory flavor. Both green papaya fruit and the latex from the tree are rich in papain, which aids digestion and are also used for tenderizing meat and other proteins. Papain has been used in medicine to treat ulcers and reduce skin adhesions following surgery, and studies have shown that it has antimicrobial properties. Papain is also used to clarify beer, to give shrink resistance to wool, manufacture of chewing gum, cosmetics, for degumming natural silk and remove hair from hides before tanning (Ward, 2011).

2.1.1 Factors Affecting Nutritional Composition of Papaya Fruit

Varying environmental conditions has great effect on papaya production. This includes temperature, rainfall, wind, light intensity, daylength and relative humidity. Soil fertility, soil pH, soil moisture content, disease and pest pressure are other factors that affect growth and development of papaya plant (Raymond, 2014). These factors also ultimately affect

papaya nutritional composition. Concentrations of sugars and vitamins in mature fruit are modulated by climate while mineral contents are closely related to soil fertility (Wall and Tripathi, 2013).

The availability and transport of carbohydrates from leaves to fruit are great determinants of papaya sugar content (Zhou *et al*., 2000). Papayas can sustain high carbon assimilation rates under well-watered, high light intensity conditions (Campostrini *et al*., 2007). As air temperature rises from 30^{o}C to 40^{o}C, leaf to air vapor pressure deficit rises, causing the stomata to close and increase of net assimilates. Plants with lower fruit load can endorse sugar concentration on the fruits (Zhou *et al*., 2000).

Light intensity and temperature determines the final content of Vitamin C (Kader, 2000). Lee and Kader (2000) observed that fruits exposed to sunlight have higher vitamin C than shaded fruits on the same plant. They also observed that soil moisture deficits, too much nitrogen levels and raised temperature resulted to lower ascorbic acid contents. Carotenoid synthesis tends to increase with nitrogen fertility (Reif *et al*., 2012). Chilling temperatures disrupts key enzymes in the carotenoid pathway hence reducing carotenoid levels in papaya fruit (Rivera- Pastrana *et al*., 2010).

2.1.2 Papaya Propagation

2.1.2.1 Sexual Propagation

Papaya is almost entirely propagated from seed in commercial cultivation (Han-xin *et al*., 2009). Seed can be collected from fruit when the flesh is soft or over-ripe. The fleshy outer layer of the seed coat, sarcotesta, which encloses the seed is removed since it hinders germination. This is done by rubbing the seed together alongside a fine-meshed screen and this is carried out under running water. Papaya has moderately recalcitrant seed that does not tolerate desiccation; the moisture content of papaya seeds can reduce to 5% after five days of open air conditions and result in total loss of viability (Janick *et al*., 2006). Papaya has a coarse fibrous root system that is quite fragile, hence containers are more

preferred for propagation rather than in a nursery bed (Janick *et al*., 2006). Germination is usually after 2 weeks and ready for transplanting at 8 to 12 leaf stage or about 6 weeks (Han-xin *et al*., 2009).

2.1.2.2 Vegetative Propagation

Asexual propagation of different papaya varieties has often been used to attain clonal plants, with traits identical to the mother plant .Vegetative propagation by means of grafting or cutting is an important practice which is widely used in majority of the fruit trees. The main aim of this technology is to retain the desirable features of the mother plant and production precocity (Costa *et al*., 2019). Schmildt *et al*., (2016) stated that use of vegetative propagation establishes 100% hermaphrodite plants for cultivars of ginoic-andromonoic populations. Female plants are retained by vegetative propagation together with male plants to ensure pollination takes place in the dioecious cultivars (Senthilkumar *et al*., 2014). Although the production cost for cutting seedlings is higher compared to seed propagated plants, effective pruning practices extends the papaya production period (Allan, 2007).

Chip-budding and whip-and-tongue grafting are the two most reliable means to clonally propagate papaya. The methods are most successful when the seedling rootstock is at least 0.5 cm diameter and actively growing (Janick, 2006). Chong *et al*., (2005) successfully conducted cleft grafting. The female trees were side cleft grafted with scion shoots harvested from hermaphrodite plant. The union was established in 2 to 3 weeks, and the female tree cut back to about 60 cm from the ground to allow the scion to develop (Ying, 2009). In order for grafting to be successful, induction chemicals for lateral shoots and the seedlings age should be standardized. Papaya mother plants that were 5 to 6 months old were sprayed with a combination of benzyl adenine (BA) at 100 ppm and giberrelic acid (GA3) at 250 ppm (Satisha and Vincent, 2023). Moreover, wedge grafting on softwood was more successful in comparison with side and cleft grafting. Plants that were grafted produced flowers and fruits at a lesser height compared to seedling plants (Satisha and Vincent, 2023).

2.1.2.3 Invitro Propagation

It has been achieved by culturing apices of mature, field grown papaya plants in Murashige and Skoog (MS) media. Chan and Teo (2000), indicated that they propagated true to sex without somaclonal reversion, and the problems of sex segregation and variation of fruit shape did not arise. Apart from greater uniformity, micro propagated plants bear fruits earlier, at lower height as well as improved yield (Chan and Teo, 2002). Debnath and Teixeira da Silva, (2007) indicated that the most reliable method of *in vitro* propagation of papaya is by shoot tip explants or axillary buds.

The modified tissue culture procedure of papaya showed that lateral and apical buds from mature mother plants and seedlings may be used as explants for *in vitro* propagation. Apical bud to be used as the explant for papaya should be treated in 0.08% mercuric for ten minutes while lateral bud 12 minutes for their surface sterilization (Podikunju, 2017). The multiplication of shoot was ideal in MS media boosted with 0.1 mg/l of 1-Naphthaleneacetic acid (NAA) and 0.5 mg/l of 6-Benzylaminopurine (BAP), rooting in 2.5 mg/l of Indole-3-butyric acid (IBA) and shoot elongation in 0.05 mg/l of NAA and 0.1 mg/l of BAP (Mumo *et al*., 2013).

2.1.3 Papaya Production Constrains in Kenya

Papaya farming is a very significant economic activity in Kenya and it provides a source of income for large and small scale (Macharia *et al*., 2017). However, yield losses have been experienced in Kenya due to pests such as mealybug among others (Kansiime *et al*., 2023). Diseases such as phytophthora which causes seedling rot and wilting of the plantles have also contributed immensely to the low yields in Kenya (Kansiime *et al*., 2023). Most farmers have shifted from papaya production to other crops. This has led to a decline of area harvested from 8,800ha to 6,052 ha from 2019 to 2022 (Food and agricultural organisation, 2023) and consequently, a decline in papaya production and yield (105,047t to 76,430.22t, 2019 to 2022) ((Food and agricultural organisation, 2023). Apart from pests and diseases challenges, papaya farming has minimal chances for export markets access

unless the international quality and safety standards are adhered to (Farmers trend, 2023). Papaya is sensitive to growing environment and the changes in the environmental factors severely affect the productivity and quality of fruits. Prolonged moisture stress will slow down the growth and encourage the production of a number of male or sterile flowers which lead to poor fruit set (Jeyakumar *et al*., 2005).

2.1.4 Benefits Of Indigenous Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular mycorrhizal fungi (AMF) are soil micro- organisms that form a symbiotic relationship with 80 to 90% of vascular plant species and 90% of agricultural plants and are globally distributed (Smith and Read, 2010). They belong to the phylum Glomeromycota and includes three classes (Archaeosporomycetes, Paraglomeromycetes and Glomeromycetes) (Tedersoo *et al*., 2018), 11 families, 25 genera and almost 250 species (Spatafora *et al*., 2016). The Glomeromycota are symbionts that depend on the carbon substrates supplied by the host plants while the fungi, through the intraradical and extraradical hyphae, root apoplast interface and arbuscules, improve the nutrients and water uptake to the host plant (Parniske, 2008). This symbiosis could possibly be the most extensive beneficial interaction between micro-organisms and plants (Parniske, 2008). Arbuscular mycorrhizal fungi have been reported to play crucial roles in plant nutrition and growth in stressed environment among other benefits (Nakmee *et al*., 2016).

2.1.4.1 Role of AMF in Nutrient Uptake by Plants

Mycorrhizae is important in plants due to the enlargement of the nutrient absorption surface area by fungal hyphae, beyond the nutrient depletion regions of the soil rhizosphere, which improves the nutrient absorption by the roots, thereby increasing primary production (Smith, 2011).

The fungal hyphal network is very efficient in nutrient uptake because of its very large surface area (Plenchette, 2005), and are thinner than the plants' roots hence able to penetrate smaller pores and absorb more nutrients (Allen, 2011). This extension of the

root absorbing zones by AMF increases the absorption surface of the plants, thus improving the access of plants to nutrients, particularly those in low concentration in the soil (Smith and Read, 2010).

Bacteria such as *Pseudomonas spp* and *Bacillus spp* interact with the fungi and possess the ability to solubilize inorganic phosphates and make them available to the plants. These organisms produce organic acids such as fumeric, lactic, succinic, acetic and gluconic acid which helps in the solubilizing effect. They are also known to produce vitamins, amino acids and growth promoting substances such as Gibberellic Acid (GA) and Indole Acetic Acid (IAA) which results in better growth of plants (Gupta *et al*., 2014).

The extra radical hyphae extend beyond this region and absorbs bio-available phosphate which is not accessible to the plant (Plenchette, 2005). The uptake of other major nutrients such as nitrogen is also enhanced in the presence of AMF especially in stressed conditions (Jansa *et al*., 2019). Mycorrhizae have also been shown to be important in the mobilization of zinc, copper, iron, manganese (Liu *et al.*, 2000); and organic nutrients (Hodge *et al.*, 2001). Additionally, the performance of peach seedlings with AMF inoculation was improved as minerals such as zinc, iron, potassium, calcium and magnesium concentrations were elevated in leaves and roots while manganese and copper were more concentrated on the roots (Wu *et al*., 2011).

Al-Hmoud and Al-Momany (2017) reported improved metabolism and growth of *Cucurbita maxima* inoculated with AMF and higher concentrations of crude fiber, fat, carbohydrates and crude protein in roots and shoots systems compared to the control treatments. Watermelon (*Citrullus lunatus*) plants inoculated with AMF improved the water use efficiency, yield of the plants and the fruits' quality (Kaya *et al*., 2003) while tomato plants with AMF had increased concentrations of organic acids,sugars and vitamin C in the fruits (Bona *et al*., 2016).

Papaya seedlings, mountain variety, inoculated with AMF had an improved growth through increase in leaf area and number, height of the plants, increased biomass

accumulation in both fresh and dry biomass and improved uptake of nutrients such as phosphorous and potassium (Chebet *et al*., 2020). Moreover, Ruth *et al*., (2011) noted that AMF symbiosis contributed 20 per cent of total plant water uptake, highlighting the importance of AMF on water status of the host plant.

2.1.4.2 Role of AMF in Management of Abiotic Stress

Plants with AMF colonization adapt to abiotic stress, and this is independent on the host plant. The symbiosis improves the growth, physiology and hydration of the plants under numerous environmental stress conditions (Kapoor *et al*., 2013). Drought, affects plants at various stages of growth. Water is the central molecule in all physiological processes of plants since it is the major medium for transporting metabolites and nutrients. Water stress lowers plant water potential and turgor to the extent that plants are restrained in executing normal physiological functions, (Seyed *et al*., 2017). Under prolonged drought, the solutes' concentrations in the cytosol and extracellular matrices rises. Cell enlargement decreases leading to growth inhibition and failure to reproduce. Consequently, abscisic acid (ABA) and compatible osmolytes like proline accumulates and this cause wilting. Drought affects stomatal closure, curbs gaseous exchange, reduces transpiration and arrests carbon assimilation (Seyed *et al*., 2011). Mycorrhizae symbiosis is a great contributor to tolerating drought in plants due to better osmotic adjustment (Kubikova *et al*., 2001) and leaf hydration (Ruiz-Lozano, 2003).

Drought reduces the productivity of plants significantly (Posta and Duc, 2020). Water stress induces stomatal closure, thereby reducing carbon dioxide influx and consequently carbon partitioning and photosynthetic activity are diminished, leading to decreased plant productivity and yield (Osakabe *et al*., 2014). However, AMF improves the performance of plant in drought stress (Balestrini and Lumini, 2018). Plants with mycorrhizal fungi deal with water stress through drought mitigation, whereby AMF indirectly enhance water uptake to the plants and drought tolerance whereby AMF improve the host plants' ability to deal with the stress (Bernardo *et al*., 2019). The fungal hyphae increases the surface area for water absorption by the plants' roots, the access to the micro soil pores is increased

and the apoplastic water flow is improved (Auge, 2001). Tomato plants grown under field condition with AMF root colonization enabled plants to grow well under water deficit conditions through effective water usage and improvement of nutrient contents (Subramanian *et al*., 2006). Abscisic acid (ABA) reduces cell water loss, induces stomatal closure and regulate stomatal conductance (Ouledali *et al*., 2019). Inoculation with mycorrhizal fungi influences the control of stomata functioning by the regulation of abscisic acid (Ouledali *et al*., 2019).

In saline environmental conditions, AMF occur naturally and contribute immensely on growth of various species of plants (Amanifar *et al*., 2019). *Lycopersicon esculentum* plant grown under mycorrhizal and saline conditions had increased plant growth, chlorophyll content, fruit weight, phosphorous and potassium concentrations, yield and antioxidant enzymes activities (Latef and Chaoxing, 2011). The mechanisms involved in salinity tolerance in plants with AMF inoculation include the ionic homeostasis (Munns and Tester, 2008), accumulation of osmoregulators such as sugars and proline and enhancement of nutrient uptake and water absorption capacity (Yamato *et al*., 2008). Moreover, AMF colonization reduces the oxidative damage in plants exposed to salinity and improves stomatal conductance (Pedranzani *et al*., 2015). Tomato plants with AMF inoculation were irrigated with saline water and had considerably increased on the plant biomass, yield and increased contents of potassium, phosphorous, iron, copper and zinc on the shoots (Al-Karaki, 2006).

Extreme temperatures negatively affects plant growth and production. Mycorrhizal inoculation improves plant performance to tolerate temperature stress by enhancing photosynthetic capacity, improving water and nutrient uptake, increasing the accumulation of osmolytes and protection against oxidative damage (Zhu *et al*., 2017). Plants with mycorrhizal colonization had developed their root system for absorption of water to prevent the damaging of the photosynthetic apparatus and to ensure high photosynthetic capacity at high temperature (Mathur and Jajoo, 2020).

In flooded conditions, AMF has been found to enhance the growth of plants through improved absorption of nutrient elements, especially phosphorous, a case study of rice (Bao *et al*., 2019). Wang *et al*., (2009) associated the improved growth of plants with mycorrhizal fungi on flooded conditions with the improved osmotic adjustment.

Inoculating plants with AMF enhanced growth and nutrition, improved soil quality, soil structure, increased plant establishment and survival on more than 80 per cent of plants growing on mining sites (Wang, 2017). The hyphal "metal binding" ability reduces the bioavailability of heavy metal elements such as cobalt, lead and cadmium (Audet and Charest, 2007). Establishing a mycorrhizal association can curb the transplanting shock which is brought about by undesirable environmental conditions at nursery and field establishment (Sebastiana *et al*., 2013). Nursery plantlets of olive trees with mycorrhizal association, specifically *Glomus intraradices* and *Glomus mosseae*, have been successfully established into barren lands under stress conditions (Estaun *et al*., 2003). Mycorrhizal fungi are crucial agents for the establishment and growth of fruit trees such as citrus (Ba *et al*., 2000).

2.1.4.3 Role of AMF in Management of Biotic Stress

Mycorrhizal colonization of roots reduces the damage instigated by plant pathogens. The protective effect of AMF, termed as mycorrhiza induced resistance (MIR), provides systemic protection against a pathogen attack (Cameron *et al*., 2013). As a result of AMF inoculation, the production of plants' antioxidant enzymes which defend plants against stresses such as pathogens, is increased (Cameron *et al*., 2013). In addition, microbial changes in the mycorrhizosphere enhances alteration in root growth and morphology, improved nutrient status of the plant as well as competition for colonization sites and photosynthates. Consequently, the improvement of the host plant growth enables the mycorrhizae to facilitate the regrowth of new tissues after pathogen attacks (Vos *et al*., 2012). Nematode infection was reduced by 45% for *Meloidogyne incognita* and 87% for *Pratylenchus penetrans* in AMF colonized plants in comparison with the controls, and this was mostly due to the alteration of the mycorrhizal plants' root exudates by AMF

(Vos *et al*., 2011). In AMF colonized soils, striga seed germination was suppressed while the number of striga seedlings emerging was reduced in cereals (Lendzemo *et al*., 2006). Manjunatha *et al*., (2018) confirmed AMF efficiency in promoting the growth of sugarcane, protection against striga infestation and reduction of the soil striga seed bank.

Plants inoculated with mycorrhizal have been observed to receive protection from pathogens in comparison with plants not inoculated with mycorrhiza (Filion *et al*., 2003). Kumari and Prabina, (2019) observed pathogen protection against *Fusarium oxysporum* on the host plant with arbuscular mycorrhizal fungi. The fungi and bacteria relations resulted in enhanced plant nutrition, growth and survival of the plants (Smith and Read, 2008). Numerous AMF species are better able to enhance the nutrient status of a host plant compared to a lone fungus. Host defenses of AMF plants may be enhanced hence reduced abundance of pathogenic fungi in roots. Increasing the abundance of AMF colonizing the root system may result in more intense competition with a pathogenic fungus. Fungal colonization by mycorrhiza influences root architecture of the host plant by causing a more profusely branched root system (Olah *et al*., 2005; Gutjahr *et al*., 2009).

The effect of AMF in control of phytophthora blight disease in Malkia F1 and JKUAT papaya hybrids, in Kenya, have not yet been verified.

2.1.5 Phytophthora Blight Disease

Phytophthora blight is among the most vital diseases which affetcs papaya. It occurs mostly during windy and humid weather conditions and is caused by *Phytophthora palmivora* fungus (Nelson, 2008). The genus, 'Phytophthora' is a very destructive pathogenic oomycetes which is responsible for worldwide economic losses in the ecosystem and agriculture as a whole (Vanegtern *et al*., 2015). There are more than one hundred and fifty species of phytophthora that have been identified and they cause diseases in crops both temperate and tropical regions (Yang *et al*., 2017). Fruit crops such as papaya and pineapple as well as root crops such as potato have lately become popular in tropical regions and are source of income to the country (Nelson, 2008). However the

phytophthora genus have posed a threat to crop production and have a significant global influence on the agricultural industry. Phytophthora blight can also be referred to root rot, stem canker, soft fruit rot or soft foot rot and the pathogen, *Phytophthora palmivora* is vastly virulent and have a significant impact on the production of crops such as papaya (Nelson, 2008).

Phytophthora reproduces asexually through resting spores, that is, chlamydospores and oospores, and sexually through dispersal spores, that is, zoospores and sporangia (Perrine-walker, 2020). Whereas chlamydospores remain viable in the soil for a lengthy duration until a favorable and conducive environments for sporulation and dispersal are achieved, zoospores and sporangia assist the phytophthora species to spread and escape from the harsh environment (Butubu, 2016). Phytophthora enters its hosts by utilizing hyphal slicing, and penetrate through the host surface at an oblique angle with slight energy. The interruption of the cytoskeleton structure allows the hyphae to enter through the crack alongside the direction of the angle, initiating the fracture on the host surface (Bronkhorst *et al*., 2021). Poor drainage and higher soil temperatures contributes greatly in the spread of this disease. Lesions are formed at the base of the stem and they result in yellow wilted leaves (Ventura *et al*., 2004). The white mycelial coat causes the lower leaves and fruits to shrink and fall prematurely (Narayanasamy, 2011).

Regulating irrigation and water systems is an important measure in controlling phytophthora diseases. Papaya orchards should not be established in flood-prone areas, forest regions with poor drainage due to the possibility of spore dispersal through water as a defense against this disease (Misman *et al*., 2022). Establishing crops on raised beds or in a highly porous soil media can reduce the invasion of the disease (Pscheidt and Ocamb, 2022). Beneficial micro organisms can promote the growth of plants and avail new approaches to combat the pathogen. They act as biocontrol by inhibiting pathogen infection directly within the host through nutrient competition mycoparasitism and antibiosis as well as indirectly through responses inherent to the host and triggering resistance (Mejia *et al*., 2008).

2.1.6 Plants Secondary Metabolites

Plant secondary metabolites are the byproducts of metabolic processes that are produced naturally and they do not have direct role in plant metabolism, growth or development but play an important role in plant defense mechanism; and enhance the growth and survival of plants during biotic and abiotic stress conditions (Zandalinas *et al*., 2017). The production and accumulation of secondary metabolites (SMs) differ within plants of similar species as well as from species to species under varying environmental conditions (Radušienė *et al*., 2012). The type and concentration of SMs synthesis is determined by the stages of plant development, genotype, physiology and prevailing environmental factors and the biosynthesis is stimulated when plants are under abiotic and/or biotic stresses such as heavy metals, salinity, drought or herbicides (Eid *et al*., 2015). During these periods of stress, the reactive oxygen species (ROS) induces oxidative stress which leads to inactivation of enzymes, lipid peroxidation and consequently the DNA is damaged (Akula and Ravishankar 2011). The lack of mobility and the immune system of plants prompts the synthesis of the SMs; whereby more than 100,000 SMs are produced from various metabolic pathways (Meena *et al*., 2017). The SMs consists of phenolics, terpenes, nitrogen and sulphur containing compounds.

Terpenes are synthesized in two major pathways; 2-C-methylerythritol 4-phosphate (MEP) pathway and mevalonic-acid (MVA) pathway (Sankari *et al*., 2019). The terpenes structures are produced by plants as toxins in defense to the huge numbers of mammals and feeding insects. Abscisic acid (ABA) is a sesquiterpene and plays regulatory roles in the initation and maintenance of bud and seed dormancy and responds to water stress by altering the membrane properties and also act as a transcriptional activator (Berli *et al*., 2010).

Phenolic compounds are synthesized in plants through the malonic acid and shikimic acid pathways (Ghasemzadeh *et al*., 2011). The malonic acid pathway has been reported in bacteria and fungi for the synthesis of phenolics (Cheynier *et al*., 2013). The enzymes that regulate phenolic levels under different stress constraints are chalcone synthase (CHS)

and phenylalanine ammonia lyase (PAL). The phenols defend the plant system against diseases and pests such as root parasitic nematodes (Wuyts *et al*., 2006)

Nitrogen-containing SMs are characterized by the presence of nitrogen molecule in their structure and amino acids such as tyrosine, tryptophan and lysine (Mehrotra *et al*., 2018). They include cyanogenic glucosides, alkaloids and non-proteins amino-acids. Most of these alkaloids are generally toxic in defense against herbivoral attack and microbial infection. The plants are not toxic but when the plants are crushed, volatile poisonous substance such as hydrocyanic acid (HCN) and hydrogen sulfide (H_2S) are readily broken down to deter feeding by insects and herbivorous (Pagare *et al*.,2015)

Sulphur containing SMs include glutathione, glucosinolate, thionins, phytoalexins, allinin and defensins. They have been linked with the plants' defence against microbial pathogens (Grubb and Abel, 2006).

CHAPTER THREE

ABUNDANCE AND DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGAL (AMF) SPORES ISOLATED FROM THE RHIZOSPHERE OF PAPAYA AND OTHER DIFFERENT CROPPING SYSTEMS IN CENTRAL KENYA

Abstract

Arbuscular mycorrhiza fungi (AMF), obligate symbionts, are important in the majority of cultivated plant species in colonizing roots and supporting plant growth in adverse climatic conditions. The abundance and quality of mycorrhizal colonization is affected by land-use types, cropping systems and climate change. On the other hand, rhizospheric mycorrhizae present in soil rhizosphere can be isolated for enhancing plant performance. One such opportunity arises in the acclimatization of seedlings for adaptation to depleted field conditions. Isolation and characterization of rhizospheric AMF species is important in evaluating the efficiency of colonization especially in plants that have not been previously evaluated such as papaya, which is well known for its high nutritional value. In this study, soils were sampled from grass, banana and papaya plants'rhizospheres, from three different papaya growing regions in Kenya; Mwea, Mitunguu and Juja. Spores were isolated using the sucrose method. Spore abundance was done using a gridded petri dish and morphologically characterized using the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM) database. Four families, 10 genera and 41 species of Glomeromycota phylum were isolated from the three sampling sites. The families of *Glomeraceae* (16 species) and *Acaulosporaceae* (14 species) dominated in Juja and Mwea Sub Counties. *Glomus spp*. isolated from the rhizosphere of banana and grass plants were the most abundant, at $p \leq 0.05$, in Juja while *Diversispora spp* (*Diversisporaceae*) was the least abundant, at $p \leq 0.05$. Mwea Sub County had the most spore abundance, at $p \leq 0.05$, compared to Mitunguu and Juja. Mycorrhizal spores isolated from the grass were the most abundant, at $p \leq 0.05$, (Mwea, 37.73; Juja, 37; Mitunguu, 35.2) compared to those isolated from banana (Mwea, 31; Juja, 25; Mitunguu, 26.6) and

papaya plants (Mwea, 32; Juja, 18.5; Mitunguu, 21.2). The results showed that AMF spore abundance and diversity varies with different locations and the associated plants.

3.1 Introduction

Arbuscular mycorrhizal fungi (AMF) belongs to glomeromycota phylum/division, from the kingdom of fungi. They are mostly found in terrestrial ecosystems and are obligate root symbionts that establish a mutualistic symbiosis with quite a number of plant species worldwide (Lekberg *et al*., 2013). The AM fungi play an important role in plant nutrition and especially phosphorous and nitrogen uptake, as well as water absorption. This is attributed to the presence of arbuscules that they produce inside the host plant's roots (Smith and Read 2008). According to Augé (2001), this aspect results in the enhancement of plant growth and the ability to withstand abiotic and biotic stresses. The colonization of the roots is arbitrated by genetic, morphological and functional relations between the AMF species and the plant (Kiriacheck *et al*., 2009).

The morphology of spores may comprise one million nuclei and they also vary in size, shape and colour (Pawlowska and Taylor, 2004). The diversity, as well as distribution of AMF species, could be affected by the farming systems of the plants and their communities (Jefwa *et al*., 2006). To accomplish adequate levels of productivity including desirable food quality, sustainable agricultural systems have been enhanced. These systems enables decreased fertilizer usage which leads to precluded environmental pollution and on the other hand, input costs are minimized (Harrier and Watson 2004; Siddiqui *et al*., 2008).

Various studies have been conducted on exploring the importance of microorganisms in boosting the soil fertility and the improved crop production and substitutes to the use of commercialized synthetic pesticides (Igiehon and Babalola, 2017). More attention is being devoted to improving the exploitation of indigenous soil microorganisms which will lead to improved soil fertility (Hamel and Strullu, 2006). Crops respond and benefit from AMF depending on various agricultural factors such as the inoculation potential of the

mycorrhizal fungi, fertilization, tillage practices as well as the reliability of the host crop on mycorrhizal colonization (Auge, 2004). Cultivation in soils with decreased levels of fertilizer enhances numerous AMF beneficial effects such as retaining of nutrients in the soil (Djuuna *et al*., 2009). There is high diversity of AMF observed around the rhizosphere in the natural habitat (Opik *et al*., 2008). The distribution of the AMF is due to their ability to withstand high levels of nutrients in various types of habitats (Porras-Alfaro *et al*., 2007). Even under the same climatic conditions and ecosystem, the AMF communities were different (Meadow and Zabinski, 2012). The survival of AMF was observed between 2001 and 2013 (3 seasons) using pyro-sequencing and it was reported that the biofertilizer was still in existence and had initiated some variations in the abundance and diversity of indigenous AMF at the end of the third season (Islam *et al*., 2021).

Due to the diversity of AMF spores, there is need to isolate, obtain the spore abundance and characterize the genus and species of the AMF spores, even under the same climatic conditions. The fungal spores can be isolated and bulked in crops such as sorghum to produce AMF inoculum which can be used as a biofertilizer in soils with less population of AMF spores to maximize the benefits of AMF on horticultural crops.

3.2 Materials and Methods

3.2.1 Description of the Study Sites

The research was conducted at Mwea, Mitunguu and Juja in Kenya, since the areas have existing established papaya orchards, which is the crop of focus in this study. The site at Mwea, in Kirinyaga County, is located at 00 42’ 0” S latitude, 370 22’ 0” E longitude and lies at 1093 M above sea level. It has an annual mean temperature of 22.71^{o}C and mean annual rainfall of 930 mm. The area has upper midland ecological zones with pellic vertisols, black cotton soils, whose colour ranges from dark grey to black and are poorly drained (Schmidt *et al*., 2006). Rice farming is common to most farmers in this region. Mitunguu, in Meru County, is located at 00 60’ 0” S latitude and 370 47’ 0” E longitude and lies at 1498 M above sea level. It has an annual mean temperature of 20.6^{o}C and mean

annual rainfall of 550 mm. The area has upper highlands ecological zones with well drained soils that are moderately fertile loam and dark brown in colour (Schmidt *et al*., 2006). Banana farming and dairy farming were the most common agricultural practices in this area. Juja, on the other hand, is in Kiambu County and has geographical coordinates of 10 11' 0" S latitude and 370 7' 0" E longitude. It lies at 1519 M above sea level. The average annual temperature is 19.6^{o}C while the mean annual rainfall is 1014 mm. The area has lower midland ecological zones with soils that are well drained humic nitisol, friable clays that are reddich brown in colour (Schad, 2016). Crops grown varied with the prevailing soil conditions. Maize farming and sisal plantations were however most common.

