EVALUATION OF *Trichoderma* spp. AND MYCORRHIZA ON GROWTH AND MANAGEMENT OF *Pestalotiopsis theae* CAUSING GREY BLIGHT IN TEA

BY

SAMUEL KIPKOGEI TANUI

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE MICROBIOLOGY IN THE SCHOOL OF SCIENCE UNIVERSITY OF ELDORET, KENYA

AUGUST, 2023

DECLARATION

Declaration by the candidate

This thesis is my original work and has not been submitted for any academic award in any institution; and shall not be reproduced in part or full, or in any format without prior written permission from the author and/or University of Eldoret.

_____ Date _____

Tanui Kipkogei Samuel

SC/PGB/030/14

Declaration by Supervisors

This thesis has been submitted with approval of the following as the supervisors.

_____ Date_____

Prof. Lizzy Mwamburi

University of Eldoret, Kenya

_____ Date_____

Dr. Evelyn Cheramgoi

KALRO-Tea Research Institute, Kenya (TRI).

DEDICATION

To my wife Josephine and children; Kiptoo, Kipkemoi, Kipkosgei, Jepchirchir, Jepkurui for their prayers, love, support and patience.

ABSTRACT

Tea is an economic important crop that provides revenue to farmers and contributes to Gross Domestic Product. Tea maintenance leaves are affected by the grey blight caused by *Pestalotiopsis theae*. *Trichoderma* species are soil fungus that freely colonizes plant roots as opportunistic, plant symbionts with ability to antagonize diseases with additional benefits to the host plant. Arbuscular mycorrhizal fungi provide relationship of symbiosis with plant roots where the host receives minerals nutrients while the fungus gets products of photosynthesis. The study evaluated population dynamics of *Trichoderma* spp. propagules in the soil and percentage colonization of mycorrhizal structures in tea roots. The impact of Trichoderma spp. and arbuscular mycorrhizal fungi on the development characteristics of selected tea clones and the investigation on *Trichoderma* spp. *in-vitro* antagonistic action on the *Pestalotiopsis theae* pathogen. In the nursery, Complete Randomized Block Design (CRBD) using three replications and eight treatments while *In-vitro* studies were conducted under Complete Randomized Design (CRD). Results after three and twelve months on the recovery of *Trichoderma* spp. varied significantly $p<0.05$) with different treatments T39, T39+AMF and T17 population was higher in clone AHP S15/10, TRFK 31/8 and TRFK 303/577. In twelve months, there was an increase in *Trichoderma* spp. population. Percentage recovery of Mycorrhizal structures (hyphae, arbuscules and vesicles) was higher with treatment T17+AMF, T4+AMF and AMF in clones AHP S15/10, TRFK 31/8, and TRFK 303/577. Population of mycorrhizal structures root colonization increased after twelve months. In all the treatments on three clones of tea, there was enhanced growth in the tested parameters (shoot length, shoot diameter, and root length). Overall growth increased in treatment T39+AMF, T4, T17+AMF, T39 and T4 in almost all the tested parameters on the different tea clones. Dry weights did not vary significantly ($p<0.05$) in all the treatments applied to three clones of tea. Antagonistic strains of isolates T17 and T4 have potentiality in controlling *P. theae* in dual culture while in volatile metabolites it was T4 and T39. To boost *Trichoderma* population, re-application may be done twice a year. Recommendation for extension of research in field conditions to include yield as a growth parameter. *In situ* studies on grey blight disease management should be carried out to ascertain bioagent control.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

AHP	African Highland Produce
AMF	Arbuscular Mycorrhizae Fungi
ANOVA	Analysis of Variance
CRD	Complete Randomized Design
CV	Coefficient of Variation
GDP	Gross domestic Product
ITC	International Trade committee
KALRO	Kenya Agriculture and Livestock Research Organization
LSD	Lowest Significant Difference
MM	Millimeters
PDA	Potato Dextrose Agar
PGI	Percentage growth Inhibition
RCBD	randomized complete block design.
RPM	Revolution per Minute
SDW	sterile distilled water

TRFK	Tea Research Foundation Kenya
TRI	Tea Research Institute
UoE	University of Eldoret

LIST OF APPENDICES

ACKNOWLEDGEMENT

I praise the Lord our heavenly father, for all the blessing and strength that helped me finish my thesis. Similar to that, I want to express my profound thanks to everyone who helped guide and support me in writing my thesis in its current form.

I want to express my gratitude to the Director General Kenya Agriculture and Livestock Research Organization (KALRO) and Tea Research Institute (TRI) Director for approving my request for a study vacation. Special thanks to my supervisors Prof. Lizzy Mwamburi and Dr. Evelyn Cheramgoi C Rugutt for their overwhelming interest in the study and useful guidance. My word of appreciation is extended to all staff, more so to the Integrated Pest and Diseases Management (IPDM) department of Tea Research Institute, Kericho for their co-operation during the field and laboratory activities of this research work. Let me not forget the support from UOE staff additionally Prof. Samuel Lutta, the Dean school of Science, Dr Emily Too, and HOD School of Science. May God bless you.

CHAPTER ONE

INTRODUCTION

Tea (*Camellia sinensis (L). O. Kuntze*) is an essential crop that provides revenue to farmers and contributes 26% to foreign exchange worth Ksh130 billion (without domestic sale it contributes 1.2 billion dollars) (I.T.C., 2022). Tea is cultivated/grown by large and small-scale farmers throughout tea growing regions in Kenya (Nyabundi *et al*., 2016). Kenya stands the third-largest tea producer in the world after India and China, provides 22% of the world's black tea, and exports around 95% of its production, with a consumption of the remaining 5% (Muthiani *et al.,* 2016). In tea production, vegetative clonal propagation methods have been used frequently (Jain and Newton 1990).

Soil microorganisms like bacteria, fungi, and protozoa are well-known to inhabit the rhizosphere or the plant fleshy tissue, hence promoting plant development and therefore can act as biofertilizers. *Trichoderma* sp. are naturally occurring, free-living fungi that inhabit soil colonizing plant roots as opportunistic, virulent plant symbionts with ability to provide protection against diseases as well as increase yield in the field (Hermosa *et al*., 2013). *Trichoderma asperellum* has significantly increased the cucumber roots' shoot length, leaf area, and dry weight. (Yedidia *et al*., 2001). Tomato seedling development was improved in an experiment by Ozbay and Newman (2004) using commercial and non-commercial strains of Trichoderma harzianum. Hormones that encourage plant development are produced, and certain strains of *Trichoderma* sp. release nutrients and organic materials from the soil. enhanced root development in the soil's top layer (Harman *et al.*, 2004).

Biofertilizers are constituents that contain living microorganisms that encourage development to seed, plant surfaces, or soil when applied. They invade the rhizosphere or inside the plants and provide or access the host plant's key nutrients. Biological fertilizer contains living organisms which raise the nutrient status of the host plant through presence in association with the plant (Vessey 2003). Studies by Roychowdhury *et al*., (2014) showed that biofertilizers have the capacity to move nutrients from an unusable state to a useable one.

Arbuscular mycorrhiza fungi (AMF) belong to Glomeromycota phylum that live in the majority of plants among Angiosperms, Gymnosperms, Pteridophytes, and some Bryophytes. Most terrestrial plants and fungi form a mutualistic relationship known as mycorrhiza, and these associations are simple to spot in certain locations. Obligatory biotrophic fungi form symbiotic associations because they must colonize the roots of host plants to complete their life cycle. They occur mostly in the majority of agricultural crops, shrubs, species of tropical trees, and certain species of temperate trees (Bagyaraj, 2014). The symbiotic relationship, which has been referred to as "biofertilizers" and "crop bio-protectors," as well as "valuable for integrated management programs of soils and crops," has significant ramifications for the cycling of nutrients in the soil (Carvajal-Muñoz *et al*., 2012). Members of the genus *Trichoderma* spp and arbuscular mycorrhiza fungi have shown promising potential as microbial inoculants that promote plant growth development and disease resistance (Tchameni *et al.,* 2011). Symbiosis is characterized by the movement in two opposite directions flow of nutrients where the plant through the chemical photosynthates, stimulates development and reproduction of the fungus, is another advantageous trait according to Smith and Read, (1997). In turn, the fungus improves the absorption of nutrients and water, particularly immobile nutrients like phosphorus that are present outside of the deficient zones of the root

rhizosphere. AMF and *Trichoderma* spp. are two helpful rhizosphere fungi that enhance plant nutrition and health (Martínez-Medina *et al*., 2011). Mycorrhizal structures abundant in the roots of plants are hyphae, arbuscular, and vesicles. The hypha penetrates the root and spreads in the root cortex while appressorium forms on the surface. Arbuscular is haustoria-like structures that emerged from the intercellular hyphae that ran longitudinally and entered the cortical cells. Vesicles serve as storage organs and are thin-walled objects of varied sizes and forms that hold oil droplets. AMF may be recognized by looking for vesicles and arbuscules in a plant's roots (Bagyaraj 2014).

Pestalotiopsis theae causes the foliar disease known as grey blight, which affects plants including tea (*Camellia sinensis*) and other crops. In both the nursery and the field, it is the most devastating leaf disease affecting growth of tea. In Southern India, it causes 17% production loss and 10 to 20% in Japan (Joshi *et al*., 2009, Chen *et al.*, 2018). Tea nurseries occupy an important position in supplying farmers with vegetative propagated tea seedlings and crop loss is enormous when the pathogen infects bare stalk and young shoots.

The current study focused on the evaluation of the potential of *Trichoderma* spp. and AMF in improving development of tea and in control of grey blight disease management in tea nursery. This will ultimately contribute to use of biocontrol and an increase in agricultural production as well as moving away from the use of chemicals in tea. Therefore, the need to screen the effectiveness of *Trichoderma* spp. and AMF for enhancement of overall growth of vegetative propagated tea cuttings. Similarly, grey blight disease attacking the leaves can be managed after evaluation of antagonistic effect with *Trichoderma* spp.

1.1 Statement of the problem

Studies of Banayo *et al*., (2012) have shown that tea is affected by diseases which lower the yields. Inadequate nutrition can cause low production in tea plants and stress which ultimately make them susceptible to attack by pests and diseases. Chen *et al.,* (2008; 2018) and Joshi *et al.,* (2009) discovered that *P. theae* pathogen of tea, which caused a 17% production loss in India, is most devastating diseases in countries growing tea. In general, chemicals employed as pesticides, fungicides, or fertilizers are utilized in the production of tea, but their excessive usage has reduced the capability of the soil to sustain plant growth, hardened the soil, contaminated the air and water, and emitted greenhouse gases, endangering human health. Due to growing concern about the extensive use of harmful agricultural chemicals, the potential use of biological pesticides has motivated significant study to develop and execute its usage (Epstein and Bassein 2003, Strobel and Daisy 2003).

The current study uses *Trichoderma* spp. and AMF to improve the growth of tea as well as test the potential of these fungi in the management of *Pestalotiopsis theae* causing grey blight disease in tea for ultimate contribution to increase tea productivity.

1.2 Justification

Using *Trichoderma* spp. as a biofertilizers has been shown to improve crop nutrition, increase fertilizer absorption, boost plant growth and greenness, and suppress a variety of plant diseases (Banayo *et al*., 2012; 2013). Higher nutrient in-take by the hyphae expanding and penetrating into nutrient depletion zones is correlated with increased plant development and improve efficiency of immobile components as a result of AMF

(Sadhana, 2014). Additionally, bio-fertilizers are sustainable, biodegradable, renewable, and eco-friendly. Once after piercing the epidermis, some *Trichoderma* strains have shown to colonize root surfaces with vigour and longevity (Harman *et al.*, 2004). Therefore, there is a need to screen the effectiveness of *T. harzianum* and AMF for enhancement of rooting and overall growth of vegetative propagated tea cuttings. In order to improve development, using these microbes as biofertilizers can be a significant step away from relying solely on chemical fertilizers. Chemical fertilizers boost agricultural yield, but their excessive usage has also had negative effects on environment and human health, including hardening soil, decreasing fertility, polluting air and water, and releasing greenhouse gases. Moreover, the high cost of fertilizers increases the cost of production and is prohibitive to most small-scale farmers. *Trichoderma* spp. is a species of rapidly proliferating fungus with persistent conidia and a wide range of substrate preferences. They are aggressive rivals in the race for nutrition and dwelling space (Naher *et al*., 2014). In many developed nations, the importance of low-input agriculture systems has increased in order to prevent environmental deterioration. Risks of contamination of environment through the use of fungicides can impact on non-target organisms, residual effects on crops and cause of chronic illness in human.

1.3 Objectives

1.3.1 General objective

To assess *Trichoderma* spp. and mycorrhiza on the growth and management *Pestalotiopsis theae* causing grey blight disease in tea grown in Kenya.

1.3.2 Specific objectives

1. To assess the population build -up of *Trichoderma species* propagules and percentage colonization of mycorrhizal structures both in the soil and tea roots respectfully.
2. To evaluate the influence of *Trichoderma species* and arbuscular mycorrhizal fungi on the growth parameters in selected clones of tea.
3. *In-vitro* evaluation of *Trichoderma species* in control of *Pestalotiopsis theae* causing grey blight disease in tea grown in Kenya.

1.3.3 Null hypotheses

1. There is no population build -up of *Trichoderma species* propagules and percentage colonization of mycorrhizal structures both in the soil and tea roots respectfully.
2. *Trichoderma species* and arbuscular mycorrhizal fungi do not influence the growth parameters in selected clones of tea.
3. *Trichoderma species* does not have antagonistic activity against *Pestalotiopsis theae* causing grey blight disease of tea grown in Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1. Tea

Camellia sinensis (L) O. Kuntze exists as perennial woody evergreen plant crop of *Theaceae* family which originated from South East Asia and China. China was the earliest country to consume tea as a non-alcoholic drink which remains now popular through the world (Liu, *et al*., 2016). Tea is consumed everyday by two thirds of people on earth even in distant places from wherever they grow, where it helps build local infrastructure and creates work for rural populations (Mukhtar and Ahmad, 2000).

2.1.1 Production of tea in Kenya

Sector of tea as a leading external exchange earner plays key role in socio economic growth in Kenya. In addition, source of livelihood for most rural communities as it contributes to poverty reduction (Kariuki *et al.,* 2022*).* Tea thrives well in the tropical volcanic soils which are rich in nutrients, well drained and red, brownish red or dark red in colour but it can still grow in less fertile soil. Tea from selected mother bushes through vegetative propagation improves rapid multiplication of materials for commercial planting. Preferable soil pH for raising tea cutting range between 4.5 to 5.5pH (Hamid *et al*., 2006).

Polythene sleeves used in vegetative propagation plants should be of 250 gauges with a dimension of 10cm width and 25cm long. The sleeves are filled to ¾ topsoil l and filled

with subsoil and packing should not be loose nor hard. A quantity of ¼ kg DAP fertilizers is mixed with eight wheelbarrows of soil. Tea cuttings should be selected from healthy and vigorous plant from predetermined cultivar mother bushes which had been left to grow freely for 5 to 6 months. Preparation of cuttings using a sharp knife is done under a shade where the very soft tips and the hard-lower parts of branches are discarded and the remaining should consist of a single leaf of 3 to 4cm below the leaf. Planting cuttings in the sleeves must never touch the soil avoiding the fingers touching the bottom and top parts and inserted into the soil at an angle so that the soils are clear of the leaf (Anon 2002, Kamunya *et al.*, 2019).

2.1.2 Constrains to Tea production

A biotic stress in tea is among constraints which affects tea production. Production of tea is unpredictable due influence of climatic variability among many factors such as drought stress is most popular due to water shortage, high temperatures and solar radiation that affect plant growth and development (Swaminathan, 2021, Ombogo, and Karanja, (2022). Biotic stress is among arthropods, mites and diseases also are constrains affecting tea production (Swaminathan, 2021).

In Kenya the important tea diseases affecting tea are Hypoxylon wood root (*Hypoxylon serpens*), Stem cankers (*Phomopsis theae*), Armillaria root (*Armillaria mellea*) and leaf spot diseases such as grey blight (*Pestalotiopsis theae*) and brown blight (*Colletotrichum camelliae*) (Kamunya *et al.*,2019).

Hypoxylon wood root causes considerable damage to tea in Kenya and spread by wind as conidia. The fungus gain entry to the host plant through wounds caused by pruning, hailstones and sun-scorch. Symptoms are sectorial death of primary branches which leads to drying and death. Rotten wood bear fruitification (stromata) of the fungus that

appears dark grey to black raised patches. Management strategies involves removal surgical/selective pruning of affected parts, avoid down pruning of tea basing on recommended pruning height, prevent sun-scorch by shading exposed branches with pruning's immediately after pruning among other strategies (Anon, 2002, Kamunya, *et al.,* 2019).

Armillaria root rot of tea has worldwide distribution as it also occurs in roots of most forest trees as epiphyte. Infection is traceable to woody debris of stumps and roots left in the soil during initial forest clearing. The fungus is spread when disease free tea roots come in contact with rhizomorphs (thread–like materials produced by the fungus). Symptoms are exhibited in the foliage through decrease in development, yellowing, premature flowering, and defoliation of leaves. At the collar region longitudinal cracking of the stem can be seen and when the bark is lifted off a white mycelial growth is found overlying the wood. In order to control grey blight disease, land preparation by uprooting trees and removal of all the root debris that might be having inoculum of the fungus. No effective chemical method for managing the disease. Biological control method has been used to control the disease through antagonistic effect **(**Anon, 2002, Kamunya, *et al*., 2019**).** Nevertheless, several *Trichoderma* strains have been known to affect pathogenic fungi like *Colletotrichum, Pythium, Verticillium, sclerotinia, Rhizoctonia, Armillaria, and Fusarium* (Ozbay and Newman 2004).

Pestalotiopsis theae (Ascomycota, Amphisphaeriaceae) diseases cause grey blight in tea (*Camellia* spp.) and several plants of financial importance (Kumhar *et al.,* 2016). Pestalotiopsis is a genus that contains multiple species that cause plant diseases, and several species have been isolated as endophytes and pathogens in *Camellia sinensis* (Maharachikumbura *et al.,* 2013). It infects the maintenance leaves, bare stalks and young shoots of tea hence affecting its quality and quantity that results in huge crop loss

of the crop in southern India and Japan (Kumhar *et al*., 2016 and Chen *et al*., 2018). In the nursery it causes stalk root and lesions on maintenance leaves/young shoots of vegetative propagated plants. Predisposing factors in the nursery are mainly too much shading and over watering. Among management strategies of grey blight is the selection of cuttings from the field which are free from healthy and vigorous growing from mother bushes. During propagation, usually the tea cuttings are treated by dipping into fungicides and when it is notice during growth, and then they are sprayed. Other studies have shown that grey blight is controlled by spraying with *Trichoderma* spp. based on it is antagonistic activity.

Development of safe and effective methods of controlling the diseases is therefore desirable. In numerous studies, *Trichoderma* spp has been demonstrated to antagonize Armillaria as well as grey blight diseases.

