UNIVERSITY OF EDUCATION, WINNEBA

MORPHOLOGICAL CHARACTERIZATION AND EVALUATION OF BIOLOGICAL CONTROL POTENTIAL OF *TRICHODERMA* SPECIES AGAINST ROOT-KNOT NEMATODES (*Meloidogyne* spp.) ON TOMATO (*Solanum lycopersicum*)

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A THESIS IN THE DEPARTMENT OF CROP AND SOIL SCIENCES EDUCATION, FACULTY OF AGRICULTURE EDUCATION, SUBMITED TO THE SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF PHILOSOPHY (PLANT PATHOLOGY) IN THE UNIVERSITY OF EDUCATIONWINNEBA

MAY, 2023

DECLARATION

STUDENT'S DECLARATION

I, Simon Twum, declare that this thesis, with the exception of quotations and references contained in published works which have all been identified and duly acknowledged, is entirely my own original work, and it has not been submitted, either in part or whole, for another degree elsewhere.

SIGNATURE: …….

DATE: …….

SUPERVISORS' DECLARATION

We hereby declare that the preparation and presentation of this work was supervised in accordance with the guidelines for supervision of thesis/dissertation/project as laid down by the University of Education, Winneba.

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(PRINCIPAL SUPERVISOR)

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DATE:…….

DR. JOSEPH ADOMAKO (CO-SUPERVISOR)

SIGNATURE:……..

DATE:……

DEDICATION

In memory of my lovely mum

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

FIGURE **PAGE**

ABBREVIATIONS

MoFA	Ministry of Food and Agriculture
FAOSTAT	Food and Agriculture Organization Statistics
OECD	Organization for Economic Cooperation and Development
CRI	Crops Research Institute
FAO	Food and Agriculture Organization
USDA	United States Development Agency
ISQAPER	Interactive Soil Quality Assessment for Agricultural Productivity and Environmental Resilience
HAL	Horticulture Australia Limited
CSIR	Council for Scientific and Industrial Research
KNUST	Kwame Nkrumah University of Science and Technology

ABSTRACT

This study was carried out to morphologically characterize and determine the biological control potential of *Trichoderma* species on root-knot nematodes attacking tomato plants. Laboratory and screen house experiments were conducted at CSIR-Crops Research Institute, Fumesua-Ghana. Ten soil samples at a depth of 20 cm were collected from vegetable farms in Western North, Bono and Ashanti regions of Ghana where tomato and other vegetables were grown for *Trichoderma* isolation while heavily galled-roots of lettuce crops were obtained from KNUST vegetable farms for extraction of root-knot nematodes. The tomato seeds were obtained from K. Badu Agrochemicals Limited and CSIR-Crops Research Institute. The *Trichoderma* and root-knot nematode juveniles' isolation were set up in Completely Randomized Design with three replicates while the screen house experiment was carried out in Completely Randomized Design with three replicates and nine treatments. Nine *Trichoderma* isolates were retrieved from the ten soil samples and morphologically characterized into four as *Trichoderma harzianum, Trichoderma virens, Trichoderma viride* and *Trichoderma asperellum*. Fresh root weight, fresh shoot weight, root galls, egg masses, final nematode population, plant height, stem girth and number of leaves of tomato plants differed significantly ($P < 0.05$) between *Trichoderma* treated and the control plants. It was concluded that *Trichoderma viride* (P34-5), *Trichoderma virens* (OKK3) and *Trichoderma harzianum* (TECH10) were the most promising species with biological control potential against root-knot nematodes on tomato plants.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Tomato (*Solanum lycopersicum*) belongs to the family of solanaceae which contains about eighty-five genera and two thousand three hundred species (Mumpi and Sobita, 2018). The cultivated tomato is the world's most highly consumed vegetable due to its status as a basic ingredient in a large variety of raw, cooked or processed foods (FAOSTAT, 2017). Tomato fruits serve as rich source of important antioxidant compounds, vitamins and minerals required in human nutrition (Nkansah *et al*., 2019). They act as a good skin and blood purifier and are helpful in gall stones. They tend to decrease urinary acidity (Anti, 2015). The crop serves as an essential and unavoidable vegetable crop in Ghana making up about 40% of total vegetable expenditure annually (Van Asselt *et al*., 2018).

In Ghana, tomato cultivation has been an important economic activity which is mainly carried out by small holder farmers in all the regions particularly around Akumadan, Agogo, Domfete, Tanoso, Tuobodom, Wenchi, Derma, Techimantia, Keta, Weija and Pwalugu (Diao, 2010; Robinson & Kolavalli, 2010). However, Tomato production is confronted with several constraints including limited availability of quality seeds, limited water supply (Melomey *et al*., 2019), high cost of fertilizers (Bortey, 2016), expensive labour operations (Robinson & Kolavalli, 2010) and diseases and pests (Aduhene-Chinbuah, 2018). This has led to a production and consumption gap resulting in traders turning to neighboring markets, especially Burkina Faso, which supplies the Ghanaian market with tomato particularly during the first half of the year (Robinson & Kolavalli, 2010; Gonzalez *et al*., 2016).

The most important among these constraints is the vulnerability of tomato crop to various diseases including plant parasitic nematodes (Lutuf *et al*., 2018). Plant parasitic nematodes are of particular concern because few farmers are aware of them although they cause severe and devastating damage to tomato crop. In most tropical regions, they are considered among the most destructive biotic constraints to vegetables production (Coyne *et al.*, 2018). Common plant parasitic nematode species occurring in tomato fields in Ghana include *Pratylenchus* spp., *Rotylenchulus reniformis, Tylenchus* spp., *Helicotylenchus multinctus* and *Meloidogyne* species (Osei *et al*., 2012; Lutuf *et al*., 2018).

Among these plant parasitic nematodes, it is *Meloidogyne* species which cause serious economic damage to tomato crops (Lutuf *et al*., 2018) and four *Meloidogyne* species (*M. incognita, M. javanica, M. arenaria* and *M. hapla*) are the most important nematode pests in tomato fields (Brandon, 2018). However, *M. incognita* was the most abundant root-knot nematodes associated with tomato plant in Ghana (Osei *et al*., 2012; Lutuf *et al*., 2018).

1.2 Statement of the Problem

Root-knot nematodes cause root dysfunction (Noling, 2019), interfering with the translocation of water and nutrients in crops (Adomako *et al*., 2017) and causing stunted crop growth, root galling and reduced fruits (Coyne *et al*., 2014). Root-knot nematodes interact with other plant pathogens to form a disease complex in which the resulting disease is much more severe (Seebold, 2014) resulting in significant economic yield loses by impacting the quantity and quality of marketable yields. Yield losses of over 70% have been recorded in farmers' fields (Nyaku *et al*., 2018) and global losses associated with root-knot nematodes is

estimated at $157 billion annually (Singh *et al*., 2015) and an estimated amount of $500 million is spent on root-knot nematodes control globally (Jaiteh *et al*., 2012).

In view of the devastating threat posed by root-knot nematodes on tomato production, management is of prime importance because; most of the tomato cultivars grown by farmers in Ghana are susceptible to this nematode. However, available management strategies to curtail root-knot nematode populations within economic thresholds are dominated by chemical nematicide use which is closely associated with environmental pollution, high cost implications, health concerns due to toxicity (Prajapati, 2020), resistance in target nematode species (Seebold, 2014), threat to groundwater due to leaching and long withdrawal periods when applied to crops (Jaiteh *et al*., 2012). The forgoing reasons have led to the withdrawal of most chemical nematicides from global and local markets making chemical strategy unattractive (Adomako *et al*., 2017), hence biocontrol.

1.3 Justification of the Study

Biological control of root knot nematodes is more natural, economical, sustainable and environmentally friendly (Mumpi and Sobita, 2018). The most widely used biocontrol agent is *Trichoderma* species which covers 90% of all antagonistic fungi used in plant protection (Akhtar *et al*., 2012). *Trichoderma* is non-toxic to human, compatible with other control methods and effective at low concentrations (Olabiyi, 2014) and also easy to isolate and culture (Uddin *et al*, 2018).

Incredible results of control have been observed with strains of *Trichoderma virens* against *Pythium ultimum* in cotton (Benitez *et al*., 2004) while *Trichoderma harzianum* reduced the number of galls, egg masses and final nematode population of *M. incognita* on okra (Lal and Rana, 2013; Kurulkar *et al*., 2019). Similarly, *Trichoderma* sp. was found to antagonize root-knot nematodes infecting soya bean (Izuogu *et al*., 2019) and pineapple (Kiriga *et al*., 2018)

In Ghana, limited studies have exploited the use of *Trichoderma* sp.as a biocontrol agent against root-knot nematodes. However, no work has reported on variations in efficacy of different isolates of *Trichoderma* sp. on root-knot nematodes attacking tomato and its effect on tomato growth.

1.4 Research Objectives

1.4.1 General objective

The General objective was to:

Characterise *Trichoderma* isolates from vegetable fields and evaluate their nematicidal potential against root-knot nematodes attacking tomato

1.4.2 Specific objectives

The Specific objectives were to:

- ✓ isolate and morphologically characterize *Trichoderma* isolates from rhizosphere soil samples of some vegetables.
- ✓ evaluate the effect of different *Trichoderma* isolates on reproduction and damage potential of root-knot nematodes on tomato crop.
- ✓ determine the effect of *Trichoderma* isolates on the growth of tomato crop.

1.5 Hypothesis

Trichoderma spp. can inhibit the growth and reproduction of root-knot nematodes on tomato thereby improving plant health and growth.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin of Tomato

The cultivated tomato is related to the wild tomatoes originating from Peru, Ecuador and other parts of South America including the Galapagos Islands (OECD, 2017). However, the centre of its domestication and diversification is unclear. Two hypotheses have however, been expressed for the original site of tomato domestication and diversification; one hypothesis puts Peru and the other Mexico. It is however, presumed that Mexico is probably the site of domestication and Peru as the centre of diversification. In Africa, the tomato plant was first introduced in West Africa, Eastern Africa and Central Africa during the 16th and 17th centuries by the Portuguese (Melomey *et al*., 2019). Tomato is grown worldwide with China and India being the major producing countries while commercial and intense cultivation is actively done in West African by Burkina Faso, Nigeria and Ghana (Aduhene-Chinbuah, 2018).

2.2 Classification and Description of Tomato Crop

The crop belongs to the genus *Solanum* within the family Solanaceae. Its classification has been the subject of much discussion and diversity of the genus has led to reassessment of the earlier taxonomic treatments. For a long time, tomatoes were known as *Lycopersicum esculentum*, but recently referred to as *Solanum lycopersicum* (OECD, 2017).

The growth habit of tomato plant varies from determinate to indeterminate. The indeterminate plants grow very high reaching 3m in height and mostly need staking but the

determinate plants stop growing at 1.5m when the flowers form at the terminal growing point (Aduhene-Chinbuah, 2018). Primary roots of tomato may grow several metres in length. The stem is fragile and angular covered by hairy and glandular trichomes that confer a characteristic smell. Tomato leaves are alternately arranged on the stem with 137.5^{o} phyllotaxy and range in shape from lobed to compound, with segments arranged pinnately (OECD, 2017). Tomato plant is characterized by its yellow flowers which occur in either a simple or complex inflorescence of about six to twelve bisexual flowers with its fruit being globular or ovoid containing fifty to two hundred seeds located inside the locular cavities enclosed in gelatinous membrane. Fruit colour of tomatoes changes from green to red, orange or yellow depending on the variety (Aduhene-Chinbuah, 2018).

2.3 Nutritional and Economic Benefits of Tomato

Tomato is one of the very important vegetable crops and its fruit forms an essential portion of the diet of numerous people in the world because of nutritional potential to human health (Aduhene-Chinbuah, 2018). Tomato fruits are rich source of important antioxidant compounds, vitamins and minerals needed in human nutrition (Nkansah *et al*., 2019). The fruit contains large quantity of water, low amounts of protein and fats as well as some carbohydrates needed by the body (OECD, 2017). It contains essential compounds such as lycopene and flavonoids that play useful role in the prevention of cancer, heart diseases, cataracts and many other common health problems (Romika, 2019). Tomatoes act as a good skin and blood purifier and are helpful in gall stones (Anti, 2015).

The crop is considered as one of the most important vegetables produced in commercial and traditional agriculture worldwide (OECD, 2017). In Ghana, there is high demand for fresh tomatoes all year round (Amoako-Adusei, 2021) as Ghana consumes approximately 440,000 tons of tomato annually (Van Asselt *et al*., 2018) hence tomato cultivation has been an important economic activity regarded as a highly profitable business and a potential area to improve the livelihood of smallholder farmers through better income earning (Arah, 2015). Commercial production is intense in the Upper East, Brong Ahafo, Northern and Greater Accra regions of Ghana which supply the market at various times of the year (Agyekum, 2015). The production from both the commercial and smallholder farms generates employment catering for over 90,000 farmers and more than 300,000 individuals in retail and wholesale areas of the subsector. Twenty five people are involved in getting tomato from farm to plate (Robinson and Kolavali, 2010).