3.2.2 Soil Collection and Analysis

Soil samples were collected from the root's rhizosphere of papaya, banana and grass plants in Mwea, Mitunguu and Juja, at a depth of 0-20 cm following a zig zag pattern across a paddock. The soil was packed in sterilized 500g soil sample bags. Soil was sampled from four different farms from each site. The selection of these farms within the different areas was based on the existing established papaya orchards, which is the crop of focus in this study. Banana and grass plants were selected based on abundance of mycorrhizal spores in the rhizospheric soils compared to papaya soils and also being the major crops that surrounded the papaya orchards in these regions. The soil content was analysed separately according to the plants and site of collection

3.2.2 Isolation of Arbuscular Mycorrhizal Fungi (AMF)

In the JKUAT laboratories, spore isolation was carried out as described by Boyno *et al*., (2023), with a few modifications. Fifty grams of soil was sampled out and placed in a 250 ml conical flask. 100ml of tap water was added and the flask was capped with a rubber cork. The mixture was agitated using the rotary shaker and left to decant for 30 s and then washed through 250 µm, 100 µm and 45 µm pore sieves. The contents of the 45 µm pore sieve were backwashed into a small sized beaker and swirled. The contents were then

quickly decanted into 50 ml centrifuge tubes and balanced by weight then centrifuged for 5 min at 1750 rpm. The supernatant was discarded and the tubes were filled with 48% sucrose solution (sucrose-227 g dissolved in 500 ml water), balanced by weight and stirred vigorously to re-suspend the precipitate then centrifuged for 15 sec at 1750 rpm. The supernatant sucrose was emptied through a 45 micromesh sieve. The spores retained on the sieve were rinsed thoroughly with distilled water to wash out the sucrose. The spores were then washed away with distilled water into gridded Petri dishes for examination.

3.2.3 Spore Abundance

The number of spores isolated from different soils were counted under a dissecting microscope (Labomed), using 10X objective lens hence a total magnification of 100 X using a gridded Petri dish and a laboratory needle. The population of the spores was expressed according to the number of spores per 50 g of the soil according to the plant and location. Six spores from every plant and location were randomly isolated using a needle and a 5 ml transfer pipette to a microscope slide. Melzer's reagent was added onto the spores and the slide was covered with a microscope coverslip. Since the microscope was fitted with a camera and connected to the computer, the spores observed were captured and measured.

3.2.4 Identification of Fungal Structures

Characterization of the spores was carried out morphologically based on the color, shape, melzer's reaction on the spore color, spore surface and size of the spore and distinguished according to descriptions provided by the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM, 2005).

3.3 Data Analysis

The tabulated data was subjected to two way analysis of variance (ANOVA) using Genstat statistical package 15th edition, while the means found to be significantly different at $p \leq 0.05$ were separated using Tukey's HSD test.

3.4 Results

3.4.1 Soil Analysis

Soils obtained from Mwea, Mitunguu and Juja areas from papaya, banana and grass rhizosphere were separately analysed. The pH, electrical conductivity (EC), total organic carbon (TOC), phosphorous (P), potassium (K), magnesium (Mg), zinc (Zn), iron () Fe and nitrogen (N) contents analysed from soils obtained from papaya rhizosphere did not differ significantly ($p \leq 0.05$) among the three areas. Soils obtained from papaya rhizosphere in Juja area had the lowest calcium contents (9.59 %) as compared to Mwea and Mitunguu areas which had 14.14 % and 13.52 % respectively. Soils obtained from banana rhizosphere in Mitunguu area had the significantly ($p \leq 0.05$) higher cation exchange capacity of 9.6% compared to Juja and Mwea areas which had 6.4% and 7.3% respectively. The pH, EC, CEC, K, Zn, Fe and N contents analysed from soils obtained from grass rhizosphere did not differ significantly ($p \leq 0.05$) among the three areas. Soils obtained from grass rhizosphere in Juja had the lowest TOC of 5.77% while Mwea and Mitunguu had 7.07% and 6.09 %. The phosphorous contents from soils obtained from grass rhizosphere were 11.91%, 10.29% and 11.37% for Juja, Mwea and Mitunguu respectively (Table 3.1).

Table 3.1: Soil Analysis of Papaya, Banana and Grass Plants from Juja, Mwea and Mitunguu Areas

Plant	Area	pH	EC	TOC	CEC	P	K	Mg	Ca	Zn	Fe	N
Papaya	Juja	6.55[a]	2.85[a]	6.86[a]	6[b]	14.61[a]	4.44[a]	3.3[a]	9.59[b]	0.045[a]	0.06[a]	0.84[a]
	Mwea	7.54[a]	2.88[a]	6.49[a]	7.5[a]	14.77[a]	4.68[a]	5.86[a]	14.14[a]	0.05[a]	0.057[a]	1.12[a]
	Mitunguu	6.19[a]	2.83[a]	5.73[a]	6.5[b]	14.85[a]	4.44[a]	4.66[a]	13.52[a]	0.027[a]	0.056[a]	0.93[a]
	LSD	1.55	0.1	1.24	0.9	0.35	0.26	1.07	2.7	0.096	0.03	0.35
	CV%	1.9	2.3	2.5	3.4	3.1	4.3	5.8	3.9	11.2	2.3	7.9
Banana	Juja	5.77[a]	2.61[a]	6.31[a]	6.4[b]	9.67[a]	5.42[a]	4.02[b]	11.08[b]	0.033[a]	0.057[a]	0.89[a]
	Mwea	5.85[a]	2.27[a]	5.84[a]	7.3[b]	9.51[a]	4.93[a]	3.84[b]	12.3[ab]	0.045[a]	0.034[b]	0.75[a]
	Mitunguu	4.83[b]	2.79[a]	5.55[b]	9.6[a]	7.74[b]	4.19[a]	6.4[a]	13.27[a]	0.04[a]	0.063[a]	1.03[a]
	Lsd	0.85	0.42	0.65	2.5	1.3	1.52	1.63	1.34	0.021	0.143	0.42
	CV%	3.1	2.6	2.8	2.2	3.0	3.2	4.2	2.8	3.1	5.2	2.7
Grass	Juja	6.82[a]	2.64[a]	5.77[b]	7.3[a]	11.91[a]	4.44[a]	3.35[b]	12.58[a]	0.029[a]	0.056[a]	0.75[a]
	Mwea	6.11[a]	2.73[a]	7.07[a]	6.6[a]	10.29[b]	4.43[a]	6.36[a]	14.04[a]	0.05[a]	0.033[a]	0.98[a]
	Mitunguu	6.73[a]	2.54[a]	6.09[a]	7.2[a]	11.37[a]	4.68[a]	6.91[a]	12.89[a]	0.04[a]	0.058[a]	0.93[a]
	LSD	1.3	0.25	1.1	1.2	0.98	0.85	2.1	2.34	0.37	0.42	0.37
	CV%	2.7	4.3	2.2	2.9	3.6	5.2	3.4	7.2	10.4	9.8	3.8
	Significance Level a*p	*	*	**	**	**	*	*	*	*	*	*

Means within each column followed by a different letter differ significantly at ($p \leq 0.05$) EC= Electrical conductivity in dS m^{-1}, TOC=Total Organic Carbon in %, CEC= Cation Exchange Capacity in %, P=Phosphorous in %, K= potassium in %, Mg=magnesium in %, Ca=Calcium in %, Zn=Zinc in %, Fe=Iron in % and N=Nitrogen in %

3.4.2 Spore Abundance

The mean number of spores obtained from the 3 locations and among the plants was significantly ($p \leq 0.05$) different. Soils obtained from Mwea exhibited the highest number of spores from the 3 plants in comparison with Mitunguu and Juja areas. Soils obtained from papaya and banana plants' rhizosphere in Mwea area exhibited significant ($p \leq 0.05$) higher number of spores in comparison with Mitunguu and Juja areas. Spores isolated from the grass plants' rhizosphere were most abundant in all areas. Papaya plant had the least mean number of spores and was significantly ($p \leq 0.05$) different among the areas while Juja and Mitunguu displayed the lowest overall number of spores, 18.5 and 21.2 respectively (Figure 3.1).

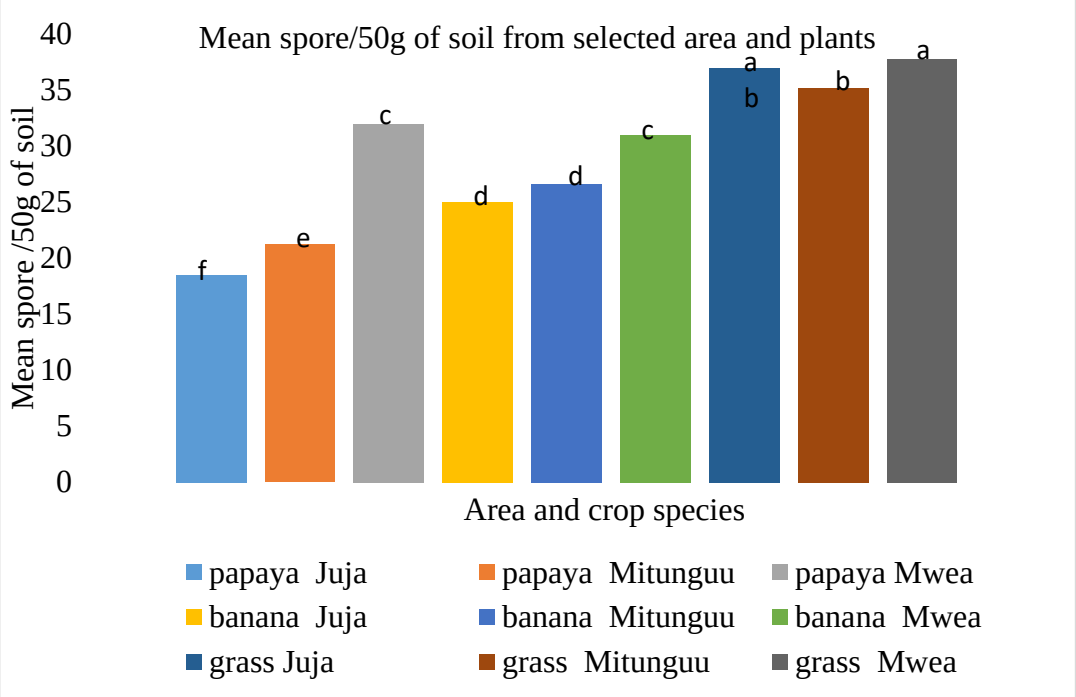


Figure 3.1: Mean Spore Abundance per 50g of Soil from the Rhizosphere of Banana, Papaya and Grass Plants Obtained from Mitunguu, Mwea and Juja Areas

Means within each bar, for the area and plants, followed by a different letter differ significantly at ($p \leq 0.05$).

3.4.3 Spore Characterization

3.4.3.1 Glomeromycota Families Distribution

Majority of the isolated spores belonged to the family of *Acaulosporaceae* (23), followed by *Glomeraceae* (19), *Gigasporaceae* (10), while *Diversisporaceae* (2), had the least number of genus and species (Table 3.2). Five out of the six spores characterized from the rhizosphere of papaya plants from Mitunguu area belonged to *Acaulosporaceae* family while the other spore belonged to *Glomeraceae* family. Five out of the six spores characterized from the rhizosphere of banana plants from Juja area belonged to *Glomeraceae* family while the other spore belonged to *Acaulosporaceae* family. Only the rhizosphere of banana from Mwea and the rhizosphere of papaya from Juja had spores belonging to *Diversispora* family.

Table 3.1: Glomeromycota Families' Distribution in the Rhizosphere of Banana, Papaya and Grass Plants Obtained from Mitunguu, Mwea and Juja Areas

Area	Plant	*Acaulosporaceae*	*Glomeraceae*	*Gigasporaceae*	*Diversisporaceae*	Number of spores
Mitunguu	Papaya	5	1			21.2
	Banana	2	3	1		26.6
	Grass	1	2	3		35.2
Juja	Papaya	3	2		1	18.5
	Banana	1	5			25
	Grass	1	4	1		37
Mwea	Papaya	4	1	1		32
	Banana	3	1	1	1	31
	Grass	3		3		37.73

3.4.3.2 Botanical Classification of the Spores

The colors of the isolated spores varied, and consisted of brown, red-brown, orange brown, bright greenish yellow among others. The diameter of the spores ranged between 42 to 84 µm while the dominant shapes of the spores were globose and sub globose (Tables 3.3 to 3.11).

Table 3.3: Morphological Characterization of Arbuscular Mycorrhizal Fungi (AMF) Obtained from the Rhizospheric Soil of Papaya from Mitunguu Area

Image of spore	Shape	Color	Melzers reaction	Size (diameter in µm)	Spore surface	Genus	Species
	Globose	Brown	Red brown	45	Granular	*Acaulospora*	*tuberculata*
	Globose	Dark greenish	Pinkish red	43.5	Granular	*Acaulospora*	*tuberculata*
	Sub globose	Brown	No reaction	51	Granular	*Acaulospora*	*foveata*
	Elliptical	Brown	No reaction	52.5	Granular	*Acaulospora*	*sp3*
	Globose	Brown	Dark brown	66	Granular	*Glomus*	*sp4*
	Ovoid	Yellow-brown	Dark red purple	61.5	Irregular	*Acaulospora*	*colombiana*

Table 3.4: Morphological Characterization of Arbuscular Mycorrhizal Fungi (AMF) Obtained from the Rhizospheric Soil of Bananas from Mitunguu Area

Image of spore	Shape	Color	Melzers reaction	Size (diameter in µm)	Spore surface	Genus	Species
	Globose	Yellow	Light purple	64.5	Granular	*Glomus*	*aggregatum*
	Elliptical	Yellow	Light purple	57	Irregular	*Glomus*	*manihotis*
	Elliptical	Orange-brown	Darker orange	58.5	Granular	*Acaulospora*	*capsicula*
	Irregular	Brown	Pinkish purple	81	Granular	*Dentiscutata*	*erythropa*
	Globose	Dark red brown	Darker orange brown	55.5	Granular	*Acaulospora*	*capsicula*
	Globose	Orange brown	No reaction	66	Granular	*Glomus*	*sp 6*

Table 3.5: Morphological Characterization of Arbuscular Mycorrhizal Fungi (AMF) Obtained from the Rhizospheric Soil of Grass from Mitunguu Area

Image of spore	Shape	Color	Melzers reaction	Size (diameter in µm)	Spore surface	Genus	Species
	Globose	Clear brown	Light pinkish red	66	Granular	*Scutellospora*	*biornata*
	Irregular	Dark brown	Light pinkish red	69	Granular	*Dentiscutata*	*reticulata*
	Elliptical	Yellow-brown	Red brown	48	Irregular	*Acaulospora*	*scrobiculata*
	Sub globose	Yellow orange	No reaction	52.5	Smooth	*Glomus*	*sp2*
	Globose	Yellow brown	Dark red purple	75	Granular	*Gigaspora*	*decipiens*
	Globose	Pale yellow brown	Dark red	63	Granular	*Rhizophagus*	*fasciculatus*

Table 3.6: Morphological Characterization of Arbuscular Mycorrhizal Fungi (AMF) Obtained from the Rhizospheric Soil of Papaya from Mwea Area

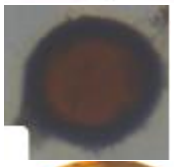
Image of spore	Shape	Color	Melzers reaction	Size (diameter in µm)	Spore surface	Genus	Species
	Irregular	Yellow-brown	No reaction	42	Irregular	*Acaulospora*	*laevis*
	Globose	Dark greyish green	Red brown	52.5	Granular	*Acaulospora*	*elegans*
	Sub globose	Orange-brown	Darker orange	69	Granular	*Acaulospora*	*excavate*
	Elliptical	Brown	No reaction	58.5	Granular	*Acaulospora*	*sp1*
	Sub globose	Bright greenish yellow	Dark red brown	73.5	Irregular	*Gigaspora*	*gigantean*
	Sub globose	Orange brown	No reaction	69	Granular	*Funneliformus*	*mosseae*

Table 3.7: Morphological Characterization of Arbuscular Mycorrhizal Fungi (AMF) Obtained from the Rhizospheric Soil of Banana from Mwea Area

Image of spore	Shape	Color	Melzers reaction	Size (diameter in µm)	Spore surface	Genus	Species
	Sub globose	Orange brown	No reaction	63	Granular	*Acaulospora*	*lacunose*
	Oblong	Yellow brown	Red brown	54	Granular	*Glomus*	*aggregatum*
	Sub globose	Orange brown	Red purple	51	Granular	*Acaulospora*	*colombiana*
	Irregular	Red brown	Dark red brown	84	Irregular	*Dentiscutata*	*heterogama*
	Sub globose	Red brown	No reaction	70.5	Granular	*Diversispora*	*epigaea*
	Globose	Light brown	Pinkish red	55.5	Granular	*Acaulospora*	*sp 4*

Table 3.8: Morphological Characterization of Arbuscular Mycorrhizal Fungi (AMF) Obtained from the Rhizospheric Soil of Grass from Mwea Area

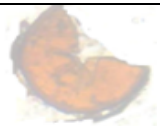
Image of spore	Shape	Color	Melzers reaction	Size (diameter in µm)	Spore surface	Genus	Species
	Sub globose	Orange-brown	Red brown	60	Granular	*Acaulospora*	*scrobiculata*
	Globose	Orange brown	Red purple	63	Granular	*Scutellospora*	*scutata*
	Sub globose	Orange brown	No reaction	51	Granular	*Acaulospora*	*laevis*
	Globose	Red brown	Red purple	54	Granular	*Acaulospora*	*denticulata*
	Ovoid	Red brown	No reaction	72	Granular	*Scutellospora*	*sp1*
	Sub globose	Red brown	Red purple	73.5	Granular	*Scutellospora*	*biornata*

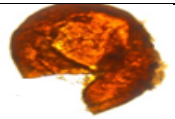
Table 3.9: Morphological Characterization of Arbuscular Mycorrhizal Fungi (AMF) Obtained from the Rhizospheric Soil of Papaya from Juja Area

Image of spore	Shape	Color	Melzers reaction	Size (diameter in µm)	Spore surface	Genus	Species
	Elliptical	Yellow brown	Red brown	46.5	Irregular	*Acaulospora*	*scrobiculata*
	Sub globose	Orange brown	No reaction	46.5	Granular	*Acaulospora*	*foveata*
	Elliptical	Orange-brown	Hyaline	54	Granular	*Diversispora*	*eburnea*
	Globose	Dark greyish green	Red brown	63	Smooth	*Acaulospora*	*elegans*
	Globose	Brown	Red brown	58.5	Granular	*Glomus*	*sp5*
	Globose	Orange brown	No reaction	57	Granular	*Glomus*	*ambisporum*

Table 3.10: Morphological Characterization of Arbuscular Mycorrhizal Fungi (AMF) Obtained from the Rhizospheric Soil of Banana from Juja Area

Image of spore	Shape	Color	Melzers reaction	Size (diameter in µm)	Spore surface	Genus	Species
	Elliptical	Yellow	Light purple	78	Granular	*Glomus*	*manihotis*
	Elliptical	Yellow	Light purple	78	Granular	*Glomus*	*manihotis*
	Globose	Orange brown	Darker orange	61.5	Granular	*Glomus*	*coronatum*
	Sub globose	Reddish brown	Darker red brown	73.5	Granular	*Septoglomus*	*deserticola*
	Sub globose	Brown	Light brown	63	Granular	*Glomus*	*sp3*
	Elliptical	Orange-brown	No reaction	63	Granular	*Glomus*	*ambisporum*

Table 3.11: Morphological Characterization of Arbuscular Mycorrhizal Fungi (AMF) Obtained from the Rhizospheric Soil of Grass from Juja Area

Image of spore	Shape	Color	Melzers reaction	Size (diameter in µm)	Spore surface	Genus	Species
	Globose	Yellow brown	Dark purple	66	Irregular	*Glomus*	*intraradices*
	Sub globose	Brown	Red brown	61.5	Smooth	*Glomus*	*sp 1*
	Globose	Brown	Red brown	72	Granular	*Septoglomus*	*constrictum*
	Irregular	Bright orange	Darker orange	63	Irregular	*Acaulospora*	*sp2*
	Globose	Red brown	Dark purple	55.5	Granular	*Septoglomus*	*deserticola*
	Globose	Cream yellow brown	Hyaline	79.5	Irregular	*Racocetra*	*sp1*

3.5 Discussion

Variations in the number of spores were recorded in this study among the plants'rhizospheric soil from different areas. The spore abundance dissimilarities could be due to abiotic factors and especially the type of soil, soil organic matter content and the amount of water in the soil. Sturmer and Siqueira, (2011) indicated that spore abundance and mean species richness were influenced by land uses. Mwea area had the highest number of spores and this could be due to poor drainage of soils, resulting to flooding compared to Mitunguu and Juja areas. A higher diversity of AMF communities were found in wetland ecosystems (Wang *et al*., 2011) and they improved the growth of plants in such conditions through improved absorption of nutrient elements (Bao *et al*., 2019). Mycorrhizal colonization contributed to the flood tolerance of *Pterocarpus officinalis* seedlings by enhancing growth of plants and phosphorous acquisition in leaves (Fougnies *et al*., 2006).

Arbuscular mycorrhizal fungi (AMF) are sturdily reliant on their host plants (Fitter, 2005) and have different levels of specific hosts pairing (Johnson *et al*., 2003; Klironomos, 2003). According to Fitter (2005), the AMF are incapable of developing independently whether in axenic culture or naturally. Arbuscular mycorrhizal fungi requires root colonization of a vascular plant for their life cycle to be complete (Brundrett, 2004).

In the current study, papaya plants' rhizospheric soil had the least mean number of spores compared to grass and banana rhizospheric soil. According to Whipps (2001), papaya plants, being a terrestrial plant, are colonized naturally by AMF. Various organisms in the soil however compete for their territory and this can lead to other different types of fungi colonizing these plants thus reducing the advantageous effects of the AMF. In this study, grass plants' rhizospheric soil had the highest number of spores. The soil spore count in the maize field was higher than that of rice, sugarcane and banana (Sankaralingam *et al*., 2016). Maize and grass belongs to the *Poaceae* family. According to Brundrett and Abbott (2002), hyphae, are structures found in mycorrhizae and are susceptible to soil disturbance as this pulls down the AMF infectious ability. Among the plants involved in

the current study, grass plants' rhizosphere was the least disturbed and this could have attributed to the high number of spores compared to banana and papaya plants' rhizosphere.

Schussler and Walker (2010) have described and named most of the AMF species according to the morphology of their spores. In the current study, spores of different genera were isolated from different plants and locations. *Acaulosporaceae* and *Glomeraceae* families dominated the three (3) areas and still among the plants. In similar environmental conditions, *Acaulospora species* and *Glomus species* have been observed to produce more spores than *Gigaspora species* and *Scutellospora species* (Zhao *et al*., 2003). Land uses influenced the richness and abundance of AMF species as *Acaulospora* and *Glomus* species were among the most abundant species regardless of land uses, that is, old and young secondary forest, agroforestry, mature pristine forest crops and sites converted to pasture (Stürmer and Siqueira, 2011). Studies administered in agricultural fields have further concluded that soil disturbance leads to drastic relocation of the AMF community (Schnoor *et al*., 2011). Maherali and Klironomos (2007) indicated that *Glomeraceae* and *Gigasporaceae* families distribute most of their biomass in the intraradical and extraradical hyphae respectively while *Acaulosporaceae* produce low biomass both intra and extraradically. Majority of the spores isolated from papaya plants' rhizospheric soil belonged to *Acaulosporaceae* family. According to Chagnon *et al*., (2013), these AMF families have characteristic life history with most species under *Acaulosporaceae* being stress tolerators, *Glomeraceae* being associated with ruderals while those in *Gigasporaceae* are competitors.

The lowest, highest and the mean of the AMF size is described according to the species and is necessary for the study of the taxonomy and ecology of spores (Oehl *et al*., 2008). Higher sizes of spores, more than 200 µm, can easily differentiate *gigasporaceae* family from others such as *glomeraceae* whose sizes are less than 200 µm (Oehl *et al*., 2006). In this study, the size of the diameter of the spores ranged from 42 to 84 µm at x10 magnification, with the larger sizes being from *gigasporaceae* family while the smaller sizes from *acaulosporaceae* family.

The shapes of spores may vary among and within species as in the case of *Funneliformis mosseae*, whose shapes vary from globose, sub-globose and irregular (Al-Qarawi *et al*., 2013). Redecker *et al*., (2013) has described nine different shapes which include: irregular, elliptical, triangular, globose, oblong, pulvinate, subglobose, ovoid and knobby. Some spores have similar ornamentation and colours but their altered shapes differentiate their species, e.g *Scutellospora calospora* and *Scutellospora dipurpurescens* (Oehl *et al*., 2008). Most of the observed shapes in this study were globose, irregular, elliptical, oblong, and ovoid.

The colors observed in this study using the Munsell color chart ranged from brown, red-brown, orange-brown and light brown. The colors of the AMF spores vary between their families, genus and sometimes species from white to black and variants such as dark, bright, pale or light (Oehl *et al*., 2008). These colors also depend on the maturity of the spore and their integrity e.g. *Acaulospora capsicula* has colors ranging from orange-brown, red-brown and dark red-brown (Schüßler and Walker, 2010). Addition of melzer's reagent on the spore changed the inner and outer colour of most of the spores while other spores did not react. During the identification of AMF species, the color of the spore is a very suitable characteristic to unravel many uncertainties of the taxonomy (Oehl *et al*., 2008).

This study showed the diversity of AMF spores isolated from papaya, banana and grass rhizosphere from Mitunguu, Mwea and Juja areas in terms of the genus and species of the glomeromycota phylum. The difference in AMF genus and species could be attributed to the different climate conditions and the plants associated with the spores. The study also revealed that *acaulosporaceae* and *glomeraceae* families were dominant in the rhizosphere of the banana, papaya and grass plants obtained from Mwea, Juja and Mitunguu areas. Since the agricultural management practices varied among the three plants and the three areas, this could affect spore abundance in the plants. Papaya plants' rhizospheric soil had low spore count and majority of the observed spores belonged to *acaulosporaceae* family which is associated with stress tolerance.

AMF species are widely distributed and they vary depending on the type of crop, the nature of the soil and also the land ecosytems. In the current study, AMF spores obtained from grass' rhizosphere were most abundant and therefore, the soil around the grass roots can be scooped and used as a growing media for various crops such as papaya which had a low spore count. Spore abundance of the soil should be considered as one of the good agricultural practices with the aim of boosting the soil with lesser spore count for maximum utilization of AMF benefits in farming.

CHAPTER FOUR

CHARACTERIZATION OF FUNGAL ISOLATES ASSOCIATED WITH RHIZOSPHERIC INDIGENOUS ARBUSCULAR MYCORRHIZAL FUNGI (AMF) FROM DIFFERENT PLANT SPECIES AND AGRO-ECOLOGIES

Abstract

Arbuscular mycorrhizal fungi (AMF) associate with plants roots and exhibit beneficial impacts such as stress tolerance, nutrient and water absorption. These functions opened the door to studying other fungi associated with AMF on the root's rhizosphere of banana, grass and papaya plants from Mwea, Mitunguu and Juja areas. The study aim was to characterize fungal isolates associated with rhizospheric indigenous arbuscular mycorrhizal fungi (AMF) from different plant species and agroecologies. A total of 30 fungal isolates were obtained from the soil samples through the pour-plate technique. The isolates had diverse microscopic morphological characteristics ranging from form, margin, color, size and surface. Physiochemical characteristics showed varied growth at different pH, temperature and salinity. The optimum growth was recorded at pH 7.0, 30^{o}C to 35℃ temperature and salinity of 0.1M to 0.5M NaCl. The Internal Transcribed Spacer (ITS**)** and AMF subunits sequences showed diversity similar to *Aspergillus spp, Ajellomyces spp, Fusarium spp, Trichoderma spp, Penicillum spp, Glomus spp* and *Diversispora spp*. The study revealed the occurrence of other fungi, rather than AMF, that could be beneficial or harmful to the plants. Therefore, the existence of AMF in the rhizospheric soil does not only benefits the host plants but also prevents the plants from detrimental effects due to the harmful fungi.

4.1 Introduction

In agriculture, fungi are among the major essential pathogens of crops as well as bio-control agents to avert and control plant diseases (Costa *et al*., 2012), control terrestrial

weeds (Machado *et al*., 2012), reduce aquatic weeds, insects and other pests (Rangel *et al*., 2018).

Arbuscular Mycorrhiza Fungi (AMF) are obligate biotrophs and requires roots of an active plant host for them to accomplish their life stages. Despite the various attempts by scientists to contrive artificial culture media to support the growth of AMF, none has been successful (Hildebrandt *et al*., 2002). Within the roots of the living plant, AMF produce arbuscules, vesicles, hyphae and spores in and out of the roots cortex (Prasad *et al*., 2017). The role of AMF in agriculture include improvement of salinity and drought tolerance of the host plant through the increase of uptake of nutrients, organic solutes accumulation and reduction of oxidative stress due to the intensified activity of dismutase, calmodulin, peroxidase, superoxide, catalase and ascorbate peroxidase (Huang *et al*., 2014). Mycorrhizal fungi are also known to block the leaching of base cation and abate the toxic effects of heavy metals such as aluminum (Finlay *et al*., 2009).

Trichoderma, a non-mycorrhizal fungi, arbitrates stress reactions in plants. They have the potential to parasitize plant pathogenic fungi by stimulating the defense response thus escalating growth of the plant (Sharma *et al*., 2019). On the other hand, *Piriformospora indica* species can stand salt stress, prompt disease resistance as well as supporting the growth of crops (Waller *et al*., 2005).

According to Photita (2001), globally, fungal diversity is significantly contributed by endophytic fungi and the distribution are massive in temperate and tropical rain forest. Endophytic fungi have been found in most of the plant species that have been studied so far (Rana *et al*., 2019; Yadav *et al*., 2019). They have already established an evenness with their plant host during the evolution and are a latent source of bioactive secondary metabolites (Tan and Zou 2001; Sonaimuthu *et al*., 2010). They enable the plant host to adapt to biotic and abiotic stresses. Plant growth is increased to resist stresses to the very extreme extent and secondary metabolites are produced (Rastegari *et al*., 2020). These secondary metabolites have been known to be the source of insecticidal, immunosuppressive, anticancer, antidiabetic and biocontrol compounds (Wekesa *et al*.,

2022) and are key solution to the health of humans, animals and plants (Yadav *et al*., 2020). Plant hosts benefit mostly by asymptomatic relationships, nonetheless, rare cases may exhibit pathogenic effects (Neubert *et al*; 2006).