2.2 *Trichoderma species*

Trichoderma species is a Deuteromycota, dematiaceous which is filamentous imperfect saprophytic fungi found in almost any soil. Aerial and soil-borne plant diseases that are commercially significant have been mycoparasitized using the fungi that have been produced and employed as some bio-control techniques (Freeman *et al.,* 200; Hajieghrari *et al.,* 2008). According to Mwangi *et al*., (2011), *Trichoderma* species promote plant development by establishing robust and continued existence after colonising the root surfaces penetrating into the epidermis. The biological control potential of *Trichoderma* Species works against a wide range of plant pathogens such as *Pythium* spp, *Fusarium* spp. and *Rhizoctonia* spp. Plant roots are known to be enhanced by microbial interactions which affect plant nutrient status and resistance to pathogens through classical mechanisms such as antibiosis, mycoparasitism, competition, and induction of plant defense responds in the plant (Chet, 1987; Altomare *et al.,* 1999;

Yedidia, *et al.,* 1999; Singh, *et al.,* 2010). Nevertheless, several *Trichoderma* strains have been identified to affect pathogenic fungi such as *Pythium, Colletotrichum, Verticillium, sclerotinia, Rhizoctonia, Armillaria, and Fusarium.* These pathogens have been extensively studied attacking crops including cotton, tomatoes, cucumbers, sugar beets, strawberries, and beans (Ozbay and Newman 2004).

Resistance to abiotic stresses and crop productivity are also enhanced through mineral absorption. Environmental factors such as light and temperature affects growth of plant. Microorganisms in the root rhizosphere are capable of exerting beneficial, neutral, or detrimental result on plant growth (Bias *et al*., 2006; Harman 2006). Studies done by Windham *et al.,* (1986) shows how production of hormones by *Trichoderma* influences plant development. Other mechanisms are by Solubilization of insoluble minor nutrients in the soil (Altomare *et al*., 1999) and enhanced uptake and translocation of less available minerals (Baker, 1989; Inbar *et al*., 1994; Kleifeld 1992). *Trichoderma* sp. comprise both enzymatic and chemical with characteristics for mycoparasitism and antagonism (Singh *et al.,* 2010). It is a bio-control agent that can also be exploited by using antibiotic metabolites secreted to fight plant diseases by pathogenic fungi which includes fungi in Ascomycota, Basidiomycota, and Oomcota. A broad array of volatile organic compounds is also produced by many other fungi as it has been shown to be produced by *Trichoderma* sp. (Schuster, and Schmoll 2010). *T. harzianum* is able to promote plant through extraction/production of Harzianic acid secondary metabolite that strongly binds iron as a result of low molecular weight organic molecules (Vinale *et al.,* 2014). According to Yedidia *et al*., (2001), the presences of *Trichoderma asperellum* have significantly increased the cucumber roots' dry weight, shoot length, and leaf area. Ozbay and Newman (2004) experiment showed Tomato seedling development enhancement using both non-commercial and commercial strains of

Trichoderma harzianum. Root development on the soil surface is increased by the production of hormones that encourage plant growth and the release of nutrients from the soil and organic matter from some strains of *Trichoderma* spp (Harman *et al.*, 2004).

2.3 Arbuscular Mycorrhizal fungi

There are several types of mycorrhizae with different associations namely ectomycorrhizal, orchidaceous mycorrhiza, ericoid mycorrhiza and arbuscular mycorrhiza (Bagyaraj, 2014). Several shrubs, tropical tree species, agricultural crops, and some temperate tree species all have arbuscular mycorrhizal associations (Bagyaraj, 2014). Arbuscular mycorrhizal fungi, which are members of the phylum Glomeromycota, develop a connection with more than 80% to 90% of terrestrial plants in addition to boosting absorption and enhancing plant fitness. Associated fungi then extract lipids and carbohydrates (Figure 2.1) from the host plant (Smith, and Read, 1997; Bias *et al.*, 2006; Veresoglou *et al.*, 2012). Ability of various mycorrhizal types to obtain both organic and inorganic sources of nutrients in the soil varies. Endomycorrhizae are known to act as organisms that promote plant development while inhibiting growth of pathogenic fungi (Figure 2.1) through their symbiotic relationship with plant roots (Sukhada *et al*., 2011). In addition to enhancing nutritional status, these microorganisms also encourage soil microbial growth and development, compete with pathogens, induce local and systemic plant defenses, and alleviate abiotic plant stress (metal toxicity, water stress) (Trillas 2009; Mwangi, 2011). Higher nutrient intake by the hyphae from the soil is correlated with increased plant development as a result of AMF. Hyphae penetrate into the nutrient-depleted zone of the soil and boost the efficiency of immobile elements (Sadhana, 2014).

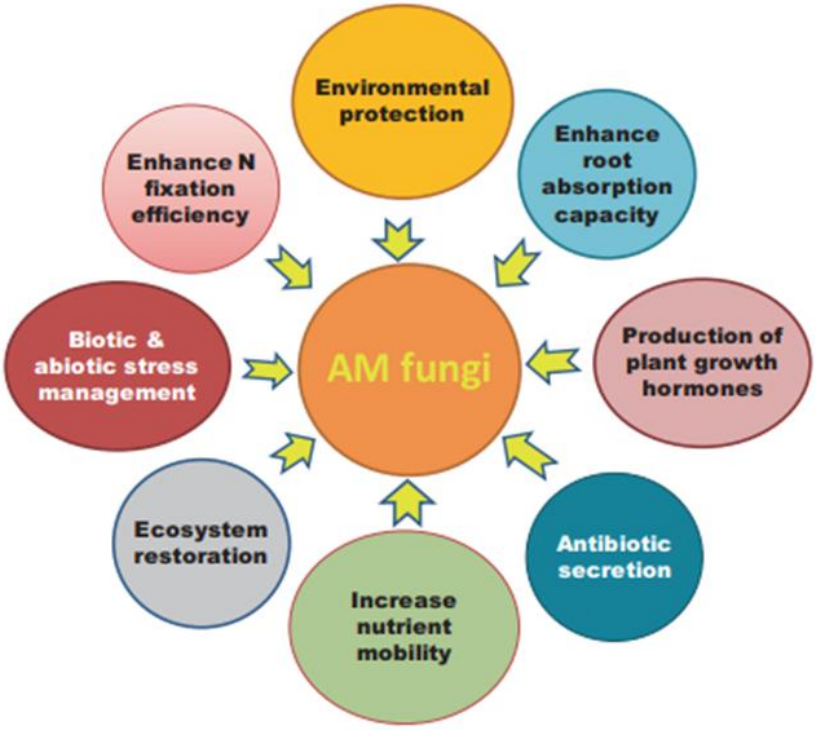

Figure 2.1: Ecological functions provided by Arbuscular Mycorrhizae Fungi. (Source: Parihar *et al.*, 2020*)*

Extramaticular Chlamydospores, thick-walled resting spores produced by AMF that can withstand harsh circumstances and germinate when the environment is suitable, are observed in soil (Bagyaraj, 2014). Appressorium forms on the surface as the hypha enters roots and extends across the root cortex. Arbuscular are haustoria-like structures that form when cortical cells are invaded by intercellular hyphae that run longitudinally. Vesicles serve as storage organs and varied sizes and forms thin-walled objects of that hold oil droplets. AMF may be recognized by looking for vesicles and arbuscules in a plant's roots (Bagyaraj, 2014). AMF asymbiotic, pre-symbiotic, and symbiotic phases are three stages in the formation of symbiosis (Figure 2.2). The asymbiotic phase involves a number of stages that ultimately lead to a germ tube and the beginning of hyphal growth (Siqueira *et al*., 1985b, Parihar *et al*., 2020). When first asymbiotic mycelium establishes contact with plant roots, pre-symbiotic phase begins. Because the

host plant produces chemotropism activators to establish membrane potential, the pre-symbiotic phase depends on them. When plant roots and AMF come in-touch physically appressorium production begins and the symbiotic phase is launched (lanfranco *et al*., 2005, Parihar *et al*., 2020). Appressorium pierce the root epidermal cell and diffuse into intra and extraradical cells during the symbiotic phase. Mycelium that is extraradical goes outside the rhizosphere and absorbs nutrients with low diffusivity (Cruz *et al.,* 2008, Parihar *et al.,* 2020).

Figure 2.2: Life cycle of Arbuscular Mycorrhizae Fungi

(Source: Parihar *et al.*, 2020*)*.

In symbiotic phase, host plant is the source of carbon and energy meant for mycorrhizal fungi which in return enhance absorption of a range of nutrients (Cruz *et al.,* 2008; Lu *et al.,* 2018 and Parihar *et al.,* 2020).

2.5 Mechanism of action for *Trichoderma* spp. and mycorrhizae

Competition for nutrients and space may be a factor in *Trichoderma* spp. enhancing plant production, growth development, and resistance to disease (Sigh *et al.,* 2010; Vinale *et al.,* 2014), mycoparasitism by coiling around the pathogen, antibiosis and

enzyme secretion (Sigh *et al.*, 2010; Tapwal *et al*., 2015). *Trichoderma* spp. is resistant to many toxic compounds hence can grow faster and produce metabolic substances. These will hinder spore germination (fungi-static) and kill cells (antibiosis), or acidifying soil which impairs the growing of the pathogen. Another bio-control mechanism is through interaction between *Trichoderma* and the pathogen (Mycoparasitism's). The hyphae coil and kill the pathogen through the production of cell wall degrading enzymes which digest the host cell (Sigh *et al., 2010*; Tapwal *et al.,* 2015).

Following colonization, arbuscular mycorrhizae trigger certain plant defence systems. Elicitation might make the plant more likely to respond quickly to a root pathogen assault via an AMF symbiosis of a particular plant defence mechanism. At the location of a pathogen assault, the production of phytoalexins, which are poisonous substances, accumulates (Siddiqui *et al*., 2008). Studies have demonstrated that AMF can increase growth development and phosphorus uptake which improve the internal status of the crop and indirectly reducing the harmful effect of the pathogen (Artursson *et al*., 2006; Sghir *et al.,* 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Geographical description of study area

Current research was carried out at Tea Research Institute Kericho which is in Timbilil Estate (Appendix i); it borders the western part of Mau Forest and it's about 10km East of Kericho town, lies in 0^{o} 22'S,35^{o} 22E and altitude of 2178m above the sea level. The area has the mean annual rainfall of 1800 mm and minimum annual mean temperate of 18°C and of a maximum 23°C. Soil is red volcanic soil that is deep, well-drained with pH 5.0-6.5 (TRFK, Clonal catalog 2002). Two experiments were carried out in the tea nursery where evaluation of *Trichoderma* spp. and mycorrhiza on growth development of tea was tested and, in the laboratory, on growth of grey blight disease control using *Trichoderma* spp.

3.2 Source of Tea cuttings

The healthy apical cuttings excised using very sharp knives were obtained from mother bushes of respective tea cultivars with their attributes that is AHP S15/10 (slow rooters), TRFK 31/8 (moderate rooters) and TRFK 303/577 (good rooters) were sourced from Timbilil tea estate. The cuttings were created from healthy young shoots that were between four and seven months old. Cuttings made were single leaf with 3 to 4 cm of stem below the leaf. The branches' very delicate ends and extremely hard bottom portions were removed.

3.3 Source of Trichoderma species

Trichoderma sp. (T4, T39, T17) which had been isolated from the soil in the tea rhizosphere and at different times, therefore the source was from stock cultures at TRI. The coding/ naming of *Trichoderma* isolates were based on number of species isolated

from tea rhizosphere soil in TRI that is code 1,2,3,4 to 40, hence T4, T17 and T39. Isolates were sub-cultured into Petri-dishes/culture plates containing Potato dextrose agar (PDA) which had been prepared by measuring 39g per 1000ml of sterile distilled water and autoclaved 121°C for 20 minutes. Inoculated plates were sealed using a Parafilm and incubated at room temperature (25°C) for two weeks to sporulate. Among these *Trichoderma* isolates only T4 had been earlier identified as *Trichoderma harzianum* while the T39 and T17 were identified during this study based on morphological keys described by Barnett *et al.,* (1972). The mycelial plugs (4 mm in diameter) were picked and placed into middle of plates or Petri dishes with PDA culture media from the 3-day-old cultures of *Trichoderma* spp. The purpose was for visual observation of cultural and morphological characteristic on the Petri dishes. Among the features noted are the rate of mycelia growth, colour and change of the medium colour. Slide culture technique was utilized for micro-morphological features to study conidiophore or Phialide form, size organization, and development. The taxonomic key for the genus *Trichoderma* was used to compare all samples or isolates of *Trichoderma* species (Barnett *et al.,* 1972; Sekhar *et al.,* 2017). Identification key for *Trichoderma* species was used to compare the species (Gams *et al.,* 2002, Naher *et al*., 2019, and Waghunde *et al*., 2016). For identification of *Trichoderma* spp. (coded T17 and T39) even though they were in TRI repository had to be done because they were the latest to be isolated but not identified in comparison with T4 (Plate 2.1and 2.2). *Trichoderma* T4 isolate had been identified earlier at Centre for Agriculture and Biosciences (C.A.B.) International Mycological Institute, as *T. harzianum*.

3.4 Identification of *Trichoderma species* based on growth characteristics and morphological observation

Growth rate (Table 3.1) and colony characteristics (Table 3.2) were determined from the three *Trichoderma* species (T4, T39, and T17) based on other studies conducted by Barnett *et al*., (1972), Gams *et al*., (2002), Devi *et al*., (2012), Kannangara *et al*., (2017), and Naher *et al*., (2019). Plates were examined daily under a light microscope (Olympus, model CH series) at low magnification (10x objective power), to observe presences of chlamydospores and conidiophore branching and apex of the conidiophore nature. Conidia shape and size, shape, and size of the phialides were recorded through examination, using a phase-contrast microscope under 100X objective lens power and photographs were taken with the Kodak Easyshare AF 5X wide optical aspheric lens, 27mm-135mm (Equiv).

3.4.1 Morphological characterization of *Trichoderma* spp.

Table 3.1: Morphological characteristics of *Trichoderma* spp. on PDA

Characteristics	Trichoderma isolates	
	Trichoderma viride	*Trichoderma harzianum*
Colony colour	-Dark bluish green 3-4 rings per plate -Bright green rings at early stage, over the plate later -Changes green to dark yellowish green after 2–3 days, no odor -Smooth surface, cottony white mycelial mat with aerial hyphae	Dark green producing tufts, yellowish green with dense white mycelia pustules -Dark green 2 rings per plate, Irregular margin -Dark Green Rings absent -Irregular Margin Yellow coloured pigment -Changes from watery white to light green in color. -Dark green, Rings absent -smooth surface, mycelial mat develops with white aerial hyphae
Colony Reverse colour	Amber -Uncoloured	Dull yellowish -uncolored ring-like zones -colorless to dull yellow, floccose, flat pustules, conidiation effuse, covering entire surface
Conidiophore's character	Lageniform, Convergent -Frequently paired, lageniform, divergent - Solitary, verticillate, more -Long, swollen in middle, like slender, and horn shaped	Frequent branching, verticillate -Highly branched and forming loose tufts -Broad, verticillate, frequent branching. Short branches phialides -Flexuous, ranches almost right angled, less extensively branched, irregular and narrower
Phialide character	-Globose to ellipsoidal -Subglobose -globose or obovoid often, perfectly	- Ampulliform, convergent to 4 phialides in whorls. -lageniform forming at dense area -Whorls of 2-6, to lageniform, short, pear-shaped, narrower at the base
Chlamydospore formation	Frequently intercalary and terminally -Infrequent, terminal and intercalary - Present	-Infrequent, internally & terminally -abundant

Source: Barnett, *et al*., (1972), Gams, *et al*., (2002), Devi, *et al*., (2012), Kannangara *et al*., (2017), and Naher *et al*., (2019

Table 3.2: Average growth development of *Trichoderma* species on PDA.

Isolate	Species	Average colony diameter (cm)			
		Day 1	Day 2	Day 3	Day 4
1	T. *harzianum*	2.90 ±0.15	7.40 ±0.07	9.00	9.00
2	*T. viridae*	2.50 ±0.03	5.60 ±0.08	6.90 ±0.08	9.00
3	*T. harzianum*	3.30 ±0.03	4.10 ±0.27	6.40 ±0.15	9.00
4	*T. viridae*	4.10 ±0.07	8.00 ±0.22	9.00	9.00
5	*T. polysporum*	2.00 ±0.10	6.10 ±0.10	7.50 ±0.08	9.00
6	*T. harzianum*	3.30 ±0.10	3.90 ±0.2	6.30 ±0.12	9.00
7	*T. viridae*	3.90 ±0.26	8.30 ±0.12	9.00	9.00
8	*T. harzianum*	3.00 ±0.13	7.40 ±0.08	9.00	9.00
9	*T. viridae*	2.60 ±0.05	4.90 ±0.1	6.70 ±0.20	9.00
10	*T. harzianum*	3.20 ±0.05	3.90 ±0.12	6.40 ±0.07	9.00

Source: Kannangara, *et al*., (2017).

3.5 Formulation of *Trichoderma species*

Inoculated plates were sealed using a Parafilm and incubation was done at room temperature (25°C) for 14 days to produce spores. Formulation of *Trichoderma* spp. was done by washing spores (Plate 3.1) each plate with 10mls sterile distilled water and straining suspension through a muslin cloth (22-25µm) into a conical flask.

Filtrates in a test tube were centrifuged at 2000 Revolution per Minute (R.P.M) for 20 minutes and supernatant was decanted leaving the spores at the bottom. Spore solutions are concentrated into one container for each *Trichoderma* isolate and each suspension was determined via haemocytometer count before incorporating with a carrier material (Kaolin powder). Depending on the number of spores obtained/ recorded, Kaolin

powder was weight and mixed evenly by spraying with spore solution to achieve $2.5x10^8$/g. The mixtures were dried and grinded using a pestle through a sieve having the smallest pore size for uniform distribution of spores which were used to inoculate sleeves in the nursery (plate 3.2b, c, and d).

Plate 3.1: Harvesting *Trichoderma* spp. from the culture plates

(Source: Author, 2022)

3.6 Application of treatments

In the nursery, (Plate 3.5a) each sleeve was treated with respective treatments that is $2.5x10^8$/g conidia of spore of *Trichoderma* isolate (Plate 3.2b, 3.2c, and 3.2d) in each sleeve except the untreated (control) while 2gm ($2.0x10^2$ propagules) of mycorrhizae (Plate 3.2a) were used in each tube.

3.7 Source of Mycorrhizae

Shubhodoya mycorrhiza (Plate 3.2a) biofertilizer (*Glomus* species) is commercially available in the market. According to the manufacturer, benefits of mycorrhizal bio fertilizer include better absorption of nutrients through increased mobilization of phosphorous, nitrogen, Sulphur, and micro-nutrients. Infective propagules per kg are

1,000,000 and application for a grown-up plant is 10-22gm per plant but 5-10gm per polybag as recommended by the manufacture for nursery bed application.

Plate 3.2: Formulated products of (A). Mycorrhizae, source;(Cosme Biotech Pvt. Ltd) (B) *Trichoderma* 39 T39) (C) *Trichoderma* 17 (T17) and (D) *Trichoderma* 4 (T4) (Source: Author, 2022)

3.8 Source of Pathogen (*Pestalotiopsis theae*)

Grey blight from tea bushes that were exhibiting leaf symptoms was collected from the field (Plate 3.3). In the laboratory, isolation was done by cutting 2cm of the infected tea leaf tissue and then surface sterilized by dipping into 70% ethanol solution for 30 seconds. The tissues were rinsed in three changes of sterilized distilled water and blotted before transferring onto PDA media in Petri dishes. Inoculated plates were incubated at room temperature (25^{o}C) for 14 days.