Ghana produces about 420,000 tons of tomatoes (FAO, 2019; MoFA, 2020) and 90% of the production is consumed locally reducing the volumes of fresh tomato imports (Agyekum, 2015). Despite the tomato supply deficit, Ghana accrued $ 31, 000 from the export of fresh and chilled tomatoes to countries such as United States of America, Niger, United Kingdom, South Africa, Belgium, Japan, United Arab Emirates and Canada in 2019 (UN Comtrade, 2021).

2.4 Tomato Production in Ghana

Tomato production occurs in almost every region mostly by small holder farmers though commercial production under irrigation occurs. The major producing regions include Upper

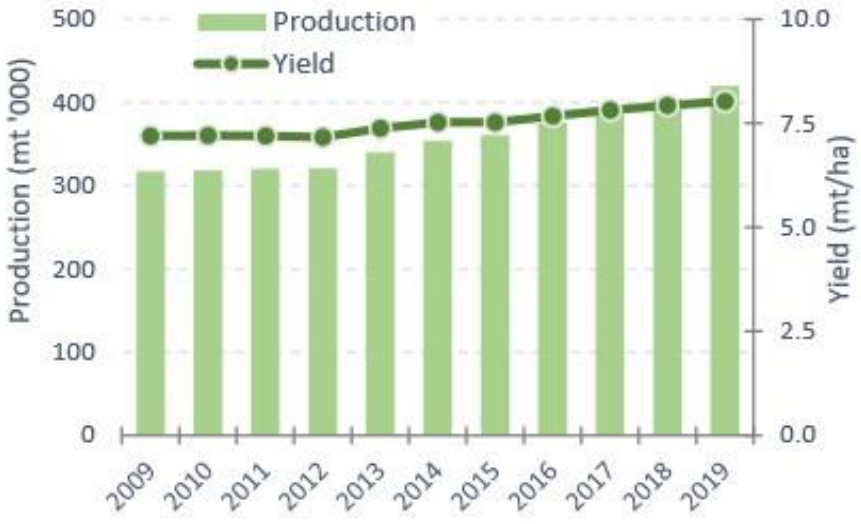
East, Northern, Brong Ahafo, Ashanti, Eastern, Greater Accra and Volta (Melomey *et al*., 2019). Tomato production in the Brong Ahafo region is centred around Domfete, Tanoso, Tuobodom, Wenchi, Derma and Techimantia while Akumadan and Agogo dominate in the Ashanti region (Robinson and Kolavalli, 2010). However, tomato production in these areas is generally rain-fed and production occurs in May to November which supplies the market with fresh tomatoes (Melomey *et al*., 2019; MoFA, 2020). Irrigated production is carried out in the dry season in the savannah zones, particularly the Upper East, Volta and the Greater Accra regions. The key production centres in these regions include Pwalugu, Vea (Upper East Region), Keta (Volta Region) and Ashiaman, Tema and Weija (Greater Accra Region). Fresh tomatoes from these centers enter the market from December to April (Van Assalt *et al*., 2018).

High yielding and quality tomato varieties grown by Ghanaian farmers in these regions are mostly imported varieties and farmers selected varieties developed from the original varieties introduced by the Portuguese (Melomey *et al*., 2019). These varieties include: Roma, Pectomech, Tropimech, Rio Grande, Jaguar, Titao, Derma, Ada Cocoa, cocoaba (MoFA, 2020), Laurano, Raki, Choco TP, Rasta (Agyekum, 2015), wosowoso (Kpodo, 2018), Petofake, Akumadan, Padma, Cobra, Power ntiawa, Konkon and BS 47 (Melomey *et al*., 2022).

These varieties grow well on most mineral soils, but prefer a deep well-drained sandy loam which is rich in organic matter. Tomato is moderately tolerant to a wide range of soil pH with optimum yield in soils with pH of 5.9 – 6.8. Tomato requires warm climate for growth and

optimum day and night temperatures of 26ºC and 12ºC respectively are ideal for crop growth (OECD, 2017). Medium rainfall is required which should be well distributed throughout the growing season at least two rains per week. An annual rainfall of 750mm evenly distributed is ideal for optimum growth (Mensah *et al*., 2013).

The total land area cultivated by these major producing regions was 47,932 hectares and the combined production volume which fed major market centres like Agbogbloshie, Makola, Techiman and Abinkyi markets stood at 420, 000 tons in 2019. This production volume has consistently increased annually at 2.7% from 318,000 tons in 2009 to the 420,000 tons recorded in 2019 (FAO, 2019; MoFA, 2020) as detailed in (Figure 2.1).



Source: FAO (2019); MoFA (2020)

Figure 2.1: Production Trends of Tomato in Ghana (2009 - 2019)

2.5 Constraints to Tomato Production

Despite the consistent annual increases, domestic production has not matched up to the ever-growing demand for fresh tomatoes hence the market is supplemented with imports from Burkina Faso especially during the first quarter of the year culminating into an import value of $ 9 million (Gonzalez *et al*., 2016; Van Asselt *et al*., 2018). The deficit resulting in the importation has been attributed to several production constraints as below:

Limited availability of quality seeds: Most tomato famers in Ghana obtain their seeds from their personal stored seeds, local markets, family and friends. However, the quality of these seeds is questionable as most of them fail to germinate or fruit poorly at maturity (Aduhene-Chinbuah, 2018). Few farmers purchase seeds of imported varieties with superior qualities from certified seed companies but at exorbitant prices (Melomey *et al*., 2019).

Limited water supply causes tomato production in Ghana to be seasonal due to the differences in the rainfall patterns causing water unavailability for all year-round planting. Apart from Upper East and Greater Accra regions where tomato production occurs during the dry season under furrow irrigation system, tomato production is generally rain fed (Melomey *et al*., 2019) hence tomato production volume is very low during the months of January to May where demand is also high leading to the importation of tomato from Burkina Faso (Van Asselt, 2018).

Fertilizers that are needed to augment the yield of tomatoes due to monoculture system of tomato production are expensive hence only few farmers are well resourced to buy the fertilizers (Bortey, 2016). In response to this, the government of Ghana, Non-Governmental

Organizations and other private sector institutions rolled out interventions to increase the productivity of farmers through fertilizer subsidy. The fertilizer subsidy program executed by the Government of Ghana, saw an increase in tomato production following its implementation however, this was not sustained (Fearon *et al*., 2015).

Labour operations such as land preparation, staking, weeding, beds preparation, pruning and harvesting are very expensive to operate in tomato production. Due to this, wholesalers are known to sponsor producers with loans and other inputs hence influencing their production practices and prices of tomatoes (Robinson and Kolavali, 2010). The high cost leaves the farmers with no alternative than reduced land area used for cultivation which affects production volumes (Arah, 2015).

Diseases and Pests: Tomato production in Ghana is closely associated with diseases and pests hindering production. Over thirty diseases caused by soil borne pathogens have been reported to infect tomato crops in Ghana (Aduhene-Chinbuah, 2018). The most common tomato diseases of economic importance are root-knot caused by nematodes, early blight, late blight, damping off and fusarium wilt caused by fungi. Bacteria diseases include bacteria canker, bacteria spot, bacteria wilt and bacteria speck while Tomato Yellow Leaf Curl Disease is caused by virus (Jaiteh *et al.*, 2012; OECD, 2017). Major pests in tomato production are aphids, grasshoppers, whiteflies, crickets, leaf miners, beetles, mites, thrips, fruit worms, beet army worms and caterpillars (Melomey *et al*., 2022).

These diseases and pests cause huge losses in tomato production (Perez *et al*., 2017). Tomato production halted in the Upper East region in 2002 due to Tomato Yellow Leaf Curl Disease and a complex of fungal pathogens. According to Melomey *et al*., (2019) more than 600

tomato farmers in the Agotime-Ziope District of the Volta region lost approximately one thousand hectares of tomato farms to various diseases. Also, early blight and late blight disease of tomato was found to be more pronounced in tomato farms in Techiman North where severe losses due to the diseases have been recorded (Amoako-Adusei, 2021). Yield losses of up to 100% have been reported. This has jeopardized the livelihood of millions of growers and other beneficiaries along the tomato value chain (Ramathani *et al*., 2021).

Notwithstanding these, root-knot nematodes have been described as malignant soil borne curse to vegetables which cause severe devastating damage to tomato crops and perhaps form the greatest biotic threat to productivity especially in the tropics. They are of particular concern because few farmers are aware of them and the associated damage they cause. The occurrence of root-knot nematodes in soil increases due to the repeated cropping of tomato or crops with similar genetic background on the same piece of land. This has resulted in the build-up of these nematodes in the soil. Besides repeated cropping, the versatile avenues of dissemination of root-knot nematodes have been found to cause their presence in newly cleared but previously uncultivated lands (Coyne *et al*., 2018). This evidence makes root-knot nematodes readily available in the soil to come into contact with plant roots. As the nematodes come into contact with plant roots, they establish permanent feeding sites within or outside the root tissues of tomato crops causing root necrosis and death, damage to the root system, resulting in the inability of the roots to support the plant.

Root-knot nematodes also provide ready avenues in plant roots for secondary pathogens (Patil and Yadav, 2021) and interact with other plant pathogens to form a disease complex in which the resulting disease is much more severe (Seebold, 2014). Nematode infection undermines the plant's resistance to other pests and diseases as nematode-infected roots deteriorate, plant health is affected resulting in greater pests and disease pressure. Adomako

et al. (2017) re-echoed the breakdown of resistance to *Fusarium oxysporum lycopersici* in the presence of *Meloidogyne incognita* which favoured the growth of bacteria and fungi in an earlier report. This interaction often leads to complete failure of the crop due to altered host physiology (Patil and Yadav, 2021).

These distortions in tomato crops by root-knot nematodes have caused severe reduction in tomato yield ranging from 28% to 68% in Zaria, Nigeria (Hassan *et al*., 2010). In Ghana, tomato yield losses of between 73% and 100% due to nematodes were reported in 1981 by Hemeng (Lutuf *et al*., 2018) and in recent times, yield losses of 70% to 100% have been recorded in tomato fields (Jaiteh *et al*., 2012; Nyaku *et al*., 2018). Root-knot nematodes increase the cost of production through increased fertilizer application and control programmes (Onkendi, 2014).

2.6 Description of Root-knot Nematodes

Root-knot nematodes belong to the genus *Meloidogyne* which contains approximately 100 described species with 22 reported to be present in Africa (Onkendi *et al*., 2014). Out of these, four species including *M. incognita, M. javanica, M. arenaria* and *M. hapla* are the most prevalent cause of serious economic damage to crops (Adomako *et al.*, 2017). The first three species highly dominate in the tropics and sub-tropics (HAL, 2014) while *Meloidogyne hapla* is more prevalent in the temperate regions (Naz *et al*., 2012). In Ghana, there have been reports and molecular confirmation of the incidence of *M. incognita, M. javanica, M. arenaria* on several crops and weeds (Osei *et al*., 2013; Onkendi *et al*., 2014; Danso and Kwoseh, 2016 and Adomako *et al*, 2016).

Root-knot nematodes are soil-dwelling microscopic, obligate sedentary endoparasites which can easily reproduce in roots of over 3,000 plant species including vegetables, fruits, field crops, ornamentals and common weeds (Seebold, 2014; Forghani and Hajihassani, 2020).

Root-knot nematodes life cycle consists of eggs captured in translucent egg case where the first stage juvenile develops and molts within the eggs before hatching into second (J2) stage juveniles (Noling, 2019). The second (J2) stage juveniles migrate either into the soil or to a different location in the root. The larva penetrates a suitable root by repeatedly thrusting its stylet into cells at the root surface to establish a permanent feeding site within the root. The roots attract the second stage juveniles due to chemicals released from host roots and meristematic zone is the most preferred site for penetration (Patil and Yadav, 2021). The second (J2) stage juveniles interfere with translocation of water and nutrients within the host plant as they begin feeding. They release enzymes into cells at the feeding site which cause an increase in cell size and number (Seebold, 2014). The second (J2) stage juveniles develop into third (J3) and fourth (J4)-stage juveniles and adult stage under suitable environmental conditions and adequate food supply, most of the adults develop into female and remain sedentary and begin to lay eggs (Adomako *et al*., 2017). The eggs hatch under suitable environmental conditions and new larvae emerge to complete the life cycle. The male reverts to the worm-shape and migrate from the roots.