Biofertilizers formulate from the living microbes and are vital sources of important nutrients for plants' growth and development. Apart from being environmental friendly and inexpensive, they enhance soil fertility and hence crop productivity (Kour *et al*., 2020). Fungal biofertilizers improve the plants' growth and development by improving the uptake of phosphorus. The most common fungi with phosphate solubilizing ability are *Candida montana*, *Trichosporon beigelii*, *Cryptococcus luteolus*, *Kluyveromyces walti*, *Zygoascus hellenicus*, *Rhodotorula aurantiaca*, *Penicillium purpurogenum var. rubrisclerotium* and *Saccharomycopsis schoenii* (Gizaw *et al*., 2017). *Aspergillus*, *Fusarium*, *Penicillium sp*. are also found in the rhizosphere of numerous plants while the genera of *Aspergillus*, *Penicillium* and *Chaetomium* are widespread (Yadav *et al*., 2018). Rhizosphere occupied with *Trichoderma species* can parasitize with other species of fungi. These species increase plant productivity, improve crop nutrition, nutrient acquirement and are generally engaged for the production of bio fertilizer which is primarily present in agricultural soils. Moreover, the metabolites produced by *Trichoderma species* work as a fungicide against disease causing fungal pathogens (Harman *et al*., 2008).

Penicillium species are extensively spread in nature through various soil environments such as cultivated, forest or dessert soil (Chandanie *et al*., 2006). Plant hormones such as indole-3- acetic acid (IAA), cytokinin and gibberellins (GA) are secreted by *Penicillium* fungus, thus promoting plant growth and are also involved in phosphate solubilization (Radhakrishnan *et al*., 2013). They also secrete antibiotics, insecticides, herbicides, anticancer compounds, antioxidants, extracellular enzymes and mycotoxins (Munns and Tester 2008).

The utmost famous plant–microbe interaction is the mutualism between arbuscular mycorrhizal fungi and host plants (Verma *et al*., 2017), yet, plants form associations with

endophytic fungi under natural occurences (Dastogeer and Wylie, 2017). Plants and microbes interaction has a key influence on the function of the plant and their community ecology (Vimal *et al*., 2017). *Ralstonia solanacearum* are microbial pathogens and they cause the clogging in the veins of the plants resulting to wilting of the plants while *Rhizoctonia spp.*and *Fusarium spp*. causes root rot which leads to death of seedlings (Naveen and Reddy 2023).The existence of AMF in the rhizospheric soil does not only benefits the host plants but also prevents the plants from detrimental effects due to the harmful fungi. Therefore molecular characterization of fungal isolates alongside mycorrhizal fungi is of major interest for the resolve of sustainable agriculture.

4.2 Materials and Methods

4.2.1 Study Area and Sample Collections

Soil samples were collected from Mwea, Mitunguu and Juja areas from the rhizosphere of papaya, grass and banana plants. Random sampling method was used to collect soil samples from different points of the farms. The collection area was registered by the global positioning system (GPS) for documentation, publication and recollection if necessary. The samples were then packaged in sterile bags and stored in cool box before transporting to Institute for Biotechnology Research (IBR) at JKUAT for further analysis.

4.2.2 Isolation of Mycorrhizal Fungi Spores from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas

Arbuscular Mycorrhiza Fungal spores were isolated from 50 g air-dried soil samples from every plant and site, by wet sieving method as described by Boyno *et al.*, (2023) with a few modifications. In brief, spores were obtained by wet sieving and decanting techniques. Approximately 250 ml of soil was suspended in ratio 1:1 of water. Heavier particles were allowed to settle for a few seconds and the liquid was decanted through a 2mm sieve. The suspension was sieved and stirred to re-suspend all particles and decanted through 5µm

sieve to obtain pure spores. The spores were then cultured on modified Potato Dextrose Agar (PDA), Himedia and incubated at 30 °C for 5 days.

4.2.3 Morphological Characterization of Isolated Fungi from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas

Fungal cultures were grown on PDA plates for 7 days at 25°C. Glass slides and cover slips were cleaned using 95% ethanol prior to application of a drop of lactophenol cotton blue onto the cleaned microscope slide. Then, a tiny pinch, about of the fungus taken from the culture plate was placed onto the dye, spread using the needle, and covered with the cleaned cover slip. Then, it was examined under the microscope, 100x magnification, and the pictures were taken (Lange and Grell, 2014).

4.2.4 Physiochemical Characterization of Fungi from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas

4.2.4.1 Growth at Different Sodium Chloride Concentrations

The fungal isolates were cultured on PDA- Himedia at various NaCl concentrations per liter (0.1 M, 0.5 M, 1.0 M, 1.5 M, and 2.0 M NaCl), according to the manufacturer and incubated at 30 °C. The growth of the fungal isolates were then observed after 7 days and recorded as either excellent (++++) average (+++), satisfactory growth (++), minimum growth (+) and no growth (-).

4.2.4.2 Growth at Various Temperatures

The fungal isolates were cultured on PDA- Himedia at varying temperatures of 20°C, 25°C, 30°C, 35°C and 40°C and 50°C (Barcenas-Moreno and Baath, 2009) and incubated at the various temperatures for 7 days. The growth of the fungal isolates was then observed and recorded as either excellent (++++) average (+++), satisfactory growth (++), minimum growth (+) and no growth (-).

4.2.4.3 Effect of pH on the Growth of the Isolates

The fungal isolates were cultured on PDA media at a varying pH of 5.0, 7.0, 8.5, and 10.0, (Rousk *et al*., 2010). The plates were incubated at 30 °C for 7 days. The growth of the fungal isolates was then observed and recorded as either excellent (++++) average (+++), satisfactory growth (++), minimum growth (+) and no growth (-).

4.2.5 Molecular Identification of Isolated Fungi from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas

4.2.5.1 DNA Extraction of Fungal Isolates

Genomic DNA of the fungal isolate was done according to the CTAB protocol described by Qadri (2013). The fungal mycelia were freeze-dried, and the cells were lysed in 10 ml of extraction buffer (CTAB). Afterward, the lysate was extracted by adding an equal volume of isopentanol/chloroform (1: 24), followed by centrifugation at 10,000 rpm for 10 min at 4°C, and the genomic DNA was precipitated from the aqueous phase in chilled isopropanol by centrifugation at 10000 rpm for 10 min at 4°C.

4.2.5.2 PCR Amplification of ITS and AML Genes

To amplify the ITS and AML genes, genomic DNA from from the fungal spores was used as a template. A pair of AML1 (5'-ATC AAC TTT CGA TGG TAGGAT AGA-3') and AML2 (5'-GAA CCC AAA CAC TTT GGT TTC C-3') (Lee *et al*., 2008) was used. The ITS4 and ITS5 regions of the genomic DNA were PCR amplified using universal ITS primers, ITS4 (5′TCCTCCGCTTATTGATATGC3′) and ITS5 (5′GGAAGTAAAAGTCGTAACAA3′). The amplification was performed using Peqlab Primus 96 PCR equipment. It was amplified in a 40 µL mixture comprising 20 µL of Master mix, 18.2 µL of PCR water, 0.4 µL of AML1/ITS4 forward primer, 0.4 µL of AML2/ITS5 reverse primer, and 1 µL of template DNA (100 ng/L DNA). The following temperature cycling profiles were applied for the reaction mixtures: A 10 min enzyme activation at 96 °C for a single cycle, which was followed by 35 cycles of 45s (seconds)

of denaturation at 95 °C, 45 s of primer annealing at 53 °C, 1 min of the chain of elongation at 72 °C, and 10 min of the chain of final extension at 72 °C. The presence and size of PCR amplicons were verified on 1.2% agarose gel and visualized under U.V. light.

4.2.5.3 Purification of PCR Amplicons

PCR amplicons were purified using the QIAquick PCR amplification kit protocol (Qiagen) according to manufacturer instructions. The PCR amplicon was sent to Macrogen Europe (Amsterdam, Netherlands) for sequencing.

4.2.5.4 Editing and Phylogenetic Analyses

The sequences were edited using ChromasPro 2.5 to trim low quality sequences. PCR amplicons (30µl) were submitted to Inqaba Biotech for Sanger sequencing. Results were analyzed using Chromas version 2.6.6. Consensus sequences were formed using Bioedit software. Nucleotide BLAST performed on NCBI where after a FASTA file was compiled using sequences with highest similarity percentage. Multiple sequence Alignment was done using CLUSTAL W in cooperated in MEGA 11 software and T92+G was found to have the lowest Akaike information criterion (AIC) and the Bayesian information criterion (BIC**)** value. A Phylogenetic tree was constructed using Maximum likelihood statistical method and ran at 1000 Bootstrap value on Tamura 3-parameter model (Tamura *et al*., 2007).

4.3 Data Analysis

Soil samples were replicated three times per plant and region. A total of 30 fungal isolates were visually characterized based on their growth at different pH, sodium chloride, and temperatures. The growth of the fungal isolates was recorded as either excellent growth (++++), average growth (+++), satisfactory growth (++), minimum growth (+), or no growth (-).The data from morphological and physiochemical characterization of the isolates were analyzed using minitab software.

4.4 Results

4.4.1 Morphological Characterization of Fungi from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas

In this study, a total of 30 fungi were isolated from the selected soil samples. The colony morphology characteristics included forms ranging from circular to rhizoid, as well as various elevations which ranged from flat, raised, umbonate, and convex (Figure 4.1, table 4.1). The isolates also exhibited differences in their margins, ranging from entire, undulate, filamentous, lobate and filiform. The color of the isolates ranged from white to black, cream-white, yellow. Their sizes ranged from small to medium and large. The surface ranged from smooth, filiform, dull, glistening, rough and wrinkle and lastly the opacity were all opaque (Figure 4.2).

The principal component analysis (PCA) of the morphological characteristics included in the PCA 1 and 2 showed varied correlation. There was diversity in terms of the color compared to opacity and form and other morphological characteristics. The margin, surface, size and elevation were clustered together (Figure 4.3).

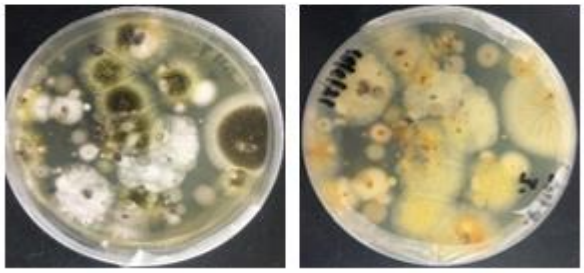


Figure 4.1: Unpurified Cultures of the Fungal Spores from the Rhizospheric Soil S4=Mitunguu, Papaya S7= Juja Papaya

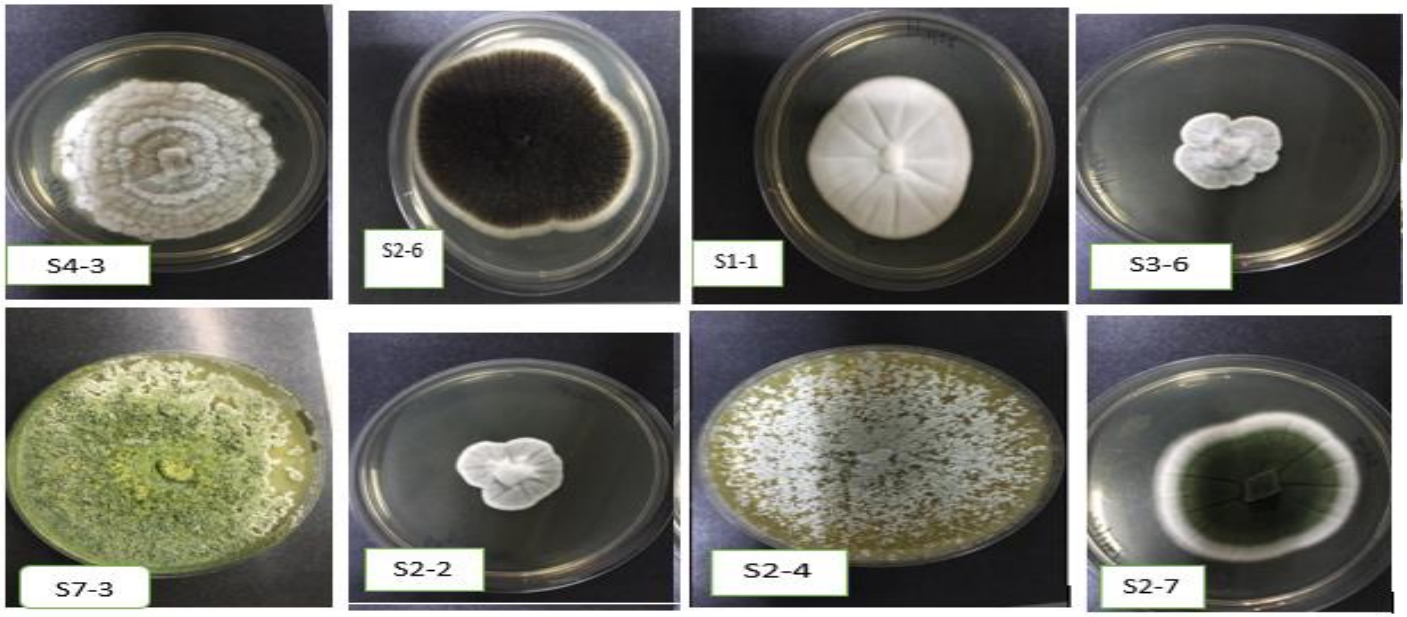


Figure 4.2: Morphology of Selected Fungi Isolated from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas

Key: S1 =Mwea Papaya, S2=Mwea Banana, s3= Mwea Grass, S4 =Mitunguu Papaya, S7 =Juja Papaya

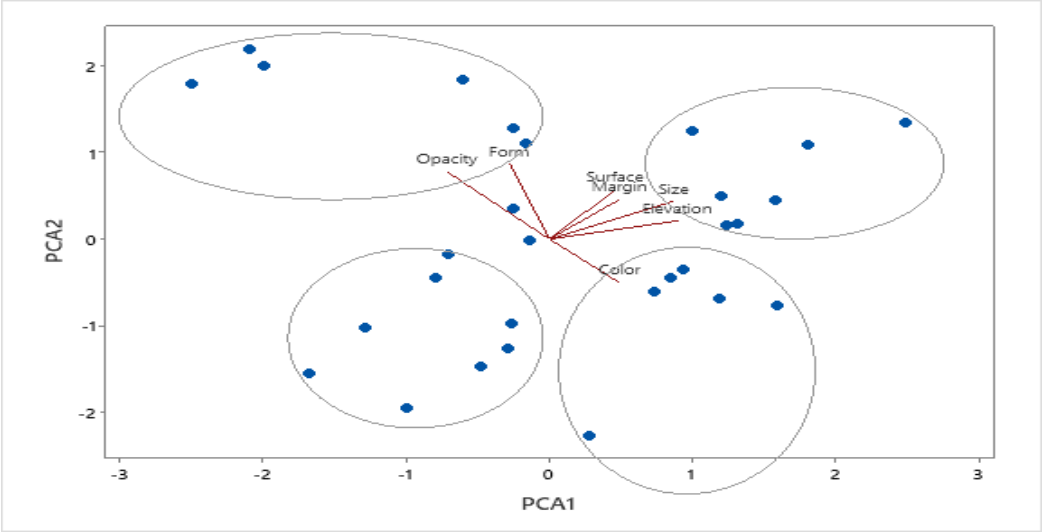


Figure 4.3: Principal Component Analysis of The Morphological Characteristics of the Fungal Isolates from The Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas

Table 4.1: The Morphological Characterization of the Fungi Isolated from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas

	Colony morphology						
Isolate	**Form**	**Elevation**	**Margin**	**Size**	**Color**	**Surface**	**Opacity**
S1-1	Circular	Flat	Entire	Medium	White	Smooth	Opaque
S1-2	Irregular	Raised	Undulate	Small	Black	Filiform	Opaque
S1-3	Circular	Flat	filamentous	Large	Yellow	Dull	Opaque
S1-5	Circular	Flat	Entire	Medium	White	Smooth	Opaque
S1-7	Filamentous	Flat	Lobate	Medium	White	Dull	Opaque
S1-8	Circular	Flat	Entire	Medium	White	Glistening	Opaque
S2-1	Rhizoid	Flat	Entire	Large	Black	Glistening	Opaque
S2-2	Rhizoid	Raised	Entire	Medium	Cream	Smooth	Opaque
S2-3	Circular	Raised	Entire	Small	White	Smooth	Opaque
S2-4	Irregular	Flat	Entire	Medium	Cream-	Smooth	Opaque
S2-5	Irregular	Umbonate	Undulate	Small	white	Smooth	Opaque
S2-6	Irregular	Flat	Filiform	Medium	Cream	Rough	Opaque
S2-7	Rhizoid	Flat	Filiform	Small	Black	Dull	Opaque
S3-1	Irregular	Raised	Lobate	Large	Black	Dull	Opaque
S3-3	Rhizoid	Raised	Lobate	Small	Black	Rough	Opaque
S3-4	Irregular	Raised	Lobate	Small	Cream	Rough	Opaque
S3-6	Irregular	Raised	Filiform	Small	Cream	Rough	Opaque
S4-2	Irregular	Raised	Entire	Medium	Cream	Dull	Opaque
S4-4	Rhizoid	Raised	Undulate	Medium	Cream	Dull	Opaque
S4-3	Irregular	Raised	Undulate	Small	Black	Wrinkle	Opaque
S5-2	Irregular	Umbonate	Lobate	Small	Cream	Wrinkle	Opaque
S5-1	Irregular	Convex	Entire	Large	Black	Wrinkle	Opaque
S6-2	Irregular	Raised	Entire	Medium	White	Wrinkle	Opaque
S6-1	Irregular	Raised	Lobate	Small	Black	Smooth	Opaque
S7-7	Circular	Raised	Lobate	Large	Cream	Smooth	Opaque
S7-3	Circular	Raised	Entire	Medium	Cream	Dull	Opaque
S7-4	Rhizoid	Flat	Entire	Small	Green	Glistening	Opaque
S8-4	Irregular	Flat	Filamentous	Medium	Black	Glistening	Opaque
S9-2	Irregular	Raised	Filamentous	Small	Black	Dull	Opaque
S10-3	Circular	Flat	Entire	Medium	Cream	Rough	Opaque

S1 =Mwea papaya, S2=Mwea banana, S3= Mwea grass, S4 =Mitunguu papaya, S5=Mitunguu banana, S6= Mitunguu grass, S7 =Juja papaya, S8= Juja banana, S9= Juja grass, S10 = Control (non rhizospheric soil).

4.4.2 Growth at different Sodium Chloride (NaCl) Concentration

The fungal isolates showed varied growth at different concentrations of NaCl. The salt concentration of 0.1 M and 0.5 M recorded the highest growth across all the isolates and the growth trend decreased with an increase in NaCl concentration (Table 4.2).

Table 4.2: The Growth of Isolated Fungi from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas, at Different Concentration of Sodium Chloride Salt

Isolate	0.1	0.5	1.0	1.5	2.0
S1-1	+++	++	+	+	-
S1-2	++	++	++	++	+
S1-3	+	++	+	+	-
S1-5	++	+++	+	-	-
S1-7	+++	++	+	+	-
S1-8	++	++	+	+	+
S2-1	+++	+	+	++	+
S2-2	+	++	+	+	-
S2-3	++	++	-	-	-
S2-4	++	++	+	++	+
S2-5	+++	+++	++	-	-
S2-6	++	++	+	+	-
S2-7	+	+++	-	-	-
S3-1	++	++	-	-	-
S3-3	++	+++	+	+	-
S3-4	+++	++	+	-	-
S3-6	++	++	++	+	+
S4-2	++	++	+	++	+
S4-4	+	+++	++	+	-
S4-3	+++	++	+	+	-
S5-2	++	++	+	+	+
S5-1	++	++	+	+	-
S6-2	++	+++	++	+	+
S6-1	++	+	+	+	-
S7-7	+++	++	+	+	-
S7-3	++	+	+	-	-
S7-4	+++	+	+	+	-
S8-4	++	+++	++	+	-
S9-2	+++	+	+	-	-
S10-3	++	++	++	+	+

The growth at varied salt concentration: ++++, excellent growth, +++ average growth, ++ satisfactory growth, + minimum growth and – no growth

S1 =Mwea papaya, S2=Mwea banana, S3= Mwea grass, S4 =Mitunguu papaya, S5=Mitunguu banana, S6= Mitunguu grass, S7 =Juja papaya, S8= Juja banana, S9= Juja grass, S10 = Control (non rhizospheric soil).

4.4.3 Growth of Isolated Fungi from The Rhizospheric Soil Of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas at Different Temperatures

Growth of fungal isolates at different temperatures is as shown in table 4. 3. The isolates were grown at temperature range of 20 °C- 50 °C. The optimum growth was recorded at temperature range of 30 °C to 35 °C. The lowest growth was observed at temperature 20 °C and 50 °C.

Table 4.3: Growth of Fungal Isolates Obtained from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas, at Different Temperature

Isolate	20 °C	30 °C	35 °C	40 °C	45 °C	50 °C
S1-1	-	++	++	+	-	-
S1-2	-	+++	+++	++	+	+
S1-3	-	+	++	+	-	-
S1-5	-	++	+	-	+	-
S1-7	+	+++	++	+	-	-
S1-8	+	++	+	+	+	-
S2-1	+	+++	++	++	+	-
S2-2	-	++	++	+	-	-
S2-3	+	++	+	-	-	-
S2-4	-	+++	++	++	+	-
S2-5	-	+++	+	-	-	-
S2-6	-	++	++	+	-	-
S2-7	-	++	+++	++	+	+
S3-1	-	++	+	-	-	-
S3-3	-	++	++	+	-	-
S3-4	+	+++	+	-	-	-
S3-6	+	+++	++	+	+	+
S4-2	+	+++	++	++	+	-
S4-4	-	++	++	+	-	-
S4-3	-	++	++	+	+	+
S5-2	+	+	++	++	+	-
S5-1	-	+++	++	+	-	-
S6-2	+	++	+	+	+	+
S6-1	-	++	+	+	-	-
S7-7	-	++	++	+	+	+
S7-3	-	++	+	-	-	-
S7-4	-	+	++	+	-	-
S8-4	-	++	++	+	-	+
S9-2	-	++	+	-	-	-
S10-3	-	+++	++	+	+	-

S1 =Mwea papaya, S2=Mwea banana, S3= Mwea grass, S4 =Mitunguu papaya, S5=Mitunguu banana, S6= Mitunguu grass, S7 =Juja papaya, S8= Juja banana, S9= Juja grass, S10 = Control (non rhizospheric soil). The growth rate at varied temperature: ++++, excellent growth, +++ average growth, ++ satisfactory growth, + minimum growth and – no growth

4.4.4 Growth of Fungal Isolates Obtained from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas at Different pH

The isolates had been obtained from normal pH environment of between 5.5 and 7.0, but they could grow at pH 8.5 and 10.0. The optimum growth was recorded at pH 7.0 with most of the isolates having average growth compared to pH 5.0, pH 8.5 and pH 10.0. The growth trend was lower at pH 5.0, increased at pH 7.0, and decreased from pH 8.5 to 10.0 (Table 4. 4).

Table 4.4: Growth of Fungal Isolates Obtained from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas at Varied pH Conditions

Isolate	pH5.0	pH7.0	pH8.5	pH10.0
S1-1	-	++	++	+
S1-2	+	+++	++	++
S1-3	-	+	-	-
S1-5	-	++	+	-
S1-7	++	+++	++	+
S1-8	+	+	-	+
S2-1	+	+	+	+
S2-2	++	++	+	-
S2-3	+	+	-	-
S2-4	-	++	+	+
S2-5	-	+++	-	-
S2-6	-	+	+	-
S2-7	-	+	++	+
S3-1	+	+	-	-
S3-3	-	+	+	+
S3-4	+	+	++	-
S3-6	+	++	-	-
S4-2	++	+++	+	+
S4-4	-	++	-	-
S4-3	-	+	+	+
S5-2	+	++	+	+
S5-1	-	++	+	+
S6-2	++	+++	++	++
S6-1	-	+++	-	+
S7-7	++	+++	+	-
S7-3	-	+	-	-
S7-4	-	++	++	+
S8-4	+	+	+	-
S9-2	-	+++	-	+
S10-3	+	++	+	-

S1 =Mwea papaya, S2=Mwea banana, S3= Mwea grass, S4 =Mitunguu papaya, S5=Mitunguu banana, S6= Mitunguu grass, S7 =Juja papaya, S8= Juja banana, S9= Juja grass, S10 = Control (non rhizospheric soil). The growth rate at varied pH: ++++, excellent growth, +++ average growth, ++ satisfactory growth, + minimum growth and – no growth.

4.4.5 Molecular Characterization of Fungal Isolates Obtained from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas

4.4.5.1 DNA Extraction of Fungal Isolates Obtained from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas

Genomic DNA was extracted from all the selected fungal isolates. All the bands were linear and approximate to the well of the loading.

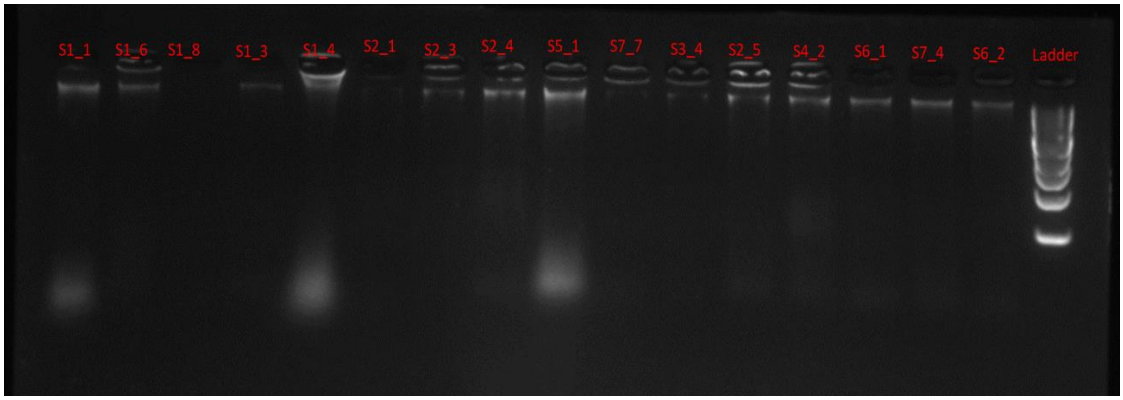


Figure 4.4: Genomic DNA Extracted from Fungal Isolates Obtained from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas

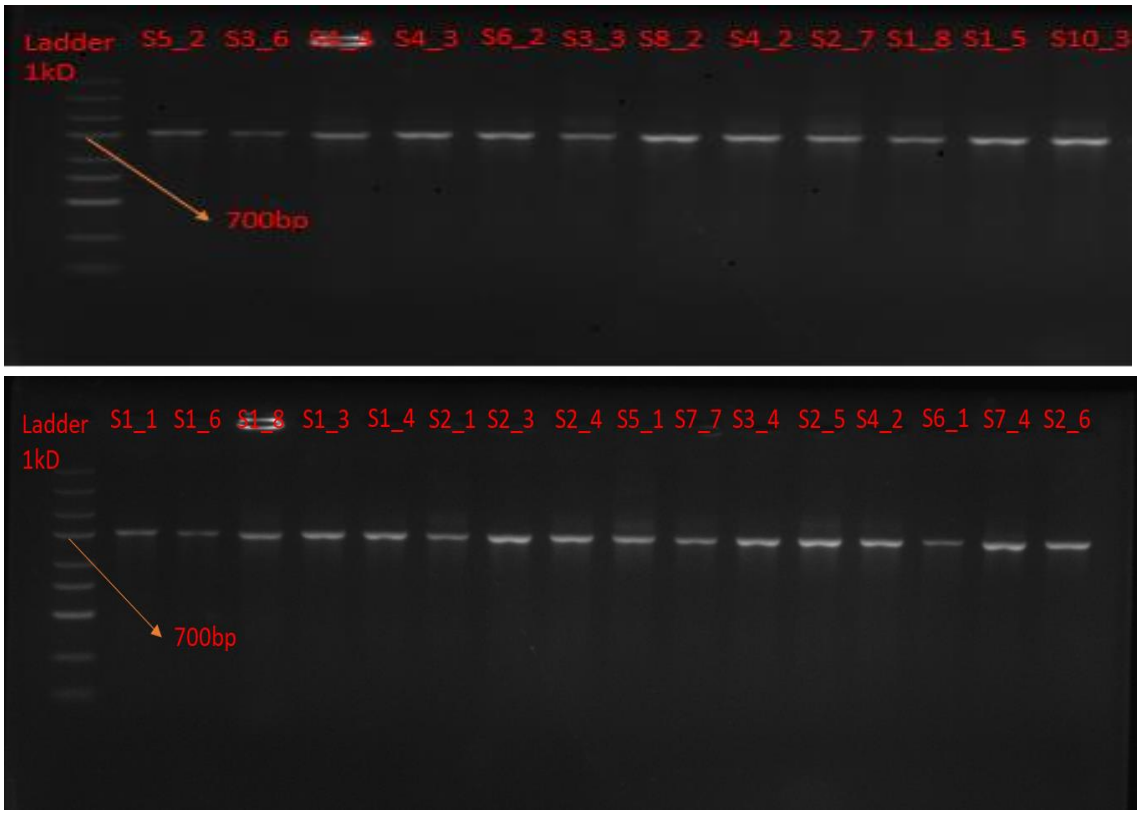


Figure 4.5: 1% Agarose Gel Showing the Size of the PCR Amplicons of Fungal Isolates Obtained from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas as Visualized after Adding Ethidium Bromide Stain

4.4.5.2 Phylogenetic Analysis of Fungal Isolates Obtained from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas

From the partial sequence, ten isolates from AMF species were analysed and among them were *Glomus etunicatum, Diversispora varaderana, Diversispora gibbose, Diversispora peridiata, Glomus clarum and Glomus lamellosum* (Figure 4.4). Three isolates (10.7%) belonged to *Aspergillus genus* with a 99.74% to 100% similarity index. Among the aspergillus group were *Aspergillus niger* and *Aspergillus cervinus*. One isolate (3.5%) was obtained from *Ajellomyces* with similarity index of 100%. *Fusarium* genera were also isolated with similarity index of 99.65% to 100% (Figure 4.6). They include *Fusarium*

solani, *Fusarium oxysporum* and other *Fusarium spp*. Four isolates (14.3%) belonging to *Trichoderma* were isolated with a similarity percentage identity of 100%. They included *Trichoderma harzianum*, *Trichoderma virens* (2) and *Trichoderma atroviride*. Lastly six isolates (21.4%) from the penicillium genus were also isolated. The isolates had percentage identity of 99.30% to 100%. They include *Penicillium brasilianum*, *Penicillium onobense, Penicillium aethiopicum and Penicillium brocae*. No novel isolates were identified since all the isolates had a similarity percentage of above 98% with the reference sequences from the National Centre for Biotechnology Information (NCBI) database (Table 4.5).