Plate 3.3: Tea leaves showing symptoms of grey blight disease

(Source: Author, 2022)

The hyphal tips transfer of the fungus radiating from the infected disc tissue was transferred onto freshly prepared PDA. After incubation at 25°C for 7-14 days, white aerial mycelia mats were formed with a circular shape and dark coloured acervuli were formed (Plate 3.4a and 3.4b). Isolated pathogen, based on cultural characteristics (white cotton-like mycelium-forming black acervuli) and conidial morphology, was identified through microscopic observations (hyphae are septate and spores are fusiform). Conidia were five celled with upper and lower cells hyaline, however, rest median three cells were dark. Sub-culturing was done until pure cultures were obtained, and then incubated for 7 days under light to enhance fungal growth and sporulation.

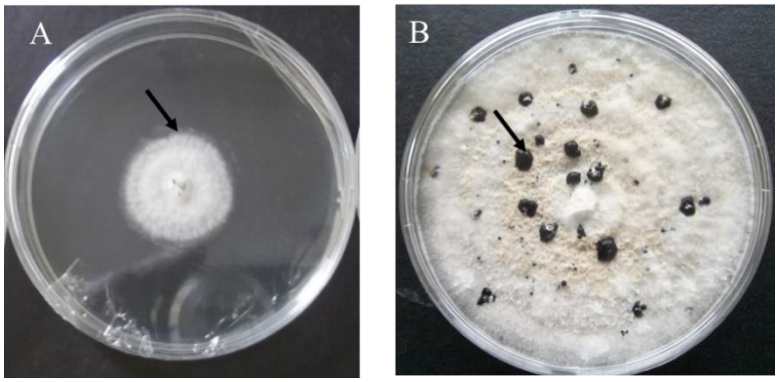

Plate 3.4: Culture plates (a) Growth of Pestalotia on PDA culture plate and (b) production of dark coloured acervuli (Source: Author, 2022)

3.9 Preparation of tea cuttings

The best shots are cut into individual cuttings, each of which has a single leaf and a stem that is 3 to 4 cm long under the leaf. Two incisions are made across the stem three to four cm below the bud, again using a sloping cut, the first immediately above the bud and sloping away from the bud. Cuttings must be ready right away, put in a water-filled container, and kept out of direct sunshine. The cuttings are planted right away after the excision in propagation polythene sleeves with excellent soil that can contain enough water and low pH since high pH causes callusing and prevent nursery plants from rooting.

3.10 Nursery establishment and experimental design

The nursery constructed was of high shade with Tinder net of 60% to reduce light intensity. Perforated Polythene sleeve measuring 10 x 25 cm (equivalent to the 10 x 4-inch polythene) was used. The sleeves were ¾ filled with topsoil and filled with sub-soil and packed fairly hard. Three replicates were used in a randomized full block design. Three clones (AHP S15/10, TRFK 31/8 and TRFK 303/577) were included in each of the eight treatments (T39 +AMF, T4, T17+AMF, T39, T4 +AMF, T17, AMF, and Control (Untreated) of respective subplots (Appendix ii). Tea cuttings were taken at random from clone AHP S15/10 (slow rooters), TRFK31/8 (moderate rooters) and TRFK 303/577 (good rooters) from mother bushes (Wachira, *et al*., 2012). There were 23 vegetative propagated tea cuttings from each clone (AHPS15/10, TRFK 31/8 and TRFK 303/577) for each treatment and replicated three times (Plate 3.5b).

3.10.1 Propagation of tea cuttings in the nursery

The healthy cuttings from mother bushes of respective clones (AHP S15/10, TRFK 31/8 and TRFK 303/577) were planted in the sleeves (Plate 3.5a). Once the whole bed was

completely planted, watering was done gently as strong jet displaces the cuttings. Hoops were used to hold transparent polythene sheet covering the tea cuttings in the nursery (Plate 3.5c). After 21 days, the polythene sheet was opened to check wetness/watering and weeding, and then covered again. In the fourth month, it was opened in a V-shaped for acclimatization then after two weeks its open half-way. Finally, the polythene sheet was removed completely after the 8 weeks and watering done twice daily in the morning and evening.

Plate 3.5: Nursery vegetative propagated tea plants. A) Planting vegetative propagated tea B) cuttings in the nursery and C). covering with a polythene sheet (Source: Author, 2022)

3.11 Data collection from nursery experiment

Data collection from vegetative propagated tea plants was through destructive sampling after 3 and 12 months. Tea plants were physically separated by placing the soil and roots on well labelled khaki bags. Soils were processed for *Trichoderma* spp. recovery while the roots were used for observation for mycorrhizal structures (arbuscular, vesicles, and hyphae). Tea was harvested /sampled at random for root length, shoot length, and stem girth of the shoot, root girth measurements and dry weights of the whole plant. The root length and shoot length measured from the point of attachments using a ruler and expressed in millimeters (mm) and, stem and root girth (mm) measured using a Viner caliper.

Dry matter/weight obtained from whole plant by drying in an oven at 60^{o}C for until constant weights are obtained. The dry weights (grams) of each tea clones were recorded using the electronic weighing balance (KERN ew/Eg-9 version 2.9)

3.11.1 Recovery of *Trichoderma species*

Trichoderma recovery from the soil after destructive sampling of the vegetative propagated tea plants was done to check population through serial dilution technique. The serial dilution was done as follows; all soil samples from rhizosphere of each treatment were mixed thoroughly then 1g was weighed into 9ml of sterile distilled water (stock solution). From the stock solution suspension, 1 ml was drawn into another tube containing 9 ml of sterile distilled water. This serial dilution technique was carried out to 10^{-3}. In the last dilution (10^{-3}), using a pipette, 0.1 ml suspension was transferred onto three Petri dishes each containing 20 ml of solidified *Trichoderma* selective medium. Spread plate method was used where a sterile bent glass rod was used to evenly spread the suspension. Inoculated plates were incubated for 18–48hours at room temperature. Daily observation was conducted, and the number of visible typical colonies of growth of *Trichoderma species* from each plate was recorded.

3.11.2 Root preparation for mycorrhizae structures observation

Roots were prepared to determine the level of abundance of mycorrhizal structures on the roots of tea Clones (AHP S15/10, TRFK 31/8 and TRFK 303/577). Percentage population of arbuscular, vesicles and hyphae mycorrhizal structures in tea clones was determined at the end of 3rd, 9th and 12th month. Vegetative propagated plant roots were washed to remove soil particles and small pieces of about 1cm of tender roots are cut and placed onto a test tube containing water waiting to be processed. In order to clear roots, water from the test tubes were decanted and replaced with 10% KOH. The roots were autoclaved for 10 minutes to clear root cells of cytoplasm and rinsed with

deionized water several times. Clearing solution was then be decanted and stained with tryptophan blue-lacto phenol (Phillips and Hayman, 1970), then left overnight for the roots to take up the stain. Root segments were mounted on a glass slide and a coverslip was placed on top, lightly pressed to expose the cells and a Compound microscope (10x, and 40x) was used to examine mycorrhizal structures (vesicles, arbuscular and hyphae). Results were then recorded based on presence or absence (- and +) of structures and finally stated as a percentage. Colonization was calculated using the following formula: percentage Colonization= (Total number of root segments colonized/Total number of root segments examined) x100 (Phillips and Hayman, 1970).

3.12 *In-vitro* antagonistic activity of *Trichoderma* spp for control of *Pestalotiopsis theae* causing grey blight disease of tea.

In the research, parasitism, competition, antagonism, and antibiosis (manufacture of non-volatile and volatile antibiotics) are some mechanisms used by bio-control agents to control illnesses (Sighn *et al.*, 2010). *Trichoderma* species were tested *in vitro* for their biological capability against the common soil-borne plant pathogen *P. theae*. The generation of volatile metabolites and non-volatile antibiotics by *Trichoderma* isolates was examined using the dual culture technique in order to determine antagonistic effect in the bio-efficacy of *Trichoderma* spp.

Antagonistic test was carried out using the dual culture technique on three isolates of *Trichoderma* (T17, T4, and T39) against plant pathogen *P. theae* as described by Morton and Strouble (1955). The mycelial discs of both actively growing fungi (*Trichoderma* spp. of 7 days-old culture and *P. theae*) were cut from the margin using a cork bore of four millimeters diameter and inoculated onto PDA media at equidistance in the center of the plates. Soil-borne plant pathogen (*P. theae*) and disc of 4mm

diameter of *Trichoderma* isolate were placed at equal distances on either side of the perimeter. For seven days, inoculated plates were incubated at 25°C. with a few minor adjustments, the production of volatile metabolites by *Trichoderma* spp. was measured as reported by Dennis and Webster (1971a), Goyal *et al*., (1994), and Al-Saeedi and Al-Ani (2014).

Impact of volatile chemicals emitted by *Trichoderma* spp. assessed using the inverted plate technique method. Fungal culture (*P. theae* and *Trichoderma* isolates) was grown separately on each PDA plates by taking a plug measuring 4mm disc each. Plates containing *Trichoderma* isolates by replacing the top with the bottom of PDA plate inoculated at the middle with *Pestalotiopsis* pathogen. The PDA filled petri dish as control, a medium with pathogens on the upper lid and no *Trichoderma* isolates at the lower lid was kept. Individually pair of Petri plates was taped together with paraffin before being incubated for four to six days.

Potato dextrose broth (PDB), is utilized as a general-purpose medium for a variety of antagonistic tests. Using Potato Dextrose Broth served the objective of capturing the non-volatile metabolite syntheses by various antagonist isolates. Test isolates of *Trichoderma* were cultivated for 21 days in 250 ml Erlenmeyer flasks with occasional shaking in 100 ml sterilized PDB (Plate 3.6).

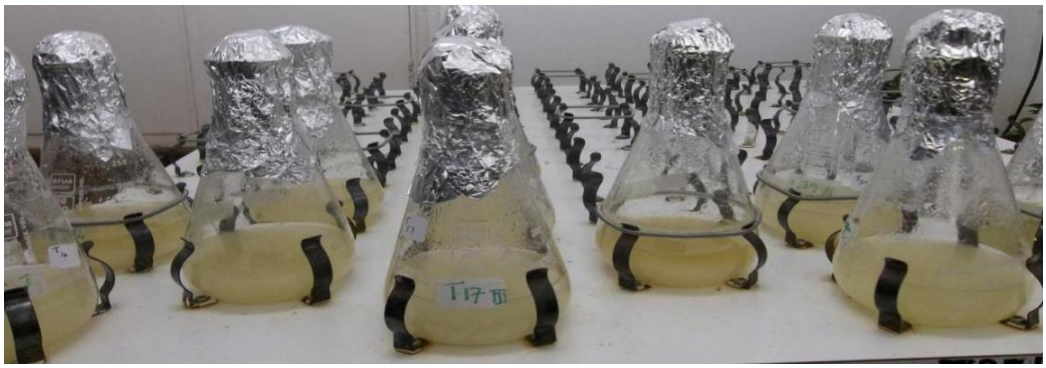

Plate 3.6: Cultures of *Trichoderma* isolates grown on PDB and periodical shaking in an orbital shaker (Source: Author, 2022)

Trichoderma spp. culture filtrate was obtained by filtering with Whatmann filter No. 1 paper to eliminate mycelia (Plate 3.7a). The culture filtrate was sterilized with Millipore membrane filter paper (Plate 3.7b) with a 0.22 m pore size (FP30/0.2 CA-S, Schleicher and Schuell Micro Science GmbH) using a vacuum pump after being centrifuged at 6000 rpm for 10 min (Plate 3.7e). Using a Buchner funnel, filtrates were trapped by conical flask (Plate 3.7c and 3. 7d).

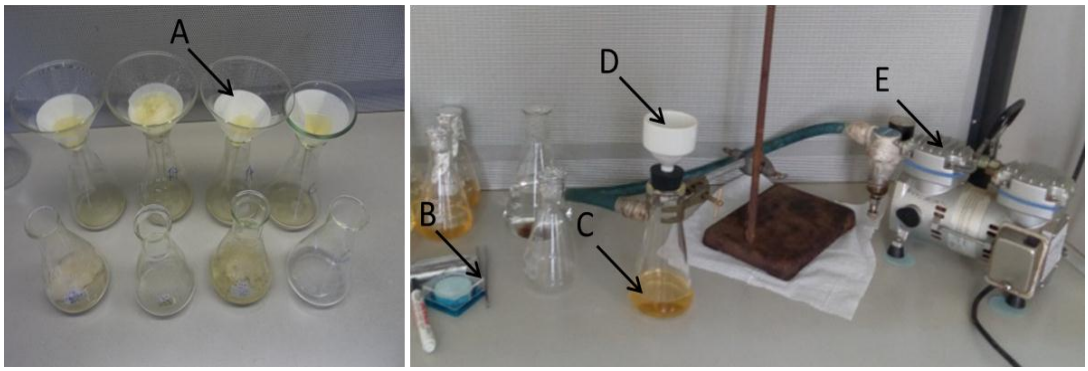

Plate 3.7: Harvesting Cultures of *Trichoderma* isolates (a) Filtration (b) Filter paper of (c) filtrates in a conical flask (d) Buchner funnel (e) vacuum pump (Source: Author, 2022)

To create a culture filtrate with a final concentration of 25% (v/v), the amount of culture filtrate of 5ml was combined with 15ml volume of melted PDA (at 40°C). Mycelial plugs (4 mm in diameter) taken from young culture of the test pathogen (*P. theae*) were added to the altered media and placed in Petri dishes which were then incubated at 25°C for 6 days. PDA medium without inclusion of antagonist's culture filtrate served as control.

3.12.1 Data Collection from laboratory experiment

A fully randomized design (CRD) was used to perform the laboratory experiment for the dual culture interaction, volatile metabolites, and repressive influence of culture

filtrates (liquid metabolites). The data on inhibition was calculated using the following formula I = (C-T/Cx100. Where, I = inhibition (%), C = colony diameter in control plate and T = colony diameter in treated plate (Vincent, 1947; Singh *et al*., 2002; Hajieghrari *et al*., 2008; Bokhara, and Perveen, 2012) to obtain percentage inhibition in comparison to mycelial growth of controls after daily measurements of radial growth of the pathogen. Daily measurements of test pathogen's growth / inhibition through the generation of non-volatile metabolites were made, and the percentage suppression of mycelial development was recorded.

3.13 Data analysis

The data from each experiment were analyzed while test of variance was calculated using analysis of variance (ANOVA) via Genstat Release 15.1 copy right 2012, and means separated using Least Significant Difference (LSD) test at 5% level of significance ($p<0.05$).

CHAPTER FOUR

RESULTS

4.1 Population dynamics of *Trichoderma spp*. and Mycorrhizal structures

4.1.1 *Trichoderma* spp. propagules population recovery from soil rhizosphere of tea roots after three months.

In the first three months *Trichoderma* spp recovery from the soil rhizosphere of tea displayed significant differences ($p<0.05$) (Appendix iii) among clones and treatments, Tea cultivar AHP S15/10 had the highest population *Trichoderma* spp recovery from treatment T39 (24,000CFU) but not significantly different with T39+AMF (11 000 CFU), T17 (17,000CFU), T4 (6,000 CFU) and T4 +AMF (3,000CFU) (Table 4.1a). This was in comparison with AMF and control plots having the lowest recovery of *Trichoderma* spp. Recovery of *Trichoderma* sp. propagules in cultivar TRFK 31/8, followed a similar trend as with cultivar AHP S15/10 where treatment T39+AMF, T39, T17, T4 and T17+AMF differed significantly ($p<0.05$) from other treatment. The *Trichoderma* spp. propagule recovery in clone TRFK 303/577 showed treatment T39+AMF (65,000 CFU) being significantly different ($p<0.05$) with T4 (10,000CFU), T39 (6,000CFU), T17 (4,000CFU), T17+AMF (3,000CFU), T4+AMF (1,000CFU), AMF (0 CFU) and control (0 CFU) (Table 4.1a).

The general observation from the results shows that combination of AMF with T39 had more recovery across all the tea clones in comparison with other treatments.

Table 4.1a: *Trichoderma* spp. mean propagules population recovery from soil rhizosphere of tea roots after three months.

	Trichoderma spp. from rhizosphere soil of tea ($1x10^3$/g CFU).		
Treatments	AHP S15/10	TRFK 31/8	TRFK 303/577
T39+AMF	11(2.58) ±0.32^{ab}	20(3.09) ±0.58^{a}	65(4.2) ±0.18^{a}
T39	24(3.26) ±0.86^{a}	30(3.47) ±0.44^{a}	6(2.05) ±0.18^{bc}
T17+AMF	17(2.97) ±0.81^{ab}	17(2.92) ±0.24^{a}	3(1.64) ±0.25^{c}
T17	4(1.75) ±0.20^{abc}	13(2.69) ±0.92^{a}	4(1.82) ±0.16^{c}
T4+AMF	3(1.52) ±0.16^{bc}	7(2.2) ±0.19^{ab}	1(1.13) ±0.14^{d}
T4	6(2.04) ±0.13^{abc}	11(2.58) ±0.44^{a}	10(2.45) ±0.23^{b}
AMF	0(0.83) ±0.14^{c}	1(1.03) ±0.34^{b}	0(0.77) ±0.07^{d}
Control	0(0.69) ±0.00^{c}	0(0.69) ±0.00^{b}	0(0.69) ±0.00^{d}

Figures in parenthesis are $\log_e$ (x+1) transformation of *Trichoderma* spp. colony forming units' recovery population from the soil. Means that do not share a letter are significantly different ($p<0.05$).

4.1.2 *Trichoderma* spp. propagules population recovery from soil rhizosphere of tea roots after one year.

In clone AHP S15/10, treatments with T39+AMF and T39 with 63 000CFU, 72 000CFU differed significantly ($p<0.05$) from other treatments (Table 4.1b). It was followed by T4 (20,000 CFU), T17 (14,000 CFU) which differed significantly ($p<0.05$) as compared with AMF (1,000CFU) and control/untreated (Appendix iv).

Recovery of *Trichoderma* spp in clone TRFK 31/8 was significantly different ($p<0.05$) in treatment T39 (81,000CFU) and T17 (74,000CFU) in comparison with the AMF and control. In treatment T4+AMF (30 000CFU), T4 (36000CFU) and T39+AMF (49,000CFU) varied significantly ($p<0.05$) with other treatments.

In clone TRFK 303/577 there was similarly significant difference ($p<0.05$) in entire treatments. *Trichoderma* spp population recovered in treatment T17 (83 000 CFU) differed significantly ($p<0.05$) from other treatment and control except in treatment T39 (62,000CFU). This implies that there were consistencies in population of *Trichoderma* spp. recovered from all the treatments and tea cultivars/clones as compared with AMF and control.

Table 4.1b: *Trichoderma* spp. mean propagules population recovery from soil rhizosphere of tea roots after one year.