The second stage juveniles are vermiform and usually measures not more than 500 µm in length and 15 µm in width. The females have globose body with a short “neck” containing their stylet, metacarpus and oesophageal gland cells and are *in situ* at maturity (Mitkowski

and Abawi, 2011). They range in length from 400 to 1000 µm. The adult females lay eggs into a gelatinous matrix attached to the posterior end (Escobar *et al*., 2015) and can produce more than 2000 eggs (Noling, 2019). The males are also vermiform and range from 1100 µm to 2000 µm in length with distinct lips and well-developed stylets. They also have visible spicules for mating as well as a blunt rounded tail. The males are unnecessary in most species since viable eggs can be produced by the female without fertilization. They are sometimes encountered when nematode population is subjected to environmental stress (Mitkowski and Abawi, 2011). Rook-knot nematodes can go through multiple generations per growing season (Seebold, 2014). One generation, from egg to egg, has been confirmed to have been completed within twenty five (25) to thirty (30) days under optimum temperature of 15 ^{o}C to 30 ^{o}C and adequate soil moisture (Patil and Yadav, 2021).

2.7 Feeding Habit and Symptoms Induced by Root-knot Nematodes

The second stage juvenile of root-knot nematodes penetrates a suitable root by using its stylet to establish a permanent feeding site within the root (Seebold, 2014). The feeding site consist of a group of cells referred to as giant cells which have been fortified by secretory proteins released by the nematodes which stimulate changes within the parasitzed cells (Escobar *et al*., 2015). The parasitized cells rapidly become multinucleate which facilitate colossal amount of protein production that are ingested by the nematodes. The giant cells also serve as nutrient sinks, passing plant nutrients to the feeding nematode through a tube secreted from the stylet into the plant cell cytoplasm which serves as a sieve to filter the cytosol ingested by the nematode. The giant cells can grow very large in size and neighboring root cells also enlarge and divide rapidly possibly due to plant growth regulator diffusion resulting in gall

formation (Mitkowski and Abawi, 2011). The galls formed on crop roots induce stunted crop growth, patchy growth, leaf chlorosis, thin or sparse foliage, wilting or leaf rolling, reduced fruit and seed size and little or no new foliage development at the onset of a new growing season as above-ground symptoms (Coyne *et al*., 2014). The galls formed may be small or large, terminal or sub-terminal and is the most obvious characteristic below ground symptom of root-knot nematode infection (Patil and Yadav, 2021).

2.8 Economic Importance of Root-knot Nematodes

Meloidogyne species are the most yield-limiting group among the plant parasitic nematodes (Forghani and Hajihassan, 2020) and ranked first among top ten plant parasitic nematodes (Jones *et al*., 2013). They pose a significant treat to crop production in agricultural crops. Huge damage is caused in economically important crop plants such as sweet potato, banana, tomato, cabbage, potato, pineapple, cassava, maize, tobacco, cowpea, okra, papaya, buchu and African spinach (Okendi, 2014). High population build-up of *Meloidogyne* species can cause high levels of crop loss. Globally, losses associated with root-knot nematodes are estimated at $157 billion annually (Singh *et al*., 2015) and an estimated amount of $500 million was spent on root-knot nematodes control globally (Jaiteh *et al*., 2012).

2.9 Management of Root-Knot Nematodes

The huge yield losses coupled with the associated high cost of tomato production imposed by root-knot nematodes as well as the urgent need to ensure global food security due to the rapid human population growth have made root-knot nematodes management critical. The practice of totally eradicating root-knot nematodes under field conditions is impossible therefore

management options are generally designed to reduce nematode population levels below damaging thresholds. Options which aim at protecting transplants and mature plants as have been reported by Adomako *et al*. (2017) include cultural, chemical and use of resistant varieties of cultivated crops.

2.9.1 Cultural management strategy

The use of cultural control strategy to manage root-knot nematodes is the most economical, environmentally sustainable and potentially most successful strategy for reducing root-knot nematodes damage. Options such as inter-cropping with antagonistic plants such as the African marigold and Sudan grass have been used to reduce soil population of many soil nematodes including root-knot nematodes due to production of chemicals that are toxic to nematodes (Mitkowski and Abawi, 2011). Crop residues are also incorporated into the soil as organic manure to improve soil structure and enhance soil biological activities (Nagaraju *et. al*., 2010). Though major effects of soil amendments have been found to enhance plant nutrients and water availability rather than significantly reducing root-knot nematode levels in the treated soils.

Crop rotation with non-host crops such as corn can be planted in fields infected with *Meloidogyne hapla* to harm their survival (Mitkowski and Abavi, 2011) though It has also been found that, short crop rotation period or halted crop rotation plan intensified root-knot nematode problem (Noling, 2019) and the difficulty in selecting a rotational crop with acceptable returns and similar labour requirements hinder crop rotation.

Fallowing vegetable fields infected with root-knot nematodes enhance natural reduction in nematode population due to non-existence of susceptible host. However, the limited land

space available for crop production and the increased demand for vegetables make fallowing an undesirable option (Adomako *et al*., 2017; Coyne *et al*., 2018).

2.9.2 Chemical management strategy

Chemical control involves the application of inorganic formulations to interfere with activities of *Meloidogyne* spp. in infested soils. Nematicides are usually the most effective and quick method of managing high levels of *Meloidogyne* spp. under field conditions. Noling (2019) observed decreased root-knot nematodes population and increased vegetable crop yield after nematicide application.

These nematicides when properly used reduce the nematode population drastically, but only temporarily; nematode populations returned to damaging levels within a season in sites replanted to a susceptible crop (Seebold, 2014). Also, due to health, environmental pollution and cost concerns as a result of high doses, nematicidal use is not a desirable choice (Nyaku *et al*., 2018).

Consistent application of synthetic nematicidal pesticides leads to increased resistance in target nematode species (Hernandez-Rosas *et al*., 2020). In addition, chemical control requires application of large amounts of chemicals with specialized equipment which makes it expensive. At the same time, some fumigants volatilize very quickly, so treated soil must have a cover to retain the fumigants in the soil long enough to be effective. Furthermore, broad-spectrum fumigants which are nervous system toxins such as Oxamyl and Fenamiphos which target root-knot nematodes nerves also affect the nervous system of human beings

causing complicated problems (Mitkowski and Abawi, 2011). Chemical nematicides are also threat to groundwater due to leaching as well as having long withdrawal periods when applied to crops which delay harvesting and marketing (Jaiteh *et al*., 2012). These negative effects have led to their withdrawal from global and local market making them readily unavailable and expensive to small scale vegetable farmers (Adomako *et. al*., 2017).

2.9.3 Use of resistant crop varieties

The use of nematode-resistant tomato crop variety is the most effective way of controlling root-knot nematodes (Noling, 2019). Resistant genotypes also protect the environment against pollution from chemical residues associated with synthetic nematicides (Adomako *et al*., 2017). Tomato varieties conferring resistance to *M. incognita* have been developed. In tomato, genetic resistance to root-knot nematodes is conferred by the *Mi* gene obtained from a wild relative of the common tomato; many other genes have also been identified that are effective against root-knot nematodes. These include the *Mi2* through *Mi8* genes from tomato and the *Me* and *N* genes from pepper (Mitkowski and Abawi, 2011). However, resistance in tomato only affects *M. incognita* hence exposing the roots to *M. hapla* and *M. javanica* infection. Also, resistance in tomato varieties has often failed as a result of heat instability of the resistant *Mi* gene. Additionally, the continuous planting of resistant tomato varieties has led to the selection for virulent races of *Meloidogyne* which has overcome resistance making it ineffective (Seebold, 2014; Noling, 2019).

2.10 Historical Application of Biological Control Agents

Biological control started with the use of macro organisms such as insects as the pest's natural enemy, taken from its original habitat and introduced into the established environment of the pests to control it (Hajek and Eilenberg, 2018). In 200 AD, the Chinese were the first to use natural enemies to control insect pests using *Oecophyllas maragdina* (ant) on *Tesseratoma papillosa* (Lepidoptera) in citrus and later in date palm in 1200 AD in Yemen (Barrat *et al*., 2017). In 1726, Reaumur recognized and used Cordyceps fungus on noctuid as the first insect pathogen.

The search for Biological Control Agents grew as in the 1840s; predators were released to control gypsy moth and garden pests in Italy. Meanwhile, in 1868, the cottony cushion scale project fueled the introduction of parasitic fly, *Cryptochaetum iceryae* and the Vedalia bettle, *Rodolia cardinalis* into California from Australia to control cottony cushion scale, *Icerya purchasi* Maskell (Barratt, 2017; Hajek and Eilenberg, 2018).

In the 1900s, biological control became prominent in New England and foreign exploration for biological control agents started with the arranged importation of parasites to the United States through the Gypsy Moth Project in 1905 to 1911 while the Lantana Weed Project in Hawaii in 1902 became the first published work on biological control on weeds and USDA laboratory for biological control was established in France in 1919 to advance the development of biological control.

In the late 1960s, the concept of Integrated Pest Management which had biological control as a core component was implemented due to the growing need to conserve the ecology and the environment making way for the commercialization of Integrated Pest Management scouting in the 1970s resulting in the extensive use of natural enemies to suppress pests in crops such as cotton, alfalfa, citrus, soybeans and other crops (Baker *et al*., 2020).

The interest in biological control grew as scientists around the world published two journals on biological control in the 1990s. The published journals served as a major reference source for the biological control community.

The research into biological control continued in the twentieth century and advancement in biotechnology and molecular biology necessitated the need to introduce microorganism such as fungi, bacteria and viruses, semio-chemicals like pheromones and natural substances of mineral of plant or animal source as biological control agents (Hulot and Hiller, 2021). Important research areas on the use of organisms recorded in scientific literature include characterization of useful species (Santos *et al*., 2016), genome analysis, insect population resistance and bio-regulatory agents (Hernandez-Rosas, 2020), determination of insecticidal effects (Sevim *et al*., 2012) and genetic manipulation and evaluation (Sansinenea *et al*, 2010).

The microorganisms targeted for use as biological control agents that have received intense research based on the areas of interest above usually belong to fungi and bacteria due to the ease with which they can be cultured (Ghazanfar *et. al*., 2018) and to date, bacteria and fungi

remain the most prominent antagonists for plant parasitic nematodes biocontrol (Xiang *et al*., 2018). Commonly focused fungi for biological control research include *Ampelomyces, Coniothyrium, Dactylella, Gliocladium, Paecilomyces* and *Trichoderma.*

It is estimated that 90% of all antagonistic fungi used in plant protection belong to the genus *Trichoderma* (Akhtar *et al*., 2012) as it is the widely studied and used component for biological control (Hulot and Hiller, 2021) and has been used commercially for several years for the control of soil, leaf or vascular pathogens (Hernandez-Rosas *et al*., 2020). The bacteria include *Bacillus, Burkholderia, Lysobacter, Pantoea, Pseudomonas* and *Streptomyces* (Pal and Gardner, 2011).

2.11 Mode of Action of Biological Control Agents

Biological control agents interact with plant and pathogens that suppress the pathogen growth through direct and indirect antagonisms (Ghazanfar *et. al*., 2018). Direct antagonism results from physical contact or a high degree of selectivity for the pathogen. In such a situation, hyper-parasitism which involves coiling around the host, formation of appressoria and breakdown of the host cell wall are employed by the biological control agent to suppress the pathogen (Shah and Afiya, 2019).

In contrast, indirect antagonism results from activities that do not involve sensing or targeting a pathogen by biological control agent (Pal and Gardener, 2011) but rather stimulation of host defense through the production of antibiotics, cell-wall degrading enzymes like lytic enzymes (Kohl *et al*., 2019). According to Kannangara *et al*. (2017), non-regulatory waste

products that may have toxic or inhibitory effects on the invading pathogen by the biological control agent are the most indirect form of antagonism (Shah and Afiya, 2019). However, other important indirect mechanism involves competition of the biological control agents with the pathogen for nutrients or infection sites. In such a situation, the biological control agents survive and takes over the more harmful competitor, the pathogen by depriving the pathogen of nutrients and water (Stenberg *et al*., 2021).

2.12 Merits of Biological Control

Biological control has been found to conserve biodiversity of soil fauna and flora as non-targeted insects and other organisms prevail after application unlike the killing and reduction of non-targeted organisms resulting from chemical pesticide application (Herz, 2020; Baker *et al*., 2020; Niggli *et al*., 2020).

The sustenance of biodiversity in agriculture facilitated flower strips to improve the effectiveness of natural enemies (Lambion and Van Rijn, 2021) and as an integral part of organic farming, biodiversity was enhanced 30% more than conventionally farmed land (European Commission, 2021).

Biological control has proved record of environmental protection and enhancement as it provides an alternative for chemical pesticides usage while leaving no toxic residues in the environment (Herz, 2020; Prajapati, 2020). It was specifically suitable for fruit, vines and potatoes where massive use of fungicides and individual broad-acting insecticides posed threat to the environment and biodiversity (Haller *et al*., 2019).

Biological control enjoins positive interplay among biodiversity, soil quality and health. Living organisms in the soil create a vital ecosystem which sieve potential pollutants and sustain healthy plant growth (Hulot and Hiller, 2021). Total pesticides contamination in conventional European Union soils affecting soil quality was found to be between 70% and 90% higher than in organic soils involving biological control (ISQAPER, 2021) confirming the decrease levels of chemical contamination (Geissen *et al*., 2021).