Table 4.5: Phylogenetic Analysis of Fungal Isolates Obtained from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas

Isolate code	Max Score	Total Score	Query Coverage	Acc No.	Next Neighbor in Blast	%ID
S1_1	909	909	100%	AB030916.1	*Aspergillus niger*	100%
S1_6	2198	2198	100%	AF038353.1	*Ajellomyces capsulatus strain UAMH 7141*	100%
S1_8	2800	2800	100%	MZ314133.1	*Aspergillus sp. isolate 7F2*	99.74%
S1_3	1471	1471	100%	OM533682.1	*Fusarium solani strain LZ09-07*	100%
S1_4	1038	1038	100%	EU750681.1	*Fusarium sp. 14012*	99.65%
S2_1	1092	1092	100%	GU048879.1	*Fusarium oxysporum isolate CAFO-IHBT*	100%
S2_3	481	481	100%	LC534254.1	*Fusarium sp. NBRC*	100%
S2_4	1112	1112	100%	MT509807.1	*Trichoderma harzianum strain KSRCT-BT-MS2*	100%
S5_1	1020	1020	100%	MT529862.1	*Trichoderma virens clone SF_586*	100%
S7_7	1020	1020	100%	MT529862.1	*Trichoderma virens clone SF_586*	100%
S3_4	1014	1014	100%	MT514373.1	*Trichoderma atroviride strain LSK_7*	100%
S2_5	1088	1088	99%	MK450675.1	*Penicillium brasilianum strain CMV002C3*	99.83%
S4_2	1554	1554	99%	MK450706.1	*Penicillium onobense strain CMV006B5*	99.30%
S6_1	798	798	100%	AY495983.1	*Penicillium aethiopicum strain CBS*	100.00%
S7_4	784	784	100%	AY495984.1	*Penicillium aethiopicum strain CBS 270.97*	100.00%
S2_6	1814	1814	100%	JF909940.1	*Penicillium aethiopicum strain CBS 484.84*	100.00%
S5_2	965	965	100%	KX253944.1	*Penicillium brocae strain S4*	99.81%
S3_6	1000	1000	100%	FN547624.1	*Glomus etunicatum*	99.64%
S4_4	1007	1007	100%	FN547624.1	*Glomus etunicatum*	99.82%
S4_3	2756	2756	99%	KT444711.1	*Diversispora varaderana isolate 7*	98.90%
S6_2	2756	2756	99%	KT444711.1	*Diversispora varaderana isolate 7*	98.90%
S3_3	1205	1205	99%	MG459189.1	*Diversispora gibbosa isolate 3-1*	99.25%
S8_4	1282	1282	100%	MG459195.1	*Diversispora peridiata isolate 5-1*	100.00%
S9_2	928	928	100%	GQ205083.1	*Glomus clarum strain DAOM 234281*	100.00%
S2_7	902	902	100%	GQ205080.1	*Glomus clarum strain DAOM 234281*	99.20%
S1_8	1051	1051	100%	JF951426.1	*Glomus lamellosum*	100.00%
S1_5	1055	1055	100%	JF951415.1	*Glomus lamellosum*	100.00%
S10_3	1068	1068	100%	AJ874118.1	*Aspergillus cervinus*	100%

S1 =Mwea papaya, S2=Mwea banana, S3= Mwea grass, S4 =Mitunguu papaya, S5=Mitunguu banana, S6= Mitunguu grass, S7 =Juja papaya, S8= Juja banana, S9= Juja grass, S10 = Control (non rhizospheric soil)

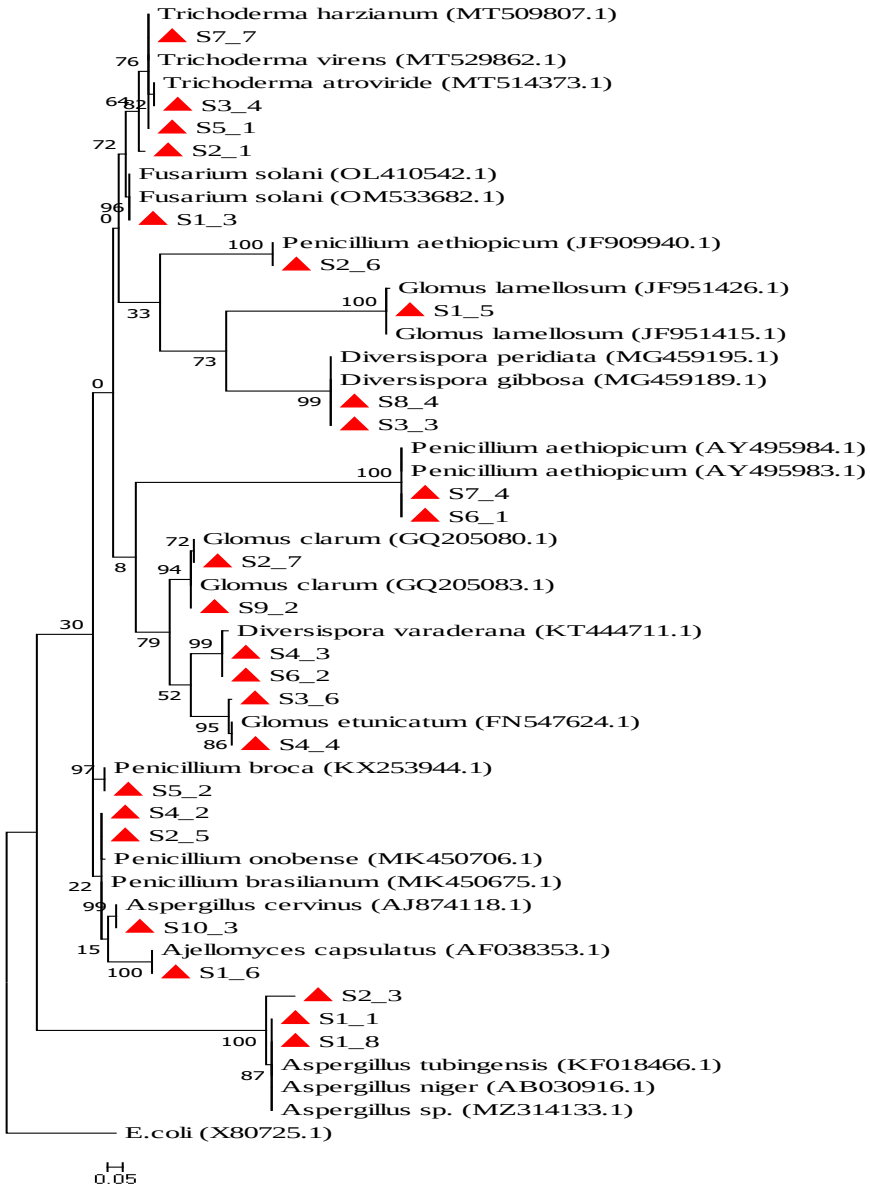


Figure 4.6: Phylogenetic Tree of Fungal Isolates Obtained from the Rhizospheric Soil of Papaya, Grass and Banana Plants from Mwea, Mitunguu and Juja Areas; Based on ITS, AML1 and AML2 sequences

Key: S1 =Mwea papaya, S2=Mwea banana, S3= Mwea grass, S4 =Mitunguu papaya, S5=Mitunguu banana, S6= Mitunguu grass, S7 =Juja papaya, S8= Juja banana, S9= Juja grass, S10 = Control (non rhizospheric soil)

4.5 Discussion

In this study, the blast analysis showed ten isolates where arbuscular mycorrhizae fungi, the main focus of the study, had a percentage identity between 98.90% and 100%. Among the isolates in this study, phylum glomeromycota, which contain all known arbuscular mycorrhizal fungi had a percentage identity between 98.90% and 100%. It contained 10 isolates as compared to ascomycota phylum which had 18 isolates comprising of *Aspergillus spp., Ajellomyces spp, Fusarium spp., Trichoderma spp. and Penicillium spp.* Further, most of the isolates had an average growth at pH 7.0 while at lower and higher pH (5.0, 8.5 and 10.0) the growth of the isolates reduced. This study also revealed that despite obtaining the isolates from temperatures ranging between 25°C and 30°C, they could grow at temperature range of 30 °C to 50 °C in the cultured PDA. However, lower growth was recorded at very low temperatures of 20°C and high temperatures of 50°C. The genomic DNA is heavy and therefore cannot move further, hence approximate to the well of loading. Moreover, all the genomic DNA from the fungal isolates had similar size and this explains why they are linear.

Similar research study on fungal isolates characterization conducted by Wang *et al*., (2017), concluded that the abundance of the soil fungi and the fungal communities differed between the bulk soils and the rhizospheric soils. The fungal phylum that dominated all the samples of the soil, from the highest, were Ascomycota, 68.7%, Zygomycota, 13.3% and Basidiomycota, 4.1%. Roots normally release organic composites due to variation of rhizospheric fungal groups which is dependent on plant hence an exceptional nutrient pool is created on the rhizosphere and is reachable to soil micro-organisms (Han *et al*., 2016). In a different study, *Penicillium spp.* (*P. raistrickii P. funiculosum P. janthinellum* and *P. erythromellis*) and *Trichoderma spp.,* (*T. pseudokoningii and T.konengii*) subjugated the rhizospheric area of established tea plants (Pandey *et al*., 2001). Majority of the fungal isolates accompanying the tea rhizospheric area displayed an extensive range of temperature and pH tolerance, properties indicating superior adaptation and survival in the rhizospheric area of the soil (Pandey *et al*., 2001). Fungi have been categorized into three groups according to their function. They include the lichens, the saprotrophs and the

mycorrhizas (Aislabie, 2013). Due to their symbiotic roles, fungi are key to the operations of the ecosystems of the terrestrial arctic vegetation, which actively grow close to the ground such as herbs, dwarf shrubs, graminoids and mosses. These plants depends highly on mutualistic relationships with arbuscular mycorrhizal fungi for their survival in these harsh environments (Bjorbaekmo *et al.,* 2010). Endophytic fungi is also abundant in the roots and some parts above the ground of arctic–alpine plants (Newsham *et al*., 2009).

The texture of the soil greatly affects the organic carbon content and this in turn determines the microbial community on the plant rhizosphere (Singh *et al*., 2007; Wang *et al*., 2009) ;while the enzyme activity of the soil is intensely linked with rhizosphere communities of the fungi (Welc *et al*., 2014). Thus, the chemical and physical properties of the soil interfere with the fungal community found on the rhizosphere (Schappe *et al*., 2017). Arbuscular mycorrhizal fungi species are distributed and vary depending on climate, land use and edaphic environments. *Glomus spp*. are the most widely distributed while *Sclerocystis spp* and *Gigaspora spp*.are commonly found in tropical soils (Singh, 2000). Thus, the variations of the fungal isolates in this study.

In this study, some of the fungi still grew at 20°C and 50°C, indicating survival at harsh temperature conditions. However, the ideal temperature range for most of the fungal isolates was found to be between 30 °C to 50 °C in the cultured PDA. Pandey *et al*., (2001) observed that majority of fungi linked with the tea bushes roots displayed a mesophillic temperature necessity. *Paecilomyces varioti* and *Aspergillus terreus* were able to grow at 50°C while *Penicillium janthinellum* and *Penicillium lanosum* could grow at 5°C. Fungal growth and activities were observed to be higher at temperatures between 25°C and 30°C for both forest-humus agricultural soils whereas the fungal activity decreased rapidly above 40°C (Pietikainen, *et al*., 2005). Interestingly, fungi dominated in high altitude soils at low temperatures (Ley and Schmidt, 2002). The main cause of soil fungistasis, inhibition of fungal growth and germination are soil microorganisms, limited carbon, production of antifungal compounds and fungal community composition (Barcenas-Moreno and Baath, 2009).

This study further revealed that an environment with too much acidity or alkalinity was not ideal for most of the fungal isolates. However, a few of the isolates could still grow at pH of 5.0, 8.5 and 10.0. In a different research study, the soil pH ranged between 4.3 and 6.1 on rhizospheric soils of different tea zones samples while 5.1 to 6.2 on non rhizospheric soils (Chen *et al*., 2006). However, AMF species have been found to be asssociated with certain characteristics of soil; for example *Acaulospora spp*. are better adapted to soils with lower pH of less than 5, that is acidic soils, while *Glomus mosseae* are mostly found in soils with high pH and fine texture while *Gigaspora spp*. are better adapted in sand dune soils (Nayak *et al*., 2019). Nevarez *et al*., (2009) observed that the soil pH determined the composition of the fungal community and this could have been due to the phylogenetic alterations between the fungal communities. Additionally, the pH values between pH 5 and pH 9 did not inhibit the growth of various fungal species. The highest vegetative growth of *Schizophyllum commune*, a basidiomycete bracket fungus, was found to be at optimum pH of 5.5 (Adejoye *et al*., 2007).

Soil microbes, fungi, archaea and bacteria are very important in the ecosystem for their varied and critical roles. Unlike archaea or bacteria, fungi are closer to animals and plants since they are eukarya, that is, their cells encompass membrane bounding nuclei with chromosomes which contain DNA (Aislabie, 2013). Due to their extreme diverse nature, fungi serve various roles in the ecosystem such as mutualists, predators, pathogens, plants endophytes as well as decomposers (Aislabie, 2013). Generally, all microbes can be found all through the soil profile; nonetheless, they are most plentiful in the rhizospheric soils of the plants, and near the macropores (Fierer *et al*., 2007). This study sought to identify and characterize other fungal isolates along with arbuscular mycorrhizal fungi (AMF) isolated from the rhizospheric soils of papaya, grass and banana plants from Mwea, Mitunguu and Juja areas.

Arbuscular mycorrhizal fungi (AMF) display plenty of benefits to the plant hosts. The current research has proved that the AMFs do not rely on their neighbouring fungi to perform their duties since they are more abundant compared to other fungi in the rhizospheric region. However other fungal isolates such as *Trichoderma spp*. have

previously been established as bio-control agents against some plant diseases while *Penicillium spp*. feeds on decaying matter in the ecosystem and are essential for the production of antibiotics, organic acids as well as cheese (Solomon *et al*., 2019). However, the overall benefits of AMF in the soil surpasses the combined importance of other beneficial fungi that coexist with AMF. The existence of *Fusarium spp*. alongside AMF in this study clearly confirms that AMF can fight soil pathogens as demonstrated by Devi *et al*., (2022) who observed the decline in severity of Fusarium wilt of tomato caused by *Fusarium oxysporum*, in a media containing *Glomus fasciculatum* and *Funneliformis mosseae* (AMF species) both in pots and field conditions.

Thus, this study enlightened the knowledge on rhizosphere fungal communities and verified coexistence of AMF with other fungal communities which could be harmful or beneficial to their surrounding. Notably, AMF population was greater compared to individual species of other fungal isolates thereby overcoming most of the adjoining negative effects from other fungi.

CHAPTER FIVE

IMPACT OF AMENDING SOIL WITH ARBUSCULAR MYCORRHIZAL FUNGI ON THE TOLERANCE TO BIOTIC AND ABIOTIC STRESS IN PAPAYA

Abstract

Arbuscular mycorrhizal fungi (AMF) are natural root symbionts. Inoculating plants with AMF provides tolerance against biotic and abiotic stressful situations thus enhancing crop productivity. The quality and yield of papaya plants have declined due to, among other causes, pathogen attack and water stress. The main objective of this study was to determine the efficacy of the isolated indigenous AMF on the management of phytophthora blight and water stress on Malkia and JKUAT papaya hybrids. Four treatments were used; compost manure, AMF inoculum, a combination of AMF and compost manure and control. Treatments were set up in a completely randomized design. Plants were subjected to water stress 4 weeks after transplanting. Papaya leaves with phytophthora blight disease were harvested from existing orchards in Juja, Mwea and Mitunguu areas and were used to obtain the pathogen inoculum, which was sprayed on the underside of some of the papaya plants' leaves. The plants that were sprayed with the pathogen inoculum were labelled as "infected plants" while the papaya plants which were not sprayed with the pathogen inoculum the "non- infected plants". Growth parameters and grading were assessed over a period of twenty weeks while chlorophyll contents, carotenoids and secondary defense subjected to analysis of variance at a significant level $p \leq 0.05$ using the GenStat 15th edition statistical package. Significantly different means were separated using the Tukey's HSD test. The height of the non-infected JKUAT hybrid with AMF and manure treatment was 60.57 cm while the infected one was 31.17 cm at 20 weeks. Two weeks after pathogen inoculation, infected JKUAT hybrid with manure treatment only had carotenoids content of 0.6mg/100g while the non infected ones had 1.5mg/100g. Non- infected JKUAT hybrids with both AMF and manure treatments had carotenoids contents of 2.2mg/100g. Defense metabolites (PAL, POD, PPO and

phenolics) significantly increased ($p \leq 0.05$) in infected plants more than non- infected plants. Infected malkia hybrid with manure treatment only had peroxidase contents of 6.4 changes in absorbance min 1g fresh weight in their roots while non - infected had 5.6 changes in absorbance min 1g fresh weight, two weeks after pathogen inoculation. Infected malkia hybrid with both AMF and manure treatment had peroxidase contents of 1.9 changes in absorbance min 1g fresh weight while non infected had 1.8 changes in absorbance min 1g fresh weight; two weeks after pathogen inoculation. Water stress affected papaya plants without AMF inoculum significantly ($p \leq 0.05$). At 20 weeks of growth, both JKUAT and malkia papaya hybrids with control and compost manure treatments, had more than 60% lesions on all parts of the plant, while only 10% lesions were recorded for plants with a combination of AMF and manure treatments. Treatment of plants with AMF combined with manure improved the performance of papaya plants infected with phytophthora blight. Although the growth rate and general performance of papaya plants under water stress appeared to decline compared to the control, addition of AMF inoculum revived them and at 20 weeks of growth, the plants with both AMF and manure treatments were still flourishing. Papaya plantlets established with AMF inoculum will thrive well under deprived water conditions and more so, a combination of both AMF and manure will prolong their lifetime.

5.1 Introduction

Papaya is among the main fruit crops grown in both tropical and sub-tropical regions and is a semi-woody fast-growing herb (Chan and Theo, 2000). Papaya is utilized in various forms such as desserts, pickles and jams while the unripe fruit can be fermented into sauerkraut, candied or cooked as a vegetable. Further research has showed that the fruit and seeds have antihelminthic and anti-amoebic activities (Okeniyi *et al.,* 2007) and the papaya latex tenderizes meat and can be used to treat burns or gangrenous wounds (Hewitt *et al.,* 2000). In Kenya, the value of papaya in 2020 was Ksh. 4.13 billion which was a 0.2% increase from 2019. However, the papaya productivity has been affected by mealy bugs and the papaya ring spot virus (HCD, 2020).

Soil microbes such as AMF play a key role in cycling of major elements such as nitrogen, phosphorous, Sulphur and this inturn leads to organic matter production (Aislabie *et al.,* 2013). These microbes produce cellular debris such as extracellular polysaccharides which is a great benefit in retaining soil structure and soil health. Fungi, archae and bacteria are among the beneficial species that offer support to the growth of the plant through intensified nutrient obtainability and by conquering pathogens attack (Aislabie *et al.,* 2013).

Mycorrhizal fungi form symbiotic associations with living plant roots hence they both mutually benefit such that the plant host receives soil nutrients and other benefits from the fungus while the plant provides carbohydrates to the fungus (Smith and Read, 2008). Arbuscular Mycorrhizal Fungi (AMF) can improve the nutrient uptake of plants and resistance to numerous stress factors (Sun *et al.,* 2018). Arbuscular Mycorrhizal Fungi are prevalent among many crop plants and colonize an estimate of 80% of all plant species (Schubler *et al.,* 2001). Gworgwor and Weber (2003), reported that *Glomus mosseae* reduced striga weed mass by 62% in sorghum field and improved the growth and shoot biomass of sorghum by 30%. Trichoderma is a non-mycorrhizal fungi identified for intermediating stress responses in plants. It has the ability to antagonize plant pathogenic fungi by stimulating the resistance response and enhancing the plant growth (Sharma *et al.,* 2019).

Arbuscular Mycorrhizal Fungi improve tolerance to pathogen attack and infections to the plants more so without intensifying on the nutrition of the plant (Borowicz, 2001). The abundance of AMF absolutely correlates with the productivity of plants and the activities within the isolates. Hence, AMF communities that are copious offer superior improvement to the plant host in bearing pathogen attack (Hoeksema *et al.,* 2010).

Growth and development of plants is affected by various stressful factors such as high temperatures, water deficit, flooding and high salinity levels (Rivero *et al*., 2022). Plants under water stress go through metabolic changes such as decrease in photosynthetic pigments absorption and physiological alterations such as reduced leaf area, reduced root:

shoot ratio, decline of the number of leaves, hence reduced transpiration and reduced rate of water absorption (Khalid *et al*., 2019). Moreover, carbon dioxide assimilation in the leaves decreases and the closure of stomata, restricting transmission through stomata and mesophyll. Consequently, Rubisco enzyme which is responsible for carbon dioxide fixation increases in order to overcome the low conductance (Guo *et al*., 2006). Arbuscular mycorrhizal fungi aids in the mitigation of deleterious variations due to water stress (Ruiz-Lozano, 2003). Association with AMF assists the plants to retain leaf water potential in order to prevent loss of turgor and plants inoculated with AMF recuperate more rapidly compared to non-inoculated after the release of water stress. The processes of transpiration and stomatal conductance are altered in AMF inoculated plants as reported in cowpea, lettuce, wheat and soybean crops (Auge, 2001). Hormones play an exceptional role in combating the reactions of plants under water stress conditions. Abscisic acid (ABA) being the main hormone associated with water stress increases in both roots and shoots during such situations (Sharp, 2002). However, ABA has been found to be regulated during water stress conditions in plants inoculated with AMF (Symons *et al*., 2012).

Papaya is vulnerable to various fungal pathogens, however, anthracnose *(Collectricum gloerosporioides*), black spot (*Asperisporium caricae*), powdery mildew (*Oidium caricae*), Phytophthora (*Phytophthora palmivora*) root and fruit rots are the main fungal pathogens (Zhu *et al*., 2004). During the wet season, phytophthora blight is a common disease of papaya, especially in poorly-drained soil, whereby the stem, fruits and roots of papaya plants are attacked. Lower leaves shows initial symptoms as a result of root rot. They turn yellow then wilt before falling prematurely as the upper leaves'color changes to light green, and emerging new leaves are normally smaller in size than healthy plants' leaves (Nelson, 2008).

Pathogen attack leads to fluctuation of plants' growth and consequently, plants produce secondary metabolites that have various functions in response to the changing environment (Berini *et al*., 2018). These defense metabolites are produced by the plants as an adaptive capacity to cope with stressful restraints during challenging growth

environment (Edreva *et al*., 2008). Disease resistance in plants is connected with the activation of various defence responses that inhibit pathogen infection. After pathogen attack, plants protect themselves through the active and passive defense mechanisms. The active defense responses include production of reactive oxygen species (ROS), phytoalexins and pathogenesis-related proteins, as well as reactive nitrogen species (RNS) that is, oxidative bursts while the passive defense mechanisms involve structural barriers which prevent colonization in the tissue. Peroxidases are associated with various vital processes of plant growth such as suberization (protects tissue from water loss and pathogen invasion), cell wall metabolism, lignification, auxin metabolism and reactive oxygen species (ROS) metabolism (Pandey *et al*., 2017).

Phenylalanine ammonia lyase (PAL) is the main enzyme in the phenylpropanoid pathway, which leads to the change of l-phenylalanine into trans-cinnamic acid with ammonia elimination. In addition, PAL is a vital enzyme in the synthesis of defence related secondary compounds such as lignins and phenols (Hemm *et al*., 2004). Polyphenol oxidase (PPO) is a plastid copper-containing enzyme which catalyzes the oxidation of phenols to quinones. PPO participates in plant defence against pathogens and pests. It is involved in noticeable reaction products when the plants are wounded or under pathogen attack (Li and Steffens, 2002). Phenolic compounds comprising of natural secondary metabolites are synthesized in plants through metabolic pathways such as phenylpropanoid pathway and are are used by plants to produce monomeric phenolic compounds such as phenolic acids and flavanoids as well as polymeric phenolic compounds like lignans, tannins, melanins and lignins (Heleno et al., 2015). Phenolic compounds aid in regulating several physiological functions in plants and are also involved in plant defense mechanisms against abiotic and biotic stress conditions (Caputi *et al*., 2012). They help plant to gain resistance against microbial pathogens by inducing position explicit vulnerable response to protect spread of infection (Cheynier *et al*., 2013).

Plants are generally overwhelmed by various pathogens and insects (Ebrahim *et al*., 2011) which has resulted to pesticides application as the main solution of regulating plant diseases (Prasannath *et al*., 2014). However, these pesticides have detrimental impacts on

the environment as well as human health necessitating the use of environmentally friendly method of disease control management such as inducing systemic resistance against plant pathogens (Prasannath and De Costa, 2015). Campostrini *et al*., (2018), reported that papaya plants' growth and fruit production are affected by water stress.The current research study sought to utilize AMF inoculum as a strategy to protect papaya plants and develop resistance against attack by phytophthora blight disease and water deficit effects during the vegetative stage of development.

5.2 Materials and Methods

52.1 Isolation and Bulking of Arbuscular Mycorrhizal Fungi (AMF)

Arbuscular mycorrhizal fungi (AMF) spores were isolated from the sampled soils described in section 3.3.2 above, using the procedure by Boyno *et al*., (2023) with minor modifications. The Sorghum, (*Sorghum bicolor*) plant was grown in 250ml pots that contained sterilized coarse sand mixed with isolated AMF spores. The sorghum seeds were spread on top of the media in the 15 cm pots and placed on a flat surface in the green house. Watering, thinning and weeding were carried out, as required, for four months. The dried shoots were pulled and disposed while the roots and the media were blended together to obtain the AMF inoculum.

5.2.2 Sowing of the Papaya Seeds

The JKUAT and Malkia F1 hybrid seeds were obtained from JKUAT seed bank and SIMLAW Seeds Company limited in Nairobi County, Kenya respectively; and germinated in forest soil and sand media in a ratio of 1:1 in a greenhouse at JKUAT. During transplantation, 4 weeks after seed sowing, 24 plants of each hybrid were treated with AMF inoculum (I), compost manure (C), combination of inoculum and compost manure (IC) and control (soil and sand medium only). The ratio of treatments and soil media used for growing the plants was 1:9. The plants were laid on completely randomized design, with three replications. The temperatures range were 32^{o}C to 38^{o}C

and light intensity range was 400nm to 700nm, 13 h light and 11 h dark. Temperature and light regulation of the greenhouse, weeding and watering of the plants were carried out as necessary.

5.2.3 Isolation and Inoculation of the Fungal Pathogen, *Phytophthora palmivora*

Leaves infected with the fungus (*Phytophthora palmivora*) were randomly selected from an existing orchard of papaya trees for the preparation of inoculum. Inoculum production was based on the Awale, (2019) method, with minor modifications. The synemmata was picked and seeded in sterilized petridishes containing sterilized V8 agar medium before incubation in the dark at room temperature for 3 days. The mycelium had covered at least 90% of the Petri dish. A single colony was harvested and crushed in sterilized 500ml distilled water to release the spores and the suspension of spores was placed on a sterilized plate containing sterilized V8 medium. The plate was sealed and stored in an incubator at 24 ° C for 14 days to allow the pathogen colony to grow.

5.24 Infecting the Papaya Plants with Fungal Pathogen, *Phytophthora palmivora*

The petri dishes containing the mycelia were flooded with sterile water and the mycelia was scraped off the medium to release the spores from the fungus. The suspension was then strained and poured into a beaker containing 500 ml of distilled water and 3 drops of tween 20 were added and thoroughly agitated. At 11 weeks after transplanting, the inoculum was sprayed onto the underside of all the papaya leaves (8 to 10 leaves) for both hybrids and all treatments (AMF, AMF and manure, manure only and the control) with 3 replications. These were the infected plants, while those that were not sprayed became the non-infected plants in this study. The infected plants remained in the greenhouse with temperatures ranging from 25°C to 30°C, relative humidity of 92 to 96% and photoperiod of 13 h light and 11 h dark.

5.2.5 Disease Estimation on the Papaya Plants

Disease severity was visually monitored in leaves inoculated with phytophthora blight spores between 11 weeks and 13 weeks after transplanting and were estimated according to Henfling (1987) disease estimation scale; the 0–9 scale (Table 5.1).