Treatments	*Trichoderma* spp. from the soil rhizosphere of tea ($1x10^3$/g CFU)		
	AHP S15/10	TRFK 31/8	TRFK 303/577
T39+AMF	$63(4.17) \pm 0.09^{a}$	$49(3.94) \pm 0.15^{ab}$	$50(3.95) \pm 0.31^{b}$
T39	$72(4.3) \pm 0.03^{a}$	$81(4.42) \pm 0.08^{a}$	$62(4.16) \pm 0.06^{ab}$
T17+AMF	$8(2.31) \pm 0.30^{c}$	$11(2.55) \pm 0.26^{c}$	$4(1.71) \pm 0.31^{d}$
T17	$14(2.76) \pm 0.11^{bc}$	$74(4.33) \pm 0.11^{a}$	$83(4.72) \pm 0.24^{a}$
T4+AMF	$8(2.33) \pm 0.03^{c}$	$30(3.48) \pm 0.26^{b}$	$10(2.52) \pm 0.44^{c}$
T4	$20(3.1) \pm 0.26^{b}$	$36(3.65) \pm 0.11^{b}$	$35(3.61) \pm 0.00^{b}$
AMF	$1(0.96) \pm 0.14^{d}$	$1(1.13) \pm 0.27^{d}$	$1(1.20) \pm 0.10^{d}$
Control	$0(0.83) \pm 0.14^{d}$	$1(1.06) \pm 0.20^{d}$	$1(1.13) \pm 0.27^{d}$

Figures in parenthesis are $\log_e (x+1)$ transformation of *Trichoderma* colony forming units' recovery population from the soil. Means that do not share a letter are significantly different ($p<0.05$)

4.2 Percentage colonization/abundance of mycorrhizal structures in roots of tea cultivars.

In the first three months, mycorrhizal structures had not colonized the roots of tea clones (AHP S15/10, TRFK 303/577and TRFK 31/8). The purpose was to determine percentage colonization of arbuscular mycorrhizal structures (arbuscular, vesicles, and hyphae) (Plate 4.8b, c, and d) in the roots of selected tea cultivars.

4.2.1 Determination of percentage colonization of arbuscular mycorrhizal structures after nine months

Evaluation of percentage abundance/colonization of mycorrhizal structures (Plate 4.8a) on selected tea cultivars after nine months in AMF plus *Trichoderma* treatments was discovered to be significantly different ($p<0.05$) in cultivars AHP S15/10, TRFK 31/8 and TRFK 303/577 (Table 4.2a).

In clone AHP S15/10, recovery of arbuscular mycorrhizal structures in treatment T17+AMF (27%), T4+AMF (21%) and AMF (17%) differed significantly ($p<0.05$) as compared with T39+AMF (2%) and control (0%) (Appendix v). The recovery percentage of the structures from roots of cultivar TRFK 31/8 showed higher in treatment AMF (24%), T4+AMF (55%), T17 +AMF (12%) and T39+AMF (7%) as compared with control/untreated plots (0%). Treatments AMF (38%), T4+AMF (17%) had the highest percentage recover of arbuscules followed by T39+AMF (7%) and T17 +AMF (2%) in comparison with control (0%) in cultivar TRFK 303/577.

Recovery of vesicles (Table 4.2a) showed significant difference ($p<0.05$) in clones TRFK 31/8 and TRFK 303/577 as compared with clones AHP S15/10. Results from treatments AMF (27%), and T17+AMF (17%), was significantly different ($p<0.05$) in

percentage recovery of vesicles as compared with control (0%), T4+AMF (2%) and T39+AMF (0%). Colonization of vesicles in clone TRFK 303/577 showed significant difference ($p<0.05$) in treatment AMF (34%) and T4+AMF (24%) in comparison with control/untreated (0%), T39+AMF (2%) and T17+AMF (0%).

The abundance of hyphae in AHP S15/10, TRFK 31/8 and TRFK 303/577 (Table 4.2a) in all the treatment was significantly different ($p<0.05$). The presence of hyphae in AHP S15/10 was 30%, 41% for AMF and T17 + AMF had the highest colonization. Treatments T39+AMF (0%), control (0%) and T4+AMF (2%) had the lowest hyphae colonization. Clone TRFK 31/8 had the highest percentage colonization of hyphae in treatment AMF (48%) and T4+AMF (17%) and T17+AMF (17%) in comparison with control (0%), T39+AMF (7%) having the lowest population. Finally, clone TRFK 303/577 treatments showed significant difference ($p<0.05$) in all the treatments. AMF (38%) T39+AMF (30%) and T4+AMF (17%) were higher without variation ($p<0.05$) in comparison with control (0%). Observation showed the results did not have significant difference ($p<0.05$) between treatment T17+AMF and control.

Table 4.2a: Population (%) of Arbuscular mycorrhizal structures from the root rhizosphere of selected tea after nine months

Mycorrhizae structures	Treatments	Mycorrhizal structures recovery (%) from tea roots		
		AHPS15/10	TRFK 31/8	TRFK 303/577
Arbuscular	T39+AMF	2(1.44) ±0.74^{b}	7(2.18) ±0.74^{ab}	7(2.18) ±0.30^{bc}
	T17+AMF	27(3.36) ±0.43^{a}	12(2.62) ±1.03^{ab}	2(1.42) ±1.09^{bc}
	T4+AMF	21(3.14) ±0.21^{a}	55(4.04) ±0.19^{a}	17(2.93) ±0.00^{ab}
	AMF	17(2.93) ±0.00^{a}	24(3.26) ±0.19^{a}	38(3.69) ±0.13^{a}
	Control	0(0.69) ±0.00^{b}	0(0.69) ±0.00^{b}	0(0.69) ±0.00^{b}
Vesicles	T39+AMF	0(0.69) ±0.00^{a}	0(0.69) ±0.00^{b}	2(1.44) ±0.74^{b}
	T17+AMF	3(1.65) ±0.96^{a}	17(2.93) ±0.00^{a}	0(0.69) ±0.00^{b}
	T4+AMF	2(1.44) ±0.74^{a}	2(1.44) ±0.74^{b}	24(3.26) ±0.19^{a}
	AMF	2(1.44) ±0.74^{a}	27(3.35) ±0.21^{a}	34(3.57) ±0.38^{a}
	Control	0(0.69) ±0.00^{a}	0(0.69) ±0.00^{b}	0(0.69) ±0.00^{b}
Hyphae	T39+AMF	0(0.69) ±0.00^{b}	7(2.18) ±0.74^{b}	30(3.48) ±0.30^{a}
	T17+AMF	41(3.76) ±0.11^{a}	17(2.93) ±0.00^{ab}	4(1.78) ±1.09^{bc}
	T4+AMF	2(1.42) ±0.74^{b}	17(2.93) ±0.00^{ab}	17(2.93) ±0.00^{ab}
	AMF	30(3.48) ±0.30^{a}	48(3.92) ±0.19^{a}	38(3.69) ±0.13^{a}
	Control	0(0.69) ±0.00^{b}	0(0.69) ±0.00^{b}	0(0.69) ±0.00^{c}

Figures in parenthesis are $\log_e$ (x+1) transformation of Mycorrhizal structures colonization (%) in the roots of tea. Means that do not share a letter are significantly different ($p<0.05$)

4.2.2 Determination of percentage colonization of arbuscular mycorrhizal structures after twelve months

Mycorrhizae recovery from the roots of selected tea cultivars was done after twelve months. Results showed significant difference ($p<0.05$) from percentage abundance of arbuscular mycorrhizal structures in clone AHP S15/10, TRFK 31/8 and TRFK 303/577.

Treatment T17+AMF (34%) was significantly different ($p<0.05$) in comparison with other treatment and control/untreated after evaluation of percentage abundance/colonization of arbuscular mycorrhizal structures on clones AHP S15/10 (Appendix vi). Root colonization in clone TRFK 31/8 showed treatment T4+AMF (30%), and AMF (24%), and T17+AMF (21%) were significantly different ($p<0.05$) when compared with treatments T39+AMF (2%) and control/untreated (0%) (Table 4.2b). Arbuscules recovery in clone TRFK 303/577 were significantly different ($p<0.05$) in treatments AMF (36%), T4+AMF (24%), and T39+AMF (24%) in comparison with and T17 +AMF (0%) control/untreated (0%).

The vesicles (Table 4.2b) in clone AHP S15/10 showed significant difference ($P<0.05$) in AMF (24%), T17+AMF (30%), and T39+AMF (36%) when compared with T4+AMF (0%), and control/untreated (0%). Cultivar TRFK 31/8 recovery of vesicles in treatment T17+AMF (21%) was significantly different ($p<0.05$) with the other treatments (T4+AMF (3%), control/untreated (0%) and T39+AMF (0%) except AMF (17%). Colonization of vesicles in clone TRFK 303/577 showed recovery in treatment AMF (48%), T4+AMF (30%) and T39+AMF (30%) which was significantly different ($p<0.05$) with control (0%), and T17+AMF (0%).

Abundance of hyphae in clone AHP S15/10 (Table 4.2b) in all the treatment was significant different ($p<0.05$). Treatments T4+AMF (30%), T17 +AMF (27%) and AMF (24%) was not statistically significant ($p<0.05$) but were significant different ($p<0.05$) compared with treatments T39+AMF (2%), and control/untreated (0%). The highest percentage colonization of hyphae from clone TRFK 31/8 was treatment T17+AMF (24%) and significantly different ($p<0.05$) with T4+AMF (3%), control (0%), and T39+AMF (0%) while not significantly different with AMF (4%). Results from treatments in clone TRFK 303/577 showed that treatments AMF (61%), was significantly different ($p<0.05$) when compared with the other treatments. Treatment T39+AMF (45%) was not significantly different ($p<0.05$) with AMF (61%). The abundance of hyphae in treatment T4+AMF (30%) and T39+AMF (45%) did not differ statistically ($p<0.05$) in comparison with treatment T17+AMF (0%) and control (0%).

Table 4.2b: Population (%) of Arbuscular mycorrhizal structures from the root rhizosphere of selected tea after twelve months

Mycorrhizae structures	Treatments	Mycorrhizal structures recovery (%) from tea roots		
		AHP S15/10	TRFK 31/8	TRFK 303/577
Arbuscular	T39+AMF	2(1.44) ±0.74^{bc}	2(1.44) ±0.74^{b}	24(3.26) ±0.19^{a}
	T17+AMF	34(3.57) ±0.38^{a}	21(3.14) ±0.21^{a}	0(0.69) ±0.00^{b}
	T4+AMF	3(1.65) ±0.96a^{bc}	30(3.48) ±0.30^{a}	24(3.26) ±0.19^{a}
	AMF	24(3.26) ±1.9^{ab}	24(3.26) ±0.19^{a}	36(3.64) ±0.38^{a}
	Control	0(0.69) ±0.37^{c}	0(0.69) ±0.33^{b}	0(0.69) ±0.36^{b}
Vesicles	T39+AMF	36(3.64) ±0.38^{a}	0(0.69) ±0.00^{c}	30(3.48) ±030^{a}
	T17+AMF	30(3.48) ±0.30^{a}	21(3.14) ±0.21^{a}	0(0.69) ±0.00^{b}
	T4+AMF	0(0.69) ±0.00^{b}	3(1.65) ±0.95b^{c}	30(3.48) ±0.30^{a}
	AMF	24(3.26) ±0.19^{a}	17(2.92) ±0.00^{ab}	48(3.92) ±0.19^{a}
	Control	0(0.69) ±0.00^{b}	0(0.69) ±0.00^{c}	0(0.69) ±0.00^{b}
Hyphae	T39+AMF	2(1.4) ±0.74^{b}	0(0.69) ±0.00^{c}	45(3.76) ±0.11^{ab}
	T17+AMF	27(3.36) ±0.43^{a}	24(3.26) ±0.19^{a}	0(0.69) ±0.00^{c}
	T4+AMF	30(3.48) ±0.30^{a}	3(1.65) ±0.96^{bc}	30(3.48) ±0.30^{b}
	AMF	24(3.26) ±0.19^{a}	4(1.78) ±1.09^{ab}	61(4.14) ±0.09^{a}
	Control	0(0.69) ±0.00^{b}	0(0.69) ±0.00^{c}	0(0.69) ±0.00^{c}

Figures in parenthesis are $\log_e$ (x+1) transformation of mycorrhizal structures colonization (%) on the roots of tea. Means that do not share a letter are significantly different ($p<0.05$)

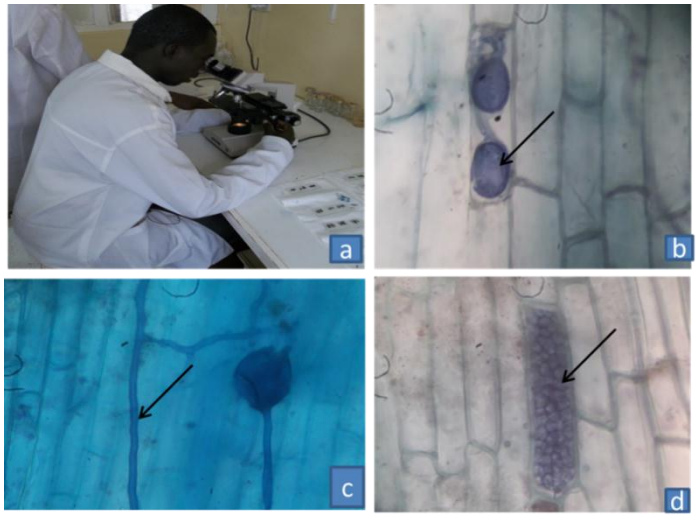

Plate 4.8: (a) Microscopic observation of Mycorrhizae structures in tea roots. (b). vesicles. (c). Hyphae. (d). Arbuscules (Source: Author, 2022)

4.3 Effect of *Trichoderma* spp and arbuscular mycorrhizal fungi on growth parameters of selected tea

4.3.1 Effect of *Trichoderma* isolates and arbuscular mycorrhizae tested on parameters of tea clone's development after three months

Results shows *Trichoderma* isolates and arbuscular mycorrhizae tested on growth parameters of tea clone's development in three months. Parameters observed were influence on growth of shoot length, root length, stem diameter, root diameter and plant dry weight (biomass) of tea clones.

In the third month (Table 4.3a), shoot length growth response was significantly different ($p<0.05$) in cultivars AHP S15/10 and TRFK 31/8 while TRFK 303/577 was not significantly different ($p<0.05$) between the other treatment (Appendix vii). The increase in shoot length of clone AHP S15/10 and TRFK 31/8 were observed to vary significantly ($p<0.05$) on treatments T17+AMF (29.3mm) and T4 (56.2mm) respectively as compare with control.

The response in growth of shoot diameter in clones of tea (AHP S15/10, TRFK 31/8 and TRFK 303/577) was significantly different ($p<0.05$) after application of treatments. Treatments T17+AMF (1.45mm) had higher shoot diameter in clone/cultivar AHP S15/10, T39+AMF (1.6mm) in clone TRFK 31/8 and T4 (1.82mm) and AMF (1.8mm) in clone TRFK 303/577 showing significant different ($p<0.05$) with other treatment (Plate 4.3a).

Growth enhances of root length showed significant difference ($p<0.05$) in clones AHP S15/10, and TRFK 303/577 as compared with clone TRFK 31/8 which did not differ significantly ($p<0.05$) Growth in clone AHP S15/10 showed that treatment T39+AMF (44.3mm) was significantly different($p<0.05$) in comparison with the other treatments and control (18.5mm). There was no significant difference ($p<0.05$) in the growth of root length of clone TRFK 31/8 between all the treatments except in the control. The results obtained from root diameter growth shows the entire tea clones (AHP S15/10, TRFK 31/8 and TRFK 303/577) being significantly different ($p<0.05$). Treatments T17+AMF (1.33mm), T17+AMF (1.10mm), and T4 (1.38mm) response on root diameter growth enhancement of clones AHP S15/10, TRFK 31/8 and TRFK 303/577 differed significantly ($p<0.05$) in comparison with other treatments and control/untreated (Plate 4.3b) (Appendix viii).

Influences of treatments on dry weights (Table 4.3c) in all the clones of tea (AHP S15/10, TRFK 31/8 and TRFK 303/577) were not significantly different ($p<0.05$) in comparison with controls in all treatments (Appendix ix).

Table 4.3a: Effect of *Trichoderma* isolates and mycorrhizae on Shoot length and diameter growth enhancement after three months

Treatments	Shoot Length (mm)			Shoot diameter (mm)		
	AHP S15/10	TRFK 31/8	TRFK 303/577	AHP S15/10	TRFK 31/8	TRFK 303/577
T39+AMF	13.5 ±7.6^{abc}	36.3 ±10.0^{ab}	49.3 ±13.3^{a}	0.94 ±0.27^{bc}	1.6 ±0.16^{a}	1.24 ±0.31^{abc}
T39	5.5 ±0.6^{c}	28 ±11.9^{ab}	32.0 ±9.10^{a}	0.85 ±0.09 ^{bc}	0.92 ±0.13^{bc}	1.3 ±0.28^{abc}
T17+AMF	29.3 ±9.1^{a}	34.2 ±6.7^{ab}	38.5 ±5.6a	1.45 ±0.23 ^{a}	0.97 ±0.12^{bc}	0.69 ±0.34^{c}
T17	7.5 ±1.4^{bc}	18.5 ±6.4^{b}	30.0 ±5.0^{a}	0.84 ±0.14 ^{bc}	1.18 ±0.33^{ab}	1.1 ±0.20bc
T4+AMF	20.2 ±11.1^{abc}	37.0 ±10.4^{ab}	41.2 ±4.7^{a}	1.15 ±0.23 ^{ab}	0.85 ±0.21^{bc}	1.45 ±0.10^{ab}
T4	23.8 ±3.1^{ab}	56.2 ±15.1^{a}	42.5 ±21.2^{a}	0.92 ±0.03 ^{bc}	1.1 ±0.0.25^{ab}	1.82 ±0.06^{a}
AMF	7 ±2.3^{abc}	43.3 ±7.9^{ab}	48.67 ±8.25^{a}	0.7 ±0.03^{c}	1.18 ±0.25^{ab}	1.18 ±0.30^{a}
Control	3.75 ±0.4^{c}	11.2 ±3.5^{b}	27.8 ±3.01^{a}	0.58 ±0.1^{c}	0.55 ±0.06^{c}	0.87 ±0.21^{bc}

Means that do not share a letter are significantly different ($p<0.05$)

Table 4.3b: Effect of *Trichoderma* spp. and mycorrhizae on root length and diameter growth enhancement after three months

Treatments	Root Length (mm)			Root diameter (mm)		
	AHP S15/10	TRFK 31/8	TRFK 303/577	AHP S15/10	TRFK 31/8	TRFK 303/577
T39+AMF	44.33 ±6.71^{a}	38.33 ±2.73^{a}	39.00 ±.8.2^{ab}	0.95 ±0.58^{abcde}	0.80 ±0.13^{ab}	0.69 ±0.13^{bc}
T39	23.83 ±2.35^{bc}	35.17 ±3.09^{a}	55.17 ±9.62^{a}	0.8 ±0.0.21^{cde}	0.96 ±0.2^{ab}	1.01 ±0.07^{ab}
T17+AMF	33.17 ±9.49^{ab}	41.67 ±3.01^{a}	36.08 ±3.49^{ab}	1.33 ±0.32^{a}	1.10 ±0.20^{a}	0.89 ±0.22^{bc}
T17	34.33 ±6.43^{ab}	33.50 ±4.07^{a}	43.75 ±4.76^{ab}	0.85 ±0.03^{bcde}	0.75 ±0.09^{ab}	0.92 ±0.16bc
T4+AMF	31.33 ±9.06abc	28.33 ±1.59^{a}	42.67 ±4.10^{ab}	0.97 ±0.03^{abc}	0.94 ±0.30^{ab}	0.94 ±0.15^{bc}
T4	39.17 ±4.05^{ab}	35.50 ±7.29^{a}	56.83 ±6.98^{a}	1.28 ±0.12^{ab}	1.03 ±0.16^{ab}	1.38 ±0.09^{a}
AMF	36.00 ±5.75^{ab}	32.17 ±4.87^{a}	50.82 ±7.16^{a}	0.97 ±0.12^{abcd}	0.97 ±0.40^{ab}	0.90 ±0.07bc
Control	18.50 ±3.82^{c}	14.00 ±2.84^{b}	21.32 ±7.52^{b}	0.51 ±0.11^{ce}	0.48 ±0.26^{b}	0.49 ±0.12c

Means that do not share a letter are significantly different ($p<0.05$)

Table 4.3c: Effect of *Trichoderma* isolates and mycorrhizae on dry mass of tea cultivars after three months

Treatments	Dry mass (g)		
	AHPS15/10	TRFK 31/8	TRFK 303/577
T39+AMF	0.62±0.02[a]	0.88±0.13 [a]	0.74±0.09 [a]
T39	0.73±0.07[a]	0.84±0.12 [a]	0.56±0.05 [a]
T17+AMF	0.61±0.06[a]	0.88±.0.09 [a]	0.75±0.12 [a]
T17	0.92±0.10[a]	0.71±0.03 [a]	0.68±0.08 [a]
T4+AMF	0.72±0.05[a]	0.68±0.04 [a]	0.72±0.05 [a]
T4	0.62±0.11[a]	0.82±0.04 [a]	0.63±0.08 [a]
AMF	0.68±0.16[a]	0.97±0.11 [a]	0.64±0.03 [a]
Control	0.64±0.10[a]	0.88±0.26 [a]	0.69±0.10 [a]

Means that do not share a letter are significantly different ($p<0.05$)

4.3.2: Effect of *Trichoderma* isolates and arbuscular mycorrhizae tested on growth parameters of tea clone's development in one year

Increase in shoot length, shoot diameter, root length, and root diameter of tea cultivars after application of treatments and determination of dry weights was done after twelve months.