Biological control agents have the capacity to influence soil microflora and biofertilizers as well as volatilization and sequestration of certain inorganic nutrients to enhance soil quality (Prajapati *et al*., 2020; Bajsa *et al.,* 2020).

The climax of biological control is the positive impact on human health due to the absence of environmental pollution and residues in soil and crop plants harvested for use as food (Baker *et al.*, 2020). Farmers do not handle toxic synthetic pesticides and application on plants when biological control is an alternative (Hulot and Hiller, 2021). Neurodegenerative diseases such as Parkinson's disease, cognitive deficits and Hodgkin lymphoma (cancer) have been identified in laboratory animals and farm workers due to chronic exposure to high doses of pesticides but not in farmers associated with biological control (Latifovic *et al*., 2020).

Though literature on the cost and influence on farm income is limited, Fowler *et al*. (2016) found in a study conducted on ex-post economic analysis where benefit-cost ratio of 14.1 to 1.0 was arrived at when biological control and no biological control situations were compared.

Biological control has largely been endorsed by scientists to reduce greenhouse gases emissions as it requires less energy and fossil fuels at the production stage or on-farm application contributing to climate change mitigation compared to chemical pesticides which contribute 5-6% of greenhouse gases emissions worldwide during production and application stages (Ritchie, 2020).

2.13 Demerits of Biological Control

The increasing specialization of biological science, industry and practice have resulted in regulatory and political hurdles which hinder biological control practice especially in the United States of America (DiTomaso *et al*., 2017). There are exaggerated claims of adverse non-target impacts and importation of exotic species to new areas (FAO, 2017) hence the need for regulations requiring risk assessments for biological control proposals (Barratt *et al*., 2017).

Biological control involving the use of microorganisms is limited by properties such as specificity which reduces the ability of biological control agents to control only one pest or pathogen though multiple pests or pathogens may be present on a crop therefore biological control is deemed to provide partial control of the pests or pathogens while biological control has been found to provide variable efficacy due to different climatic conditions under which Biological Control Agents are applied (Hernandez-Rosas *et al*., 2020).

2.14 *Trichoderma* as Biological Control Agent

Trichoderma was first reported as a biological control agent in the early 1930s (Ghazanfar *et al*., 2018). It is a diverse group of free-living fungi in the family *Hypocreaceae*, commonly present in all agricultural soils (Shah and Afiya, 2019). *Trichoderma* species produce three types of propagules: hyphae, chlamydospores and conidia (Yang *et al*., 2011). Conidia are normally focused due to their higher production (Panahian *et al*., 2012).

Trichoderma species are successful antagonists having biocontrol abilities against economically important plant parasitic soil-borne pathogens (Kushwaha and Verma, 2014). Several mechanisms have been described as responsible for their biological control activity. Mycroparasitism involving parasitizing, detecting, growing and colonizing the pathogen is largely recognized as the direct mechanism (Harman, 2011). Detection and colonization of plant pathogen is done through chemotropism, lysis of the pathogen's cell wall, aspersoria formation, and production of cell wall degrading enzymes and peptaibols as well as parasitizing the pathogens cell wall content (Nusaibah and Musa, 2019). *Trichoderma* interacts with root-knot nematodes through mycoparasitism by trapping and killing them at different life stages in the soil or root system (Forghani and Hajihassani, 2020).

Trichoderma employ competition for space and nutrient resources such as sugar to suppress pathogen growth (Montoya-Gonzalez *et al*., 2016). *Trichoderma* has a strong ability to mobilize and use soil nutrients, making it more efficient and competitive than many other soil microbes by producing secondary metabolites capable of inhibiting the growth and other activities of the pathogen.

Trichoderma produces and secretes siderophores which help it to grow in conditions that are poor in iron by signaling residual immobilized iron. Siderophores suppress the growth of plant pathogens by depriving it of iron and also solubilize iron to make it available to the plant for growth. *Tricoderma harzianum* produces the highest amount of siderophores among the species while *Tricoderma reesei* can bio-synthesize cis-fusarinine, a major siderophore (Nusaibah and Musa, 2019).

Production and secretion of nematicidal secondary metabolites like peptaibols, terpenes, polyketides, gliotoxin, gliovirin, tricholin, harzianic acid, viridian, gliosoprnins, heptelidic acid, 6-pentyl-α-pyrone and massoilactone (Mukherjee *et al*., 2013) some of which serve as antibiotics that are toxic to the invading pathogen. Secretion of lytic enzymes by *Trichoderma* species has also been confirmed by several *in-vitro* and *in vivo* studies (Tian *et al*., 2014). These compounds deter colonization of the pathogen from competing with *Trichoderma* (Nusaibah and Musa, 2019). *Trichoderma* produces plant-like hormones such as cytokinins, indole-3-acetic acid and gibberellins which stimulate root growth by increasing the absorptive surface of plant roots (Sikder and Vestergard, 2020; Nusaibah and Musa, 2019).

Trichoderma has the ability to induce systemic and local resistance against a wide range of plant pathogens. (Shoresh *et al*., 2010). *Trichoderma* induce the synthesis of regulatory proteins in plants especially under certain disease stress conditions, where these regulatory

proteins identify pathogen effectors and activate the plant's defense systems (Saenz-Mata *et al*., 2012).

Species of *Trichoderma* can control and antagonize broad range of economically important plant phytopathogenic fungi, bacteria, nematodes and viruses (Ghazanfar *et al*., 2018). *Trchoderma asperellum* had biological control activity over *Phytophthora capsici, Phytophthora megakarya* and *Rhizoctonia solani* (Segarra *et al*., 2012). *Trichoderma virens* have been employed in the protection of cotton seedlings against *Phythium ultinium* (Izuogu *et al*., 2019). They also revealed the antagonistic and suppressive ability of *Trachoderma* against root-knot nematodes in their study.

Trichoderma adopted repellent activity reducing second stage juveniles (J2s) of root-knot nematodes attraction to roots (Le *et al*., 2016), attenuated or delayed development of adult root-knot nematode females and reduction of their fecundity (Martinuz *et al*., 2013). Kurulkalr *et al*. (2019) recorded that isolated *Trichoderma harzianun* parasitized the egg and juveniles of *Meloidogyne inconita* and reduced nematode populations when applied as soil application and seed treatment on infected okra. *Trichoderma* strains have also been proven effective both as plant growth promoter against *Meloidogyne incognita* on pepper (Herrera-Parra *et al*., 2017).

Results of *Trichoderma longibrachiatum* evaluated shown a strong lethal effect on the *Meloidogyne incognita* when second stage juveniles (J2s) were exposed for 14 days to 1 x 10^5 to 1 x 10^7 conidia/ml (Zhang *et al*., 2015). *Trichoderma asperellum* applied on

commercial pineapple production reduced galling, egg masses and egg numbers of *M. javanica* and also increased fresh weight of roots (Kariga *et al*., 2018). Root colonization by *Trichoderma* prevented *M. incognita* performance both locally and systemically at multiple stages such as invasion, gall formation and reproduction (Marti nez-Medina *et al*., 2017). In Ghana, *Trichoderma viride* has been used successfully by Apiah-kubi *et al*. (2018), to control root-knot nematode population on yam and black pod disease of cocoa (Larbi-Koranteng, 2021).

2.15 *Trichoderma* Ecology

Trichoderma species are fungi residing in almost all agricultural soils containing decaying plant material (Izuogu *et al*., 2019). The growth and distribution of *Trichoderma* species are affected by climatic conditions. Shah and Afiya (2019) reported that mesophilic temperatures of 25-35^{o}C and wide range of pH of 5.5 to 8.5 favour the growth and sporulation of *Trichoderma* species but in general, *Trichoderma* is more ubiquitous in acidic soil condition (Carreras-Villasenor *et al*., 2012). Some species of *Trichoderma* showed more tolerant behavior with excessive moisture as compared to others. *Trichoderma citrinoviride* has been reported in South East Asia but not found in India due to difference in ecological conditions (Ghanzanfar *et al*., 2018).

2.16 Isolation Media for *Trichoderma*

The growth of *Trichoderma* has been screened on different culture media for various studies using available, relatively cheaper supporting media such as Corn Meal Agar, Oat Meal Agar, Potato Dextrose Agar, Czapek's Dox Agar, Special Nutrient Media, Carrot Agar, Rose

Bengal Agar and Selective media (Shah and Afiya, 2019). However, Potato Dextrose Agar favour the growth of *Trichoderma* species over other fungi hence preferred for easy culture and identification of *Trichoderma* isolates over rapidly growing fungi that may overlap it (Montoya-Gonzalez *et. al*., 2016). Khalili *et al*. (2020) reported that PDA medium prepared from fresh potatoes was best for radial growth and sporulation of *Trichoderma viride* and dehydrated PDA medium of centron showed the best growth of *Trichoderma harzianum*. Shahid *et al.* (2011) studied the growth of *Trichoderma* species on different media and reported that PDA showed excellent growth.

2.17 Morphological Characterization of *Trichoderma*

Morphological characteristics have been employed to characterize and distinguish *Trichoderma* species since 1969 (Gams and Bissett, 2002). They serve as a primary method of identification (Ghazanfar *et al*., 2018) and it remains as a potential method to identify *Trichoderma* species (Sharma and Singh, 2014). The characterization of *Trichoderma* plays an essential role to determine its potential level to inhibit plant pathogenic growth (Soesanto *et al*., 2014). Two techniques involving visual observation on petri dishes and micro-morphological studies in slide culture have been employed in morphological characterization of *Trichoderma* species based on certain defined key characteristics (Rahman *et al*., 2011).

The characteristics of fungal colony that have been used to identify isolates as *Trichoderma* include growth pattern, growth rate and colour. Species- level identification of *Trichoderma* isolates have been done based on the colour of the colony, formation of chlamydospores,

conidiophores, phialides characters and shape of conidia (Sekhar *et al*., 2017) and pigmentation, pustules formation and odours (Gams and Bissett, 2002; Hui, 2013).

Generally, Colony colour ranges from green to dark green depending on the species. Conidia colour varies from species to species but typically green or may be gray, white, yellow, brownish and sometimes colourless (Shah and Afiya, 2019). Conidia shape vary from globose to subglobose, ellipsoidal, obovoidal or short cylindrical with the basal end truncated and tapering (Schuster *et al*., 2010) while few species have smooth conidia (Ghanzanfar *et al*., 2018).

Conidiophores are indefinite, branched or unbranched hyphae bearing phialides laterally or terminally (Kumar and Sharma, 2016) and the phaialids are irregularly verticillate, bearing clusters of divergent, often irregularly bent and flask-shaped (Bastakoti, 2017). The phialides may be cylindrical or near sub-globose. They are held in divergent verticils at the end of the conidiophores, or in whorls below the septa along the conidiophores and branches. They may be held irregularly, paired, or in solitary (Samuels *et al*., 2002).

Pigmentation can be clearly seen in rich culture medium such as Potato Dextrose Agar. The colonies are white with scattered blue-green or yellow-green patches becoming noticeable when conidia are formed. Occasionally, concentric rings produced by these patches can be observed. Reverse of the colonies are pale, tan or yellowish (Rex *et al*., 2001). Some species produce odour of coconut and mold (Kumar and Sharma, 2016) while others produce aromatic and musty odours (Schuster, 2010).

2.18 Molecular Characterization of *Trichoderma*

The taxonomic consideration based solely on morphology may be associated with confusion caused by environmental conditions hence to improve the reliability of morphological characters and to resolve the confusion, molecular characterization should be ideally undertaken (Devi *et al.*, 2012) to compensate for the limitations of morphological characterization (Kannangara *et al*., 2017).

Recent advances in molecular techniques have made molecular characterization popular. Molecular methods based on genotypic characters which give fast, highly specific, effective and potentially more precise results (Badali and Nabili, 2012). Modern techniques commonly used in molecular characterization of fungi include immunological methods which are based on antigen-antibody reaction coupled with a fluorescent dye or enzyme (Mostafa *et al*., 2012). This method is flawed with difficult and expensive to produce highly specific antibodies while proven data show that it is reliable only at the genus level. In this regard, nucleic acid-based probe technology which allows determination of closely related species and detect the minute quantity pathogen when no visible sign is present. Additionally, Polymerase Chain Reaction (PCR) technology which involves synthesizing of the specific part of DNA in million copies through alternate cycles of denaturation, annealing, elongation by using specific primers. PCR includes methods such as multiple PCR, nested PCR, real-time PCR, reverse transcriptase (RT)-PCR and DNA bar coding (Aslam *et al*. 2017).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of Experimental Area

The experiment was conducted at the CSIR-Crops Research Institute, Fumesua-Ghana. Fumesua is situated in the Ejisu Municipal of the Ashanti Region (latitude 6^{o}43'N; longitude 1^{o}36'W) with an elevation of 228 meters above sea level (MoFA, 2013). It falls within the forest zone with a bi-modal rainfall pattern. Average annual temperature is 21.1 ^{o}C with annual rainfall of 3313mm (MoFA, 2022). The soil is characterized by Orthi-ferric acrisol, Offon series (Atta-Darkwa *et al*., 2020). The experiment was conducted between February and September, 2021.