Table 5.1: Disease Estimation Scale Based on Lesion Percentage on the Whole of Papaya Plants

Scale	0	1	3	5	7	9
Lesion %	0%	10%	10% and 20%	20% and 30%	30% and 60%	>60%
lesion area of the whole plant	No disease	<10%.	Between 10% and 20%	Between 20% and 30%	Between 30% and 60%	>60%

5.2.6 Water Stress Estimation

Papaya plantlets from both Malkia F1 and JKUAT hybrids and with all the treaments were deprived of water from 4 weeks after transplanting. However, equal amounts of water was added in very minimal amounts every two weeks for continuous physiological processes within the plants. Water stress symptoms was visually monitored on the whole plant between 4 weeks and 20 weeks after transplanting and were estimated according to Henfling (1987) estimation scale; the 0–9 scale (Table 5.1). Data collected included stem girth circumference, height of the plants, number of leaves and grading of the whole plant.

5.27 Determination of Defense-Related Enzymes

Papaya leaves were sampled for the evaluation of defense enzymes for phytophthora blight one and two weeks after pathogen inoculation. One gram of papaya leaf samples was homogenized in 1.5 ml of 50 mM Tris HCl buffer (pH 7.5) at 4 ° C in liquid nitrogen and centrifuged at 18,000 rpm for 20 minutes. The resulting supernatant was collected in sterilized 2 ml Eppendorf tubes and stored in a deep freezer (− 20 ^{o}C) for further use as a

crude enzyme extract. This enzyme extract was used for the peroxidase (POD), phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) assay.

Phenylalanine ammonia-lyase (PAL) activity was estimated based on the production of trans-cinnamic acid (Gerbore, *et al*., 2014). Enzyme activity was expressed as µg cinnamic acid h - 1g - 1 fresh plant weight. PPO activity was tested by measuring the change in the intensity of the colour of catechol oxidation products (Asaka, *et al*., 1996). The activity of the PPO enzyme was expressed as a change in absorbance at 495 nm per min 1 per g 1 of fresh plant weight. For the POD assay, 0.5 ml of crude enzyme extract was taken in a cuvette, and subsequently, 0.5 ml of 1% guaiacol solution and 1.5 ml of 50 mM Tris buffer (pH 7.5) were added. The reaction was then started by adding 0.5 ml of 1% H_2O_2, and Δ change in absorbance at 470 nm was recorded at an interval of 30 s for 3 min. A unit of peroxidase enzyme activity was expressed as the change in absorbance min -1 per g -1 of fresh weight (Hammerschmidt, *et al*., 1982). The total phenolic content in fresh papaya leaf and root tissue was analyzed using the Folin-Ciocalteu colourimetric method (Alabouvette *et al*., 2007). The optical density of the developed blue colour was measured at 725 nm. The phenolic content in the plant tissue was expressed as µg catechol− 1g− 1 fresh plant weight.

52.8 Chlorophyll Content Determination

One gram of newly developed leaves was sampled from all treatments and the 2 hybrids, 12 and 13 weeks after AMF inoculation and 1 and 2 weeks after pathogen introduction. They were crushed completely in a pestle with a mortar in 50ml pure acetone and clean sand was added to assist in the crushing. The content was sieved using cotton wool into a beaker; and the solution obtained in the beaker was used to determine the chlorophyll content at different wavelengths (663nm and 645 nm) using spectrophotometer and calculated as follows; Chl a= 11.75 *A663nm - 2.35 * A645nm, Chl b = 18.61*A645nm – 3.96A663nm, Total chlorophyll content = Chl a +Chl b (Brix, 2009).

5.2.9 Carotenoids Determination

Carotenoids contents were determined on papaya plants with the fungal pathogen infection and the non infected plants by a modified chromatographic procedure, one and two weeks after pathogen introduction. A sample of 5g was crushed in a pestle with a mortar. Hydroflorosupercel was added and extracted using 50ml cold acetone before filtering using glass funnel until the residue whitened. Partitioning was carried out using 25ml of petroleum ether. Saponification followed by adding an equal amount of extract into 3ml of 10% KOH in methanol, and a few drops of 0.1% butylated hydroxytoluene in petroleum ether. Anhydrous sodium sulphate was added to remove water and further concentration was done using a rotary evaporator. The total carotenoids content was calculated as follows ; (A × Volume (ml) × 10^4)/ A1% 1cm × sample weight (g); where A= absorbance; volume = total volume of extract; A1% 1cm= absorption coefficient of β−carotene in petroleum ether (2592) (Rodriguez and Kimura, 2004).

5.2.10 AMF Root Colonization

Papaya roots were sampled from JKUAT and Malkia F1 hybrids and from all treatments 12 and 13 weeks after AMF inoculation. The roots were evaluated for root colonization according to the Stoian and Florain (2009) procedure. Root samples were assessed for frequency and intensity of mycorrhizal colonization according to Stoian *et al*., (2019) and the assessment of mycorrhizal colonization (Rufykiri *et al*., 2000) with minor modifications.

5.3 Data Analyses

All data were subjected to two-way analysis of variance (ANOVA) using GenStat statistical package 15th edition. Means found to be significantly different at $p \leq 0.05$ were separated using Tukey's HSD test. DNA sequencing was carried out in Macrogen Europe (Amsterdam, Netherlands) and the sequences were edited using Chromas pro then blasted on National Centre for Biotechnology Information (NCBI) using nucleotide blast.

5.4 Results

54.1 Total Chlorophyll Content and Total Carotenoids

Infecting papaya plants with phytophthora blight affected the total chlorophyll content and carotenoids content. The AMF inoculum treated non-infected Malkia F1 hybrid plants had a chlorophyll content of 44.5 mg/g, one week after phytophthora blight infection (PBi) while those infected with PBi had a chlorophyll content of 33.2 mg / g. Chlorophyll levels gradually reduced with time to 31.9 mg/g and 33.7mg/g for infected and non-infected plants, respectively, two weeks after PBi (Table 5.2). JKUAT hybrid with AMF and manure treatments had chlorophyll contents of 44.4 mg/g and 54.4 mg/g for infected and non-infected plants, respectively, one week after PBi (Table 5.2). Carotenoids levels exhibited a similar trend. There was a significant difference ($p < 0.05$) in carotenoids levels between treatments for both infected and non-infected plants. The infected Malkia F1 hybrid with the treatment of AMF inoculum and compost manure had significantly ($p < 0.05$) the highest carotenoids content, 1.93 mg/100g, one week after the infection with phytophthora blight (PBi) and 0.847 mg/100 g, 2 weeks after PBi (Table 5.2).

Table 5.2: Total Chlorophyll Content (mg/ g) and Carotenoids (mg/100g) of Phytophthora Blight Infected Papaya Hybrids Treated with AMF and Compost Manure, One and Two Weeks after Inoculation.

		One week after phytophthora blight inoculation				Two weeks after phytophthora blight inoculation			
		Inoculated plants		Non-inoculated plants		Inoculated plants		Non-inoculated plants	
Hybrids	**Treatments**	**Total chlorophyll**	**Carotenoids**	**Total chlorophyll**	**Carotenoids**	**Total chlorophyll**	**Carotenoids**	**Total chlorophyll**	**Carotenoids**
JKUAT	Control	34.1^{f}	0.8^{g}	46.1^{f}	1.0^{e}	31.3^{h}	0.5^{e}	35.2^{e}	1.3^{g}
	AMF	52.1^{c}	1.8^{c}	63.9^{b}	1.8^{c}	49.7^{c}	0.7^{c}	55.3^{b}	1.9^{c}
	AMF and manure	44.4^{d}	1.9^{b}	54.4^{d}	1.9^{b}	41.4^{e}	0.8^{b}	46.1^{d}	2.2^{b}
	Compost manure	58.7^{b}	1.2^{e}	69.1^{a}	1.4^{d}	55.2^{b}	0.6^{d}	58.4^{ab}	1.5^{e}
Malkia F1	Control	44.9^{d}	0.9^{f}	56.6^{c}	1.0^{e}	47.2^{d}	0.5^{e}	49.5^{c}	1.4^{f}
	AMF	33.2^{f}	1.7^{d}	44.5^{f}	1.8^{c}	31.9^{g}	0.7^{b}	33.7^{e}	1.9^{c}
	AMF and manure	40.3^{e}	1.94^{a}	51.7^{e}	2.1^{a}	39.4^{f}	0.8^{a}	44.8^{d}	2.4^{a}
	Compost manure	60.5^{a}	1.7^{d}	69.7^{a}	1.7^{c}	60.3^{a}	0.6^{d}	61.2^{a}	1.8^{d}
	ANOVA (p - values)								
	Hybrids	<.001	<.001	<.001	<.001	<.001	<.001	0.006	<.001
	Treatments	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
	Hybrids × treatments	<.001	<.001	<.001	<.001	<.001	0.021	<.001	<.001

Means within each column followed by a different letter differ significantly at ($p \leq 0.05$) AMF= arbuscular Mycorrhizal Fungi, ANOVA= Analysis of Variance

5.4.2 Defense-Related Enzymes and Phenolic Contents

Enzymes and phenolic content were significantly ($p < 0.05$) affected by infecting papaya plants with phytophthora blight. The levels of phenylalanine ammonia lyase (PAL) of both leaves and roots differed significantly ($p < 0.05$) between the treatments for both infected and non-infected plants one and two weeks after PBi. PAL levels increased significantly two weeks after PBi for both infected and non-infected hybrids and in all treatments for both leaves and roots. The infected hybrid JKUAT with compost manure treatment had 255.7 cinnamic acid h / g for the leaves and 23.65 cinnamic acid h / g for the roots one week after PBi and this gradually increased to 278 cinnamic acid h / g and 28.29 cinnamic acid h / g for leaves and roots, respectively, 2 weeks after PBi. Non-infected plants had significantly ($p < 0.05$) lower levels of PAL compared to infected plants in all treatments (Table 5.3).

Polyphenol oxidase (PPO) increased significantly ($p < 0.05$) in infected plants more than non-infected plants. The content also differed significantly ($p < 0.05$) between the treatments for both leaves and roots. Infected JKUAT hybrid with AMF inoculum and compost manure treatment had 44.9 changes in absorbance min/g compared to non-infected JKUAT hybrid with AMF and manure (JKIC) treatment, 34.9 changes in absorbance min/g recorded from the leaves. Malkia control had 86.5 changes in absorbance min/g and 78.5 changes in absorbance min/g for infected and non-infected plants recorded from the leaves one week after PBi (Table 5.4). Malkia with AMF treatment had 15.1 changes in absorbance min/g and 12.6 changes in absorbance min/g for infected and non-infected plants recorded from the leaves two weeks after PBi (Table 5.4). A similar trend was observed in the phenolics (Table 5.5) and peroxidase (POD) contents (Table 5.6) for the leaves and roots of infected and non-infected plants.

Table 5.3: Phenylalanine Ammonia-Lyase (PAL) Content (Cinnamic Acid h / g) of Phytophthora Blight Infected Papaya Hybrids Treated with AMF and Compost Manure, One and Two Weeks after Inoculation

		One week after phytophthora blight inoculation				Two weeks after phytophthora blight inoculation			
		Inoculated plants		Non-inoculated plants		Inoculated plants		Non-inoculated plants	
Hybrids	**Treatments**	**PAL (Leaves)**	**PAL (Roots)**	**PAL (Leaves)**	**PAL (Roots)**	**PAL (Leaves)**	**PAL (Roots)**	**PAL (Leaves)**	**PAL (Roots)**
JKUAT	Control	302.5^{a}	26.3^{a}	274.8^{a}	25.3^{a}	321.0^{a}	31.6^{a}	313.3^{a}	29.2^{a}
	AMF	186.3^{e}	16.5^{b}	162.7^{e}	15.4^{c}	201.1^{e}	21.4^{b}	198.4^{e}	15.5^{e}
	AMF and manure	102.8^{g}	6.9^{c}	67.8^{g}	5.9^{de}	123.1^{g}	13.1^{c}	109.9^{g}	11.7^{f}
	Compost manure	255.7^{c}	23.7^{a}	215.2^{c}	20.7^{b}	278.0^{c}	28.3^{a}	257.8^{c}	22.6^{c}
Malkia F1	Control	283.2^{b}	25.6^{a}	252.4^{b}	19.6^{b}	301.1^{b}	30.3^{a}	290.7^{b}	25.6^{b}
	AMF	122.8^{f}	6.7^{c}	98.1^{f}	5.6^{e}	141.1^{f}	11.5^{c}	134.9^{f}	9.2^{g}
	AMF and manure	74.7^{h}	9.4^{c}	44.6^{h}	7.0^{d}	95.1^{h}	15.1^{c}	88.9^{h}	9.3^{g}
	Compost manure	220.5^{d}	16.4^{b}	176.3^{d}	14.4^{c}	241.1^{d}	21.6^{b}	221.1^{d}	18.7^{d}
	ANOVA (p - values)								
	Hybrids	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
	Treatments	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
	Hybrids × treatments	<.001	<.001	<.001	<.001	<.001	<.001	<.001	0.002

Means within each column followed by a different letter differ significantly at ($p \leq 0.05$) AMF= arbuscular Mycorrhizal Fungi, ANOVA= Analysis of Variance

Table 5.4: Polyphenol Oxidase (PPO) Content (Changes in Absorbance in min-1 g-1 Fresh Weight) of Phytophthora Blight infected Papaya Hybrids Treated with AMF and Compost Manure, One and Two Weeks after Inoculation

		One week after phytophthora blight inoculation				**Two weeks after phytophthora blight inoculation**			
		Inoculated plants		Non-inoculated plants		Inoculated plants		Non-inoculated plants	
Hybrids	Treatments	PPO (Leaves)	PPO (Roots)	PPO (Leaves)	PPO (Roots)	PPO (Leaves)	PPO (Roots)	PPO (Leaves)	PPO (Roots)
JKUAT	Control	76.2^{a}	6.4^{a}	67.2^{a}	5.3^{a}	91.1^{a}	8.1^{a}	88.1^{a}	7.4^{a}
	AMF	52.8^{cd}	0.1^{d}	46.1^{b}	0.1^{h}	67.8^{d}	1.1^{d}	63.6^{e}	1.1^{e}
	AMF and manure	44.9^{e}	0.7^{cd}	34.9^{c}	0.6^{e}	75.4^{cd}	1.7^{d}	72.4^{d}	1.6^{e}
	Compost manure	56.2^{c}	5.5^{a}	46.2^{b}	4.2^{b}	81.1^{bc}	7.8^{a}	75.2^{c}	6.6^{b}
Malkia F1	Control	67.7^{b}	1.6^{bc}	61.7^{a}	0.92^{d}	86.5^{ab}	3.3^{c}	78.5^{b}	3.21^{d}
	AMF	13.9^{f}	2.6^{b}	10.3^{d}	1.9^{c}	15.1^{f}	5.3^{b}	12.6^{h}	4.3^{c}
	AMF and manure	9.6^{f}	0.2^{d}	8.6^{d}	0.2^{g}	21.1^{f}	1.1^{d}	15.3^{g}	1.2^{e}
	Compost manure	46.4^{de}	0.5^{d}	42.1^{b}	0.3^{f}	57.8^{e}	1.5^{d}	55.9^{f}	1.1^{e}
	ANOVA (*p* - values)								
	Hybrids	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
	Treatments	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
	Hybrids × treatments	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Means within each column followed by a different letter differ significantly at ($p \leq 0.05$) AMF= arbuscular Mycorrhizal Fungi, ANOVA= Analysis of Variance

Table 5.5: Phenolic Content (µg Catechol−1g−1 Fresh Weight).of Phytophthora Blight Infected Papaya Hybrids Treated with AMF and Compost Manure, One and Two Weeks after Inoculation.

		One week after phytophthora blight inoculation				Two weeks after phytophthora blight inoculation			
		Inoculated plants		Non-inoculated plants		Inoculated plants		Non-inoculated plants	
Hybrids	**Treatments**	**Phenol Leaves**	**Phenol Roots**	**Phenol Leaves**	**Phenol Roots**	**Phenol Leaves**	**Phenol Roots**	**Phenol Leaves**	**Phenol Roots**
JKUAT	Control	405.3^{a}	851.2^{a}	396.3^{a}	803.2^{a}	421.2^{a}	873.2^{a}	404.6^{a}	853.1^{a}
	AMF	121.6^{d}	642.2^{d}	106.6^{d}	628.4^{e}	141.1^{e}	668.6^{e}	136.3^{e}	604.6^{e}
	AMF and manure	104.0^{f}	527.1^{f}	98.6^{e}	507.7^{g}	123.3^{f}	543.6^{g}	117.5^{f}	538.7^{g}
	Compost manure	218.9^{c}	713.1^{c}	204.5^{c}	688.1^{c}	238.4^{c}	739.7^{c}	226.7^{c}	702.4^{c}
Malkia F1	Control	238.4^{b}	727.8^{b}	222.3^{b}	706.3^{b}	231.2^{c}	750.7^{b}	228.5^{c}	720.4^{b}
	AMF	113.6^{e}	568.5^{e}	102.8^{de}	558.3^{f}	153.1^{d}	604.0^{f}	147.7^{d}	587.8^{f}
	AMF and manure	89.1^{g}	172.0^{g}	81.9^{f}	153.9^{h}	105.2^{g}	203.1^{h}	98.6^{g}	188.8^{h}
	Compost manure	215.9^{c}	647.6^{d}	206.6^{c}	636.9^{d}	263.4^{b}	684.8^{d}	249.0^{b}	651.6^{d}
	ANOVA (*p* - values) Hybrids	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
	Treatments	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
	Hybrids × treatments	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Means within each column followed by a different letter differ significantly at ($p \leq 0.05$) AMF= arbuscular Mycorrhizal Fungi, ANOVA= Analysis of Variance

Table 5.6: Peroxidase (POD) Content (Changes In Absorbance min 1g Fresh Weight) of Phytophthora Blight Infected Papaya Hybrids Treated with AMF and Compost Manure, One and Two Weeks after Inoculation

		One week after phytophthora blight inoculation				Two weeks after phytophthora blight inoculation			
		Inoculated plants		Non-inoculated plants		Inoculated plants		Non-inoculated plants	
Hybrids	**Treatments**	**POD (Leaves)**	**POD (Roots)**	**POD (Leaves)**	**POD (Roots)**	**POD (Leaves)**	**POD (Roots)**	**POD (Leaves)**	**POD (Roots)**
JKUAT	Control	121.9^{a}	9.9^{a}	112.7^{a}	8.9^{a}	142.4^{a}	11.5^{a}	128.2^{a}	11.7^{a}
	AMF	57.2^{f}	1.3^{f}	45.1^{f}	1.1^{e}	78.3^{e}	2.3^{d}	72.9^{f}	2.0^{d}
	AMF and manure	78.9^{c}	0.3^{g}	65.3^{d}	0.2^{e}	99.2^{c}	1.3^{d}	91.5^{c}	1.5^{d}
	Compost manure	71.3^{d}	6.5^{c}	68.3^{c}	5.8^{b}	91.1^{d}	8.4^{b}	86.9^{d}	5.6^{c}
Malkia F1	Control	92.1^{b}	7.5^{b}	88.6^{b}	6.3^{b}	102.3^{b}	8.8^{b}	99.1^{b}	7.9^{b}
	AMF	31.4^{h}	3.5^{e}	27.7^{h}	2.5^{d}	47.2^{f}	5.4^{c}	40.6^{h}	4.6^{c}
	AMF and manure	37.3^{g}	0.7^{fg}	33.7^{g}	0.3^{e}	50.0^{f}	1.9^{d}	43.4^{g}	1.8^{d}
	Compost manure	63.7^{e}	5.5^{d}	61.5^{e}	4.5^{c}	80.2^{e}	6.4^{c}	76.6^{e}	5.6^{c}
	ANOVA (*p* - values)								
	Hybrids	<.001	0.109	<.001	0.001	<.001	0.199	<.001	0.217
	Treatments	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001
	Hybrids × treatments	<.001	<.001	<.001	<.001	<.001	<.001	<.001	<.001

Means within each column followed by a different letter differ significantly at ($p \leq 0.05$) AMF= arbuscular Mycorrhizal Fungi, ANOVA= Analysis of Variance

5.4.3 Assessment of Growth of Papaya Hybrids Infected with Phytophthora Blight

There was a significant difference ($p < 0.05$) between the treatments in infected plants for height, leaf number and stem girth of all plants. At week 20, the Malkia F1 hybrid with AMF inoculum and compost manure treatment (MIC) was the tallest with 32.17 cm, while the Malkia F1 control hybrid was 15.6 cm. The infected JKUAT hybrid with AMF inoculum and compost manure treatments (JKIC) was 31.17 cm, while the JKUAT control was 15.6 cm at 20 weeks. The number of leaves and the stem girth had a similar trend. JKIC and MIC had significantly ($p < 0.05$) higher number of leaves and stem girth compared to other treatments while the Malkia F1 and JKUAT controls had the lowest values, which increased gradually with time (Figure 5.1).

5.4.4 Phytophthora Blight Lesion Grading

Papaya plants with a combination of AMF inoculum and compost manure treatments were not affected by the fungi (*Phytophthora palmivora*) during the period. At 8 weeks after transplanting, the Malkia F1 hybrid control was the most affected with a lesion affecting between 20% and 30% of the entire plant (grade 5) while at 20 weeks, both the JKUAT hybrid control and the Malkia F1 hybrid control had more than 60% coverage of the whole plant, grade 9. The JKUAT hybrid with AMF inoculum treatment and the Malkia F1 hybrid with AMF inoculum treatment had a lesion between 20% and 30% of the whole plant at 20 weeks (Figure 5. 1, figure 5.2).

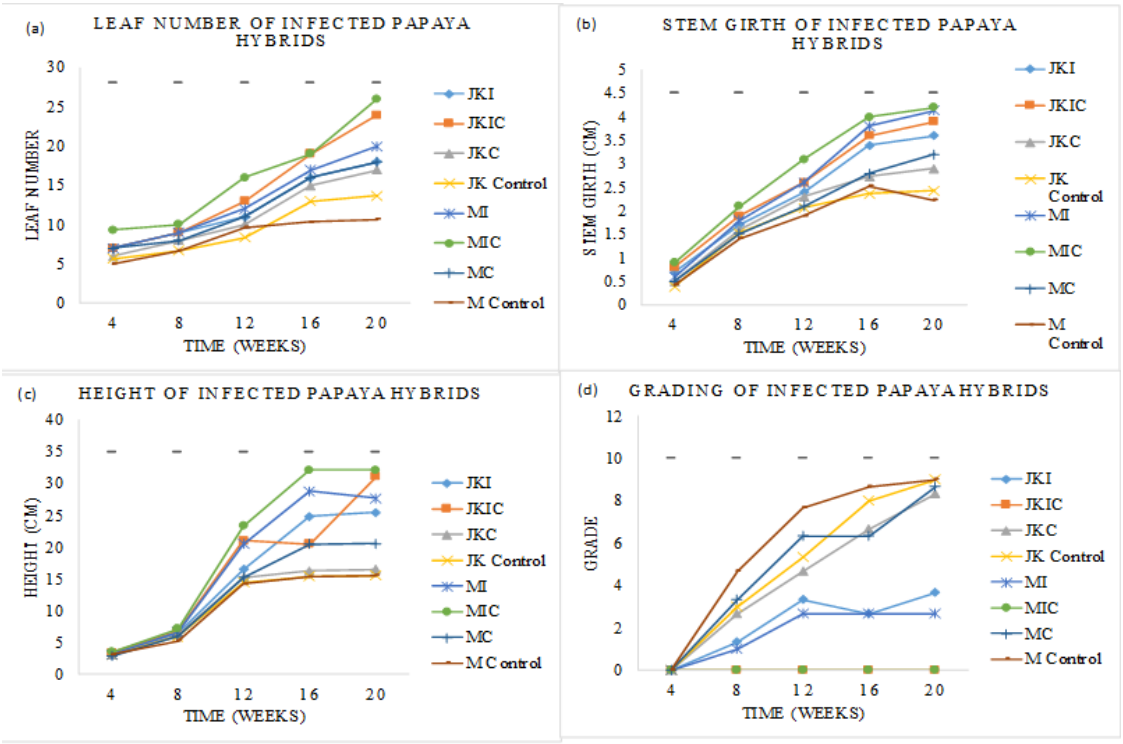


Figure 5.1: Growth of two Papaya Hybrids under Different Soil Amendments and Infected with Phytophthora Blight a) Number of Leaves, b) Stem Girth d) Plant Height and e) Severity Score

Key: JKI=JKUAT hybrid with AMF inoculum, JKIC= JKUAT hybrid with AMF inoculum and compost manure, JKC= JKUAT hybrid with compost manure JK Control = JKUAT hybrid with control treatment, MI= Malkia F1 hybrid with AMF inoculum, MIC= Malkia F1 hybrid with AMF inoculum and compost manure, MC= Malkia F1 hybrid with compost manure treatment, M Control = Malkia F1 hybrid with control treatment

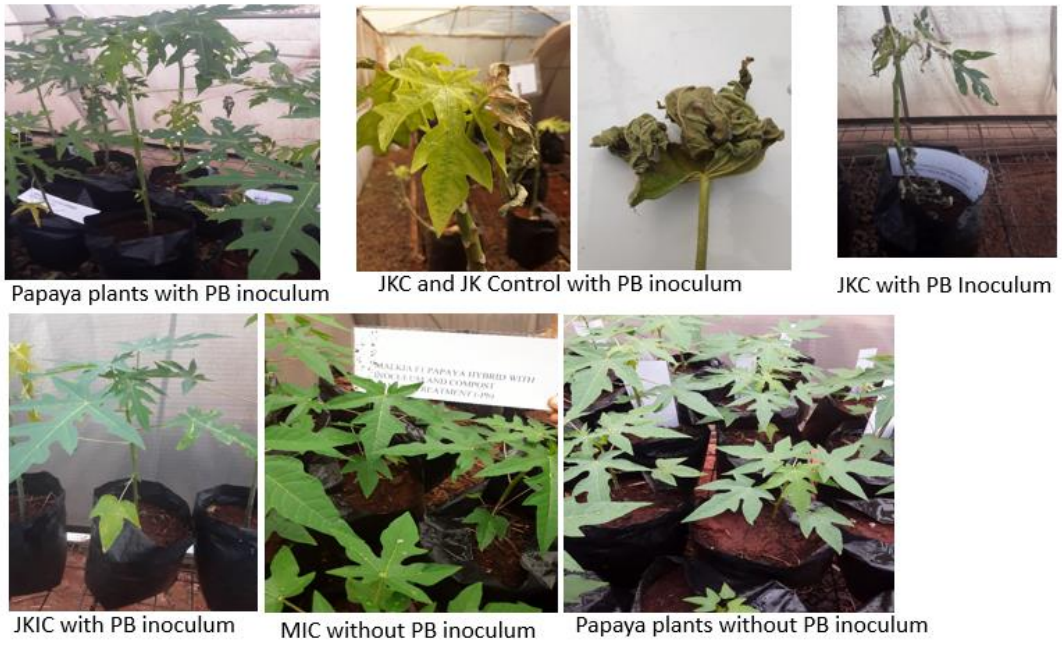


Figure 5.2: Symptomatic Observations of Inoculated and Non Inoculated Papaya Hybrid Response to PB Infection

Key: PB=phytophthora blight, JKC=JKUAT hybrid with composted manure treatment**,** JK=JKUAT, JKIC= JKUAT hybrid with AMF inoculum and compost manure treatment, MIC= Malkia hybrid with AMF inoculum and compost manure treatment

5.4.5 Assessment of Growth for Water Stressed Plants

Inoculating papaya plants with AMF significantly ($p < 0.05$) affected their growth. The height of the stressed plants with AMF inoculum treatment grew significantly ($p < 0.05$) taller as compared to non - inoculated plants. At 4 weeks, Malkia F1 hybrid with both AMF and compost manure treatments had a height of 3.467cm while at 20 weeks 27.67cm. Malkia F1 hybrid with control treatment had a height of 2.9cm and 4.8cm at 4 weeks and 20 weeks respectively. The leaf numbers of JKUAT hybrid with both AMF inoculum and compost manure treatments and JKUAT hybrid with control treatment was 17.67 and 10.33 respectively. Stem girth differed significantly ($p < 0.05$) with time and depending on the treatment. The stem girth of Malkia F1 hybrid with AMF inoculum only

was 0.68cm and 3cm at 4 weeks and 20 weeks respectively. Stem girth of Malkia F1 and JKUAT control treatments was 0.28cm and 0.317cm at 4 weeks respectively (Figure 5.3).

5.4.6 Water Stress Lesion Grading

Papaya plants with the different treatments did not show any lesions due to water stress at 4 weeks, hence they all scored 0%. At 8 weeks, both Malkia F1 and JKUAT hybrids with AMF inoculum and compost manure treatments had 0% grading while the control treatments had scored 4, that is, above 20% lesion on all parts. At 20 weeks, both control and compost manure treatments had a score of 9, that is, above 60% lesions in the 2 hybrids while combination of AMF inoculum and compost manure treatments had a score of 1, that is, only 10% lesion on all parts (Figure 5.3).