Growth of shoot length in clone AHP S15/10 and TRFK 303/577 shows that there was no significant difference ($p<0.05$) in all the applied treatments (Appendix x). On the other hand, the responses on growth in clones TRFK 31/8 was able to show significant difference ($p<0.05$) in treatments T17 (163.6mm), T17+AMF (152mm), T4+AMF (158mm), T39 (158mm), T39+AMF (153mm) and AMF (99mm) as compared with T4 (141mm) and control (99mm) (Table 4.4a).

Growth of shoot diameter (Table 4.4a) in clones AHP S15/10 and TRFK 303/577, all were statistically different ($p<0.05$), with treatment T39+AMF (2.64mm) and T17+AMF (2.8mm) giving the highest respectfully in comparison with control. In clone TRFK 303/577 all treatment did not vary in growth statistically ($p<0.05$).

Cultivars AHP S15/10, TRFK 31/8 and TRFK 303/577 growth of root length enhancement after application of treatment showed significant differences ($p<0.05$). Treatment T39 (271mm) in clone AHP S15/10 and T39 (262mm) in clone TRFK 303/577 was significantly different ($p<0.05$) as compared with other treatments and control. The growth of root length in all the treatment was varied significantly ($p<0.05$).

Increase in root diameter growth in clone AHP S15/10 was significantly different ($p<0.05$) in treatment T39+AMF (1.44mm) and AMF (1.41mm) as compared with the control (0.92) and other treatments. In clone TRFK 303/577, treatment T4+AMF (1.66mm) was significantly different ($p<0.05$) with other treatments and control (1.09mm) (Table 4.4b). There was no significant difference ($p<0.05$) between the treatments and untreated/control in clone TRFK 31/8 in the growth of root diameter (Appendix xi).

The results from dry weight (Table 4.4c showed that the treatment AMF (1.46g), T4+AMF (1.3mm), T39+AMF (1.33mm) and T39 (1.30mm) in clone AHP S15/10 was significant heavier ($p<0.05$) statistically in comparison with other treatments and control (0.84g). In clones TRFK 31/8 and TRFK 303/577, all treatments and control/untreated were not statistically different ($p<0.05$) (Appendix xii).

Table 4.4a: Effect of *Trichoderma* isolates and mycorrhizae on Shoot length and diameter growth enhancement after one year

Treatments	Shoot Length (mm)			Shoot diameter (mm)		
	AHP S15/10	TRFK 31/8	TRFK 303/577	AHP S15/10	TRFK 31/8	TRFK 303/577
T39+AMF	155 ±4.30[a]	153.7 ±8.2[a]	156.3 ±4.88[a]	2.64 ±0.05[a]	2.8 ±0.18[a]	2.61 ±0.03[a]
T39	161 ±24.5[a]	158.4 ±5.0[a]	154.3 ±3.37[a]	2.38 ±0.04[ab]	2.7 ±0.28[ab]	2.96 ±0.06[a]
T17+AMF	159 ±5.89[a]	161.9 ±6.3[a]	152.4 ±16.9[a]	2.50 ±0.10[ab]	2.8 ±0.12[a]	2.60 ±0.22[a]
T17	136 ±8.94[a]	163.6 ±11.8[a]	156.2 ±22.3[a]	2.37 ±0.69[ab]	2.5 ±0.058[ab]	2.60 ±0.33[a]
T4+AMF	162 ±3.39[a]	158.4 ±16.0[a]	169 ±12.6[a]	2.36 ±0.09[ab]	2.6 ±0.13[ab]	2.72 ±0.11[a]
T4	164 ±10.1[a]	141.7 ±16.1[ab]	166.4 ±2.33[a]	2.43 ±0.05[ab]	2.6 ±0.2[ab]	2.68 ±0.09[a]
AMF	163 ±23.79[a]	161.3 ±29.0[a]	138.0 ±18.0[a]	2.51 ±0.20[ab]	2.8 ±0.37[ab]	2.84 ±0.15[a]
Control	126 ±4.94[a]	99.0 ±27.2[b]	124.4 ±23.2[a]	2.26 ±0.05[b]	2.3 ±0.20[b]	2.58 ±0.03[a]

Means that do not share a letter are significantly different ($p<0.05$)

Table 4.4b: Effect of *Trichoderma* isolates and mycorrhizae on Root length and diameter growth enhancement after one year

Treatments	Root Length (mm)			Root diameter (mm)		
	AHP S15/10	TRFK 31/8	TRFK 303/577	AHP S15/10	TRFK 31/8	TRFK 303/577
T39+AMF	254.4 ±13.1^{ab}	222.2 ±17.46^{a}	262.6 ±8.86^{a}	1.44 ±0.09^{a}	1.4 ±0.10^{a}	1.60 ±0.19^{ab}
T39	271.8 ±35.3^{a}	203.1 ±16.8^{a}	240.8 ±21.6^{ab}	1.19 ±0.07^{ab}	1.5 ±0.30^{a}	1.49 ±0.12^{ab}
T17+AMF	219.8 ±10.1^{ab}	205.3 ±15.2^{a}	244.7 ±17.7^{ab}	1.32 ±0.12^{ab}	1.3 ±0.06^{a}	1.52 ±0.17^{ab}
T17	206.7 ±10.2^{b}	223.1 ±23.2^{a}	219.4 ±18.3^{abc}	1.10 ±0.03^{ab}	1.2 ±0.15a	1.48 ±0.31^{ab}
T4+AMF	241.9 ±24.1^{ab}	213.9 ±12.1^{a}	231.9 ±14.0^{ab}	1.21 ±0.06^{ab}	1.2 ±0.08^{a}	1.66 ±0.19^{a}
T4	246.1 ±17.9^{ab}	218.0 ±5.93^{a}	214.4 ±10.3^{abc}	1.10 ±0.05^{ab}	1.3 ±0.17^{a}	1.47 ±0.05^{ab}
AMF	225.9 ±15.6^{ab}	199.2 ±4.6a	210.6 ±19.5^{bc}	1.41 ±0.27^{a}	1.6 ±0.17^{a}	1.56 ±0.18^{ab}
Control	222.8 ±19.8^{ab}	145.2 ±3.6^{b}	172.2 ±3.64^{c}	0.92 ±0.22^{b}	1.1 ±0.15^{a}	1.09 ±0.10^{b}

Means that do not share a letter are significantly different ($p<0.05$)

Table 4.4c: Effect of *Trichoderma* isolates and mycorrhizae on dry mass of tea cultivars after one year

Treatments	Dry mass (g)		
	AHP S15/10	TRFK 31/8	TRFK 303/577
T39+AMF	1.33±0.10[a]	1.76±0.19[a]	1.77±0.07[a]
T39	1.30±0.16[a]	1.60±0.14[a]	1.79±0.22[a]
T17+AMF	1.11±0.13[ab]	1.65±0.15[a]	1.72±0.11[a]
T17	1.17±0.08[ab]	1.33±0.11[a]	1.96±0.15[a]
T4+AMF	1.30±0.10[a]	1.37±0.11[a]	1.86±0.07[a]
T4	1.17±0.14[ab]	1.45±0.18[a]	1.88±0.13[a]
AMF	1.46±0.10[a]	1.72±0.12[a]	1.71±0.13[a]
Control	0.84±0.04[b]	1.61±0.0.26[a]	1.71±0.15[a]

Means that do not share a letter are significantly different ($p<0.05$)

4.4 Screening *in-vitro* antagonistic activity of *Trichoderma* species against grey blight (*Pestalotiopsis*).

Using quantitative and qualitative characters taken for morphological characterization it was possible to identify/characterize *Trichoderma* isolates. Characteristics of colony colour of *Trichoderma* isolate (T17) was progressing from white to yellow and the reverse was colourless to pale at the center (Table 4.5, and Plate 4.9 {2c, d}). Conidiophore's character was irregularly branched with ampliform, convergent Phialide (Plate 4.9 {2a}). Chlamydospores occur either terminally or intercalary in position to the hyphae (Plate 4.9 {2b}). Growth rate is 7.3cm (Table 4.6) and spores shape is sub globose to ovoid with a size of 3.5X3µm and the spores germinated in 13-15hrs (Table 4.5).

Trichoderma isolate T39, colony observation was turning from whitish to green while reverse was light green and clear at the center (Plate 4.9{1c, d}). Conidiophore's character was broad verticulate infrequently branching and Lageniform divergent Phialide while spores are sub globose to ovoid in shape and sizes of 3.3 X 3µm. Chlamydospores are terminal and intercalary and abundant (Plate 4.9 {1b}). Spore germination was 13-15hr (Table 4.5 and 4.7). In comparison, micro morphological and macro morphological characteristics observed were similar with results from other studies as shown in Tables 2.1 and 2.2 respectfully. Therefore, *Trichoderma* isolates T17 and T39 were classified and characterized to be *T, viride* because of similar macro and micro-morphological features.

Table 4.5: Morphological characteristics of *Trichoderma* spp., on PDA media observed in 7 days

Characteristics	*T. harzianum* T4	*Trichoderma* T17	*Trichoderma* T39
Colony colour	Dark green	White –yellow	White-green
Colony Reverse colour	Dull Dark Green with a ring	Colourless to Pale at the center	Light green and clear at the center
Conidiophore's character	Broad verticulate frequently branching	Irregularly branched	Broad verticulate infrequently branching
Phialide character	Ampulliform (Flask shaped) convergent	Ampulliform (Flask shaped) convergent	Lageniform (Flask shaped) divergent
Conidia shape, Size and Colour	Sub globose to obovoid 4X3µm Pale green conidiation	Sub globose to obovoid 3.5X3µm Hyaline	Sub globose to obovoid 3.5X3µm Pale green conidiation
Chlamydospores formation	Terminal and intercalary, frequent	Terminal and intercalary, abundant	Terminal and intercalary, abundant
Spore germination	13-15h	13-15h	13-15h

Table 4.6: Average development of *Trichoderma* spp., on PDA

Trichoderma spp.	Average growth in diameter (cm)		
	Day 2	Day 3	Day 4
T4	2.53b	5.33b	7.55b
T39	2.76a	5.90a	8.40a
T17	2.00c	4.70c	7.30c
C.V. (%)	5	2.2	1.7
LSD ($p<0.05$)	2.1	2.1	2.3

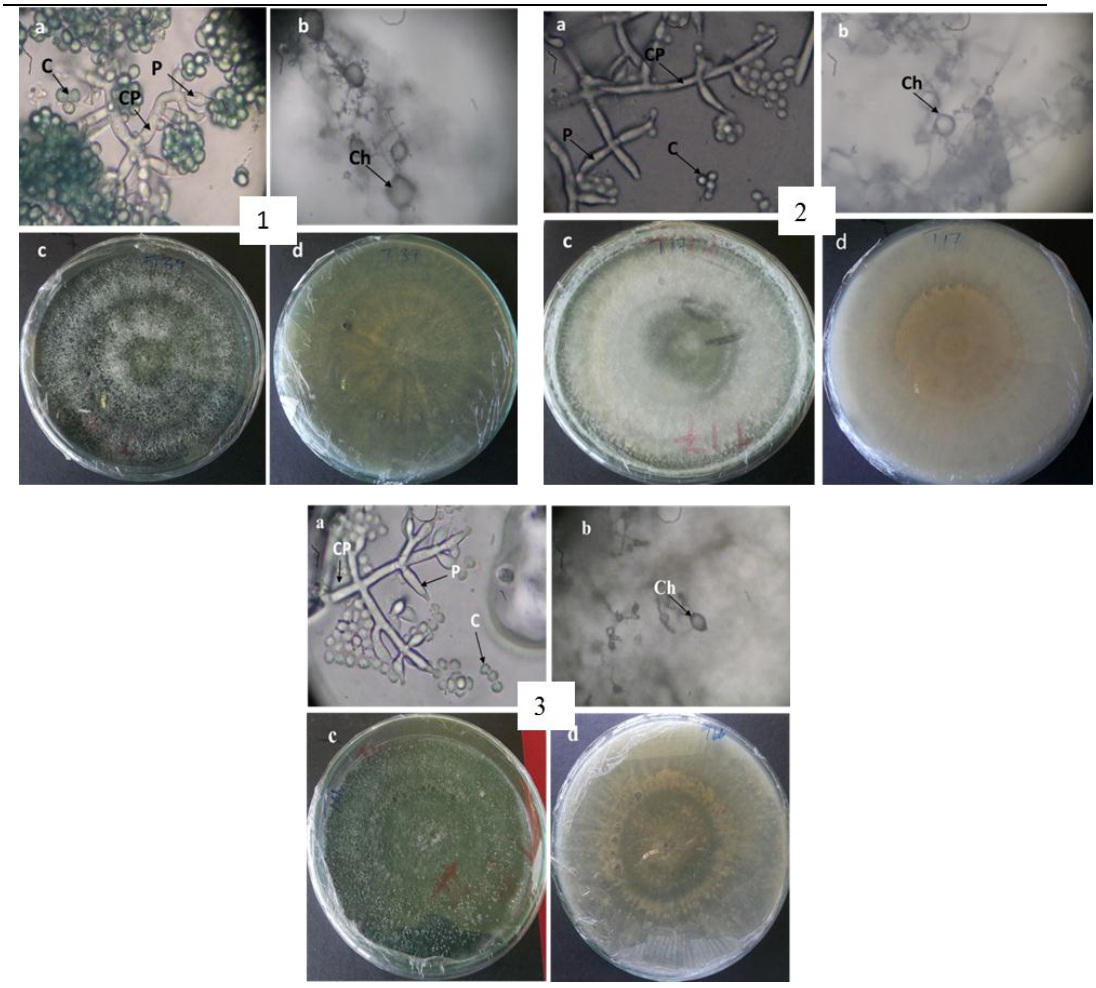

Plate 4.9: *Trichoderma* isolate [1: T39, 2: T17, 3: *T. harzianum*] *showing* [a]. Conidia (C), Phialides (P), and Conidiophores (CP), [b]. Chlamydospores (Ch), [c] colony growth, [d] colony growth reverse (Source: Author, 2022)

4.4.1 Efficacy of *Trichoderma* spp. on growth of *P. theae* in dual-culture plate assay

The results in Table 4.7 on percentage growth inhibition (PGI) in dual culture showed that inhibition of the pathogen (*P. theae)* by all the three *Trichoderma* isolates T4, T39, and T17 varied significantly ($p<0.05$) in day four, fifth and sixth. *Trichoderma* isolate T4 and T17 had significantly difference ($p<0.05$) with higher percentage inhibition of 38.1% and 28.8% in day 4. Percentage inhibition of Pestalotia in day 5 was high for T4 (47.8%) but not significantly different ($p<0.05$) to T17 (42.8%) while in day 6 isolate T17 (57%) gave the highest inhibition but not significantly different ($p<0.05$) to isolate T4 with 51% (Plate 4.10) (Appendix xiii).

Table 4.7: Average growth inhibition (%) of *P. theae* by *Trichoderma* spp. in dual culture

Trichoderma isolates	Percent inhibition of Pestalotia (Days after Incubation)		
	Day 4	Day 5	Day 6
T4	38.12 ± 23.6^{a}	47.8 ± 15.7^{a}	51.9 ± 6.4^{a}
T39	17.98 ± 3.6^{b}	31.61 ± 11.2^{b}	36.64 ± 4.1^{b}
T17	28.75 ± 10.1^{ab}	42.8 ± 4.5^{a}	57.25 ± 6.8^{a}
Control	0 ± 0.0^{c}	0 ± 0.0^{c}	0 ± 0.0^{c}

Means that do not share a letter are significantly different ($p<0.05$)

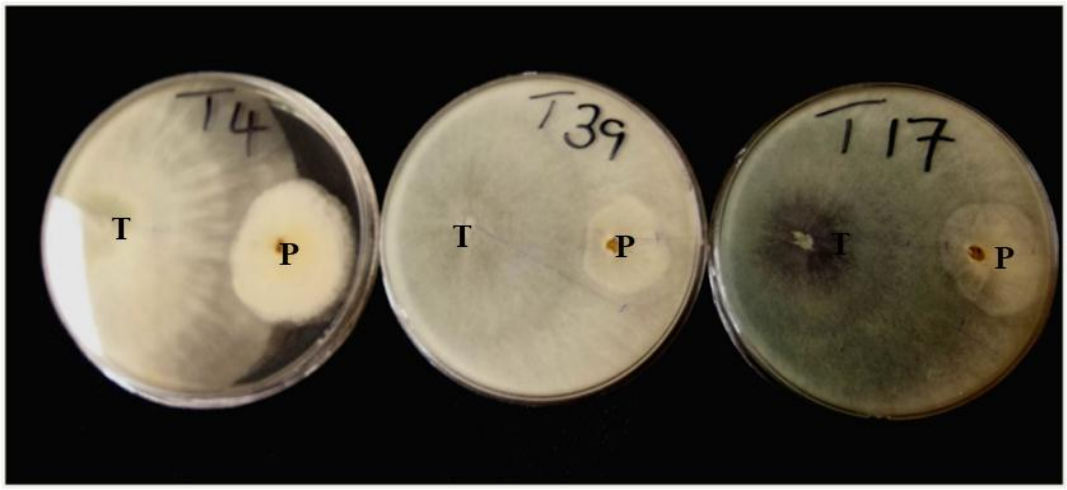

Plate 4.10: Antagonistic activity of *Trichoderma* isolates (T4, T39, and T17) {T} on *P. theae* {P} through dual culture techniques (Source: Author, 2022)

4.4.2 Effect of volatiles on *P. theae* vegetative growth using an inverted plate method

The study on the effect of volatiles on *P. theae* vegetative growth using inverted plate method (Table 4.8) showed inhibition from day 4 to 7 in different *Trichoderma* isolates. Percentage growth inhibition (PGI) showed that inhibition of the pathogen *P. theae* by all the three *Trichoderma* isolates T4, T39, and T17 did not vary significantly **(**$p<0.05$**)** in day 4 and 5. On the sixth day (Appendix xiv), Isolate T4 and T39 had significantly ($p<0.05$) higher in percentage inhibition (38.2 and 32.5%) compared to T17 (20.2%). Treatment T4 in the seventh day had the highest inhibition (45.7%) followed by T39 (33.5%) and T17 (23.6%) in descending order (Plate 4.11).