3.2 Activity 1: Isolation and Characterization of *Trichoderma* Isolates

3.2.1 Soil sampling for Trichoderma *species isolation*

Soil samples at a depth up to 20 cm were collected from ten farms in the Bono, Ashanti and Western North regions of Ghana. The locations (Table 3.1) were selected based on favourable climatic conditions such as mesophilic temperature and massive cultivation of vegetables with shallow and widespread rooting system coupled with relatively high amount of organic matter content in the soils which induce *Trichoderma* growth, sporulation and abundance. Soil samples were collected from the root zone of crops as described as by Montoya-Gonzalez *et al*. (2016). The soil samples were collected from around the root zone of tomato, garden eggs, okro and cabbage crops in a zig-zag manner through zone sampling with a hand trowel. Fifteen core samples were taken and mixed to get a composite sample of 500 g for each. Collected soil samples were placed in clean sturdy bags, labeled clearly, kept

cool in an insulated cool box and transported to the Mycology Laboratory of CSIR-Crops Research Institute, Kumasi and stored at 25^{o}C in refrigerator until isolation.

Table 3.1: Sample Codes, Sources and Crop Fields of *Trichoderma* species

Sample Code*	Sources of Isolates	District	Region	Crop Field
ESM1	Essam	Bia West	Western North	Tomato
YMT2	Yawmatwa	Bia West	Western North	Okro
OKK3	Oseikojokrom	Bia West	Western North	Garden Eggs
MANZ4	Manzannouan	Bia West	Western North	Okro
P34-5	Pillar 34	Bia West	Western North	Garden Eggs
WFO6	Wamanafo	Dormaa East	Bono	Cabbage
WE7	Wamfie	Dormaa East	Bono	Garden Eggs
WIA8	Wiamoase	Asante Mampong	Ashanti	Garden Eggs
MAM9	Asante Mampong	Asante Mampong	Ashanti	Garden Eggs
TECH10	Gaza, KNUST	Oforikrom	Ashanti	Cabbage

*The soil samples were coded according to the names of the towns in Ghana where they were picked from for the studies.

3.2.2 Media preparation

Potato dextrose agar media was prepared by dissolving 19.5 g of Oxoid Potato Dextrose Agar powder in 500 ml of de-ionized water contained in reagent bottle. The suspension was mixed thoroughly by stirring with stirring rod until the powder completely dissolved. The mixture was properly sealed with cork and autoclaved at 121 ^{o}C for 20 minutes.

The sterilized media was allowed to cool under a laminar flow hood and amended with 250 mg Chloramphenicol to suppress bacteria growth. Thereafter, 25 ml of the molten potato dextrose agar was poured into sterilized petri dishes arranged inside the lamina flow and were allowed to cool at room temperature for use.

3.2.3 Isolation and identification of **Trichoderma** *isolates*

Trichoderma isolates were isolated using the Serial Dilution Technique as described by Johnson and Curl (1972). One gram of soil from each composite sample was weighed and mixed with 9ml of distilled water in centrifuge tubes and thoroughly mixed to form suspension. The suspension was allowed to stand for five minutes after which, aliquots of 1 ml of the supernatant from each bottle was diluted with 9ml of sterilized distilled water to attain a tenfold dilution. The dilutions were successfully done to 10^{-3} and aliquot of 1ml of the suspension of 10^{-3} from each tube was pipetted and spread on Potato Dextrose Agar (PDA) plates and incubated at 28± 2^{o}C five days. Well-defined colonies resembling *Trichoderma* morphology were isolated onto fresh Potato Dextrose Agar (PDA) plates and incubated at 28± 2^{o}C for four days. A further subculture was done and incubated for seven days and examined for colonies that resembled *Trichoderma* species morphology. Individual fungal colonies were isolated and purified by single spore isolation as per the procedure described by Choi *et al.* (1999).

3.2.4 Production of Trichoderma single spore

Single spore isolation for *Trichoderma* was achieved as per the procedure described by Choi *et al*. (1999). Seven-day old fungal culture on Potato Dextrose Agar was removed from the substrate surface using fine forceps and broken open in sterilized water in order to form a

spore suspension. The homogenized suspension was then transferred with sterilized pipette onto prepared water Agar plates. The inoculated Agar plates were incubated at 28^{o}C for one day. Thereafter, well isolated colonies germinating from single conidium were picked with a sterilized inoculation needle and transferred onto newly prepared water agar plates and incubated at the incubation room which served as the source of inoculum for the morphological characterization and for the screen house work. Three replicates were maintained for each isolate in a Completely Randomized Design.

3.2.5 Morphological characterization of **Trichoderma** *isolates*

Trichoderma isolates cultured on Potato Dextrose Agar plates were incubated at 28± 2^{o}C for 14 days. The plates were visually examined daily for pattern of conidiation, formation of conidia pustules, colony colour, colony reverse colour, colour pigmentation and colony growth. Pictures of colonies were taken at three, seven and fourteen days. The colony radius was measured with meter rule at 24-hour interval from the edge of the inoculum plug while culture smell was detected using Odour Identification Test Procedure by Mueller *et al*. (2006). Slides were prepared from the incubated isolates in distilled water from pustules for microscopic examination using Optika Compound microscope. After mounting the slide with the cover slip on the microscope, morphological characters like branching pattern and angle to main axis of conidiophores, phialide arrangement, conidia shape, conidia colour and formation of chlamydospores were observed. Photographs of the observed structures were taken using the Optika Compound Microscope Imager. Species identification was done based on the Morphological and Taxonomic Keys provided by Gams and Bissett (2002).

3. 3 Activity 2: Extraction of Root-knot Nematodes from Soil and Root Samples

Lettuce roots showing symptoms of root galling were collected from vegetable farms at the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. The root samples were gently rinsed under low running tap water to remove soil particles attached to the roots. The roots were placed in sturdy bags, labeled and kept in a refrigerator at CSIR-Crops Research Institute Nematology Laboratory for root-knot nematodes extraction.

The root-knot nematodes eggs from the roots were extracted using modified Hussey and Barker (1973) method while modified Baermann tray method as described by Whitehead and Hemming (1965) was used to extract the second stage juveniles. The roots were rinsed, dabbed dry and cut into 1-2 cm sections with scissors. The cut roots were placed in a blender and were intermittently macerated for three minutes and the partially macerated roots transferred into a conical flask where 50 ml of 0.5% sodium hypoclorite (NaOCl) solution was added. The conical flask with the content was shaken with hands for two minutes. The content was poured onto a 106 µm sieve nested over a 45 µm sieve and washed under slow running tap water to collect the eggs into beakers. The nematodes eggs-water suspension was allowed to stand on laboratory benches at room temperature to ensure hatching into juveniles. The fine macerated root residual on the sieves were scooped with spatula onto a 2-ply tissue paper lined in plastic sieves resting in plastic plates filled with water and incubated for two days to extract juveniles that remained in them. Two days after incubation, the juvenile-water suspension was poured into beakers and allowed to stand overnight for the nematode juveniles to settle below. Water was decanted to 40ml for standardization.

The number of juveniles in the aqueous suspension was determined by using Novex Holland Compound microscope and tally counter. An aliquot of one milliliter of each juvenile-water suspension was pipetted onto the counting tray after blowing air with the pipette through the suspension to homogenize it. All the juveniles in the counting tray were systematically counted following the gridlines on the tray.

Counting was done three times per each sample, the average calculated and the root-knot nematode population needed was estimated for inoculation.

3.4 Activity 3: Screening of *Trichoderma* Isolates against Root-knot Nematodes

3.4.1 Preparation of Trichoderma *inoculum*

Six morphologically distinct *Trichoderma* isolates from the laboratory study were selected and screened against root knot nematodes. Conidia of each selected *Trichoderma* isolate was harvested by carefully scraping the surface of the media with flame-sterilized inoculating needle after flooding the surface of each plate with sterilized distilled water into beakers containing 10ml of sterilized distilled water to obtain mycelia-conidia-water suspension. The suspension was filtered through sterilized muslin cloth placed in a funnel into 250 ml conical flask to remove mycelia fragment. The suspension was further diluted to different volumes in the conical flask to until less concentrated spores were obtained. Spore suspension of each of the selected isolates was pipetted on improved Neubaaer haemocytometer and the spore concentration in the suspension of each isolate was adjusted to 1.7×10^7spores/ml under an Optika Compound microscope.

3.4.2 Soil sterilization

Soil was sterilized using the barrel steam sterilization method. Sandy loam soil in a ratio of one part of sand to three parts of loamy soil (1:3) for three hours at 102 ^{o}C with gas as source of heat. The soil was kept in jute bag and placed in the upper chamber of the barrel. Steam supplied by hot water in the lower chamber of the barrel sterilized the soil. The sterilized soil was collected into clean plastic containers and allowed to cool for one week before use as recommended by Coyne and Ross (2014).

3.4.3 Nursing and transplanting of tomato

Clean Pectomech and Pectofake tomato seeds which are marketed as susceptible to root-knot nematodes infection (Osei *et al*., 2012) were obtained from K. Badu Agrochemicals Limited, Kumasi and CSIR-Crops Research Institute, Fumesua. The seeds were nursed thinly in two pots filled with sterilized sandy loam soil. The transplants were watered when necessary with tap water using watering can to ensure seedling establishment and growth. Three weeks old, tomato seedlings were then transplanted into 10-litre size bottom perforated pots filled with sterilized sandy loam soil to two-third the volume of the pots. Prior to transplanting, watering was reduced to harden the seedlings to avoid transplanting shock. The pots were watered to ensure successful establishment of the seedlings. Each pot contained two seedlings but thinned-out to one prior to root-knot nematode inoculation. All agronomic practices were carried out to ensure proper growth and development until harvesting.

3.4.4 Inoculation of tomato roots

The potted tomato seedlings were inoculated with 1000 second stage of root-knot nematode juveniles per pot two weeks after transplanting as recommended by Coyne and Ross (2014). The egg-water suspension contained in beakers was discharged near the root zone using pipette through three holes made in a triangular pattern with pencil. The seedlings were watered prior to inoculation and two days after inoculation.

3.4.5 Application of treatments

The following treatments were evaluated.

Table 3.2: Treatments Applied to Tomato Plants at the Screen House

Treatment	**Description**
Control (T_0)	1000 juveniles/plant
OKK3 (T1)	1000 juveniles/plant + 1.7×10^7 spores/ml of isolate OKK3
MANZ4A (T2)	1000 juveniles/plant + 1.7×10^7 spores/ml of isolate MANZ4
P43-5 (T3)	1000 juveniles/plant + 1.7×10^7 spores/ml of isolate P34-5)
WFO6 (T4)	1000 juveniles/plant + 1.7×10^7 spores/ml of isolate WFO6)
MAM9 (T5)	1000 juveniles/plant + 1.7×10^7 spores/ml of isolate MAM9
TECH10 (T6)	1000 juveniles/plant + 1.7×10^7 spores/ml of isolate TECH10
CARBOFURAN (T7)	1000 juveniles/plant + 3g/plant of Carbofuran
MANZ4B (T8)	1.7×10^7 spores/ml of isolate MANZ4

The experiment was set up in Completely Randomized Design with three replicates. Two days after inoculating tomato roots with root knot nematodes, 10ml of *Trichoderma* spore-water-suspension containing 1.7×10^7 conidia of *Trichoderma* was applied to the root zone of each tomato plant while three grams of carbofuran was weighed on an electronic balance and placed on three petri dishes for application at the screen house as a treatment to serve as a chemical check. The carbofuran was applied in the soil near the root zone through three holes made in the soil in a triangular pattern. The carbofuran was covered with surrounding soil and firmed.

3.4.6 Crop management

Tap water was applied to the growing plants two days after *Trichoderma* inoculation using beaker. However, watering was done as when and how necessary depending on the soil moisture conditions during the vegetative and flowering stages of growth of the tomato plants. Manual weed control using the hand by pulling the weeds as they appeared in the pots was done. Stakes were placed in the pots close to the plants through the soil to provide support. This was done 28 days after transplanting. Depending on the growth of the plants, some pots were provided with two stakes

3.4.7 Measurement of growth parameters

Plant height was measured from the base of the plant to the tip using meter rule. Measurement was done at 30, 44, 58, and 72 days after transplanting while the stem girth was measured by placing vernier calipers around the upper base of the stem of the plants. Measurement was done at 30, 44, 58, and 72 days after transplanting and the number of

leaves were counted branch per branch for all the plants. Counting was done at 30, 44, 58, and 72 days after transplanting.

3.4.8 Harvesting and harvest data collection

Harvesting of tomato plants was done at 80 days after transplanting. The pots were watered prior to harvesting to enhance easier removal of plants from the soil in the pots. The plants were removed with hand trowel from the pots and the roots were rinsed in water, dab dried and galling score was done. Thereafter, the roots and shoots were cut with scissors while soil samples from their respective pots were sampled.