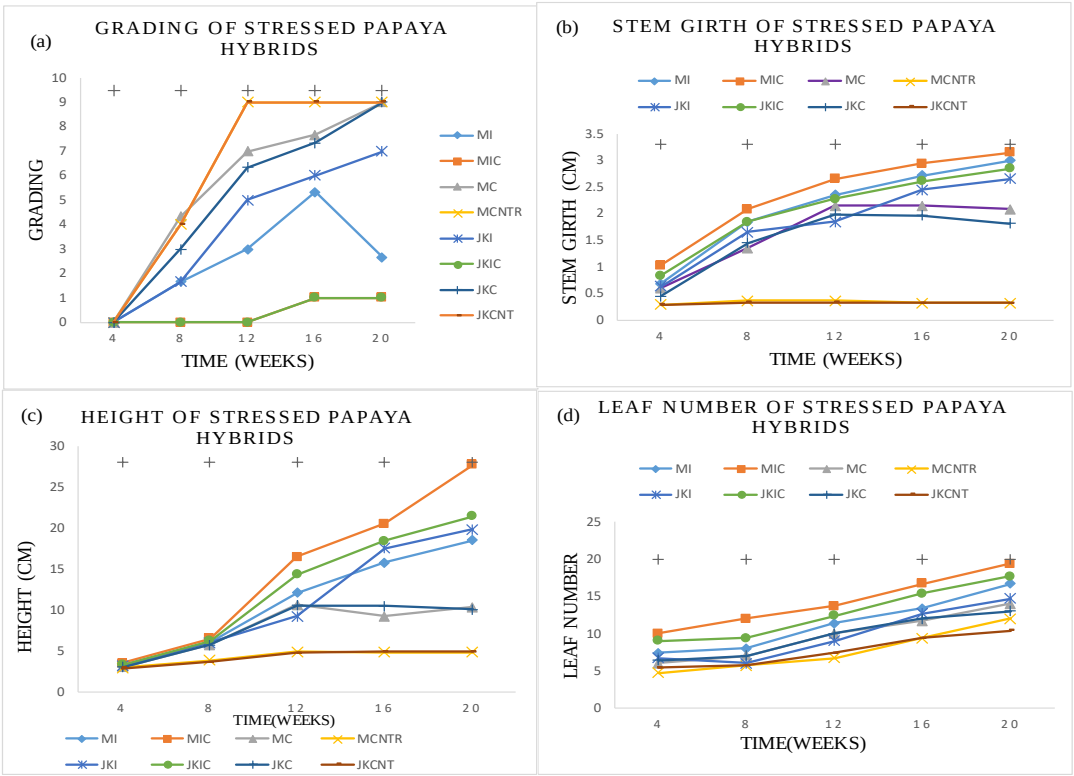


Figure 5. 1: Grading, stem girth, height and leaf number of stressed papaya hybrids (a-d)

Key: JKI=JKUAT hybrid with AMF inoculum, JKIC= JKUAT hybrid with AMF inoculum and compost manure, JKC= JKUAT hybrid with compost manure JK Control = JKUAT hybrid with control treatment, MI= Malkia F1 hybrid with AMF inoculum, MIC= Malkia F1 hybrid with AMF inoculum and compost manure, MC= Malkia F1 hybrid with compost manure treatment, M Control = Malkia F1 hybrid with control treatment

5.4.7 Arbuscular Mycorrhiza Fungi (AMF) Colonization

Arbuscular mycorrhiza fungi (AMF) inoculated papaya had significantly ($p < 0.05$) higher percentage root colonization compared to controls and compost manure treatments. The JKUAT hybrid with a combination of AMF inoculum and compost manure treatments had root colonization of 64.67% at 12 weeks and 68% at 13 weeks, while the JKUAT control had 26% and 30.33% root colonization at 12 and 13 weeks, respectively (Figure 5.4).

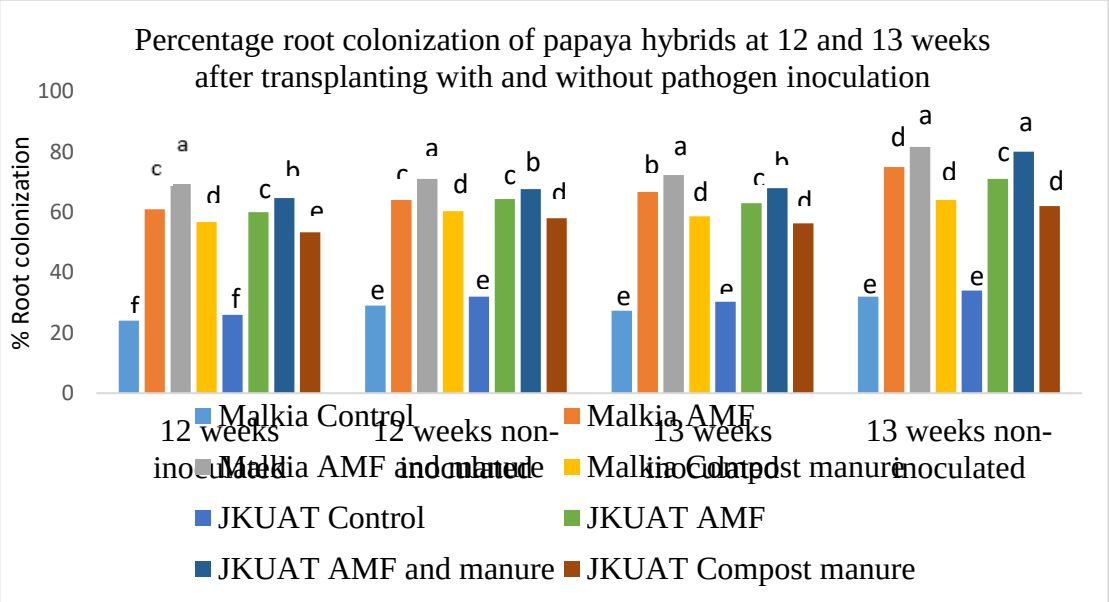


Figure 5.4: Percentage AMF Root Colonization of Papaya Hybrids at 12 and 13 Weeks after Transplanting; with and without Pathogen Inoculum

Means within each bar, for the weeks and inoculation status, followed by a different letter differ significantly at ($p \leq 0.05$)

5.5 Discussion

This study outlined the tolerance levels to phytophthora blight and water stress in JKUAT and Malkia F1 *Carica papaya* hybrids inoculated with arbuscular mycorrhizal fungi (AMF), combination of AMF and manure and manure only treatments. Plants infected with phytophthora blight disease contained lower levels of chlorophyll contents compared to non - infected ones. Two weeks after phytophthora blight infection (PBi), the total chlorophyll levels lowered further on the infected plants. Chlorophyll is the utmost important of the pigments, and crucial for the oxygenic change of light energy to the chemical energy which is stored and influences the biosphere (Richardson *et al*., 2002). Leaf chloroplasts contain antennae pigments which absorbs solar radiation to the responsive centre pigments, which discharge electrons and photochemical process kicks off (Richardson *et al*., 2002). Sun *et al*., (2021) observed negative effects on the growth of maize plants with AMF colonization due to stress and this could have been due to reduced growth of AMF structures hence inhibition of hyphal growth and minimal spore germination (Salloum *et al*., 2018). Biotic or abiotic stress leads to reduced supply of carbohydrate by host plants (Tyagi *et al*., 2017). However, the leaf chlorophyll content of maize under stress increased in plants with AMF inoculation compared to plants without AMF colonization (Sun *et al*., 2021). This outcome was consistent with this study results.

Papaya plants inoculated with AMF inoculum and compost manure treatments had higher chlorophyll contents as opposed to the controls. Leaf pigmentation can be related openly to stress physiology. Chlorophylls mostly decline under stressful conditions and during senescence (Jespersen *et al*., 2016). The amount of chlorophyll content in leaves gives the amount of nutrient status of the leaves indirectly since considerable leaf nitrogen is incorporated in chlorophyll (Moran *et al*., 2000). Aseri *et al*., (2008) reported that the highest total chlorophyll contents was detected in *Punica granatum* plants inoculated with *G. mosseae* and *A. brasilense*. Plants colonized by AMF resulted to increased nitrogen and consequently higher chlorophyll contents (De Andrade *et al*., 2015).

In this study, carotenoids contents were affected by the phytophthora blight infection. The levels were lower in plants that were infected with the disease. Moreover, treating plants with inoculum and compost manure boosted their carotenoids levels for both infected and non-infected plants. Arbuscular mycorrhizal fungi enhanced growth and development of red and green leaf lettuce through increased levels of chlorophylls, tocopherols and all major carotenoids and therefore mycorrhization emerged as a dependable technique to improve the nutritional value of vegetables (Baslam, *et al*., 2013). Among the biocolor isoprenoids, carotenoids are the utmost important, synthesized in photosynthetic plastids and sink organs and are responsible for red, orange and yellow colors found naturally in plants (Rosas and Stange 2016). Hart *et al*., (2015) reported that AMF enhance the nutritional quality of crops through production of carotenoids while Bona *et al*., (2017) recorded improved sugar concentrations, carotenoids, vitamin C, organic acids as well as enlarged size of tomatoes grown with AMF inoculum.

Phytophthora blight slowed the growth rate of the plants and especially plants with compost manure treatment only and the controls. Non - infected Malkia F1 hybrid with inoculum and compost manure treatment performed better than all other treatments and in comparison to JKUAT hybrid in this current study. AMF are very efficient in facilitating plants to absorb nutrients from soils with little or no nutrients due to soil degradation (Kayama and Yamanaka, 2014). Tomato plants that were inoculated with AMF showed increased leaf area and higher contents of calcium, potassium, phosphorus and nitrogen contents (Balliu *et al*., 2015). Pathogen attack by fungi resulted to damage of the cell membrane, nucleus fragmentation and cytoplasm condensation (Dehgahi *et al*., 2015). Consequently, photosynthetic activity reduced and this could be due to the declined thylakoid membrane proteins and decreasing leaf soluble protein (Weintraub and Jones, 2010). This explains the reduced growth rate of the infected papaya plants and the severe wilting scenario observed on the infected plants without AMF treatment. Plants under fungal attack showed symptoms of necrosis and leaf wilting and this was as a result of reduced chlorophyll content (Dehgahi *et al*., 2015).

Papaya plants under water stress conditions had suppressed growth in the current study. However, AMF inoculated seedlings performed better as compared to non- inoculated plants. Li *et al*., (2004) observed that water melon (*Citrullus lanatus*) seedlings that were inoculated with AMF fungi increased transpiration rate, net photosynthetic rate and water-use efficiency in a drought stricken field in Shandong province, China. Papaya plants with *Glomus spp* were exposed to water stress and were found to have an improved growth and nutrition (Mohandas 2012). Root colonization by *Gigaspora margarita* in high levels were linked with this enhanced water-stress tolerance. The AMF inoculated plants take up water through the hyphae and water relations of these plants is improved (Ruiz-Lozano and Azcón, 2000). The roots of AMF inoculated plants were more colonized during water stressed period and this may be connected to a decline in ethylene concentration as reduced ethylene levels endorses the AMF activity root's rhizosphere. Establishment of mycorrhiza in water stressed papaya plants controls the ethylene levels, a strategy to reduce damage in plants due to water stress (Mahouachi *et al*., 2007, Ishii *et al*., (2006).

The performance of papaya plants with inoculum and compost manure treatments for infected and non- infected plants was superior compared to other treatments. This was further evidenced by the production of secondary metabolites, peroxidase (POD), phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) and phenolic compounds in greater amounts on the stressed plants in this study. These plants included the diseased and the plants that were not inoculated with AMF inoculum. Secondary defense metabolites that were studied included peroxidase (POD), phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (PPO) assay. Plants boost defense responses by inducing activities of defense enzymes which includes phenylalanine ammonia lyase, peroxidase, polyphenol oxidase, chitinase and β-1,3- glucanase and which can reduce the rate of disease spread due to pathogen attack (Deborah *et al*., 2001; Kumari and Vengadaramana, 2017).

The infected papaya plants produced more of POD, PAL, PPO and phenolic contents compared to non-infected papaya plants in both leaves and roots; and as time progressed, more of these metabolites were produced. The interaction between plant host and AMF

activates plant defense responses especially during early root colonization (García-Garrido and Ocampo, 2002). Combination of systemic acquired resistance (SAR) and induced systemic resistance (ISR) can intensify defense against pathogens resistance through both pathways (Choudhary, 2007). SAR is induced systemically through inoculation with necrotizing pathogens (Prasannath *et al*., 2014). Some microorganisms which promotes growth of plants might arouse defense activity and plant resistance enhancement against soil borne pathogens (Whipps *et al*., 2001). On the other hand, AMF can improve the concentration of macro and micro-nutrients hence increasing photosynthate production and consequently, biomass accumulation increases (Chen *et al*., 2017; Mitra *et al*., 2019). Generation of superoxide radicals and oxidation burst occur during development of hypersensitive response in the interactions between plants and pathogen (Hajiboland *et al*., 2010). According to Latef and Chaoxing (2011), AMF colonization enhanced the enzymatic activities such as catalase and peroxidase even in saline conditions.

During pathogen attack, the reactive oxygen species (ROS) is produced in plant tissues (Singla *et al*., 2019). Consequently, photo-oxidative damage occurs to biomolecules and other internal cellular structures (Mittler, 2017). Plants with AMF inoculation retort to such by inducing a plethora of biochemical changes related with stress signaling and this activates their defense pathways. The induced defense mechanism includes lignins, flavonoids, phenolic compounds, polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL) peroxidases (POX) catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD), accumulation of phytoalexins and tannins (Debona *et al*., 2012; Akter *et al*., 2015). The phytoalexins accumulate at the infection site during the pathogen attack, and inhibit the fungal growth as well as other pathogens in-vivo (Bizuneh 2020). The polyamines (PAs) act as a protective barrier to pathogens by alteration of their cellular activities (Hussain *et al*., 2011)). The interactions are not only restricted to the nutrients exchange but also discrepancy progressive and spatial stimulation of defense mechanisms (García-Garrido and Ocampo 2002). A distinction and complete alteration in the

manifestation of four defense genes was observed in pathogenic interaction of arbuscular mycorrhizal in beans within all tissues (Guillon *et al*., 2002).

Papaya plants grown under combination of AMF inoculum and compost manure treatments were not suppressed by phytophthora blight disease and tolerated the harsh water stress conditions. Their growth, carotenoids and chlorophyll levels were enhanced despite exposure to pathogen infection. Therefore, inoculation with AMF enhanced absoption of nutrients and equipped the host plant to fight and / or cope with the pathogens and other stress factors in its environment.

CHAPTER SIX

EFFECTS OF AMENDING SOIL WITH ARBUSCULAR MYCORRHIZAL FUNGI ON GROWTH AND DEVELOPMENT OF SELECTED CARICA PAPAYA L. HYBRIDS IN KENYA

Abstract

Arbuscular Mycorrhizal Fungi (AMF) are naturally occurring root symbionts known to improve the uptake of essential nutrients to host plants due to their extra-radical hyphae. However, the latent effect of indigenous AMF inoculation on the growth of papaya hybrids JKUAT and Malkia has not been ascertained. This study evaluated the effect of AMF inoculation on the growth characteristics of these hybrids at vegetative stages of growth. A greenhouse experiment consisting of four treatments (AMF inoculum, compost manure, a combination of AMF inoculum and compost manure and a control (non-treated seedlings) and the two hybrids was set up in a completely randomized design. The experiment was replicated six times. Spores were isolated from rhizospheric soil samples and bulked in sorghum plant for four months to obtain AMF inoculum. At 2 and 3 leaves papaya seedling stage, all the treatments were introduced. Height of the plant, leaf length, stem girth, number of leaves and root colonization were assessed and recorded every 4 weeks for a period of 20 weeks. The collected data was subjected to two way analysis of variance (ANOVA) at $p \leq 0.05$ level of significant and significant means separated using Tukey's HSD test in Genstat statistical package 15th edition. The results showed that plants treated with a combination of compost manure and inoculum were significantly different in all growth parameters tested, $p \leq 0.05$, compared to compost alone and the control treatments. Malkia hybrid treated with a combination of compost manure and AMF had the highest plant height of 3.55 cm at 4 weeks which increased to 53.2 cm at 20 weeks. Root colonization increased significantly at $p \leq 0.05$ in all the treatments. At 4 weeks, both JKUAT and malkia hybrids with control treatment had the lowest colonization percentage of 15.33% and 11.67% respectively. JKUAT and malkia hybrids with AMF treatment had 55.67 % and 58.33 % respectively at 8 weeks which increased

to 70.67% and 73.33% respectively at 20 weeks. Roots biomass of JKUAT hybrid with both AMF inoculum and compost manure treatment was 49g while the control was 11.80g at 20 weeks. At 4 weeks, Malkia and JKUAT hybrid with both AMF and manure treatments had leaves biomass of 11.5g and 7.7g respectively which had increased to 28.2g and 23.2g respectively at 20weeks. Soil media amendment with AMF inoculum enhanced the growth of the papaya seedlings as evidenced by the performance and the biomass of the plants. Moreover, combining AMF inoculum and compost manure yielded superior results on the overall growth of the papaya seedlings.

6.1 Introduction

Arbuscular mycorrhizal fungi (AMF) are soil micro-organisms that form associations with roots of majority of plant species (Abbott and Lumley, 2014). The AMF plays a major role in processes associated with soil aggregation, acquisition of nutrients by plants and in ecosystem function (Abbott and Lumley, 2014). Arbuscular mycorrhizal fungi forms symbiotic relationships with over 80 % of terrestrial plant species (Wang and Qiu 2006). This symbiotic relationship is characterised by its association with phosphorus, nitrogen, sulfur and micronutrient uptake by host plants and the enhancement of water uptake through the extra radical fungal hyphal networks (Brundrett, 2002).

The symbiosis can also prompt physiological and molecular signals at subcellular levels, modifying the structure of the plant community thus escalating plant tolerance to several abiotic and biotic stresses. Ectomycorrhizal (ECM) fungi form associations with only 3 % of terrestrial plant families (Smith and Read 2008). When a symbiosis occurs, both ECM and AMF can demand 20–40% of photosynthetically fixed Carbon supplied by their host plants to complete their life cycle (McNear, 2013). In return, AMF supplies nutrients including phosphorous, nitrogen and zinc to the plant through the arbuscules; the nutrient exchange sites (Balestrini and Bonfante, 2005). Mycorrhizal plants usually display better performance compared to non-mycorrhizal plants in high-input agricultural systems where nutrients are limited (Janos, 2007). Inoculation with AMF becomes effective in plants when it is introduced during the early plant development stages and thereafter

colonization by AMF follows root establishment of the already inoculated seedlings and the plant will be extensively mycorrhizal. Plants survival rate during acclimatization phase is highly enhanced, plant growth and development is stimulated and overall high production of the plants at vegetative and flowering stages. Plants inoculation with indigenous AMF similarly aids the general growth of the plants, nitrogen fixation and phosphorous acquisition by plants (Jeffries *et al*., 2003).

Papaya (*Carica papaya*) is a fruit with commercial significance due to its high nutritive and medicinal value (Pinnamaneni, 2017). Papaya crop is majorly propagated by seeds and grows well in warm places. It requires full sunlight and well-drained soils (Heena and Sunil, 2019). The cost of certified papaya seeds is however very high hence increasing percentage germination for more health seedling becomes a challenge to papaya farmers (Bhardwaj, 2013).

Growing media is an essential part of majority of horticultural crops as it directly affects germination, enlargement and proper function of the rooting system (Abad *et al*., 2002). An appropriate propagation media serves as a nutrient and water reservoir for the plants, provides adequate anchorage and allows sufficient gaseous exchange between the roots and their substrates (Abad *et al*., 2002). According to Wilson *et al*., (2001) superiority of seedlings is mostly dependent on the constituents of growing media. Agbo and Omaliko (2006) stated that potting media used in the nursery influences the quality of the resultant seedlings and this in turn increases the yield (Baiyeri, 2006).

Various biofertilizers have been used and among them is rhizobium which enhanced root nodulation in cowpea and this reduced the need to incorporate nitrogen fertilizer in the soil media (Anitha *et al*., 2004). Further experiments with inoculation with vesicular arbuscular mycorrhizal (VAM) fungus facilitated more efficient uptake of phosphorous from the soil and boosted absorption of water, potassium, nitrogen and micronutrients in the inoculated cowpea plants (Anitha *et al*., 2004). Fungus such as *Trichoderma harzianum* has been found to be beneficial in the plants' roots development hence enhancing effective mineral absorption from the soil and resistance to stresses due to

abiotic elements hence improved crop productivity (Harman 2000). Mycorrhizal fungi act as a solid sink for photosynthates and also condense the nutrient imbalances in cowpea plant (Muthukumar and Udaiyan, 2000). Inoculation of plants with mycorrhizal fungi in revegetation systems not only help plant establishment but also improves soil physical, chemical and biological properties, thus contributing to improved soil quality. Progress has been achieved in exploring the use of microorganisms for enhancement of soil fertility and ultimately increased crop productivity. Growth of root system is suppressed in soils with inadequate drainage and the plants become more prone to soil borne diseases (Stirling *et al*., 2016). Organic matter is incorporated into the soil to enrich it with enough nutrients for the plants and improve on the rooting system as well as increasing resistance against pest and disease attacks (Akanbi *et al*., 2002).

Greater emphasis is being placed in order to enhance exploitation of indigenous soil microbes such as AMF, which will contribute to soil fertility and increase plant growth and protection, especially in papaya (Abbott and Lumley, 2014). Inoculating soil media with mycoohizal has improved the growth and nutrients contents in fruits such as strawberries (Bona *et al*., 2015). The indigenous AMF has not been incorporated with the soil media for the growth of JKUAT and Malkia F1 papaya hybrids and therefore this research seek to explore the benefits of incorporating indigenous AMF inoculum, a cost effective and non hazardous approach, in the soil media for the growth and development of *Carica papaya* plants in Kenya.

6.2 Materials and Methods

6.2.1 Isolation of Arbuscular Mycorrhizal Fungi (AMF)

In the JKUAT laboratories, spore isolation was carried out as described by Boyno *et al*., (2023), with minor modifications. Fifty grams of soil was sampled out and placed in a 250 ml conical flask. 100ml of tap water was added and the flask was capped with a rubber cork. The mixture was agitated vigorously and left to decant for 30 seconds and then washed through 250 μm, 100 μm and 45 μm pore sieves. The contents of the 45 μm pore

sieve were backwashed into a small sized beaker and swirled. The contents were then quickly decanted into 50 ml centrifuge tubes and balanced by weight then centrifuged for 5 min at 1750 rpm. The supernatant was discarded and the tubes were filled with 48% sucrose solution (sucrose-227 g dissolved in 500 ml water), balanced by weight and stirred vigorously to re-suspend the precipitate then centrifuged for 15 seconds at 1750 rpm. The supernatant sucrose was emptied through a 45 micromesh sieve. The spores retained on the sieve were rinsed thoroughly with distilled water to wash out the sucrose. The spores were then washed away with distilled water into grid calibrated Petri dishes for examination.

6.2.2 Bulking of Arbuscular Mycorrhizal Fungi (AMF) Inoculum

Sorghum, *Sorghum bicolor* (L.) Moench, a fast-growing annual grain plant, was used as the trap culture crop. Disposable glasses, 300ml, were perforated and half filled with sterile coarse sand mixed with the isolated AMF spores. Sorghum seeds were planted and placed on greenhouse whose temperatures ranged between 36^{o} C to 41^{o} C and the relative humidity 60 % to 90 %. The plants were watered as necessary and grown for 4 months before obtaining a sample of roots and medium from the sorghum rhizosphere for preparation of AMF inoculum.

6.2.3 Establishment of the Papaya Plants with Arbuscular Mycorrhizal Fungi

Papaya seeds of JKUAT and Malkia F1 hybrids were planted in a mixture of sterilized soil and sand media at a ratio of 1:1. During transplanting, 2 and 3 leaves stage, sterilized soil media was used and AMF inoculum, compost manure or a combination of the AMF inoculum and the compost manure (1:1) were used as the treatments. The treatments were combined with the sterilized soil at a ratio of 1:9 (1 part of treatment to 9 parts of the soil). Sterilized soil media only was used for the controls. Eighteen plants from each hybrid were grown in each treatment and watering of the plants was done when necessary.

6.2.4 Root Colonization by Arbuscular Mycorrhizal Fungi (AMF)

Three plants per each treatment were assessed for root colonization. The roots of the papaya subjected to all the treatments were assessed for root colonization at 20 weeks after transplanting, according to the procedures of Stoian and Florain (2009). The frequency and intensity of mycorrhizal colonization was done using the subjective visual technique by Stoian *et al*., (2019).The roots samples were put in aqueous solution of 2.5% potassium hydroxide before autoclaving at 120^{o}C for 3 to 5 minutes. The samples were then placed in an oven at 70^{o} C for one hour and then rinsed in several changes of water to remove KOH. Alkaline hydrogen peroxide was added to the samples and placed in an oven at 70^{o} C for 20 minutes so as to remove phenolic substances. The samples were then rinsed with tap water and then acidified with 1% HCL for 30 minutes and without rinsing, the samples were stained in an acidic glycerol solution containing 0.05% trypan blue. They were then placed in the oven at 70^{o} C for one hour. The stain was decanted and de-staining solution comprising of acid glycerol was added. The root samples were then mounted on slides under a compound microscope to assess the frequency and intensity of mycorrhizal colonization.

6.2.5 Assessment of Seedling Growth and Statistical Analysis

Seedlings were assessed for height, length and number of the leaves, stem girth, root colonization, roots biomass, shoots biomass and leaf biomass. The data was obtained and recorded every 4 weeks for a period of 20 weeks. Three plants were randomly uprooted from every treatment and from the 2 hybrids (JKUAT and Malkia F1). The roots were carefully and thoroughly cleansed to remove any debris and soil particles. They were then air dried, carefully cut from the stem, weighed and recorded as root biomass. The upper portion of the plant was weighed and recorded as shoot biomass. All leaves were then plucked from the shoots and weighed separately to obtain leaves biomass. The tabulated data was subjected to two- way analysis of variance (ANOVA) using Genstat statistical package 15th edition, while the means found to be significantly different at $p \leq 0.05$ were separated using Tukey's HSD test.

6.3 Results

6.3.1 Effect of AMF Inoculation, Compost Manure and a Combination of AMF Inoculation and Compost Manure on Papaya Hybrid Growth and AMF Colonization

Media amendment on the nursery had significant ($p \leq 0.05$) effects on the growth of JKUAT and Malkia F1 papaya hybrids and also between the hybrids. A combination of AMF inoculum and compost manure significantly ($p \leq 0.05$) increased the height of Malkia F1 hybrid (53.2cm) compared to all other treatments as at 20 weeks after transplanting. The stem girth of JKUAT hybrid with AMF treatment was 0.833 cm at 4 weeks and 4.63cm at 16weeks while JKUAT hybrid with both AMF and manure treatments was 1.13 cm at 4 weeks and 5.63 cm at 16 weeks.

At 4 weeks, JKUAT hybrid with AMF treatment only and compost manure treatment only had 43.33% and 23.33% root colonization respectively. Malkia F1 hybrid with a combination of AMF inoculum and compost manure treatments had the highest percent AMF colonization (81.3%) followed by JKUAT hybrid plants inoculated with AMF and compost manure (78%) while the non-inoculated JKUAT and Malkia F1 control plants had significantly ($p \leq 0.05$) lower percent AMF colonization of 32.33% at 20 weeks. (Figure 6.1, figure 6.2).

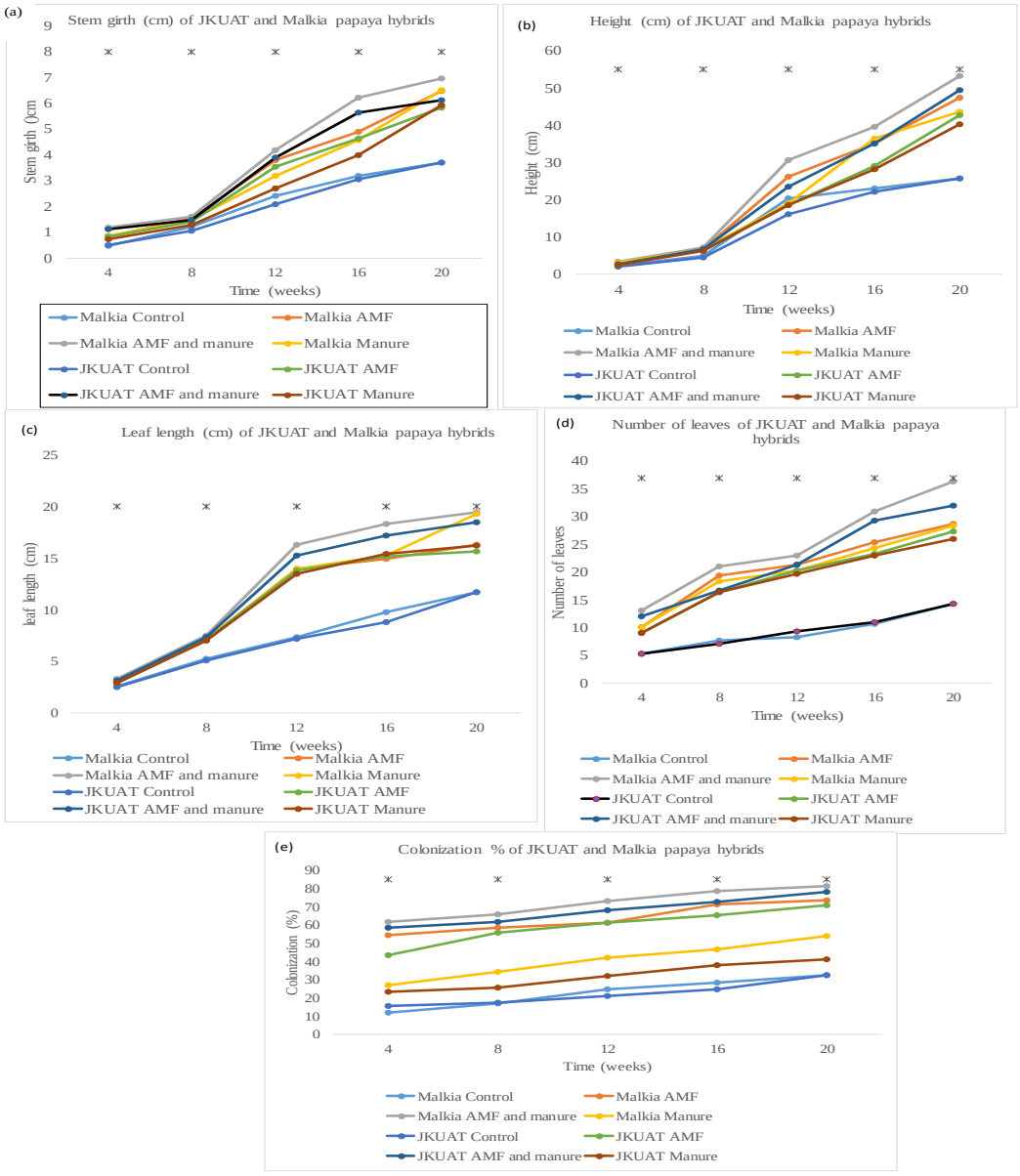


Figure 6.1: Growth of Two Papaya Hybrids under Different Soil Amendments (a) Stem Girth, (b) Plant Height, (c) Length of Longest Leaf, (d) Number of Leaves and (e) % Colonization of JKUAT and Malkia F1 Papaya Hybrids

6.3.2 Leaves biomass

A gradual increase in leaves weight over time was observed in all the treatments. Malkia F1 hybrid treated with a combination of AMF inoculum and compost manure treatment

was significantly different ($p \leq 0.05$) from JKUAT hybrid with a similar treatment during the entire period. At 4 weeks, Malkia F1 hybrid with a combination of AMF and manure treatments had leaves biomass of 18.99g as compared to the control which had 5.07g. This weight gradually increased to 32.5g for Malkia F1 hybrid with AMF and manure treatments and 15.2g for the control at 20 weeks. Leaves biomass of JKUAT hybrid with compost manure treatment plant weighed 21.48 g at 20 weeks while JKUAT control weighed 14.83g. At 12 weeks, JKUAT hybrid with compost manure treatment weighed 14.3g while JKUAT hybrid with both AMF and manure treatments weighed 22.04g (Table 6.1).