Table 4.8: Average growth inhibition (%) of *P. theae* by volatiles produced by *Trichoderma* spp. (Inverted plate method)

Trichoderma Isolates	Percentage inhibition of Pestalotia			
	Day 4	Day 5	Day 6	Day 7
T4	24.1(16.7) ±1.6[a]	33.0(29.6) ±1.3[a]	38.2(38.3) ±2.0[a]	45.7(51.2) ±0.42[a]
T39	28.2(22.3) ±4.6[a]	30.5(25.7) ±1.8[a]	32.5(28.8) ±1.7[a]	33.5(30.5) ±2.28[b]
T17	25.1(18.0) ±2.1[a]	24.1(16.7) ±4.3[a]	20.2(11.9) ±4.9[b]	23.6(16.0) ±1.63[c]
Control	0(0) ±0b	0(0) ±0b	0(0) ±0c	0(0) ±0d

Figures in parenthesis are Arcsine transformation of percentage growth inhibition of *P. theae.*

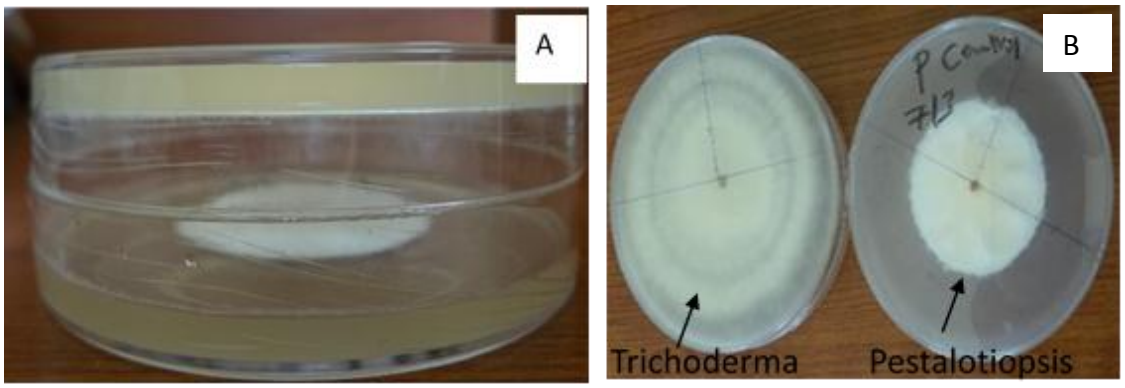

Plate 4.11: Antagonistic activity of *Trichoderma* isolates on *P. theae*. (A) Top – *Trichoderma* spp and Bottom- *Pestalotiopsis* (B). Aerial view of top lid and the bottom lid with pathogens was maintained as control.

4.4.3 Effect of antagonistic activity through the production of antifungal liquid metabolites (culture filtrates).

Trichoderma isolate percentage growth inhibition (PGI) in culture filtrate plate technique (Plate 4.12) showed that inhibition of the pathogen *P. theae* by all the three *Trichoderma* isolates (T4, T39, and T17) varied significantly ($p<0.05$) in day 5 while day 2 to day 6 did not vary except with the control. Treatment T17 and T39 had significant difference ($p<0.05$) percentage inhibition (28.9%) and (26.5%) respectively compared to T4 (27.4%) in day 5 (Table 4.9) (Appendix xv).

Table 4.9: Effect of culture filtrates (non-volatiles) of *Trichoderma* spp against vegetative growth of *P. theae*

Trichoderma spp	Percentage inhibition of Pestalotia				
	day 2	day 3	day 4	day 5	day 6
T17	24.8(17.5) ±4.40 [a]	28.4(22.6) ±3.30 [a]	29.7(24.5) ±1.64[a]	28.9(23.3) ±0.99[a]	29.2(23.8) ±1.3[a]
T4	26.9(20.4) ±2.46 [a]	26.6(20.2) ±2.44 [a]	29.73(24.5) ±0.59[a]	27.4(21.2) ±1.17[ab]	28.0(22) ±0.68[a]
T39	23.3(15.6) ±3.67 [a]	23.6(20.0) ±5.38[a]	27.6(21.4) ±1.72a	26.5(19.9) ±0.72[b]	24.8(17.5) ±2.15[a]
Control	0±0.0[c]	0±0.0[c]	0±0.0[c]	0±0.0[c]	0±0.0[c]

Figures in parenthesis are Arcsine transformation of percentage growth inhibition of *P. theae.* Means that do not share a letter are significantly different ($p<0.05$)

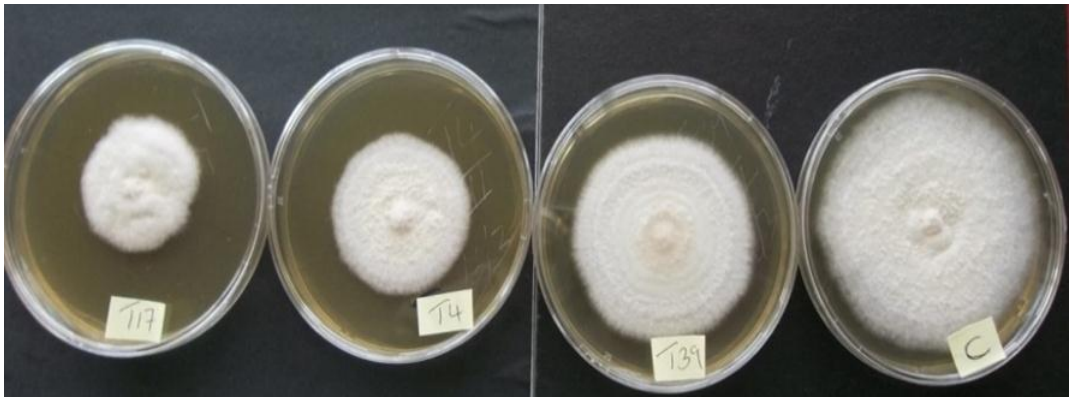

Plate 4.12: Antagonistic activity of *Trichoderma* isolates on *P. theae* through the production of non-volatiles liquid metabolites techniques (Source: Author, 2022)

CHAPTER FIVE

DISCUSSION

5.1 Population build-up of *Trichoderma* spp. in the soil

Trichoderma spp. propagules population analyses after three and twelve months revealed an increase in recovery. This might be because *Trichoderma* spp. plays an important role in colonizing a variety of ecological niches and becoming an efficient biocontrol agent of plant-pathogenic fungi. The findings of this study revealed that the population of *Trichoderma* spp. was lower after three months compared to a higher recovery after twelve months. It showed variation increase in population of *Trichoderma* spp from Treatment T39+AMF, T39, T17 +AMF and T17 in the three clones (AHP S15/10, TRFK 31/8 and TRFK 303/577) for the first three months as well as in the twelve months. The increase in *Trichoderma* spp. population in the tea clone's rhizosphere was as result of colonization and adaption to the environment.

5.2 Abundance of mycorrhizal structures in tea roots

The discovery of a few entrance sites and spores in these trials supported an attempt to infect tea roots. According to Graham and Syvertsen's (1985) theory, host plants with coarse roots and sparse root hairs are more likely to form mycorrhizae associations. In the study the population of arbuscular, vesicles and hyphal increase was not noticed in the first three months until the 9^{th} and 12^{th} month. Abundance of mycorrhizal structures development in the selected tea clone (AHP S15/10, TRFK 31/8 and TRFK 303/577) roots showed varied results after nine and twelve months respectfully. Findings from the 9^{th} month exhibited arbuscular structures higher colonization/abundance in the roots of tea (AHP S15/10, TRFK 31/8 and TRFK 303/577) were from treatments T17+AMF, T4

+AMF and AMF when compared with control. Recovery of vesicles mycorrhizal structure tea clones (AHP S15/10, TRFK 31/8, and TRFK 303/577) varied with treatments T17 + AMF, AMF, and AMF being the highest in comparison with control. Hyphae recovery in clones of tea gave the same results as for vesicles after treatment. Other studies by Wu (2019) demonstrated mycorrhizal root colonization in tea plants having 32.7 ± 3.50% which was almost similar to what obtained in current study. Lu *et al.* (2018), showed extent of mycorrhizal colonization to be a measure of dependency of crop and the subsequent effect on growth response. The overall percentage recovery of mycorrhizal structures in the three clones of tea was high at the end experiment (12^{th} month) due to good root establishment.

All the treatments showed different effects on mycorrhizae structures colonization (abundant in the roots of plants are hyphae, Arbuscules, and vesicles). Appressorium forms on the exterior as hypha enters root and spreads into root cortex. Arbuscules are haustoria-like structures created from the intercellular space that runs longitudinally and allows hyphae to enter cortical cells. Vesicles serve as storage organs and are thin-walled objects with varied sizes and hold oil-droplets.

5.3 Evaluation of *Trichoderma* spp. and arbuscular mycorrhizal fungi on growth parameters and plant dry weight of selected tea clones

In a similar experiment, Harman (2004) demonstrated that some strains of *Trichoderma* are able to stimulate root development in the soil surface by producing hormones that promote plant growth, releasing nutrients from the soil, and secreting organic materials. In the current study, all the treatments applied on three tea cultivars (AHP S15/10, TRFK 31/8 and TRFK 303/577) were effective in enhancing growth parameters (shoot length, shoot diameter, and root length). Growth of the selected tea clones responded

differently with treatments as per each parameter. On the other hand, dry weights of three tea clones did not vary. Studies by Harman (2000) also showed that *T. harzianum* had ability of enhancing root growth development in maize. These observations agreed with those obtained in the current study despite the differences in the parameters and time periods (3 months and 12 months). This may be as a result of aggressiveness in growth and colonisation. On the other hand, combination of both *Trichoderma* and AMF has also increased efficacy in growth enhancement. Research by Yedidia, *et al.*, (2001) showed presence of *Trichoderma asperellum* led to increase in shoot length, leaf area and dry weight of cucumber roots significantly. Tomato seedling development was enhanced in an experiment by Ozbay and Newman (2004) using commercial and non-commercial strains of *Trichoderma harzianum*. Other treatments (*Trichoderma* spp.) in the current experiment had the same impact on the development of vegetatively propagated tea cultivars. The first three months' tea cuttings/plants roots had not developed properly. Arbuscules Mycorrhizae Fungi (AMF) application in combination of *Trichoderma* spp. was ineffective in promoting tea plant development. Each quarter produced distinct effects, as seen by the response in shoot and root length and diameter in tea cultivar improvement. *Trichoderma* spp. colonization commonly improves root growth and development, agricultural yield, and tolerance to abiotic stressors through augmentation of mineral absorption, according to studies by Li *et. al.*, (2015) and Singh and Zaidi (2017). In the current experiment, Arbuscules mycorrhizae fungi (AMF) and members of the genus *Trichoderma* emerged as promising groups of microbial inoculants that induce plant growth development and resistance to disease which is in agreement with study conducted by Tchameni *et al.*, (2011).

AMF can be recognized by the presence of vesicles and arbuscules in a plant's roots (Bagyaraj, 2014). Mycorrhizal structures presences in the root system of host plant

improve growth. This relates to promotion of water and nutrition absorption, enhancement of stress tolerant, root morphological modification and improvement of both endogenous hormones level and of soil physico-chemical properties (Lu *et al.* 2018).

5.4 Antagonistic activity of *Trichoderma* species against *Pestalotiopsis theae*

In the current study, the experiments comprised three study techniques; namely dual culture, inverted plate and non-volatile liquids metabolites. The goal was to assess *Trichoderma* spp. ability to prevent the development of pathogens (*P. theae*) under *in vitro* conditions. *Trichoderma* spp. may use nutrition and competitions for space, production of volatile and non-volatile antibiotics, according to studies by Hajieghrari *et al.,* (2010), which this work has demonstrated. Two interacting organisms (*Trichoderma* spp. and *P. theae* pathogen) in dual culture showed the mean percentage suppression in radial growth, which was attributed to an inhibitory chemical secreted by one or both organisms through the competitive mechanism (Dennis and Webster 1971b; Tapwal *et al*., 2015). In the current investigation, *Trichoderma* spp. showed a variation in their antagonistic potential by inhibiting the development of *Pestalotia* isolates in the dual culture technique. Antibiosis, which manifested as an inhibitory zone in the Petri dishes, was the most prevalent form of action seen. In the sixth day, there was change in antagonistic ability between the isolates. These could have been through inhibition of pathogen either through competition of nutrients and space as result of fast growth rate. The other method may have been through antibiosis through production of non-volatile metabolites impeding the growth of the pathogen. Finally, direct attack by the *Trichoderma* spp. through production of cell-wall- degrading enzymes like glucarases, chitinase and many others as mycoparasitism. Mycelial growth was slowed as a result

of the release of toxic metabolites *Trichoderma* spp (T17) at the end of the 6th day showed high percentage inhibition to *Pestalotiopsis theae* as compared to other *Trichoderma* isolates T4 and T39. Rahman *et al*., (2013) used several methods in the laboratory to assess the effectiveness of various *Trichoderma* strains against *Colletotrichum capsici.* According to the study, *T. harzianum* exhibited the capacity to act as an antagonist by preventing the growth development of mycelium, conidial germination, and elongation of the germ tube. *Trichoderma* spp. effectively competed for space and resources with harmful fungus by growing considerably more quickly than them, which could have prevented *P. theae* from proliferating. Ambuse *et al*., 2012) investigated ten *Trichoderma* species using a dual culture approach against susceptible and unaffected isolates of *Alternaria tenuissima*. The showed that up to 80% antagonistic activity by *T. viride, T. koningii,* and *T. pseudokoningii* was effective.

Because *Trichoderma* spp. are important source of secondary metabolites, they have been employed as plant diseases biological control agent. Production of antibiotic and low molecular-weight compounds inhibits growth development of phytopathogenic fungi (Meliani *et al.,* 2017, Shah *et al.,* 2019). Inverted plate technique indicated presence of volatiles released by *Trichoderma* spp. that inhibited *P. theae,* which acted through antibiosis. Microbial specific or non-specific metabolite, lytic enzyme, volatile chemical, or other harmful agent, such as hydrogen cyanide, ethylene, ketones, or aldehyde, can act as a mediator of antibiosis, which is antagonism (Meena *et al.*, 2017). Inhibition of *Pestalotia* at the end of the 7th day showed T17 as the lowest inhibiter in this mode (16%) followed by T39 (30%) while T4 had the highest of 51%. The method by which Trichoderma spp. produces powerful volatile metabolites to combat *Pestalotiopsis theae* led to the detection of a volatile inhibitor. *Trichoderma* species have been shown to produce diffusible volatile antibiotics that are effective in vitro

against fungal plant pathogens. In accordance with the current work, *Trichoderma* isolate T4 showed the maximum inhibition which is in agreement with results by Claydon *et al.,* (1987) that *T. harzianum* produces volatile chemicals (Alkyl pyrenes) that have antifungal activity.

Culture filtrates (liquid volatiles) of *Trichoderma* spp. against vegetative growth of *P. theae* tested for inhibition until the 6th day. The best inhibition was exhibited by T17 (24%) followed by T39 (22%) and the lowest was T4 (20%). Another study was carried out by Choudhary and Reena (2012), and used liquid culture filtrate technique, dual culture method to screen 19 isolates (T. *harzianum*, T. *viride*, and T. *koningii*) against F. *oxysporum* f. sp. lentils, which are responsible for wilt, and found a major inhibition of pathogen development. According to the current investigation, the proliferation of P. *theae* pathogenic fungus is adversely impacted as a result of non-volatile metabolites generated by *Trichoderma* spp. Dubey's (2006) research; T. *harzianum* produces non-volatile compounds that prevent *F. o. f. sp. ciceri* from producing chickpea wilt. In addition, technique proposed by Dennis and Webster (1971a); Quimio and Cumagun (2001) showed that *Trichoderma* isolates had the capacity to produce non-volatile substances which is in agreement with the results. An efficient biocontrol agent for fungi that cause plant damage has been found to be *Trichoderma* spp. (Chet, 1987; Altomare *et al.*, 1999; Yedidia *et al.*, 1999; Singh *et al.*, 2010).

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The investigation on bio-assay of *Trichoderma* Colony Forming Units recovery from the soil showed the maximum population of *Trichoderma* spp. to be T39+AMF, T17+AMF and T39 in the three tea clones root rhizospheres. Percentage colonization of AMF structures at the root of tea was higher after twelve months. The results indicated that the percentage recovery of mycorrhizae structures (arbuscules, vesicles and hyphae) was from treatment AMF, T17 +AMF and T4+AMF for the 9^{th} and 12^{th} months.

It is evident from results that treatment T4 (*Trichoderma harzianum*), T39, T39+AMF and T17+AMF enhanced growth of tea clones changing within the 3^{rd} and 12^{th} months period.

Trichoderma Isolates which were tested suppressed mycelial growth of the pathogen (*P. theae*) with differences in their abilities. The two antagonistic *Trichoderma* isolates (T17 and T39) have potentiality in controlling *P. theae* in dual culture technique as well as culture filtrates (liquid metabolites) method. *T. harzianum* (T4) have better inhibition in inverted plate (volatile metabolite) technique. Production of toxic volatile metabolites by all *Trichoderma* isolates has important results in decreasing the development of *P. theae*.

6.2 Recommendation

1. Re-application of the treatments/product to be done in order boost/increase the population of propagules and colonization of mycorrhizae structures.

2. It is clear from the current study that combining *Trichoderma* spp. and AMF was able to improve the growth of tea cuttings in the nursery. A long-term field experiment will demonstrate the possible role that bio-fertilizers could play in promoting growth and controlling disease.

3. In-vitro antagonistic action of *Trichoderma* species verses *Pestalotiopsis theae* sheds light on ways of controlling the pathogen. The current results reveal that *Trichoderma* spp. produces volatile metabolites, which antagonize *P. theae.* This research work has provided information for further studies not only for *Pestalotiopsis theae* but also for other plant diseases attacking tea. Parasitism nutrient and niche competition should be conducted too to enriched knowledge on the *Trichoderma* spp. antagonisms.

4. Studies to be extended to the field conditions to include yield as a growth parameter and manifestation of grey blight disease bioagent control.

REFERENCES

Ambuse MG, Chatage VS, and Bhale UN. (2012). Influence of *Trichoderma* spp. against *Alternaria tenuissima* inciting leaf spot of Rumex Acetosa L. *Biosci Discovery*, 3(2):259–262.

Al-Saeedi, S. S., and Al-Ani, B. M. (2014). Study of antagonistic capability of *Trichoderma harzianum* isolates against some pathogenic soil borne fungi. *Agriculture and Biology Journal of North America*, *5*(1), 15-23.

Altomare, C., Norvell, W. A., Björkman, T., and Harman, G. E. (1999). Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum Rifai* 1295-22. *Appl. Environ. Microbial*, *65*(7), 2926-2933.

Anon, T. R. F. K. (2002). Tea grower's handbook. *Tea Research Foundation of Kenya*,*5*, *5*.