Fresh shoot and root weight were measured on electronic balance. Roots of the harvested tomato plants were assessed visually for galls. The number of galls on each root were counted with the aid of tally counter and scaled into root gall index as described by Taylor and Sasser (1978) (Appendix A). The roots were stained in an aqueous solution of Phloxine B for 15 minutes which aided the visibility of the egg masses. The stained roots were placed on tissue papers lined in plastic plates for counting. The egg masses were counted and recorded.

The final nematode population for each treatment was obtained by the summation of the nematode population in the roots of the tomato plants and the respective soil samples and subsequently, the reproductive factor which is the final nematode population over the initial nematode population was calculated for each treatment.

3.5 Experimental Design

The laboratory and the screen house experiments were set up in Completely Randomized Design (CRD) with three replicates for each isolate and treatment.

3.6 Statistical Analysis

The quantitative data collected were subjected to Analysis of variance Test (ANOVA) using GenStat statistical package (18th edition). The least significant difference (LSD) at 5% level of probability was used to compare the means where significant differences existed between the treatments. Where necessary data were transformed using square root transformation of $\sqrt{(x + 0.5)}$, where x was the mean count to comply with the assumption of normal distribution especially in nematodes count while cluster analysis of the qualitative data on colony colour, colony reverse colour, culture smell, conidiophores character, conida shape, conidia colour, colour pigmentation and phialide arrangement were analysed using WPGMA approach in DARWIN 6.0.21.

CHAPTER FOUR

4.0 RESULTS

4.1 Identification and Characterization of *Trichoderma* Isolates

Fungal species were successfully isolated from soil samples and were identified as *Trichoderma* spp. based on macro-morphological (colony) characters such as colony colour, colour pigmentation, formation of concentric rings, culture smell and micro-morphological (microscopic) characters such as conidia colour, conidia shape, conidiophores and phialide characters.

Results from the macroscopic observation of the isolates showed dark green colony colour and colourless reverse colony colour for all the isolates (Figure 4.2). However, yellow colour pigmentation was observed in some isolates while culture smell was found to be either malt, indistinct or coconut-like. On the other hand, microscopic examination of the isolates showed conidia produced were globose, obovoid, ellipsoidal or globose to obovoid in shape (Figure 4.3). Phialides were essentially ampuliform, lageniform and subulate (Figure 4.4) in character whilst highly branched, moderately branched and infrequently branched conidiophores were observed.

4.2 Macro-Morphological (Colony) Characters

4.2.1 Variations in Colony Diameter of Trichoderma isolates

Similar growth rate ($P < 0.05$) was recorded when *Trichoderma* isolates were cultured on Potato Dextrose Agar at 28^{o}C and relative humidity of 73%.

Table 4.1: *Trichoderma* species identified using macroscopic and microscopic characters compared with Gams and Bisset (2002) morphological keys

Isolate Code	Cluster	*Trichoderma* species
YMT2	ST1	*Trichoderma harzianum*
OKK3	ST2	*Trichoderma virens*
MANZ4	ST2	*Trichoderma virens*
P34-5	ST3	*Trichoderma viride*
WO6	ST2	*Trichoderma virens*
WFE7	ST1	*Trichoderma harzianum*
WIA8	ST3	*Trichoderma viride*
MAM9	ST4	*Trichoderma asperrellum*
TECH10	ST1	*Trichoderma harzianum*

Key: ST1, ST2, ST3 ST4 represent morphological groups

ST1 = Morphological group of *Trichoderma harrzianum*, ST2 = Morphological group of *Trichoderma virens*. ST3 = Morphological group of *Trichoderma viride*, ST4 = Morphological group of *Trichoderma asperrellum.*

However, isolate MANZ4 sampled from Manzannouan showed remarkable growth rate of 4.25 cm one day after inoculation and fully colonized the 8.5cm vented PDA plates on day two. On the other hand, isolate WFE7 sampled from Wamfie in the Dormaa East District grew at slightly slower rate (Figure 4.1) compared to the other isolates though it fully colonized the plates on day three making it different from the other isolates.

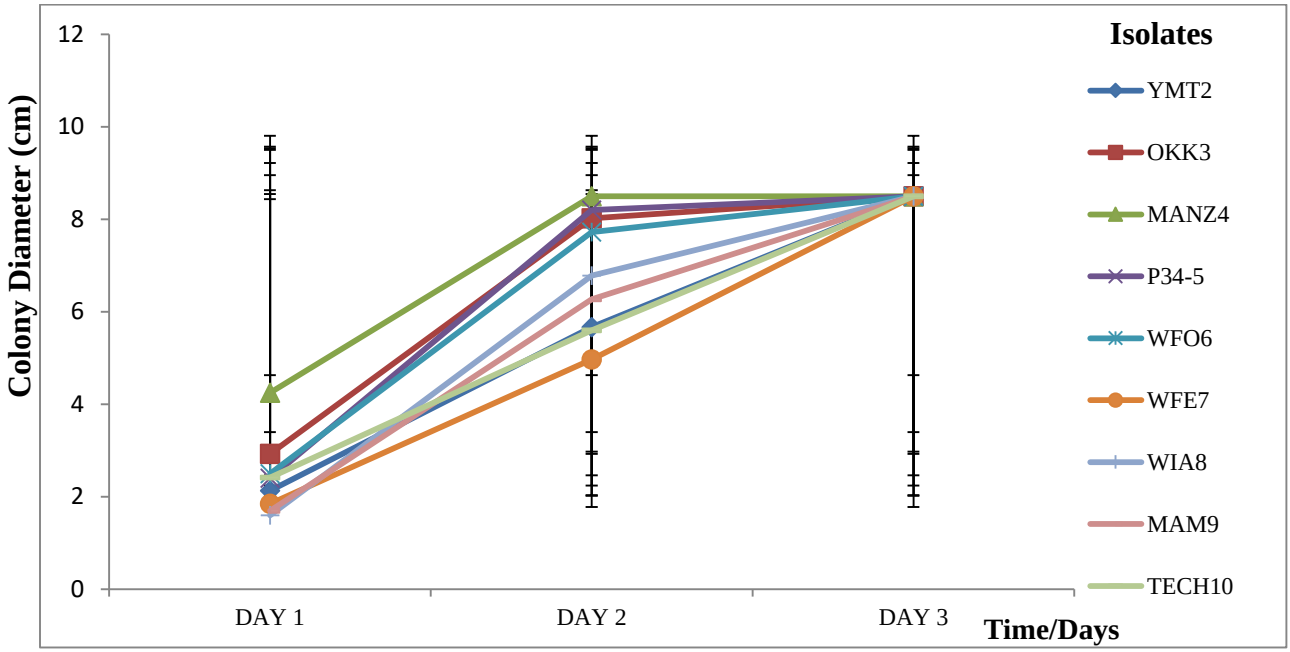


Figure 4.1: Colony diameter of *Trichoderma* Isolates Cultured on Potato Dextrose Agar at 28ºC after 3 days

4.2.2 Variations in colony growth pattern of Trichoderma isolates

Variations in colony growth pattern of *Trichoderma* isolates were observed. The isolates exhibited four colony patterns based on the growth on PDA media and hence grouped into four morphological groups which were named ST1, ST2, ST3 and ST4 (Figure 4.2). Isolates of ST1 exhibited very rapid colony growth and fully colonized the PDA plates on day 3. The colonies initially formed whitish to greenish mycelia which later changed into dark green around the central part upon maturation. Aerial hyphae formed near the margins of the plates. Conidia was predominantly effused and powdery spreading around the original inoculum plug to form 3 concentric rings making them distinct from the other morphological groups (Figure 4.2A). The colony growth of isolates in ST2 was very rapid reaching a diameter of 8.5cm on day 2 and fully colonized the 8.5cm vented pates on day 3 (Figure 4.1). The

colonies were initially seen as pale green at day 3 but gradually changed to dark green at day 14. Conidiation was predominantly effused and powdery and formed on aerial hyphae evenly distributed over the entire surface of the plates while the isolates produced yellow diffusible pigments called sorbicillinoids, a secondary metabolite on the Potato Dextrose Agar media which served as an important distinguishing character for identification (Figure 4.2B). The colony growth was fast and fully colonized the PDA plates on day 3 in isolates of ST3. The mycelium was white and turned into dark green upon maturation. Green conidia were formed within the centre of the colony and distributed over the entire surface of the plate (Figure 4.2C) whilst the colony growth was fast and fully colonized the PDA plates on day 3. More white pustules mycelium which later turned to dark green were formed and spread on the plates. Conidia was predominantly effused (Figure 4.2D).

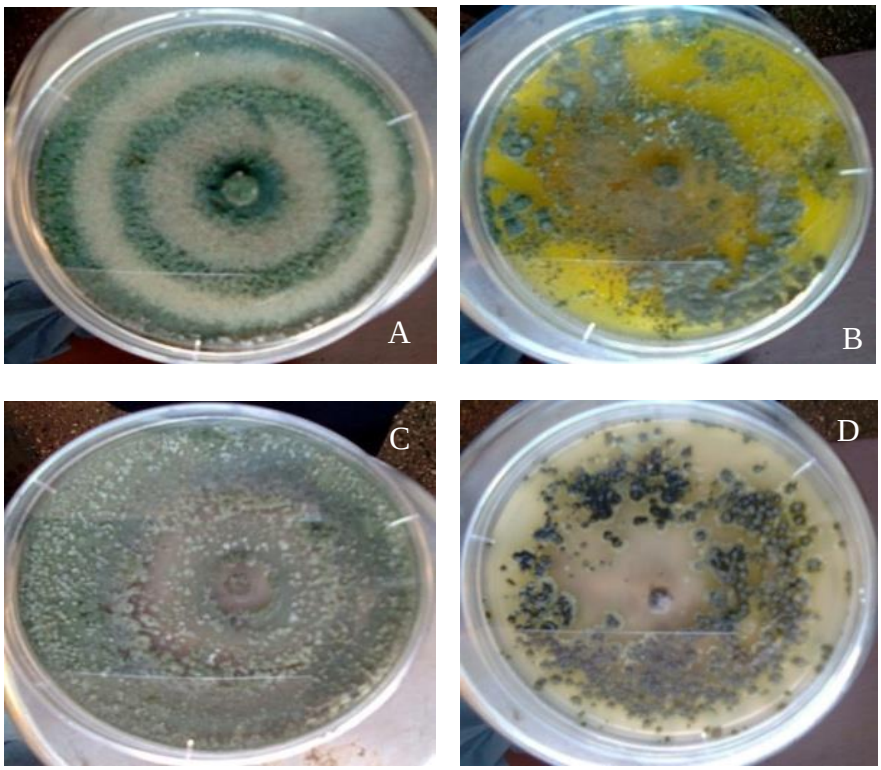


Figure 4.2: Various colony types produced by *Trichoderma* isolates cultured on PDA media A-Colony of morphological group ST1, B-Colony of morphological group ST2, C-Colony of morphological group ST3, D-Colony of morphological group ST4.

4.2.3 Variations in culture smell of Trichoderma isolates

Variations in culture smell of *Trichoderma* isolates were observed among the morphological groups. The malt culture smell was unique for isolates in ST1. Meanwhile, isolates P34-5 and WIA8 in ST3 as well as MAM9 in ST4 produced coconut odour while the remaining isolates produced indistinct odour (Table 4.2). It is however important to note that, although MAM9 in ST4 produced coconut-like smell just like ST3, the globose to obovoid conidia shape and the subulate shaped phialides distinguished it from isolates in ST3 which shown ellipsoidal conidia shape and less lageniform and solitary phialides.

Table 4.2: Macro-morphological (Colony) Characters of *Trichoderma* Isolates

Isolate Code	Colony Colour	Colony Reverse Colour	Pigmentation	Concentric Rings	Culture Smell
YMT2	Dark Green	Colourless	-	Formed	Malt
OKK3	Dark Green	Colourless	Yellow	-	Indistinct
MANZ4	Dark Green	Colourless	Yellow	-	Indistinct
P34-5	Dark Green	Colourless	-	-	Coconut
WFO6	Dark Green	Colourless	Yellow	-	Indistinct
WE7	Dark Green	Colourless	-	Formed	Malt
WIA 8	Dark Green	Colourless	-	-	Coconut
MAM9	Dark Green	Colourless	-	-	Coconut
TECH10	Dark Green	Coluorless	-	Formed	Malt

NB: (-) absence of the parameter observed

4.3 Micro-Morphological (Microscopic) Characters

4.3.1 Variations in conidia colour and shape of Trichoderma isolates

Conidia were found to be green for all the isolates under Optika compound microscope. However, WFE7 shown pale green colour. Three conidia shapes in the form of globose, ellipsoidal and globose to obovoid were observed under microscopic examination among the morphological groups. Globose shape was found in morphological groups ST1 and ST2 (Table 4.3). However, ellipsoidal shaped was seen in morphological group ST3 while ST4 produced globose to obovoid shape (Table 4.3 and Figure 4.3).