Table 6.1: Leaves Biomass (g) of JKUAT and Malkia F1 Papaya Hybrids with AMF Inoculum, Compost Manure and a Combination AMF Inoculum and Compost Manure

Hybrid	Treatments	4 weeks	8 weeks	12 weeks	16weeks	20 weeks
Malkia F1	Control	5.07^{e}	7.6^{f}	12.6^{f}	13.5^{e}	15.2^{d}
	AMF	11.5^{c}	15.1^{c}	20.3^{c}	27.5^{b}	28.2^{b}
	AMF and Manure	18.99a	22.5^{a}	24.99^{a}	30.3^{a}	32.5^{a}
	Compost manure	8.5^{d}	12.3^{d}	16.3^{d}	20.4^{c}	22.7^{c}
JKUAT	Control	4.7^{e}	7.1^{f}	11.6^{f}	12.8^{e}	14.8^{d}
	AMF	7.7^{d}	11.3^{de}	16.8^{d}	18.5^{d}	23.2^{c}
	AMF and Manure	15.2^{b}	18.3^{b}	22.04^{b}	27.3^{b}	30.5^{a}
	Compost manure	6.07^{e}	10.2^{e}	14.3^{e}	17.95^{d}	21.5^{c}
	ANOVA (*p* - values)					
	Hybrids	<.001	<.001	<.001	<.001	<.001
	Treatments	<.001	<.001	<.001	<.001	<.001
	Hybrids × treatments	<.001	<.001	0.003	<.001	<.001

Means within each column followed by a different letter differ significantly at ($p \leq 0.05$). AMF= arbuscular Mycorrhizal Fungi, ANOVA= Analysis of Variance

6.3.3 Shoot Biomass

The total weight of the shoots increased gradually with time in all the treatments. JKUAT hybrid with the combination of AMF inoculum and compost manure treatment was significantly different ($p \leq 0.05$) from JKUAT hybrid with compost manure treatment during the entire period. JKUAT hybrid with compost manure treatment had a weight of 32.99 g while JKUAT hybrid treated with a combination of AMF inoculum and compost manure had 50.98 g at 16 weeks. Non-treated control plants (JK Control and M Control),

had the least shoot biomass weight throughout the data collection period. At 20 weeks, Malkia F1 hybrid with both AMF and manure treatments had shoots biomass of 65.1g while Malkia F1 hybrid with manure treatment only weighed 39.1g (Table 6.2).

Table 6.2: Shoots Biomass (g) of JKUAT and Malkia F1 Papaya Hybrids with AMF Inoculum, Compost Manure and a Combination AMF Inoculum and Compost Manure

Hybrid	Treatments	4weeks	8 weeks	12 weeks	16weeks	20 weeks
Malkia F1	Control	15.5^{e}	18.9^{e}	22.5^{c}	26.3^{f}	28.07^{f}
	AMF	27.4^{b}	40.9^{ab}	44.8^{a}	50.3^{bc}	54.4^{bc}
	AMF and Manure	33.5^{a}	41.5^{a}	46.2^{a}	52.4^{a}	65.1^{a}
	Compost manure	20.2^{d}	24.8^{d}	32.5^{b}	37.5^{d}	39.1^{d}
JKUAT	Control	14.2^{e}	17.6^{e}	20.7^{c}	24.4^{g}	26.2^{f}
	AMF	25.8^{c}	38.8^{c}	45.1^{a}	49.3^{c}	53.3^{c}
	AMF and Manure	28.2^{b}	39.2^{bc}	44.5^{a}	50.98^{ab}	56.4^{b}
	Compost manure	18.9^{d}	24.5^{d}	28.5^{b}	32.99^{e}	35.8^{e}
	ANOVA (*p* - values) Hybrids	<.001	<.001	0.008	<.001	<.001
	Treatments	<.001	<.001	<.001	<.001	<.001
	Hybrids × treatments	<.001	0.121	0.134	<.001	<.001

Means within each column followed by a different letter differ significantly at ($p \leq 0.05$). AMF= arbuscular Mycorrhizal Fungi, ANOVA= Analysis of Variance

6.3.4 Root Biomass

Malkia F1 and JKUAT hybrids inoculated with AMF had significantly ($p \leq 0.05$) higher root mass throughout the growth assessment period compared to the compost manure treatments and the controls (Figure 6.2). JKUAT hybrid with AMF inoculum treatment was significantly different ($p \leq 0.05$) from Malkia F1 hybrid with AMF inoculum at 12 weeks after inoculation; that is, 16.4g and 18.8 g respectively. At 20 weeks, JKUAT hybrid with AMF treatment had roots biomass of 23.5g while JKUAT hybrid with both AMF and manure treaments weighed 49g (Table 6.3).

Table 6.3: Roots Biomass (g) of JKUAT and Malkia F1 Papaya Hybrids with AMF Inoculum, Compost Manure and a Combination AMF Inoculum and Compost Manure

Hybrid	Treatments	4weeks	8 weeks	12 weeks	16weeks	20 weeks
Malkia F1	Control	4.07^{ef}	4.4^{ef}	5.3^{f}	8.3^{g}	12.5^{g}
	AMF	9.5^{c}	14.2^{c}	18.8^{c}	26.3^{c}	32.6^{c}
	AMF and Manure	24.7a	36.4^{a}	41.4^{a}	48.3^{a}	58.5^{a}
	Compost manure	4.7^{e}	5.7e	8.7^{e}	14.8^{e}	18.2^{e}
JKUAT	Control	3.8^{f}	4.1^{f}	4.7^{f}	8.1^{g}	11.8^{g}
	AMF	8.3^{d}	11.6^{d}	16.4^{d}	21.4^{d}	23.5^{d}
	AMF and Manure	18.5^{b}	29.8^{b}	37.97^{b}	41.6^{b}	49^{b}
	Compost manure	3.3^{f}	4.9^{ef}	6.5^{f}	11.1^{f}	16.1^{f}
	ANOVA (*p* - values) Hybrids					
		<.001	<.001	<.001	<.001	<.001
	Treatments	<.001	<.001	<.001	<.001	<.001
	Hybrids × treatments	<.001	<.001	0.019	<.001	<.001

Means within each column followed by a different letter differ significantly at ($p \leq 0.05$) while means with a similar letter in a column do not differ significantly at ($p \leq 0.05$).

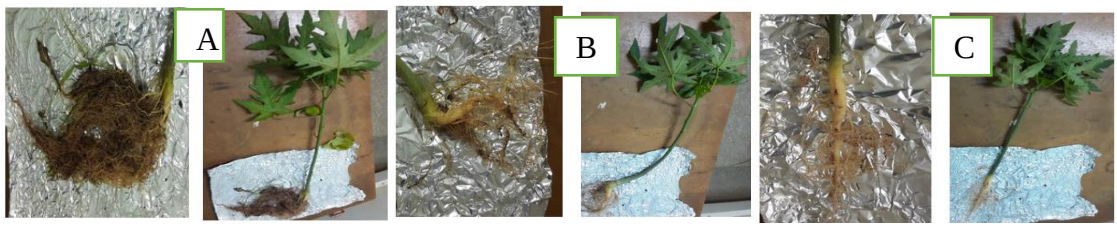


Figure 6.2: Effects of Roots and Shoots Development due to Soil Amendments

Key: A = AMF and compost manure treatments, B = AMF treatment and C = compost manure treatment

6.4 Discussion

In this study, papaya plants treated with compost manure which majority of farmers in the Mwea, Juja and Mitunguu regions of Kenya use both as the soil media accompaniment and a top dresser did not respond as effectively as AMF treatment or a combination of AMF and compost manure. Papaya plants with AMF inoculum treatment grew more

vigorously and stronger compared to those with compost manure treatment and the control. Moreover papaya plants with a combination of AMF and compost manure treatment had significantly more enhanced growth compared to other treaments. This could be due to the combination of beneficial effects of AMF and compost manure in the soil.

The rate of emergence of new leaves and branches as well the average length of the leaves was higher on the plants treated with both AMF and compost manure suggesting a synergistic effect between the AMF and compost manure. Disturbed and eroded soil has been found to contain extremely low amounts of nitrogen, phosphorous, pH and organic matter content (Shrestha Vaidya *et al.*, 2008). According to Geetha and Fulekar (2008), when organic matter was added to eroded soil, AM spore count increased and this led to soil stabilization as well as effective plants establishment.

The increased stem girth in plant inoculated with both AMF and compost manure in this study may also suggest a compost induced AMF colonization providing avenues for greater nutrient exploration and uptake by the plant. The combined effects of mycorrhiza provides a great mass of external mycelium that can extend even beyond the rhizospheric area of the plant, searching for more water and nutrients from the soil. The absorbed minerals are then directed to the intraradical mycelium and transported to the host plant (Ramos *et al.*, 2009). This may also explain why the roots, shoots and the leaves biomass of the plants treated with both AMF and compost manure performed better than plants treated with either compost manure or AMF alone.

The effects of AMF on the growth of plants have been studied in crop species such as *Sorghum bicolor* (L.) Moench (Nakmee *et al*., 2016), *Solanum lycopersicum* L. (Bona *et al*., 2016), fruit trees such as *Musa acuminata* Colla (Rodríguez-Romero *et al*., 2005) and *Citrullus lanatus* (Thunb.) (Ban *et al*., 2011) and in all the studied species, AMF improved plant growth parameters and the uptake of major nutrients such as phosphorus and nitrogen especially in stressed conditions (Jansa *et al*., 2019). The growth stimulation is associated with the fact that AMF extends the absorbing network far beyond the nutrient

depletion zones of the roots' rhizosphere, which allows access to a larger volume of soil (Smith and Smith, 2011). The hyphae are thinner than plants' roots and are able to penetrate micropores (Allen, 2011), increases the total absorption root surface area and absorb more nutrients especially those whose ionic forms have a poor mobility rate and are in low concentration (Smith and Read 2010).

Previous studies on combination of compost manure and mycorrhiza showed that the hyphae of the mycorrhiza and the roots of the plants contribute independently to the stability of soil aggregates and their overall effects on the plants is enhanced on their combination (Sharif *et al*., 2009). The shoot biomass and uptake of micro nutrient of wheat crop increased significantly with inoculation of AMF and in combination with poultry manure (Sharif *et al*., 2009). Combining AMF with compost manure enriched the production of Argan plants' shoot biomass (El Mrabet *et al*., 2014). Inoculation of AMF combined with compost has potential to improve copper, manganese, zinc and iron uptakes by plant (Jan *et al*., 2014). Potassium and calcium uptake of maize plants increased on soil media with AMF combined with manure (Astiko *et al*., 2013) while Rutkowska *et al*., (2014) reported increased concentrations of iron, boron and zinc in the soil solution with farmyard manure. The enrichment of AMF could be intensified using addition of organic matter since both combinations improved soil physico-chemical properties due to the lowering of the soil pH and favorable water- air balance (Warnock, *et al*., 2007).

The weight of shoots and leaves biomass was significantly higher on papaya plants with a combination of AMF and manure treatments as compared to other treatments in this study. Mycorrhizas are known to increase the absorption of elements such as phosphorous, and other major elements found in the soil as well as zinc and copper absorption especially in nutrient scarce conditions. These elements provide the roots, leaves and stems of the plants with the required nutrients for their effective development thereby increasing the yield through the intensified dry matter (Roy-Bolduc and Hijri, 2011). Compost manure on the other hand is a beneficial source of organic matter for crop production. However, low application of the compost manure may steer low crop yields due to deficiency of

essential nutrients whereas excess application results to the leaching of nitrates and phosphorous unavailability to the plants (Termorshuizen *et al*., 2004).

In the current study, the effect of amending the soil media with AMF inoculum was clearly indicated on the roots which significantly differed on the percentage levels of root colonization depending on the treatment and papaya hybrid. The overall performance indicated that the Malkia F1 papaya hybrid portrayed better results in the vegetative phase of growth compared to the JKUAT papaya hybrids. According to Tim Jumah (2022), Malkia F1 papaya variety is currently the most preffered papaya hybrid in Kenya due to its qualities including high yielding, large size and fast maturity. Useful effects of incorporating AM fungi on soil media have been stated in various plants, such as apple rootstocks, (Schubert and Lubraco, 2000) and the rootstocks of pistachio, (Kafkas and Ortas, 2009). Mycorrhiza also enables plants to bear with saline and dry conditions, along with other biotic and abiotic stress factors (Pozo *et al*., 2010). Studies have shown that grapevines grown on sterilized media that was inoculated with AMF developed faster than those in non-inoculated media (Ozdemir *et al*., 2010). Shrestha Vaidya *et al*., (2008) concluded that organic amendments boost AMF spore production. Studies have also indicated that combination of soil as the basic medium, sand for porosity, organic matter to enrich the soil and trichoderma to reduce the incidence of soil borne diseases executed superior results on the performance of papaya seedlings (Rakibuzzaman *et al*., 2019). Plant roots morphology can be affected by various factors and among them is AMF and photosynthetic rate of host plants (Heinonsalo *et al*., 2016). Inoculation with *Acaulospora scrobiculata* or *Funneliformis mosseae* in trifoliate orange improved root morphological traits compared to non-inoculated plants (Wu *et al*., 2016). The total root length and volume of tea plants improved upon AMF inoculation while the root hair number and length decreased (Shao *et al*., 2018). On the other hand, AMF colonisation had insignificant effects on root hair length and root diameter in a temperate forest, suggesting that the mycorrhizal role in root morphology depends on plant hosts (Eissenstat *et al*., 2015).

In this study, amending the soil with AMF inoculum and compost manure treatments improved the growth of papaya plants during the vegetative stage. The plants had a larger stem circumference and grew more vigorously as compared to other treatments. Boosting the soil media with AMF inoculum has proven to improve papaya growth and performance and can therefore be recommended as a biofertilizer in establishment of papaya plants.

CHAPTER SEVEN

THE EFFECT OF AMENDING SOIL WITH ARBUSCULAR MYCORRHIZAL FUNGI ON PHYTO-ACCUMULATION OF PROXIMATES, SELECTED MINERALS, ASCORBIC ACID, TOTAL CAROTENOIDS AND TOTAL POLYPHENOLS IN JKUAT AND MALKIA F1 CARICA PAPAYA HYBRIDS

Abstract

Papaya (*Carica papaya* L.) is a climacteric fruit with a resilient and distinctive aroma. The consumption of the fruit is global due to its high nutritive and medicinal values. However, there has been low production of quality papaya fruits due to unavailability of crucial mineral elements in the soils. The current study therefore focuses on the effect of indigenous arbuscular mycorrhizal fungi (AMF) as a biofertilizer on the quality of papaya fruits. The papaya seeds from JKUAT and Malkia papaya hybrids were sown in trays and transplanted at 3 leaves stage into 5 litre pots within the green house. Four treatments were used; AMF inoculum only, compost manure only, combination of AMF inoculum and compost manure and control where only soil and sand media. The treatments were added into the soil media of the papaya plantlets every 4 weeks after first transplanting until they were 20 weeks old. They were then transplanted to 100 litre containers, where completely randomized design was used and replication of six papaya plants for each treatment and hybrid. Watering, weeding and cooling the green house with water fumes was carried out when necessary; as the papaya plants grew until the fruits attained physiological maturity. The fruits were separately harvested and ripened to a predetermined stage. They were then analysed for proximates, minerals (nitrogen, phosphorous, potassium, magnesium, calcium, iron and zinc), ascorbic acid, total carotenoids and total polyphenols. Data obtained was subjected to two-way ANOVA at $p \leq 0.05$ significance level; means were separated using Tukey's HSD test in Genstat's 15th edition. JKUAT hybrid with AMF inoculum treatment had 3.07%crude fibre and 8.42mg/100g phosphorous content while JKUAT hybrid with both AMF inoculum and manure treatments had 4.9 % crude fibre

and 9.88 mg/100g phosphorous content. JKUAT and Malkia hybrids with compost manure treatment had potassium content of 98.31mg/100g and 109.4 mg/100g respectively while the controls had 31.58 mg/100g and 35.32mg/100g respectively. Incorporating soil media with manure and AMF inoculum improved the nutritive quality of papaya fruits and this was contingent on papaya hybrids.

7.1 Introduction

Intake of both vegetables and fruits on regular basis reduces risks of chronic ailments such as cataract, Alzheimer's disease, cancer, cardiovascular disease, stroke, among others (La Vecchia *et al*., 2001). Papaya displays a burst of respiration and ethylene production during ripening and hence, considered a climacteric fruit (Manenoi *et al*., 2007). The nutritional content of papaya fruits changes during the ripening stage and this is mostly due to carotenoids synthesis whose levels elevate during usual ripening (Martins *et al*., 2016). Papaya fruit provides fibers as well as basic and complex sugars (fructose, glucose and sucrose) (Shiga *et al*., 2009). Degradation of crude fiber provides the commercially desired softness of the fruits'flesh (Shiga *et al*., 2009) while the sugar levels, which contribute to the sweetness of the fruit, vary during ripening process (Gomez *et al*., 2002). Additionally, the fruit provides vitamin C which is continually synthesized as the fruits develop (Souza *et al*., 2008).

Low mycorrhizal colonization, that is, less than 20%, due to factors such as environmental or genetic affects the yield and quality of fruits (Wang *et al*., 2019). Inoculating with AMF enhanced the absorption of water and mineral nutrients by citrus roots as well as endorsing photosynthetic intensity and chlorophyll synthesis, resulting to accumulation of fruit sugars (Zou *et al*., 2021).

Microbes occur through the soil profile although they are most abundant around macropores, plants' rhizosphere and on the surface soils (Fierer *et al*., 2007). The diversity and abundance of microbes are associated with the organic matter of the soil and are peak in the top 10 cm from the soil surface and reduces with depth. (Aislabie, *et al*., 2013).

Naturally, plant roots interacts with soil organisms which can have either beneficial, neutral or pathogenic effects (Callaway *et al*., 2004). Some of the beneficial organisms that have been recognized are arbuscular mycorrhizal fungi (AMF) and they have an influence on the plants' community productivity, structure and diversity in a natural environment (Stein *et al*., 2009). They are the utmost common symbiotic fungi, related with various species of plants (Smith and Read, 2008). Arbuscular mycorrhizal fungi rely entirely on the carbon provided by the host plant and on the other hand, the plant obtains additional nutrients, protection against pathogens and enhanced water relations from AMF (Auge 2001). Additionally, mycorrhizal association brings about accumulation of water in greater amounts to the host plants, particularly when water supply is limited and hence improved growth of plants (Goicoechea *et al*., 2004).

The mechanisms by which AMF protects pathogen attack include enhanced nutrient status, alterations in root branching and root morphology, infection sites competition and the plant defense mechanisms becomes activated (Heinonsalo *et al*., 2016). Mycorrhizal formation enhances exploration of greater volumes of soils and the absorption and translocation of nutrients to the plants due to the extraradical mycelium, hence the plants are able to take up more nutrients especially those with restricted mobility in soil, such as phosphorus as well as trace elements (Gupta *et al*., 2000). Arbuscule, part of AMF, is the nutrient exchange site, finely branched and it penetrates through the root cell of the plants (Aislabie, *et al*., 2013).

The quantity and the quality of roots exudates with mycorrhizal formation inhibits pathogen growth in soil (Norman and Hooker 2000) and also interferes with hormonal levels such as ethylene, cytokinins, auxins and abscisic acid (Torelli *et al*., 2000). The fruiting process is elicited by gibberellins and auxin (Serrani *et al*., 2007; De Jong and Vriezen, 2009). The roots and nodules of *Vigna mungo* produced Indole-3-acetic acid (IAA) as a result of synergistic effect of AMF and rhizobia (Chakrabarti *et al*., 2010). The symbiosis of AMF can also arouse the synthesis of secondary metabolites in plants which increases tolerance to biotic and abiotic stresses in plants and are helpful to the well-being of human health through their antioxidant activity (Seeram, 2008).

Arbuscular mycorrhizal fungi can be applied directly to the plant roots, soil, seed or the seedlings and thereafter, they mobilize and access the nutrients to the plants (Pal *et al*.., 2015). According to Rai *et al*., (2013), AMF can be applied alone or blended with the natural fields, so as to benefit the plants' overall growth and development directly or indirectly (Kour *et al*., 2019). The roots of epiphytes, herbs, shrubs, aquatics, xerophytes, hydrophytes, trees, terrestrial and aquatics plants develop mycorrhizal associations when grown with the inadequate vital elements such as nitrogen, sulphur, zinc, copper, iron, phosphorus and boron (Rastegari *et al*., 2020).

Eradication of AMF communities leads to various challenges with plant establishment and existence (Madawala, 2021). This fungi can be eliminated through chemical or mechanical soil disturbance since the functioning and vigor of the AMF is reduced. Disturbance of the soil reduces the abundance of the spores and the colonization of roots especially to exotic AMF isolates that have not adjusted to soil environments as compared to indigenous isolates (Madawala, 2021). Consequently, isolation of indigenous AMF is a latent biotechnological means for plants' inoculation especially in disturbed ecosystems (Berruti, *et al*., 2014). The current research sought to improve the nutritive quality of papaya fruits through amendment of soil using AMFinoculum.

7.2 Materials and Methods

7.2.1 Establishment of the Papaya Plants

The seeds of JKUAT and Malkia F1 hybrids were sown in sterilized mixture of soil and sand media at a ratio of 1:1. At 2 and 3 leaf stage, the seedlings were transplanted to 500g pots which contained sterilized soil media combined with treatments; AMF inoculum, compost manure, and a combination of the AMF inoculum and the compost manure at a ratio of 1:9. The control media comprised of sterilized soil media only. The plants were replicated six times from each hybrid and each treatment and routine cultural practices of the plants were carried out when necessary. The plantlets were transplanted to 2 litre pots 4 weeks after first transplanting and the treatments were added to the growing media every

4 weeks. When the seedlings were 20 weeks old, they were transplanted into 100 litre containers with three quarter full soil media and placed in a greenhouse. Routine cultural practices were carried out until the fruits were physiologically mature, five months after transplanting. The skin color was full yellow to red and a very soft pulp (Sebedi *et al*., 2022). The fruits were then harvested by twisting them until the stalk snapped off and carefully arrmged in crates according to the varieties and treaments. They were then taken to the JKUAT laboratory for analysis.

7.2.2 Determination of Proximate Composition

7.2.2.1 Determination of Moisture Content

Empty moisture dishes were weighed and recorded. Five grams of papaya fruit pulp was weighed and put into the dishes, dried into the oven at 105 °C for 4 hours. The samples were then removed from the oven and cooled in a desiccator, weighed and recorded (AOAC, 1995). Percentage moisture content was calculated as follows;

% Moisture content =

$$\frac{Weight\ of\ papaya\ sample\ before\ drying - weight\ of\ papaya\ sample\ after\ drying}{\text{Weight of sample before drying}} \times 100$$

7.2.2.2 Determination of Ash Content

Crucibles were preconditioned in the oven, cooled in a desiccator and weighed. Approximately 5 g of papaya fruit pulp was accurately weighed in triplicate into the weighed crucibles then charred using flame until all smoke was removed. The samples were then transferred into a muffle furnace and incinerated at 550 °C until white ash was obtained. The remains were cooled in a desiccator, weighed and recorded (AOAC 1995). Ash content was expressed as percentage of the original sample weight on dry weight basis as follows:

$$\% \text{ crude ash} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

7.2.2.3 Crude Protein Determination

Crude protein was determined using the semi-micro Kjeldahl method (AOAC, 1995). Two grams of the papaya fruit sample was weighed into a digestion flask together with a combined catalyst of 5 g of potassium sulphate (K_2SO_4), 0.5 g of copper sulphate ($CuSO_4$) and 15 mL of concentrated sulphuric acid (H_2SO_4). The mixture was digested in a fume hood until the color changed to blue-green. The contents were cooled and transferred into a 100 ml volumetric flask which was topped up to the mark using distilled water. A blank digestion composing of acid and catalyst was performed concurrently. 10 ml of the topped-up digest was added into a distilling flask and washed with 2 ml of distilled water. Fifteen milliliters of 40% sodium hydroxide (NaOH) was added and also washed with 2 ml distilled water. Distillation was performed to obtain a distillate of 60 ml in volume. Into the distillate, mixed indicator was added, followed by titration with 0.02N hydrochloric acid (HCl) until color changed to green. All determinations were performed in triplicate. The titres were recorded and protein content determined following the formula below:

$$\% \text{ Nitrogen } = (\text{Titre for sample} - \text{titre for blank}) \times \text{N} \times \text{f} \times 0.014 \times \frac{100}{\text{S}} \times \frac{100}{\text{V}}$$

N = Normality of standard hydrochloric acid (HCl) solution (0.02)

f = factor of standard HCl solution

V = Volume of diluted digest taken for distillation (ml)

S = weight of sample taken (g)

% Protein = Nitrogen × protein factor (6.25)

7.2.2.4 Crude Fibre Determination

Two grams of papaya fruit pulp was weighed into a conical flask. 200 ml of sulphuric acid (1.25% H_2SO_4) was poured into the flask and boiled for 30 minutes. The mixture was then filtered into another conical flask after which the residue was washed thoroughly with hot distilled water to wash away the acid. 200 ml of 1.25% NaOH was added to the washed residue and the same process, above, repeated. The mixture was again filtered using glass wool and 15 ml of 1% hydrochloric acid (HCl) solution was used to rinse the residue followed by another rinsing with hot distilled water to rinse away the acid from residue. The residue was then washed using 10 ml petroleum ether in a fume hood. The residue was transferred to labelled crucibles and air-dried for 30 minutes. The residue in porcelain crucibles were then dried in a hot-air oven at 100°C for 1 hour before transferring to a desiccator to cool for 15 minutes. The crucible weight was recorded (W1). The crucibles were then incinerated in a muffle furnace at 550 °C for 3 hours. The crucibles and samples were then cooled in a desiccator and weighed (W2) (AOAC, 1995). Crude fibre was calculated as follows:

$$\%\ \text{Crude fibre} = \frac{W1 - W2}{S} \times 100$$

W1= crucible weight after oven

W2= crucible weight after ignition

S= sample weight

7.2.2.5 Determination of Crude fat

Crude fat was determined using Soxhlet method according to (AOAC, 2000). Extraction flasks were conditioned in the oven for 1 hour at 105 °C then cooled in a desiccator to room temperature and weighed. Five grams of pre-dried papaya fruit samples were weighed into extraction thimbles and covered with defatted cotton wool. The thimbles

were placed in thimble support holders and fixed into the extraction unit. Fat extraction was done using petroleum ether and extraction proceeded for 8 hours. The extraction solvent was removed through rotary evaporation then the extracted fat was put to dry in an air oven at 105 °C for 30 min. The extraction flasks were cooled in a desiccator and the final weight of the flasks with the extracted fat taken. Fat content in percentage was calculated as follows:

$$\% \text{ fat} = \frac{\text{weight of extracted fat (g)}}{\text{weight of sample (g)}} \times 100$$

7.2.2.6 Carbohydrate Determination

Carbohydrate content was determined by difference method according to Alam *et al*., (2006):

$$\% \text{ Carbohydrate} = 100 - (\% \text{ moisture content} + \% \text{ crude fibre} + \% \text{ ash content} + \% \text{ fat content} + \% \text{ protein})$$

7.2.3 Determination of Selected Minerals (Nitrogen (N), Potassium (K), Phosphorous (P), Magnesium (Mg), Iron (Fe), Calcium (Ca) and Zinc (Zn)

Minerals were analysed using the AOAC (1996) method. Five grams of the pulp was charred in the oven for 30 minutes then put in a muffle furnace at 550°C for eight hours to ash. The ash was allowed to cool and diluted with 10ml of 1N hydrochloric acid. The mixture was then filtered and diluted with 100ml of distilled water. Calcium, magnesium, zinc and iron were analysed using atomic absorption spectrophotometer, Potassium was analyzed using flame emission photometer, phosphorous was determined using spectrophotometer while nitrogen was determined using the same procedure for crude protein described in section 7.3.2.3 above.