Artursson, V., Finlay, R. D., and Janson, J. K. (2006). Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. *Environmental microbiology*, *8*(1), 1-10.

Bagyaraj, D. J. (2014). Mycorrhizal fungi. In *Proc Indian Natl Sci Acad* (Vol. 80, No. 2, pp. 415-428).

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., and Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu. Rev. Plant Biol.*, *57*, 233-266.

Baker, R. (1989). Improved Trichoderma spp. for promoting crop productivity. Trends in Biotechnology, 7(2), 34-38.

Banayo, N. P. M., Cruz, P. C., Aguilar, E. A., Badayos, R. B., and Haefele, S. M. (2012). Evaluation of biofertilizers in irrigated rice: Effects on grain yield at different fertilizer rates. *Agriculture*, *2*(1), 73-86.

Barnett, H. L., & Hunter, B. B. (1972). Illustrated genera of imperfect fungi. *Illustrated genera of imperfect*

Bokhara, N. A., and Perveen, K. (2012). Antagonistic action of *Trichoderma harzianum* and *Trichoderma viride* against *Fusarium solani* causing root rot of tomato. *African Journal of Microbiology Research*, *6*(44), 7193-7197.

Carreras-Villaseñor, N., Sánchez-Arreguín, J. A., and Herrera-Estrella, A. H. (2012). *Trichoderma:* sensing the environment for survival and dispersal. *Microbiology*, *158*(1), 3-16.

Carvajal-Muñoz, J. S., & and Carmona-Garcia, C. E. (2012). Benefits and limitations of bio fertilization in agricultural practices. *Livestock Research for Rural Development*, *24*(3), 1-8.

Carvalho, D. D. C., Inglis, P. W., de Ávila, Z. R., Martins, I., Muniz, P. H. P., and de Mello, S. C. (2018). Morphological characteristics and genetic variability of *Trichoderma* spp. from conventional cotton crop soils in Federal District, Brazil.

Embrapa Recursos Genéticos e Biotecnologia-Artigo em periódico indexado (ALICE).

Carvalho, D. D., Lobo Junior, M., Martins, I., Inglis, P. W., and Mello, S. (2014). Biological control of Fusarium oxysporum f. sp. phaseoli by *Trichoderma harzianum* and its use for common bean seed treatment. *Tropical Plant Pathology*, *39*(5), 384-391.

Chen Y, Zeng L, Shu N, Ziang M, Wang H, Huang Y and Tong H. 2008. Pestalotiopsis like organisms causing grey blight disease of Camellia sinensis in China. Plant Disease 98: 102–06

Chen, L., Zhou, Z. X., and Yang, Y. J. (2007). Genetic improvement and breeding of tea plant (*Camellia sinensis*) in China: from individual selection to hybridization and molecular breeding. *Euphytica*, *154*(1-2), 239-248.

Chen, Y., Zeng, L., Shu, N., Jiang, M., Wang, H., Huang, Y., and Tong, H. (2018). *Pestalotiopsis*-like species causing gray blight disease on *Camellia sinensis* in China. *Plant disease*, *102*(1), 98-106.

Chet I, Inbar J. (1994). Biological control of fungal pathogens. *Applied Biochemistry and Biotechnology*, 48:37-43.

Chet, I. (1987). *Trichoderma*: application, mode of action, and potential as biocontrol agent of soil borne plant pathogenic fungi. *Innovative approaches to plant disease control*, 137-160.

Choudhary S, R. M. (2012). In-vitro antagonism of indigenous *Trichoderma* isolates against phytopathogens causing wilt of lentil. *Int J Life Sci Pharma Res.*, 2(3):195–202.

Claydon, N., Allan, M., Hanson, J.R., and Avent, A.G. (1987). Antifungal alkyl pyrenes of *Trichoderma harzianum*. *Transactions of the British Mycological Society*, 88: 503-513.

Cruz C, Correia P, Ramos AC, Carvalho L, Bago A, Martins-Loução MA (2008) Arbuscular mycorrhiza in physiological and morphological adaptations of Mediterranean plants. In: Varma A, Hock B (eds) Mycorrhiza: biology, genetics, novel endophytes and biotechnology. Springer, Berlin, pp 729–749

Dennis, C. Webster, J. (1971a). Antagonistic properties of species group of Trichoderma I. *Hyphal interaction. Trans. Br.Mycol. Soc.*, 57: 41-48.

Dennis C, Webster J. (1971b). Antagonistic properties of species groups of *Trichoderma*-II. Production of volatile antibiotics. *Trans Br Mycol Soc.*, 57:47–48.

Devi, P., Prabhakaran, N., Kamil, D., Pandey, P., and Borah, J. L. (2012). Characterization of Indian native isolates of *Trichoderma* spp. and assessment of their bio-control efficiency against plant pathogens. *African Journal of Biotechnology*, *11*(85), 15150-15160.

Dubey SC, Suresh M, Singh B. (2007). Dubey SC, Suresh M, Singh B (2007). Evaluation of Trichoderma species against Fusarium oxysporum f.sp. ciceros for integrated management of chickpea wilt. *Biological Control,* 40:118-127.

Dubey, S. C., and Suresh, M. (2006). Randomly amplified polymorphic DNA markers for Trichoderma species and antagonism against Fusarium oxysporum f. sp. ciceris causing chickpea wilt. *Journal of phytopathology, 154*(11- 12), 663-669.

Elad, Y. (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. *Crop Protection*, 19:709-714.

Epstein, L., and Bassein, S. (2003). Patterns of pesticide use in California and the implications for strategies for reduction of pesticides. *Annual Review of Phytopathology*, *41*(1), 351-375.

Freeman S, Minz D, Kolesnik I, Barbul O, Zreibil A, Maymon M, Nitzani Y, Kirshner B, Rav-David D, Bilu A, Dag A, Shafir S, and Elad Y. (2004). *Trichoderma* biocontrol of Colletotrichum acutatum and Botrytis cinerea, and survival in strawberry. European Journal. *Plant Pathology*, 110:361-370.

Gams, W., Bissett, J., and MALIK, A. F. (2002). Morphology and identification of. *Trichoderma and Gliocladium*, 3-31.

Goyal S.P., Jandiak C.L. and Sharma V.P (1994). Effect of weed fungi metabolites on the mycelial growth of A. bisporus (Lang.). Imbach. Mushroom Research. 3: 69: 69-74.

Hajieghrari B, Torabi-Giglou M, Mohammadi MR, Davari M. (2008). Biological potential of some Iranian *Trichoderma* isolates in the control of soil-borne plant pathogenic fungi. *African Journal of Biotechnology*, 7 (8):967-972.

Hamid, F. S., Ahmad, T., Khan, B. M., Waheed, A., and Ahmed, N. (2006). Effect of soil pH in rooting and growth of tea cuttings (Camellia sinensis L.) at nursery level. *Pakistan Journal of Botany*, *38*(2), 293.

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) *Trichoderma* species-opportunistic, virulent plant symbionts. Nat Rev Microbiology 2:43-56

Harman, G. E. (2000). Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma harzianum* T-22. *Plant disease,* 84(4), 377-393.

Harman, G. E. (2006). Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology*, *96*(2), 190-194.

Hermosa, R., Rubio, M. B., Cardoza, R. E., Nicolás, C., Monte, E., and Gutiérrez, S. (2013). The contribution of Trichoderma to balancing the costs of plant growth and defense. *Int. Microbiol*, *16* (2), 69-80.

Inbar, J., Abramsky, M., Cohen, D., & Chet, I. (1994). Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. European Journal of Plant Pathology, 100(5), 337-346.

International Tea Committee. (2022). Annual bulletin statistics for 2018. *International tea committee.*

Jain, S. M., and Newton, R. J. (1990). Prospects of biotechnology for tea improvement. *Proceedings of the Indian National Science Academy. Part B, Biological Sciences*, *56*(5-6), 441-448.

Joshi, S. D., Sanjay, R., Baby, U. I., and Mandal, A. K. A. (2009). Molecular characterization of Pestalotiopsis spp. associated with tea (*Camellia sinensis*) in southern India using RAPD and ISSR markers.

Kamunya, S., Ochanda, S., Cheramgoi, E., Chalo, R., Sitienei, K., Muku, O, and Bore, J. K. (2019). Tea Growers Guide.

Kannangara, S., Dharmarathna, R. M. G. C. S., and Jayarathna, D. L. (2017). Isolation, identification and characterization of Trichoderma species as a potential biocontrol agent against *Ceratocystis paradoxa*. *Journal of Agricultural Sciences–Sri Lanka*, *12*(1).

Kariuki, G. M., Njaramba, J. & Ombuki, C. (2022). Tea Production Response to Climate Change in Kenya: An Autoregressive Distributed Lag Approach. *African Journal of Economic Review*, *10*(1), 2-26.

Kleifeld, O., & and Chet, I. (1992). Trichoderma-plant interaction and its effect on increased growth response. *Plant soil*, *144*(2), 267-272.

Kumhar, K. C., Babu, A., Bordoloi, M., Benarjee, P., and Rajbongshi, H. (2016). International Journal of Current Research in Biosciences and Plant Biology. *Int. J. Curr. Res. Biosci. Plant Biol*, *3*(4), 20-27.

Lanfranco, L., Novero, M., and Bonfante, P. (2005). The mycorrhizal fungus Gigaspora margarita possesses a CuZn superoxide dismutase that is up-regulated during symbiosis with legume hosts. *Plant Physiology*, *137*(4), 1319-1330.

Li, R. X., Cai, F., Pang, G., Shen, Q. R., Li, R., and Chen, W. (2015). Solubilization of phosphate and micronutrients by *Trichoderma harzianum* and its relationship with the promotion of tomato plant growth. *PLoS One*, *10*(6), e0130081.

Liu, Z. W., Wu, Z. J., Li, X. H., Huang, Y., Li, H., Wang, Y. X., & Zhuang, J. (2016). Identification, classification, and expression profiles of heat shock transcription factors in tea plant (Camellia sinensis) under temperature stress. *Gene*, *576*(1), 52-59.

Lü, L. H., Zou, Y. N., and Wu, Q. S. (2018). Relationship between arbuscular mycorrhizas and plant growth: improvement or depression? In *Root Biology* (pp. 451-464).

Maharachchikumbura, S. S., Chukeatirote, E., Guo, L. D., Crous, P. W., Mckenzie, E. H., and Hyde, K. D. (2013). *Pestalotiopsis* species associated with *Camellia sinensis* (tea). *Mycotaxon*, *123*(1), 47-61.

Malusà, E., Pinzari, F., and Canfora, L. (2016). Efficacy of biofertilizers: challenges to improve crop production. In *Microbial inoculants in sustainable agricultural productivity* (pp. 17-40). Springer, New Delhi.

Martinez-Medina, A., Roldán, A., and Pascual, J. A. (2011). Interaction between arbuscular mycorrhizal fungi and *Trichoderma harzianum* under conventional and low input fertilization field condition in melon crops: growth response and Fusarium wilt biocontrol. *Applied Soil Ecology*, *47*(2), 98-105.

Meena, M., Swapnil, P., Zehra, A., Dubey, M. K., and Upadhyay, R. S. (2017). Antagonistic assessment of *Trichoderma* spp. by producing volatile and non-volatile compounds against different fungal pathogens. *Archives of Phytopathology and Plant Protection*, *50*(13-14), 629-648.

Meliani, A., Bensoltane, A., Benidire, L., and Oufdou, K. (2017). Plant growth-promotion and IAA secretion with *Pseudomonas fluorescens* and *Pseudomonas putida*. *Research* and *Reviews: Journal of Botanical Sciences*, *6*(2), 16-24.

Morton, D. J. and Strouble, W. H. (1955). Antagonistic and stimulatory effect of soil microorganism upon *Sclerotium rolfsii*. *Phytopath.45,* 417-420.

Mukhtar, H., and Ahmad, N. (2000). Tea polyphenols: prevention of cancer and optimizing health. *The American journal of clinical nutrition*, *71* (6), 1698S-1702S.

Muthiani, M. A., Wanyoko, J. K., Wachira, F. N., Kamunya, S. M., Chalo, R. M., Kimutai, S., and Karori, S. (2016). The potential use of Kenyan tea cultivars in the development of high value diversified products. *International Journal of Tea Science (IJTS)*, *12*(1/2), 30-48.

Mwangi, M. W., Monda, E. O., Okoth, S. A., and Jefwa, J. M. (2011). Inoculation of tomato seedlings with *Trichoderma harzianum* and arbuscular mycorrhizal fungi and their effect on growth and control of wilt in tomato seedlings. Brazilian Journal of Microbiology, 42(2), 508-513.

Naher, L., Syawani, N., Amieza, N., Kamarudin, A. B., and Karim, S. M. R. (2019). *Trichoderma* species diversity in rhizosphere soils and potential antagonism with *Fusarium oxysporum*. Bioscience Journal, 35(1).

Naher, L., Yusuf, U. K., Ismail, A., & Hossain, K. (2014). Trichoderma spp.: a biocontrol agent for sustainable management of plant diseases. *Pak. J. Bot*, *46*(4), 1489-1493.

Nyabundi, K. W., Owuor, P. O., Netondo, G. W., and Bore, J. K. (2016). Genotype and environment interactions of yields and yield components of tea (Camellia sinensis) cultivars in Kenya.

Ombogo, O., and Karanja, A. M. (2022). Regression Models in Forecasting Crop Yield under Climate Change Scenarios. *International Journal of Innovative Research and Development*, *11*(3).

Ozbay, N., and Newman, S. E. (2004). Biological control with *Trichoderma* spp. with emphasis on *T. harzianum*. *Pakistan Journal of Biological Sciences*, *7*(4), 478-484.

Parihar, M., Chitara, M., Khati, P., Kumari, A., Mishra, P. K., Rakshit, A. and Jatav, S. S. (2020). Arbuscular mycorrhizal fungi: Abundance, interaction with plants and

potential biological applications. In *Advances in Plant Microbiome and Sustainable Agriculture* (pp. 105-143). Springer, Singapore.

Phillips, J.M. and P.S. Hayman, 1970. Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brazilian Mycol. Soc., 55: 158–161.

Rahman, M. A., Razvy, M. A., and Alam, M. F. (2013). Antagonistic activities of *Trichoderm*a strains against *chili anthracnose* pathogen.

Quimio, A. J., & Cumagun, C. J. (2001). Module 2: Biocontrol fungi with emphasis on Trichoderma. In *Training Course on Tropical Fungi, College, Laguna (Philippines), 20-22 Oct 1999*. MSPI.

Roychowdhury, D., Paul, M., and Banerjee, S. K. (2014). A review on the effects of biofertilizers and biopesticides on rice and tea cultivation and productivity. International Journal of Science, Engineering and Technology, 2(8), 96-106.

Sadhana, B. (2014). Arbuscular Mycorrhizal Fungi (AMF) as a Biofertilizer-a Review. *Int. J. Curr. Microbiol. App. Sci*, *3*(4), 384-400.

Samynathan, R., Shanmugam, K., Nagarajan, C., Murugasamy, H., Ilango, R. V. J., Shanmugam, A., .and Thiruvengadam, M. (2021). The effect of abiotic and biotic stresses on the production of bioactive compounds in tea (*Camellia sinensis* (L.) O. Kuntze). *Plant Gene*, *27*, 100316.

Sangoyomi, T. E. (2004). Post-harvest fungal deterioration of yam (Dioscorea rotundata Poir) and its control.

Schuster, A., and Schmoll, M. (2010). Biology and biotechnology of *Trichoderma*. *Applied microbiology and biotechnology*, *87*(3), 787-799.

Sekhar, Y. C., Ahammed, S. K., Prasad, T. N. V. K. V., and Devi, R. S. J. (2017). Identification of *Trichoderma* species based on morphological characters isolated from the rhizosphere of groundnut (Arachis hypogaea L). *Int J Sci Environ Technol*, *6*, 2056-2063.

Sghir, F., Chliyeh, M., Touati, J., Mouria, B., Touhami, O. A., Filali-Maltouf, A., and Douira, A. (2014). Effect of a dual inoculation with endomycorrhizae and *Trichoderma harzianum* on the growth of date palm seedlings. *Int. J. Pure App. Biosci*, *2*(6), 12-26.

Shah, M. M., Sharif, U., and Buhari, T. R. (Eds.). (2019). *Trichoderma: The Most Widely Used Fungicide*. BoD–Books on Demand.

Siddiqui, Z. A., and Futai, K. (Eds.). (2008). *Mycorrhizae: sustainable agriculture and forestry* (p. 359). Dordrecht: Springer.

Singh, A., & Islam, M. N. (2010). ISSN-In Progress-ISSN Center Paris, France Int. J. Expt. Agric. 1 (1): 20-25 (July 2010) *in vitro* evaluation of *Trichoderma* spp. AGAINST Phytophthora nicotianae. *Int. J. Expt. Agric*, *1*(1), 20-25.

Singh, R., Singh, B. K., Upadhyay, R. S., Rai, B., and Lee, Y. S. (2002). Biological control of Fusarium wilts disease of pigeon pea. *The Plant Pathology Journal*, *18*(5), 279-283.

Singh, U. S., and Zaidi, N. W. (2017). *Trichoderma*—an Impeccable Plant Health Booster. In *Biopesticides and Bio agents* (pp. 33-58). Apple Academic Press.

Siqueira JO, Sylvia D, Gibson J, and Hubbel D (1985b) Spores, germination, and germ tubes of vesicular- arbuscular mycorrhizal fungi. Can J Microbiol 31:965–997

Smith, S. E., & Read, D. J. (1997). Growth and carbon economy of VA mycorrhizal plants. *Mycorrhizal symbiosis*, 105-125.

Srinivasa, N., Devi, T. P., Sudhirkumar, S., Kamil, D., Borah, J. L., and Prabhakaran, N. (2014). Bio efficacy of *Trichoderma* isolates against soil-borne pathogens. *Afr. J. Microbiol. Res*, *8*, 2710-2723.

Strobel, G., and Daisy, B. (2003). Bioprospecting for microbial endophytes and their natural products. *Microbiol. Mol. Biol. Rev.*, *67*(4), 491-502.

Sukhada, M., Manjula, R., and Rawal, R. D. (2011). Evaluation of arbuscular mycorrhiza and other biocontrol agents against Phytophthora parasitica var. nicotinate infecting papaya (*Carica papaya* cv. Surya) and enumeration of pathogen population using immune techniques. *Biological Control*, *58*(1), 22-29.

Tapwal A, Thakur G, Chandra S, and Tyagi A. (2015). In-vitro evaluation of *Trichoderma* species against seed-borne pathogens. *Int J Biol Chem Sci.*, 1(10):2349–2724.

Tchameni, S. N., Ngonkeu, M. E. L., Begoude, B. A. D., Nana, L. W., Fokom, R., Owona, A. D. & Kuaté, J. (2011). Effect of *Trichoderma asperellum* and arbuscular mycorrhizal fungi on cacao growth and resistance against black pod disease. *Crop protection*, *30*(10), 1321-1327.

TRFK, Clonal catalog (2002

Trillas, M. I., and Segarra, G. (2009). Interactions between nonpathogenic fungi and plants. *Advances in Botanical Research*, *51*, 321-359.

Van der Heijden, M. G., Klironomos, J. N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., and Sanders, I. R. (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability, and productivity. *Nature*, *396*(6706), 69.