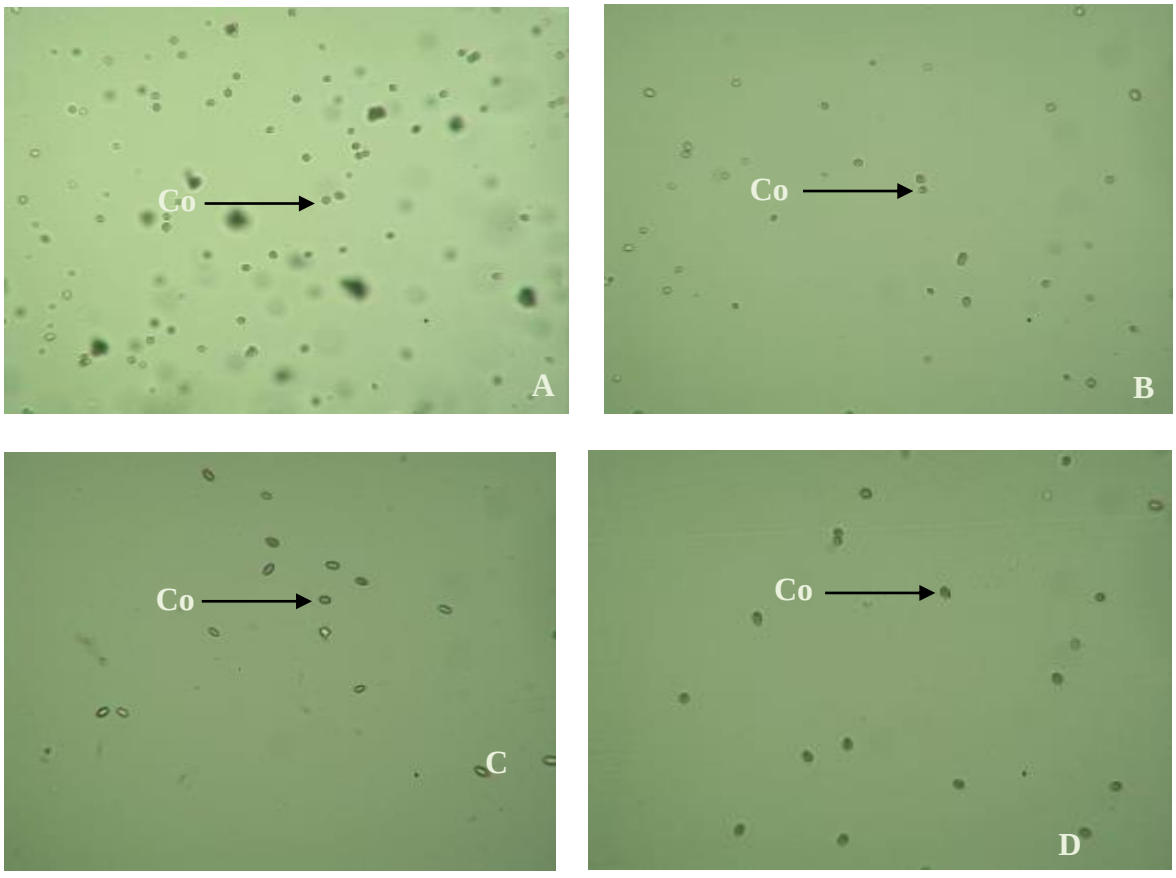


Figure 4.3: Microscopic view of conidia produced by *Trichoderma* isolates under Optika Compound Microscope. A-Morphological group ST1, B-Morphological group ST2, C-Morphological group ST3, D-Morphological group ST4, Co-Conidia

4.3.1 Variations in conidiophores and Phialide character of Trichoderma isolates

Variations in conidiophores and phialides character of *Trichoderma* isolates were observed among the morphological groups. Conidiophores of YMT2, WFE7 and TECH10 in ST1 were found to be highly to moderately branched, divergent and dentritic. Isolates OKK3, MANZ4 and WF06 in ST2 shown conidiophores arising in clusters from aerial mycelium and branching towards the tip. On the other hand, isolates P34-5 and WIA8 in ST3 shown infrequently branching and verticillate conidiophores while isolate MAM9 in ST4 shown moderately branched and dilated conidiophores. Phialides were bottle shape, protruded at the middle and narrower at the base and arising singly for morphological group ST1. Morphological group ST2 produced lageniform to ampulliform in closely appressed verticels while isolates P34-5 and WIA8 in ST3 shown less lageniform and mostly solitary phialides but isolate MAM9 in ST4 dominated with subulate shape (Table 4.3 and Fig 4.4).

4.4 Cluster Analysis

The isolates showed similarity in colony diameter, colony colour, colony reverse colour and conidia colour but differed in pigmentation, concentric ring formation, culture smell, conidia shape, conidiophores and phialide characters. Based on the results obtained, the nine isolates were grouped into four main clusters (Figure 4.5) confirming the four morphological groups (Figure 4.2).

Table 4.3: Micro-morphological Characters of *Trichoderma* Isolates

Isolate Code	Conidia Colour	Conidia Shape	Conidiophore Character	Phialide Character
YMT2	Green	Globose	Highly branched, divergent and dendritic	Ampulliform and narrower at base
OKK3	Green	Globose	Arising in clusters and branching towards the trip	Lageniform to ampulliform
MANZ4	Green	Globose	Arising in clusters and branching towards the trip	Lageniform to ampulliorm
P34-5	Green	Ellipsoidal	Infrequently branching and verticillate	Less lageniform to ampulliform
WF06	Green	Ellipsoidal	Arising in clusters and branching towards the trip	Ampulliorm and narrower at base
WFE7	Pale Green	Globose	Highly branched, divergent and verticillate	Ampulliform and narrower at base
WIA8	Green	Ellipsoidal	Infrequently branching and verticilllate	Less lageniform and solitary
MAM9	Green	Globose to Obovoid	Moderately branched	Subulate-shaped
TECH10	Green	Globose	Highly branched, divergent and dendritic	Ampulliform and narrower at base

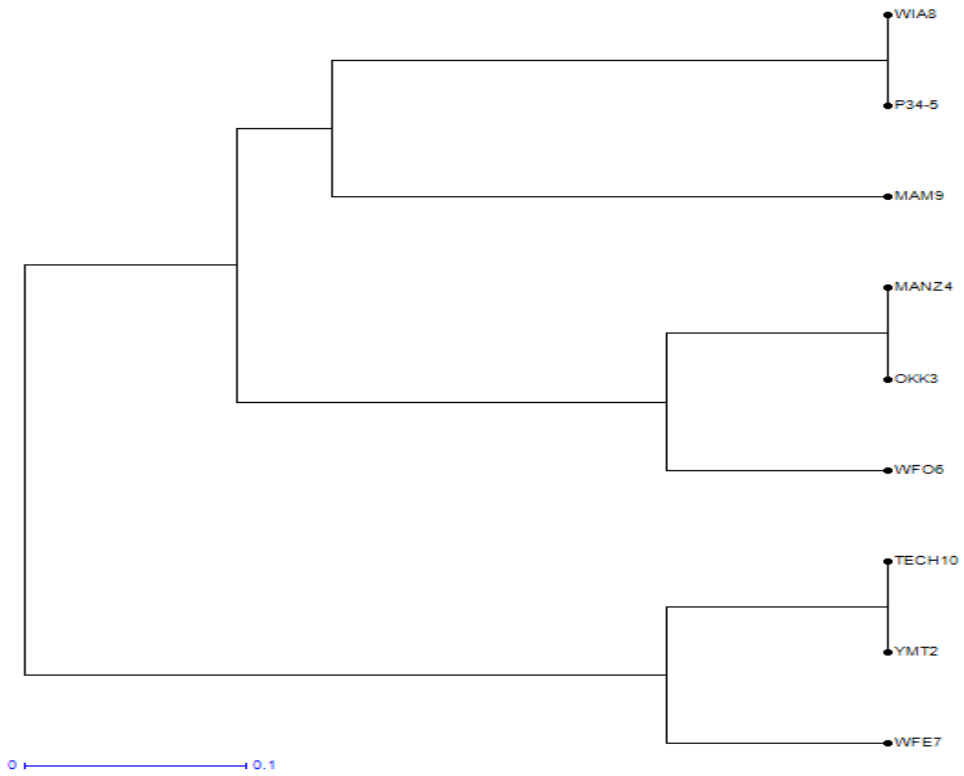


Figure 4.5: Dendrogram showing the clustering of *Trichoderma* isolates using the WPGMA approach in DARWIN 6.0.21

4.5 Effects of *Trichoderma* Isolates on Growth of Tomato Crop

There were variations in the plant height, stem girth and number of leaves recorded between the treatments. Plants treated with *Trichoderma* isolates showed significant difference ($P \leq 0.05$) in plant height and number of leaves compared to control and carbofuran treated plants (Figures 4.6, 4.7 and 4.8).

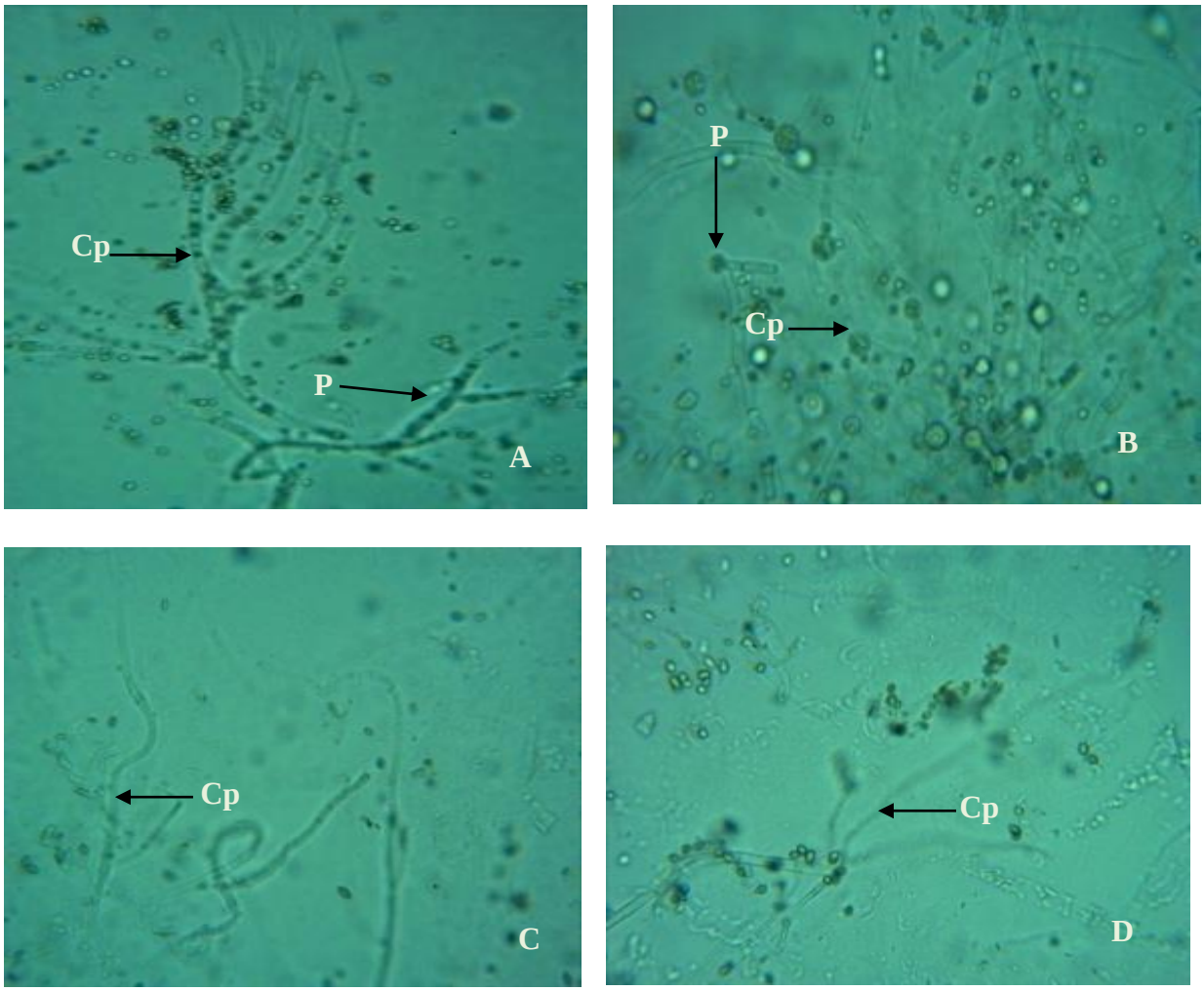


Figure 4.4: Microscopic view of Conidiophores and Phialides produced by *Trichoderma* isolates under Optika Compound Microscope. A-Morphological group ST1, B-Morphological group ST2, C-Morphological group ST3, D-Morphological group ST4, Cp - Conidiophore, P-Phialide.