7.2.4 Determination of Ascorbic Acid

Ascorbic acid content of the papaya fruit juice was determined by visual titration according to AOAC (1996) methods. Five milliliters of the juice was topped up with 10% trichloroacetic acid (TCA) in 100ml volumetric flask. The indicator (2, 6-dichlophenolindophenol) was titrated into 10ml of the fruit juice until pink color appeared. Ascorbic acid content was calculated as follows: Ascorbic acid (mg/100g) = (A-B) X C X 100/S X (50/5) Where A = volume in ml of indophenol solution used in the sample. B = Volume (in ml) of indophenol solution used for the blank, C = Mass (in mg) of ascorbic acid equivalent to 1 ml of standard indophenol solution. S = Weight of the sample taken (in ml)

7.2.5 Determination of Total Phenolic Content

The total phenolic contents of the papaya fruit pulp were estimated using the Folin Ciocalteau reagent as described by Singleton and Rossi, (1965). The calibration curve was plotted using the absorbance results read after mixing 1 ml aliquots in the concentrations of 50, 100, 150, 200, 250, 300, 350, 400 and 450 mg/ml Gallic acid solutions with 5.0 ml of Folin Ciocalteu reagent (diluted tenfold) and 4.0 ml of sodium carbonate solution (75 g/l). The absorbance was measured after 30 minutes at 765 nm. For the fruit pulp, 1 ml was mixed separately with the same reagents, as performed for constructing the calibration curve. After 1 h, the absorbance was measured to determine the total phenolic contents in both extracts separately using the formula,

$C = C_1 * V/m$

where C = total phenolic content in mg/g, in GAE (Gallic acid equivalent), C1 = concentration of Gallic acid established from the calibration curve in mg/ml, V = volume of extract in ml, and m = weight of the fruit pulp in g.

7.2.6 Determination of Total Carotenoids

Total carotenoid content was determined by a modified chromatographic procedure (Heionen, 1990). A sample of 5g of the papaya fruit was crushed in a pestle with a mortar. A spatula of hydroflorosupercel was then added and then extracted using 50ml cold acetone and filtered using glass funnel until the residue became white. Partitioning procedure followed using 25ml of petroleum ether in a separating funnel. Saponification was carried out by adding an equal amount of extract into 3ml of 10% (potassium hydroxide) KOH in methanol and a few drops of 0.1% butylatedhydrotoluene in petroleum ether. Sodium sulphate (anhydrous) was added to remove water and further concentration was carried out using a rotary evaporator. The carotenoids content was determined using HPLC (Model LC-10AS, Shimadzu Corp., Kyoto, Japan), having the following conditions; Mobile phase: acetonitrile: methanol: dichloromethane (70: 10: 20), Injection volume: 10µL, Flow rate: 1.0 ml/min, Column: ODS 150; Oven temperature: 35 ^{o}C. The carotenoid content was calculated as follows: Total carotenoids (mg/100g) = A × Volume (ml) × 10^4 / A1%1cm×sample weight (ml) Where A= absorbance; volume = total volume of extract (25 ml); A1%1cm = absorption coefficient 2500 (for mixtures).

7.3 Results

7.3.1 Proximates

The crude fibre content was significantly different ($p \leq 0.05$) among the treatments in JKUAT and Malkia F1 papaya hybrids. JKUAT hybrid with both AMF and compost manure treatments had 4.9% crude fibre and 1.35% in the treatment of compost manure only. Malkia F1 hybrid with AMF and compost manure treatments had crude fibre of 5.7% and 2.0% in compost manure only. Carbohydrates (CHO) contents were also significantly different ($p \leq 0.05$) among the treatments. JKUAT hybrid with AMF and compost manure treatments had the least amount of CHO (2.69%) while the controls had the highest amount of CHO (6.97%). Malkia F1 hybrid with compost manure treatment had the highest amount of CHO (7.98%) while Malkia F1 hybrid with AMF and compost manure

treatment had the lowest amount of CHO (1.93%). There were interactions between the hybrids on CHO contents. There were interactions between hybrids and among the treatments on ash and oil contents. Malkia F1 hybrid with AMF and manure treatment had the highest moisture content of 90.6%, followed by compost manure treatment on Malkia F1 hybrid, 88.4%. There were interactions between hybrids and among the treatments on the moisture contents levels. The proteins content in control treatments on Malkia F1 hybrid was significantly different ($p \leq 0.05$) from other treatments with 0.11% protein content (Table 7.1).

Table 7.1: Effect of Soil Amendment on Moisture, Ash, Proteins, Crude Fibre, Oil and Carbohydrate Contents of JKUAT and Malkia F1 Papaya Fruits

Hybrid	Treatments	Moisture content (%)	Ash (%)	Proteins (%)	Crude fibre (%)	Oil content(%)	CHO (%)
Malkia F1	Control	90.0^{ab}	0.33^{a}	0.11^{a}	1.41^{f}	1.3^{a}	6.86^{ab}
	AMF	90.1^{ab}	0.43^{a}	0.076^{cd}	4.3^{c}	1.37^{a}	3.69^{c}
	AMF and Manure	90.6^{a}	0.35^{a}	0.09^{bc}	5.7^{a}	1.36^{a}	1.93^{c}
	Compost manure	88.4^{b}	0.22^{a}	0.08^{bcd}	2.0^{e}	1.29^{a}	7.98^{a}
JKUAT	Control	90.19^{ab}	0.36^{a}	0.069^{d}	1.13^{g}	1.29^{a}	6.97^{ab}
	AMF	90.0^{ab}	0.84^{a}	0.077^{cd}	3.07^{d}	1.28^{a}	4.74^{bc}
	AMF and Manure	90.19^{ab}	0.83^{a}	0.097^{ab}	4.86^{b}	1.33^{a}	2.69^{c}
	Compost manure	89.6^{ab}	0.5^{a}	0.071^{d}	1.35^{fg}	1.65^{a}	6.79^{ab}
	ANOVA (p - values)						
	Hybrids	0.431	0.11	<.001	<.001	0.606	0.658
	Treatments	0.018	0.546	<.001	<.001	0.680	<.001
	Hybrids × treatments	0.215	0.809	<.001	<.001	0.456	0.253

Means within each column followed by a different letter differ significantly at ($p \leq 0.05$)

7.3.2 Minerals

Amending the soil media with AMF inoculum significantly ($p \leq 0.05$) affected the mineral contents of the papaya fruit. JKUAT hybrid with AMF inoculum treatment had phosphorous and potassium content of 8.42mg/100g and 109.4mg/100g respectively as compared to compost manure treatment which had 6.39 mg/100g and 98.31mg/100g respectively. Malkia F1 hybrid with both AMF inoculum and compost manure treatments had phosphorous and potassium content of 10.48mg/100g and 117.3mg/100g respectively as compared to compost manure treatment which had 7.4mg/100g and 103.3mg/100g respectively. The calcium content in JKUAT hybrid with both AMF inoculum and compost manure treatments was 19.45mg/100g while the controls had 8.21mg/100g. Iron

content in Malkia F1 hybrid with AMF inoculum was 0.4mg/100g while the control had 0.13mg/100g (Table 7.2).

Table 7.2: Effect of Soil Amendment on Nitrogen, Phosphorous, Potassium, Calcium, Magnesium, Iron and Zinc Contents of JKUAT and Malkia F1 Papaya Fruits

Hybrid	Treatments	Nitrogen (mg/100g)	Phosphorous (mg/100g)	Potassium (mg/100g)	Calcium (mg/100g)	Magnesium (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)
Malkia F1	Control	0.002^{d}	2.85^{f}	35.3^{f}	8.44^{f}	10.5^{f}	0.13^{d}	0.01^{e}
	AMF	0.012^{bc}	9.32^{b}	112.6^{b}	16.7^{c}	25.4^{cd}	0.42^{b}	0.06^{c}
	AMF and Manure	0.014^{ab}	10.5^{a}	117.3^{a}	18.5^{b}	27.6^{b}	0.51^{a}	0.09^{b}
	Compost manure	0.013^{bc}	7.4^{d}	103.3^{d}	15.4^{e}	22.1^{e}	0.38^{bc}	0.04^{d}
JKUAT	Control	0.001^{d}	2.45^{f}	31.58^{g}	8.2^{f}	9.9^{f}	0.1^{d}	0.012^{e}
	AMF	0.012^{bc}	8.42^{c}	109.4^{c}	16.6^{cd}	27.3^{bc}	0.42^{b}	0.07^{bc}
	AMF and Manure	0.016^{a}	9.88^{b}	115.7^{a}	19.5^{a}	31.1^{a}	0.53^{a}	0.12^{a}
	Compost manure	0.011^{c}	6.39^{e}	98.3^{e}	15.8^{de}	23.7^{de}	0.36^{c}	0.05^{cd}
	ANOVA(*p*-values)							
	Hybrids	0.445	<.001	<.001	0.075	<.001	0.192	<.001
	Treatments	<.001	<.001	<.001	<.001	<.001	<.001	<.001
	Hybrids × treatments	0.024	0.074	0.037	0.025	0.002	0.053	0.01

Means within each column followed by a different letter differ significantly at ($p \leq 0.05$)

7.3.3 Ascorbic Acid Content

Ascorbic acid content was significantly ($p \leq 0.05$) different among the treatments. JKUAT hybrid with both AMF inoculum and compost manure treatments had ascorbic acid content of 62.6 mg/100g while compost manure treatment had 46.93mg/100g. Malkia F1 hybrid with AMF inoculum and compost manure treatments had ascorbic acid content of 68.32mg/100g while compost manure treatment had 52.6 mg/100g (Table 7.3).

7.3.4 Total Polyphenols Contents

Soil amendment with AMF inoculum significantly ($p \leq 0.05$) affected the total polyphenols contents among the treatments. The total polyphenol contents in JKUAT hybrid with both AMF inoculum and compost manure treatments was 58.6 mg GAE/100g while compost manure treatment had 48.2mg GAE/100g and the control 42.8 mg GAE/100g. Malkia F1 hybrid with AMF inoculum treatment had total polyphenol contents of 53.6 mg GAE/100g while fruits from compost manure treatment 46.6 mg GAE/100g of total polyphenols. There were interactions between the hybrids.

7.3.5 Total Carotenoids Content

Total carotenoids contents in the fruits were affected significantly ($p \leq 0.05$) by AMF inoculation in the soil media. Malkia F1 hybrid with AMF inoculum treatment had total carotenoids contents of 3.4 mg/100g while the controls had 1.06mg/100g. JKUAT hybrid with both AMF inoculum and compost manure treatments had the highest ($p \leq 0.05$) total carotenoid contents of 4.2mg/100g while the controls of both JKUAT and Malkia F1 hybrids had the lowest ($p \leq 0.05$) carotenoids contents of 1.05mg/100g and 1.1 mg/100g respectively (Table 7.3).

Table 7.3: Effect of Soil Amendment on Ascorbic Acid, Total Polyphenols and Total Carotenoids of JKUAT and Malkia F1 Papaya Fruits

Hybrid	Treatments	Ascorbic acid (mg/100g)	Total polyphenols (mg GAE /100g)	Total carotenoids (mg/100g)
Malkia F1	Control	22.8^{f}	42.6^{e}	1.1^{f}
	AMF	58.1^{c}	53.6^{b}	3.4^{c}
	AMF and Manure	68.3^{a}	58.9^{a}	3.99^{ab}
	Compost manure	52.6^{d}	46.6^{d}	2.35^{e}
JKUAT	Control	20.5^{f}	42.8^{e}	1.05^{f}
	AMF	54.6^{d}	51.2^{bc}	3.79^{b}
	AMF and Manure	62.6^{b}	58.6^{a}	4.2^{a}
	Compost manure	46.9^{e}	48.2^{cd}	2.9^{d}
	ANOVA (*p* - values)			
	Hybrids	<.001	0.653	<.001
	Treatments	<.001	<.001	<.001
	Hybrids × treatments	0.005	0.087	<.001

Means within each column followed by a different letter differ significantly at ($p \leq 0.05$)

7.4 Discussion

In this study, crude fibre and carbohydrates differed among the treatments while ash content and oil contents did not show any differences among the treatments in both JKUAT and Malkia F1 hybrids. Oil contents in this study did not differ among the treatments in the 2 hybrids and this observation was similar to Cabral *et al*., (2014) who reported insignificant differences among cultivars of the papaya fruits whose average value was 0.38 g/100 g. Higher oil contents in papaya fruits indicate that they are poor sources of energy (Garcia-Diaz *et al*., 2012). The range of ash content in both JKUAT and malkia papaya hybrids and in all treatments was 0.22% and 0.84%. Low ash contents in the fruit pulp shows that the total inorganic mineral content is also low (Cabral *et al*., 2014).

In the current study, there were interactions between the hybrids and among the treatments on moisture content levels. Malkia F1 hybrid with a combination of AMF and manure treatments had the highest moisture content level while Malkia F1 hybrid with compost manure treatment had the lowest moisture content level. There was no significance difference among all the treatments in JKUAT hybrid. The higher moisture levels in Malkia F1 hybrid with both AMF and manure could be attributed to the ability of

mycorrhizal plants to retain more moisture in comparison with non–mycorrhizal plants (Auge, 2004). Hence, Malkia F1 hybrid could have a more superior trait than JKUAT hybrid in terms of moisture retention in the fruits when AMF and manure are used in the soil media. However, when plants are grown under moisture stress, AMF helps to retain fruit succulence and prompt drought tolerance in plants (Yadav *et al*., 2015). Plants induced with mycorrhiza are able to retain higher water tissue content and this leads to improved phenological, morphological and physiological characteristics of mycorrhizal plants (Wu and Xia, 2006). Mycorrhizal plants had more moisture contents in okra fruits signifying the vital role of AMF in plant water relations (Kumar *et al*., 2015). Inoculating plants with mycorrhiza improves water utilization and nutrient uptake particularly minerals with low soil mobility such as phosphorous, zinc and copper and this enhances plants' tolerance to environmental stresses (Aguirre *et al*., 2011).

The present study revealed high carbohydrates contents in non- inoculated treatments compared to inoculated ones and also low proteins levels were recorded in all the treatments. Carbohydrates are necessary for the incorporation of ammonia in amino acids and accelerate proteins biosynthesis (Bulgari *et al*., 2015). Elevated protein contents in plant leaves has been found to be associated with increased carbohydrate concentrations (Abbas, 2013).The protein contents are as a result of amino acids that are used for the biosynthesis of protein after being incorporated by the plant (Abbas, 2013). The hyphal network of AMF improves the soil quality through enhancement of soil particle aggregation and soil erosion is reduced, reduces leaching of nutrients from the soil thus promoting nutrient retention and the risk of ground water contamination is decreased (Tavarini *et al*., 2018). Regulation of root biomass by AMF improves uptake and translocation of nutrients and this results to an increase in proteins and total carbohydrates (Lucini *et al*., 2019). Some fruits accumulate starch that breaks down into sugars during the ripening stage, through catabolic degradation, but this is an exception with papaya fruit. Therefore, papayas that are left on-tree until late harvesting stages have more consumer benefits that is, they obtain a continuous supply of sucrose produced by the photosynthesis in the leaves, (Sharma *et al*., 2008).

In the current study the mineral contents of the fruits was enhanced through AMF inoculation, as evidenced by Malkia F1 and JKUAT papaya hybrids with AMF inoculum as compared to the controls and the media containing compost manure only. Phosphorous, potassium, magnesium, calcium and iron contents greatly improved in the papaya fruits upon AMF inoculation into the soil media. Arbuscular mycorrhizal fungi has the ability to absorb nitrogen, potassium, phosphorous, sulphur, zinc and calcium from the soil and translocate it to related plants. This development is as a result of the increased absorptive surface area of the roots (Nouri *et al*., 2014). Oliveira *et al*., (2005) observed that formation of AMF correlated with calcium, copper, iron and zinc in guarana (*Paullinia cupana*) and magnesium, calcium, copper and phosphorous in cupuacu (*Theobroma grandflorum*). Inoculating cucumber plant with *Glomus mosseae*, AMF species, brought about detection of elements such as zinc, copper and phosphorous which are essential for plant growth and this indicates that the fungal mycelium absorbed these elements for the plant (Lee and George, 2005). Trindade *et al*., (2001) also recorded increased absorption of potassium, copper and phosphorous in papaya seedlings after *Gigaspora margarita*, AMF species, was inoculated. Mycorrhizal inoculation increased zinc contents in tomato fruits (Cavagnaro *et al*., 2007) and Giovannetti *et al*., (2012) observed increased phosphorous, zinc and lycopene contents in AMF inoculated tomato fruits.

In this study, ascorbic acid contents increased in the papaya fruits of both JKUAT and Malkia F1 hybrids through AMF inoculation. The controls had the least amounts of ascorbic acid contents in both hybrids. Higher concentration of ascorbic acid were observed on the fruits of inoculated strawberry plants (Bona *et al*., 2015). Accessibility of vitamin C biosynthesis substrates and also high levels of sugars particularly fructose and glucose in inoculated plants could lead to upsurge of ascorbic acid contents (Cruz-Rus *et al*., 2011).

The current study showed an increase of phenolic compounds after AMF inoculation. The interactions between the hybrids and the treatments indicate the differences in phenolic compounds within the hybrid and not necessary due to the effect of any of the treatments. These results concurred with total phenolic contents of six papaya cultivars studied by

Farina *et al*., (2020) which varied between 38.6 mg GAE/100 g and 60.2 mg GAE/100 g. Application of AMF in the soil media during transplanting increased the total phenolic compounds of the three studied cultivars of lettuce (Baslam *et al*., 2011). Generally, papaya fruit pulp contains polyphenolic compounds (Karunamoorthi *et al*., 2014). Phenolic compounds accumulation depends on the physiology of the fruit and this is the outcome of the balance between biosynthesis and metabolic phases, for instance catabolism and turnover, (Oufedjikh *et al*., 2000). Phenols contribute to the taste and color of fruits and moreover, they possess antimutagenic and anticarcinogenic activities, (Reddy *et al*., 2010). Mycorrhizal inoculation alters both primary and secondary metabolisms of the plant, and this stimulates production of phytochemicals in the plant (Sbrana *et al*., 2014). Phenolic compounds have been reported to contain great antioxidant potential, hence useful effect to human health (Yuan *et al*., 2003).

The present study showed that total carotenoids contents were enhanced by mycorrhization. In accordance with our results, Ulrichs *et al*., (2008) recorded higher carotenoids and lycopene content in tomato fruits of plants inoculated with *Glomus sp*. Inoculation with AMF also improved the carotenoids quantities of outer leaves of lettuce (Baslam *et al*., 2013). In mycorrhizal tomato, carrots and parsley fruits, carotenoids content increased significantly as compared to non-inoculated plants as was observed by Regvar *et al*., (2003). The final nutritional composition of climacteric fruits such as papaya is as a result of physiological and chemical changes that occur during pre and post-harvest periods (Roberts and Dixon, 2008). Total carotenoids contents in the flesh of papaya fruit increases during maturation stage (Rodriguez-Amaya, 2016). The inoculation can be reckoned to be a good strategy to boost the symbiosis benefits and improve the fruits' nutritional status. In papaya, the main types of carotenoids found are β-cryptoxanthin, β-carotene, lycopene and lutein and are major bioactive compounds (Wall, 2006).

The present study has verified the importance of AMF inoculation to papaya seedlings during transplanting and boosting the soil media with AMF inoculum for improved nutrient contents in papaya fruits for JKUAT and Malkia F1 papaya hybrids. Thus, for enhancement of nutritive value of papaya fruits, soil media should be amended using AMF

inoculum and this will be more effective if the amendment is done at the seedling and vegetative stage of growth.

CHAPTER EIGHT

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

The current research assessed the efficacy of indigenous AMF on the performance, tolerance to phytophthora blight, water stress and nutritional quality of JKUAT and Malkia F1 papaya hybrids. Moreover, the AMF spores and other fungi associated with AMF were morphologically and molecularly characterized. This study has proved that boosting organic manure with AMF generated better performance in papaya seedlings regardless of the hybrid. This study reports for the first time the isolation and bulking of indigenous AMF inoculum obtained from rhizospheric soil and roots of papaya, grass and banana plants from Juja, Mwea and Mitunguu areas. The inoculum was incorporated in the soil media to improve on the growth of JKUAT and Malkia F1 papaya hybrid seedlings, control of phytophthora blight disease and improving the fruit quality of JKUAT and Malkia F1 papaya hybrid. This study therefore offers a more environmentally friendly soil amendment, for papaya seedling growth and establishment. Furthermore, papaya hybrid enhanced with AMF inoculum and compost manure was found to develop resistance against phytophthora blight disease and tolerance to water stress. Ascorbic acid and carotenoids contents were enhanced on papaya plants with AMF inoculation. Boosting existing soil media of plants with AMF inoculum prior to establishing plants can yield better results in overall plant performance and yield.

Most farmers commonly use inorganic substitutes to improve the soil nutrient status in order to boost crop growth and development. However, the rise on the environmental costs are a great concern due to the negative effects such as water contamination (surface and groundwater) increased pest resistance, soil erosion, greenhouse gas emissions and reduced biodiversity (Tilman *et al*., 2002). The provision of adequate, healthy and safe food by evading environmental degradation are the utmost key issues the world is facing under current and the anticipated climate change (Timsina, 2018). Microbial or bio fertilizers, containing organisms such as fungi can be applied to soils, seeds or decomposting materials to escalate the micro organism population and hasten the

microbial processes such as phosphate solubilisation or atmospheric nitrogen fixation (Mamaril, 2004). Increasing the amount of nutrients, providing adequate water, control of pests, diseases and weeds have been considered as major contributors of high crop yields (Timsina *et al*., 2018). Bio fertilizers such as AMF, contributes indirectly to plant nutrients' availability by obtaining them from surroundings such as soil organic matter and atmospheric nitrogen (Mamaril, 2004).

Inoculating the papaya seedlings with AMF tendered a crucial opportunity in formation of the symbiosis before transplanting. The plants survived the acclimatization associated with many seedlings and the overall performance was increased. Arbuscular mycorrhizal fungi (AMF) reduced application of fertilizers that have previously been considered crucial for effective growth of papaya fruits. Moreover, the land ecosystems with unavailability of essential elements such as calcium, magnesium, phosphorous, potassium and zinc will be enhanced through AMF inoculation. In addition, combining compost manure and AMF inoculum resulted to better levels of the analyzed nutrients as compared to AMF inoculum only in JKUAT and Malkia F1 papaya hybrids. The application of AMF inoculum in soils in which manure is also being applied develops symbiotic interaction and extensive root morphology that improve effectiveness of acquisition of immobile nutrients (Yang *et al*., 2023).

Isolation of AMF spores and bulking of the AMF inoculum involves a fathomable protocol and thereafter used as a biofertilizer and this will not only improve the plants' growth and performance but also minimize use of pesticides and other chemicals which are unfriendly to the environment.

8.1 Recommendations

1) The research recommends utilization of grass rhizospheric soil to boost the soil media used for papaya growth since it had the most abundant number of AMF spores compared to banana and papaya rhizospheric soil.

2) This study recommends utilization of bulked AMF inoculum in combination with compost manure as soil amendment for the growth of papaya plants.
3) The inoculation of the AMF inoculum should be done both at seeding and seedling stages for effective growth.
4) Boosting the soil media with a combination of AMF inoculum and compost manure can be recommended for control of phytophthora blight disease in papaya plants, tolerance in water stress and improved nutritive quality of papaya fruits, especially ascorbic acid and carotenoids.

8.1.1 Recommendations for Further Studies

1) Root morphology is improved and becomes extensive through AMF colonization. Therefore, the recommended spacing of inoculated plants should be further investigated on papaya plants to ensure maximal utilization of the spores in the soil.
2) The current study focused on the effect of AMF on phytophthora blight disease, thus, the effect of AMF on other papaya diseases such as anthracnose and papaya ringspot virus should also be studied
3) Nevertheless, in this context, AMF inoculation of seedlings of papaya fruit plants can reduce the seedling development period, fertilizer usage is minimised, increases productivity of papaya nurseries for commercialization and also enhances survival and growth rates after transplantation and during acclimatization. Therefore, other benefits of AMF inoculation such as exposure to high salinity levels, tolerance to flooding and heavy metals exposure could be investigated on the seedlings and fruits of JKUAT and Malkia F1 hybrids, as well as other papaya hybrids.

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APPENDICES

Appendix I: Analysis of Variance (ANOVA) Table for Spore Abundance in Juja, Mitunguu and Mwea for Papaya, Banana and Grass Plants

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.0000	0.0000	0.00	
Area	2	243.2853	121.6426	240.87	<.001
Treatments	2	775.7573	387.8786	768.06	<.001
Area.Treatments	4	130.9885	32.7471	64.84	<.001
Residual	16	8.0802	0.5050		
Total	26	1158.1113			

Appendix II: Analysis of Variance (ANOVA) Table for Roots Biomass of JKUAT and Malkia F1 Papaya Hybrids at 20 Weeks Growth

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	2.8763	1.4381	3.60	
Treatments	3	6180.4025	2060.1342	5158.82	<.001
Hybrid	1	171.6815	171.6815	429.91	<.001
Treatments.Hybrid	3	94.3468	31.4489	78.75	<.001
Residual	14	5.5908	0.3993		
Total	23	6454.8980			

Appendix III: Analysis of Variance (ANOVA) Table for Leaf Length of JKUAT and Malkia F1 Papaya Hybrids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.82583	0.41292	12.19	
Treatments	3	182.45792	60.81931	1795.72	<.001
Hybrid	1	8.05042	8.05042	237.69	<.001
Treatments.Hybrid	3	8.18125	2.72708	80.52	<.001
Residual	14	0.47417	0.03387		
Total	23	199.98958			

Appendix IV: Analysis of Variance (ANOVA) Table for Root Colonization of JKUAT and Malkia F1 Papaya Hybrids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	8.083	4.042	1.58	
Hybrid	1	130.667	130.667	50.93	<.001
Treatments	3	8627.333	2875.778	1120.95	<.001
Hybrid.Treatments	3	137.333	45.778	17.84	<.001
Residual	14	35.917	2.565		
Total	23	8939.333			

Appendix V: Analysis of Variance (ANOVA) Table for Moisture Contents of JKUAT and Malkia F1 Papaya Hybrids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.8196	0.4098	0.93	
Hybrid	1	0.2907	0.2907	0.66	0.431
Treatments	3	6.2753	2.0918	4.74	0.018
Hybrid.Treatments	3	2.2375	0.7458	1.69	0.215
Residual	14	6.1831	0.4417		
Total	23	15.8063			

Appendix VI: Analysis of Variance (ANOVA) Table for Crude Fibre Contents of JKUAT and Malkia F1 Papaya Hybrids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.008400	0.004200	0.74	
Hybrid	1	3.390017	3.390017	600.76	<.001
Treatments	3	62.596950	20.865650	3697.71	<.001
Hybrid.Treatments	3	0.711683	0.237228	42.04	<.001
Residual	14	0.079000	0.005643		
Total	23	66.786050			

Appendix VII: Analysis of Variance (ANOVA) Table for Carbohydrates Contents of JKUAT and Malkia F1 Papaya Hybrids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	1.0788	0.5394	0.55	
Treatments	3	102.1007	34.0336	34.55	<.001
Hybrid	1	0.2020	0.2020	0.21	0.658
Treatments.Hybrid	3	4.4916	1.4972	1.52	0.253
Residual	14	13.7925	0.9852		
Total	23	121.6656			

Appendix VIII: Analysis of Variance (ANOVA) Table for Ascorbic Acid Contents of JKUAT and Malkia F1 Papaya Hybrids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.7288	0.3644	0.55	
Treatments	3	6418.1788	2139.3929	3217.73	<.001
Hybrid	1	112.1473	112.1473	168.67	<.001
Treatments.Hybrid	3	13.0427	4.3476	6.54	0.005
Residual	14	9.3083	0.6649		
Total	23	6553.4058			

Appendix IX: Analysis of Variance (ANOVA) Table for Carotenoids Contents of JKUAT and Malkia F1 Papaya Hybrids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.008725	0.004362	0.58	
Treatments	3	32.416679	10.805560	1446.14	<.001
Hybrid	1	0.445538	0.445538	59.63	<.001
Treatments.Hybrid	3	0.237613	0.079204	10.60	<.001
Residual	14	0.104608	0.007472		
Total	23	33.213163			

Appendix X: Analysis of Variance (ANOVA) Table for Polyphenols Contents of JKUAT and Malkia F1 Papaya Hybrids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.939	0.470	0.29	
Treatments	3	850.210	283.403	177.65	<.001
Hybrid	1	0.336	0.336	0.21	0.653
Treatments.Hybrid	3	12.839	4.280	2.68	0.087
Residual	14	22.334	1.595		
Total	23	886.658			

Appendix XI: Analysis of Variance (ANOVA) Table for Potassium Contents of JKUAT and Malkia F1 Papaya Hybrids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.4924	0.2462	0.31	
Treatments	3	26754.9267	8918.3089	11302.62	<.001
Hybrid	1	69.2920	69.2920	87.82	<.001
Treatments.Hybrid	3	8.8238	2.9413	3.73	0.037
Residual	14	11.0467	0.7890		
Total	23	26844.5816			

Appendix XII: Analysis of Variance (ANOVA) Table for Nitrogen Contents of JKUAT and Malkia F1 Papaya Hybrids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	8.820E-08	4.410E-08	0.06	
Treatments	3	6.103E-04	2.034E-04	284.46	<.001
Hybrid	1	4.419E-07	4.419E-07	0.62	0.445
Treatments.Hybrid	3	9.185E-06	3.062E-06	4.28	0.024
Residual	14	1.001E-05	7.151E-07		
Total	23	6.300E-04			

Appendix XIII: Analysis of Variance (ANOVA) Table for Phosphorous Contents of JKUAT and Malkia F1 Papaya Hybrids

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.04651	0.02325	0.57	
Treatments	3	194.78468	64.92823	1583.91	<.001
Hybrid	1	3.19010	3.19010	77.82	<.001
Treatments.Hybrid	3	0.35311	0.11770	2.87	0.074
Residual	14	0.57389	0.04099		
Total	23	198.94830			