Veresoglou, S. D., Chen, B., and Rillig, M. C. (2012). Arbuscular mycorrhiza and soil nitrogen cycling. *Soil Biology and Biochemistry*, *46*, 53-62.

Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and soil*, *255*(2), 571-586.

Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Woo, S. L., Nitro, M., Marra, R. and Manzanillo, G. (2014). *Trichoderma* secondary metabolites active on plants and fungal pathogens. The Open Mycology Journal, 8(1).

Vincent, J. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, 159-850.

Waghunde, R. R., Shelake, R. M., and Sabalpara, A. N. (2016). *Trichoderma*: A significant fungus for agriculture and environment. *African journal of agricultural research*, *11* (22), 1952-1965.

Windham, M., Elad, Y., & Baker, R. (1986). A mechanism for increased plant growth induced by Trichoderma spp. *Phytopathology*, 76(5), 518-521.

Wu, Q. S., Shao, Y. D., Gao, X. B., Xia, T. J., and Kuča, K. A. M. I. L. (2019). Characterization of AMF-diversity of endosphere versus rhizosphere of tea (*Camellia sinensis*) crops. *Indian Journal of Agricultural Sciences*, *89*(2), 348-352.

Yedidia, I., Benhamou, N., and Chet, I. (1999). Induction of defense responses in cucumber plants (*Cucumis sativus L*.) by the biocontrol agent *Trichoderma harzianum*. *Applied and environmental microbiology*, *65*(3), 1061-1070.

Yedidia, I., Srivastva, A. K., Kapulnik, Y., and Chet, I. (2001). Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants. *Plant and soil*, *235*(2), 235-242.

APPENDICES

APPENDIX I: A map showing Study area (Source: Author, 2022)

APPENDIX II: Field layout showing eight treatments and three clones of tea as sub-treatments replicated three times

RI		RII		RIII	
Main treatments	Sub treatments	Main treatments	Sub treatments	Main treatments	Sub treatments
T39+AMF	S15/10	T4+AMF	S15/10	T39	S15/10
	31/8		31/8		31/8
	303/577		303/577		303/577
T4	S15/10	T39+AMF	S15/10	T4+AMF	S15/10
	31/8		31/8		31/8
	303/577		303/577		303/577
T17+AMF	S15/10	T4	S15/10	T17	S15/10
	31/8		31/8		31/8
	303/577		303/577		303/577
T39	S15/10	Control	S15/10	AMF	S15/10
	31/8		31/8		31/8
	303/577		303/577		303/577
T4+AMF	S15/10	T17+AMF	S15/10	T39+AMF	S15/10
	31/8		31/8		31/8
	303/577		303/577		303/577
T17	S15/10	AMF	S15/10	Control	S15/10
	31/8		31/8		31/8
	303/577		303/577		303/577
AMF	S15/10	T39	S15/10	T17+AMF	S15/10
	31/8		31/8		31/8
	303/577		303/577		303/577
Control	S15/10	T17	S15/10	T4	S15/10
	31/8		31/8		31/8
	303/577		303/577		303/577

APPENDIX III: ANOVA tables of *Trichoderma* spp. mean propagules population recovery from soil rhizosphere of tea roots after three months

Variate AHP S15/10					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	2	1.2622	0.6311	0.79	
Treatment	7	17.6916	2.5274	3.17	0.031
Residual	14	11.1566	0.7969		
Total	23	30.1104			
Variate: TRFK 31/8					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	1.2784	0.6392	0.87	
Treatment	7	21.9289	3.1327	4.27	0.010
Residual	14	10.2816	0.7344		
Total	23	33.4889			
Variate: TRFK 303/577					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.0491	0.0245	0.16	
Treatment	7	24.5803	3.5115	23.12	<.001
Residual	14	2.1267	0.1519		
Total	23	26.7561			

APPENDIX IV: ANOVA tables of *Trichoderma* spp. mean propagules population recovery from soil rhizosphere of tea roots

Variate: AHP S15/10					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.06406	0.03203	0.36	
Treatment	7	34.84894	4.97842	56.69	<.001
Residual	14	1.22948	0.08782		
Total	23	36.14248			
Variate: TRFK 31/8					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.05912	0.02956	0.31	
Treatment	7	38.63665	5.51952	58.57	<.001
Residual	14	1.31941	0.09424		
Total	23	40.01517			
Variate: TRFK 303/577					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.9371	0.4685	4.06	
Treatment	7	48.7287	6.9612	60.29	<.001
Residual	14	1.6166	0.1155		
Total	23	51.2824			

APPENDIX V: ANOVA tables of mean colonization of Mycorrhizal structures on selected tea clones after nine months

Analysis of variance

AHP S15/10
Variate: Arbuscular

Source of variation	d.f.	s.s.	m.s.	v.r.	F PR.
Reps stratum	2	1.4207	0.7103	1.72	
Treatment	4	16.6437	4.1609	10.06	0.003
Residual	8	3.3078	0.4135		
Total	14	21.3721			

TRFK 31/8
Variate: Arbuscular

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	1.668	0.834	0.80	
Treatment	4	18.980	4.745	4.57	0.033
Residual	8	8.311	1.039		
Total	14	28.959			

TRFK 303/577
Variate: Arbuscular

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	2.0155	1.0077	1.70	
Treatment	4	16.8308	4.2077	7.11	0.010
Residual	8	4.7360	0.5920		
Total	14	23.5822			

AHP S15/10
Variate: Vesicles

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	3.095	1.548	1.00	
Treatment	4	1.629	0.407	0.26	0.894
Residual	8	12.380	1.548		
Total	14	17.105			

TRFK 31/8
Variate: Vesicles

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.3394	0.1697	0.42	
Treatment	4	18.7742	4.6935	11.53	0.002
Residual	8	3.2580	0.4072		
Total	14	22.3716			

TRFK 303/577
Variate: Vesicles

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	1.0405	0.5202	1.25	
Treatment	4	23.3367	5.8342	13.98	0.001
Residual	8	3.3391	0.4174		
Total	14	27.7163			

AHP S15/10
Variate: Hyphae

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	1.4543	0.7272	2.34	
Treatment	4	27.1099	6.7775	21.85	<.0.001
Residual	8	2.4819	0.3102		
Total	14	31.0461			

TRFK 31/8
Variate: Hyphae

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.4291	0.2145	0.55	
Treatment	4	17.1850	4.2962	11.02	0.002
Residual	8	3.1196	0.3899		
Total	14	20.7336			

TRFK 303/577
Variate: Hyphae

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	2.0004	1.0002	1.40	
Treatment	4	19.0570	4.7642	6.67	0.012
Residual	8	5.7113	0.7139		
Total	14	26.7687			

APPENDIX VI: ANOVA tables of Arbuscular mycorrhizal structures (%) from the root rhizosphere of selected tea after one year

AHP S15/10
Variate: Arbuscular

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	1.646	0.823	0.80	
Treatment	4	18.421	4.605	4.48	0.034
Residual	8	8.232	1.029		
Total	14	28.298			

TRFK 31/8
Variate: Arbuscular

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.0719	0.0360	0.07	
Treatment	4	18.8952	4.7238	8.86	0.005
Residual	8	4.2661	0.5333		
Total	14	23.2332			

TRFK 303/577
Variate: Arbuscular

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.59922	0.29961	3.49	
Treatment	4	26.46867	6.61717	77.18	<.001
Residual	8	0.68586	0.08573		
Total	14	27.75374			

AHP S15/10
Variate: Vesicles

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.0342	0.0171	0.09	

Treatment	4	27.8303	6.9576	35.21	<.001
Residual	8	1.5810	0.1976		
Total	14	29.4455			
TRFK 31/8					
Variate: Vesicles	.				
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	1.6425	0.8212	1.59	
Treatment	4	16.6024	4.1506	8.05	0.007
Residual	8	4.1267	0.5158		
Total	14	22.3716			
TRFK 303/577					
Variate: Vesicles					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.3891	0.1945	1.72	
Treatment	4	31.3357	7.8339	69.30	<.001
Residual	8	0.9043	0.1130		
Total	14	32.6291			
AHP S15/10					
Variate: Hyphae					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.2098	0.1049	0.17	
Treatment	4	19.9919	4.9980	8.02	0.007
Residual	8	4.9878	0.6235		
Total	14	25.1896			
TRFK 31/8					
Variate: Hyphae					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	1.218	0.609	0.42	
Treatment	4	13.326	3.331	2.31	0.146
Residual	8	11.562	1.445		
Total	14	26.106			
TRFK 303/577					
Variate: Hyphae					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.15864	0.07932	1.26	
Treatment	4	35.26194	8.81548	140.17	<.001
Residual	8	0.50314	0.06289		
Total	14	35.92372			

APPENDIX VII: ANOVA tables results of *Trichoderma* isolates and mycorrhizae on shoot length and diameter growth enhancement after three months

AHP S15/10

Variate: Shoot Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	552.48	276.24	3.41	
Treatments	7	1907.45	272.49	3.36	0.025
Residual	14	1134.60	81.04		
Total	23	3594.53			

TRFK 31/8

Variate: Shoot Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	216.8	108.4	0.36	
Treatments	7	4141.4	591.6	1.97	0.133
Residual	14	4212.2	300.9		
Total	23	8570.4			

TRFK 303/577

Variate Shoot Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	761.7	380.8	3.19	
Treatments	7	1414.8	202.1	1.69	0.190
Residual	14	1671.0	119.4		
Total	23	3847.5			

TRFK 303/577

Variate: Shoot Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	761.7	380.8	3.19	
Treatments	7	1414.8	202.1	1.69	0.190
Residual	14	1671.0	119.4		
Total	23	3847.5			

AHP S15/10

Variate: Shoot diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.62547	0.31273	7.00	
Treatments	7	1.53664	0.21952	4.91	0.006
Residual	14	0.62578	0.04470		
Total	23	2.78789			

TRFK 31/8

Variate: Shoot diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.89443	0.44721	5.58	
Treatments	7	1.79031	0.25576	3.19	0.031
Residual	14	1.12141	0.08010		
Total	23	3.80615			

TRFK 303/577

Variate: Shoot diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	1.0102	0.5051	3.81	
Treatments	7	2.5135	0.3591	2.71	0.054
Residual	14	1.8581	0.1327		
Total	23	5.3818			

APPENDIX VIII: ANOVA tables results of *Trichoderma* isolates and mycorrhizae on root length and diameter growth enhancement after three months

AHP S15/10
Variate: Root Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1090.65	545.32	8.70	
Treatments	7	1418.83	202.69	3.23	0.029
Residual	14	877.35	62.67		
Total	23	3386.83			

TRFK 31/8
Variate: Root Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	55.02	27.51	0.53	
Treatments	7	1484.00	212.00	4.09	0.012
Residual	14	724.81	51.77		
Total	23	2263.83			

TRFK 303/577
Variate: Root Length

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	172.1	86.1	0.59	
Treatments	7	2804.5	400.6	2.75	0.051
Residual	14	2042.3	145.9		
Total	23	5018.9			

AHP S15/10
Variate: Root diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.36974	0.18487	3.31	
Treatments	7	1.45727	0.20818	3.73	0.017
Residual	14	0.78234	0.05588		
Total	23	2.60935			

TRFK 31/8
Variate: Root diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.05266	0.02633	0.31	
Treatments	7	0.82997	0.11857	1.40	0.278
Residual	14	1.18151	0.08439		
Total	23	2.06414			

TRFK 303/577
Variate: Root diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Reps stratum	2	0.10520	0.05260	0.96	
Treatments	7	1.35853	0.19408	3.53	0.021
Residual	14	0.77043	0.05503		
Total	23	2.23416			

APPENDIX IX: ANOVA tables results of *Trichoderma* isolates and mycorrhizae on dry mass of tea cultivars after three months

Variate: AHP S15/10					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps' stratum	2	0.00120	0.00060	0.02	
Treatment	7	0.22054	0.03151	1.05	0.444
Residual	14	0.42167	0.03012		
Total	23	0.64341			
Variate: TRFK 31/8					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.09701	0.04851	1.08	
Treatment	7	0.18792	0.02685	0.60	0.750
Residual	14	0.63076	0.04505		
Total	23	0.91570			
Variate: TRFK 303/577					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.06726	0.03363	2.02	
Treatment	7	0.08750	0.01250	0.75	0.635
Residual	14	0.23305	0.01665		
Total	23	0.38782			

APPENDIX X: ANOVA tables result of *Trichoderma* isolates and mycorrhizae on shoot length and diameter growth enhancement after one year

Shoot length AHP S15/10					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	2368.4	1184.2	2.65	
Treatment	7	4323.7	617.7	1.38	0.286
Residual	14	6251.0	446.5		
Total	23	12943.1			
TRFK 31/8 Variate: shoot length					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	1994.3	997.2	1.14	
Treatment	7	9838.6	1405.5	1.61	0.212
Residual	14	12208.4	872.0		
Total	23	24041.4			
TRFK 303/577 Variate: shoot length					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	1687.1	843.6	1.26	

Treatment	7	4530.5	647.2	0.97	0.490
Residual	14	9354.6	668.2		
Total	23	15572.2			

AHP S15/10
Variate: shoot diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.07565	0.03782	1.48	
Treatment	7	0.30056	0.04294	1.68	0.192
Residual	14	0.35694	0.02550		
Total	23	0.73315			

TRFK 31/8
Variate: shoot diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.04009	0.02005	0.41	
Treatment	7	0.62773	0.08968	1.84	0.157
Residual	14	0.68213	0.04872		
Total	23	1.34995			

TRFK 303/577
Variate: shoot diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.30481	0.15241	2.24	
Treatment	7	0.38995	0.05571	0.82	0.588
Residual	14	0.95296	0.06807		
Total	23	1.64773			

APPENDIX XI: ANOVA tables result of *Trichoderma* isolates and mycorrhizae on root length and diameter growth enhancement after one year

Variate: root length
AHP S15/10

Source of variation	d.f.	s.s.	m.s.	v.r.	F.pr.
Reps stratum	2	6060.9	3030.4	3.28	
Treatment	7	9472.8	1353.3	1.47	0.257
Residual	14	12925.4	923.2		
Total	23	28459.1			

TRFK 31/8
Variate: Root length

Source of variation	d.f.	s.s.	m.s.	v.r.	F.pr.
Reps stratum	2	900.3	450.1	0.74	
Treatment	7	13412.6	1916.1	3.15	0.032
Residual	14	8525.5	609.0		
Total	23	22838.3			

Variate: root length
TRFK 303/577

Source of variation	d.f.	s.s.	m.s.	v.r.	F.pr.
Reps stratum	2	1864.9	932.4	1.38	
Treatment	7	15685.6	2240.8	3.32	0.027
Residual	14	9447.4	674.8		
Total	23	26997.9			

Variate: root diameter
AHP S15/10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.14528	0.07264	1.28	
Treatment	7	0.64625	0.09232	1.63	0.208
Residual	14	0.79472	0.05677		
Total	23	1.58625			

TRFK 31/8
Variate: root diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.26271	0.13135	1.77	
Treatment	7	0.54267	0.07752	1.04	0.445
Residual	14	1.04007	0.07429		
Total	23	1.84545			

TRFK 303/577
Variate: root diameter

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.54009	0.27005	3.82	
Treatment	7	0.61773	0.08825	1.25	0.341
Residual	14	0.98880	0.07063		
Total	23	2.14662			

APPENDIX XII: ANOVA tables results of *Trichoderma* isolates and mycorrhizae on dry mass of tea cultivars after one year

Variate: AHP S15/10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.01018	0.00509	0.12	
Treatment	7	0.71771	0.10253	2.44	0.074
Residual	14	0.58793	0.04199		
Total	23	1.31582			

Variate: TRFK 31/8

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.04360	0.02180	0.24	
Treatment	7	0.52717	0.07531	0.83	0.580
Residual	14	1.27056	0.09075		
Total	23	1.84133			

Variate: TRFK 303/577

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.15740	0.07870	1.49	
Treatment	7	0.16935	0.02419	0.46	0.849
Residual	14	0.73948	0.05282		
Total	23	1.06623			

APPENDIX XIII: ANOVA tables results on average growth inhibition (%) of *P. theae* by *Trichoderma* spp. in dual culture

Variate: Day 4					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps' stratum	3	1517.5	505.8	4.95	
Treatment	2	812.7	406.4	3.98	0.080
Residual	6	613.2	102.2		
Total	11	2943.5			
Variate: Day 5					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	3	1120.23	373.41	9.43	
Treatment	2	549.81	274.91	6.95	0.027
Residual	6	237.49	39.58		
Total	11	1907.53			
Variate: Day 6					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	563.25	187.75	4.69	
Treatment	2	915.44	457.72	11.43	0.009
Residual	6	240.26	40.04		
Total	11	1718.94			

APPENDIX XIV: ANOVA tables results on average growth inhibition (%) of *P. theae* by volatiles produced by *Trichoderma* species (Inverted plate method)

Variate: Day 4					
Source of variation	d.f.	s.s.	m.s.	v.r.	F.pr.
Reps stratum	3	132.69	44.23	1.95	
Treatment	3	2020.69	673.56	29.71	<.001
Residual	9	204.06	22.67		
Total	15	2357.44			
Variate: Day 5					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	3	93.25	31.08	1.44	
Treatment	3	2712.25	904.08	41.89	<.001
Residual	9	194.25	21.58		
Total	15	2999.75			
Variate: Day 6					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	3	162.69	54.23	2.45	
Treatment	3	3456.19	1152.06	51.96	<.001
Residual	9	199.56	22.17		
Total	15	3818.44			
Variate: Day 7					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	3	25.188	8.396	1.06	
Treatment	3	4512.688	1504.229	189.18	<.001
Residual	9	71.562	7.951		
Total	15	4609.438			

APPENDIX XV: ANOVA tables results of culture filtrates (non-volatiles) of *Trichoderma* spp against vegetative development of *P. theae*

Variate: day 2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	196.020	98.010	10.61	
Treatment	2	19.327	9.663	1.05	0.431
Residual	4	36.953	9.238		
Total	8	252.300			

Variate: day 3

Source of variation	d.f.	s.s.	m.s.	v.r.	F. pr.
Rep stratum	2	246.536	123.268	17.49	
Treatment	2	35.362	17.681	2.51	0.197
Residual	4	28.191	7.048		
Total	8	310.089			

Variate: day 4

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	30.056	15.028	10.40	
Treatment	2	8.682	4.341	3.01	0.160
Residual	4	5.778	1.444		
Total	8	44.516			

Variate: day 5

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	15.0289	7.5144	14.24	
Treatment	2	8.3089	4.1544	7.87	0.041
Residual	4	2.1111	0.5278		
Total	8	25.4489			

Variate: day 6

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	19.549	9.774	1.81	
Treatment	2	31.796	15.898	2.95	0.163
Residual	4	21.551	5.388		
Total	8	72.896			

APPENDIX XVI - SIMILARITY REPORT

DrillBit

The Report is Generated by DrillBit Plagiarism Detection Software

Submission Information

Author Name	Samuel Kipkogei Tanui
Title	EVALUATION OF Trichoderma spp. AND MYCORRHIZA O..
Paper/Submission ID	951997
Submission Date	2023-09-05 13:56:44
Total Pages	99
Document type	Thesis

Result Information

Similarity 7 %

Sources Type

Report Content

Exclude Information

Quotes	Excluded
References/Bibliography	Excluded
Sources: Less than 14 Words Similarity	Not Excluded
Excluded Source	0 %
Excluded Phrases	Not Excluded

A Unique QR Code use to View/Download/Share Pdf File