The highest plant height of 88.08cm was recorded by *Trichoderma* treatment P34-5 whilst a height of 67.07cm was recorded in the control treatment while carbofuran recorded the largest stem girth of 0.97cm followed by treatment P34-5 but the least girth was recorded by

TECH10 and the control. Isolate P34-5 recorded the highest number of leaves of 155.80 while the control recorded 99.30 as the least number of leaves (Figures 4.6, 4.7 and 4.8).

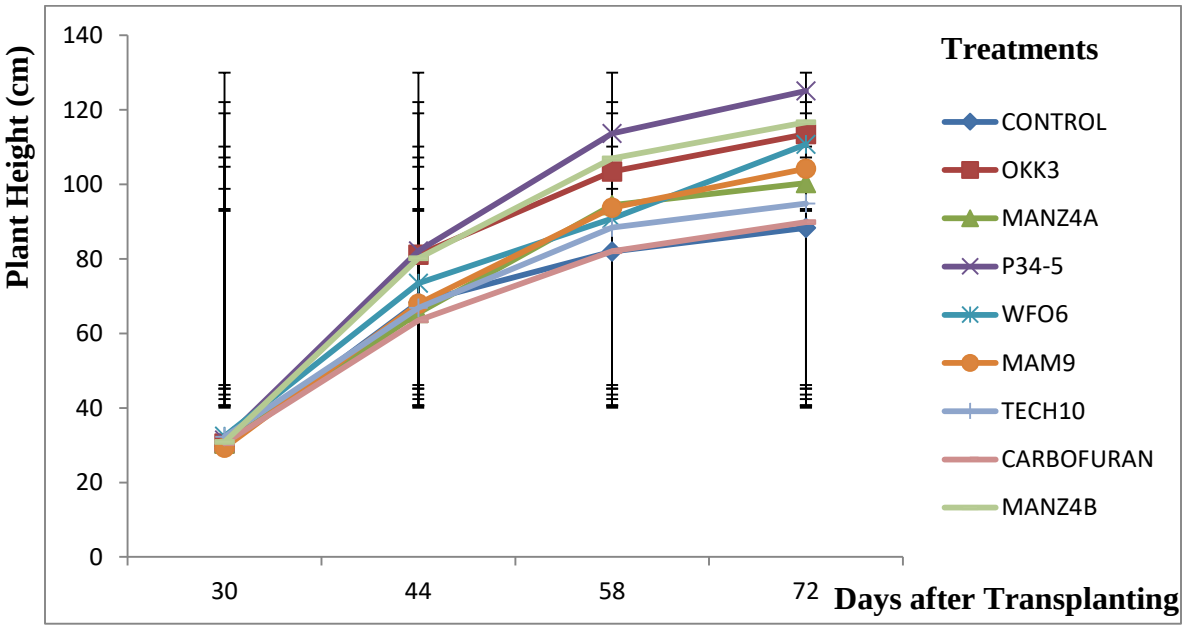


Figure 4.6: Effect of *Trichoderma* Isolates on Plant Height of Root-knot Nematode Inoculated Tomato Plants

All the *Trichoderma* treated plants performed better ($P < 0.05$) than the control and carbofuran (Figure 4.6). However, treatment P34-5 recorded the highest plant height which was significantly different ($P < 0.05$) from the control and carbofuran but similar to the other *Trichoderma* treatments

4.6 Effect of *Trichoderma* isolates on Root-knot Nematodes

Mean galling, mean egg masses, number of juveniles and corresponding reproductive factors differed ($p < 0.05$) between the treatments (Tables 4.4, 4.5, 4.6 and Figure 4.9). All the plants inoculated with *Trichoderma* isolates and carbofuran performed better than the non-

inoculated control. The maximum reduction of galls per treatment was observed in OKK3 followed by P34-5 while the maximum mean gall score per treatment was recorded in the control.

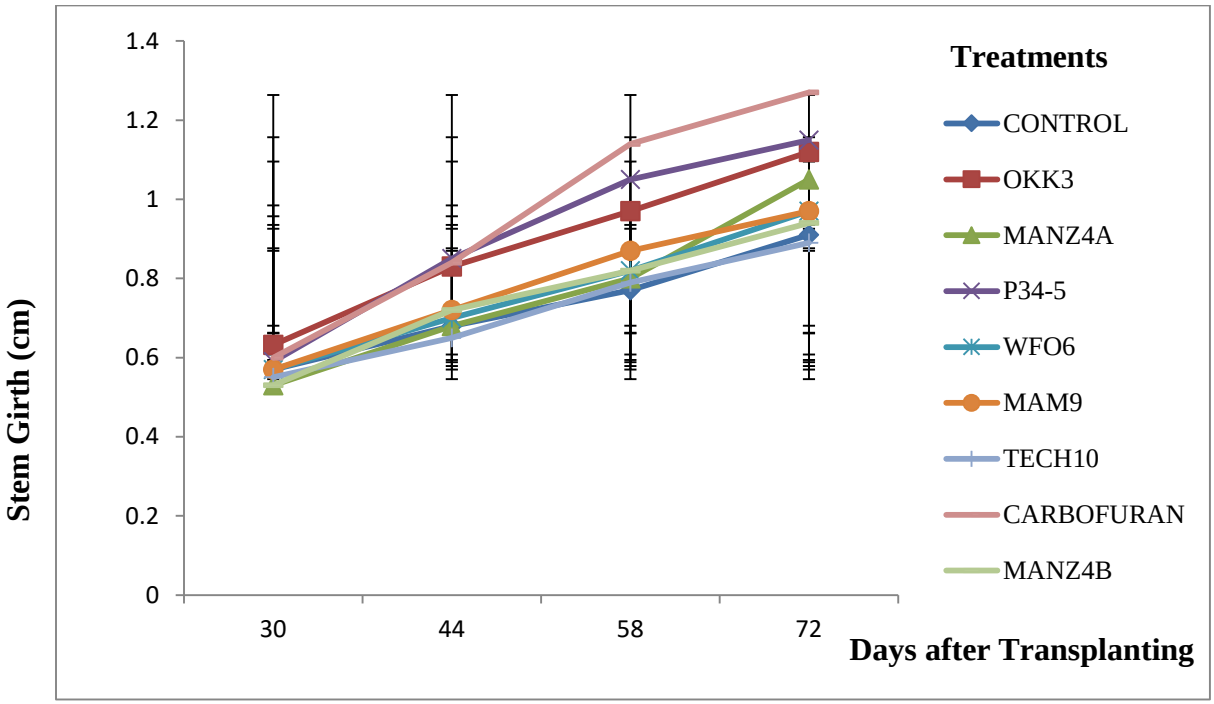


Figure 4.7: Effect of *Trichoderma* Isolates on Stem Girth of Root-knot Nematode Inoculated Tomato Plants

Carbofuran performed better and was significantly different ($P < 0.05$) from the control, but similar to *Trichoderma* treatments MANZ4A, WFO6, TECH10 and MANZ4B (Figure 4.7)

All the *Trichoderma* treatments performed better than the control with P34-5 recording the highest number of leaves which was significantly different ($P < 0.05$) from the control (Figure 4.8). However, *Trichoderma* treatments OKK3, MANZ4A, WFO6 and MAM9 performed similar to carbofuran. However, it is important to note that although MANZ4B

recorded the least mean gall score of 0.00, it served as a check therefore, was not inoculated with second stage juveniles of root-knot nematodes as a result; absolutely no galls were formed on the roots.

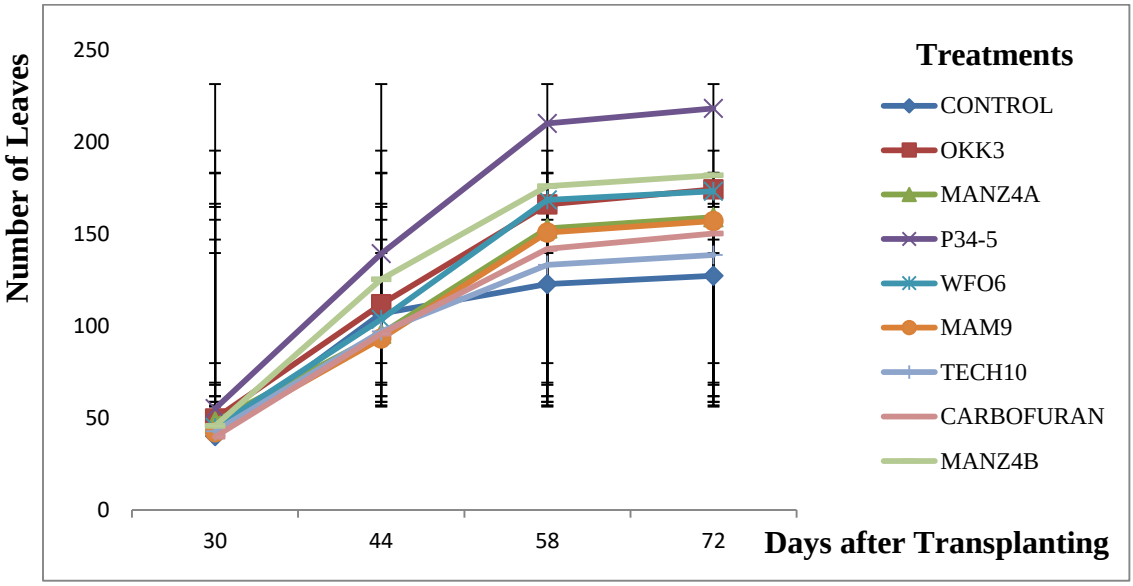


Figure 4.8: Effect of *Trichoderma* Isolates on Number of Leaves of Root-knot Nematode Inoculated Tomato Plants

Between the *Trichoderma* treatments, MAM9 recorded the minimum reduction of galls but performed better than the control (Table 4.4). It is also important to note that, although galls were formed on tomato roots inoculated with *Trichoderma*, the sizes in the form of a few spherical swellings as observed were relatively smaller as compared to the non-inoculated control which shown heavily and severely convoluted swellings under similar growth conditions indicating that *Trichoderma* has the potential to impair the growth and development of second stage juveniles of root-knot nematodes.

Table 4.4: Effect of *Trichoderma* isolates on Root-knot Nematode Juveniles and Galling of Root-knot Nematode Inoculated Tomato Plants

Treatments	Mean Galling (0-5)	Mean Number of Juveniles (Transformed) *	**Reproductive Factor
CONTROL	4.67^{e}	91.93^{c} (8451.20)	2.91
OKK3	1 .33^{ab}	30.85^{a} (951.15)	0.97
MANZ4A	3.17^{d}	62.45^{b} (3899.00)	1.97
P34-5	1.67^{bc}	27.73^{a} (768.28)	0.88
WFO6	3.50^{de}	64.82^{b} (4201.00)	2.05
MAM9	3.83^{de}	43.01^{ab} (1849.45)	1.36
TECH10	3.17^{d}	30.79^{a} (947.25)	0.97
CARBOFURAN	3.00^{cd}	21.55^{a} (464.00)	0.68
MANZ4B	0.00^{a}	0.71^{a} (0.00)	0.71
LSD (5%)	1.43	12.09	
CV (%)	2.40	5.91	

*$\sqrt{(x + 0.5)}$, Where x is the mean number of juveniles

**$R_f < 1$ implies no reproduction and $R_f > 1$ implies reproduction (Seinhorst, 1967)

Means followed by the same letters in the same column are not significantly different ($P > 0.05$) but means bearing different letters in the same column are significantly different ($P < 0.05$)

(a) Control (b) Carbofuran (c) *Trichoderma* Isolate

Figure 4.9: (a) Control treatment, (b) Carbofuran treatment and (c) *Trichoderma* isolate treatment.

4.7 Effect of Different *Trichoderma* Isolates on Fresh Weights of Root-knot Nematode Inoculated Tomato Crop

All the *Trichoderma* and carbofuran treated plants were highly significantly different ($P < 0.05$) from the control. Treatment P34-5 recorded the lightest root weight of 5.68 g while the control recorded the heaviest root weight of 18.08 g. It is important to note that although, MANZ4B recorded the lightest root weight of 2.49 g and the heaviest shoot weight of 22.81 g (Table 4.6), it served as a check therefore, was not inoculated with second stage juveniles of root-knot nematodes as a result; no galls were formed on the roots.

Table 4.5: Effect of *Trichoderma* isolates on Mean Root-knot Nematode Egg Masses produced on Roots of Tomato Plants

Treatments	Mean Number of Egg Masses/Root System (Transformed)*
CONTROL	14.53[b] (211.17)
OKK3	1.96[a] (3.35)
MANZ4A	10.73[ab] (114.70)
P34-5	4.06[a] (16.00)
WFO6	11.08[ab] (122.37)
MAM9	7.00[a] (48.50)
TECH10	6.67[a] (44.00)
CARBOFURAN	6.18[a] (37.67)
MANZ4B	0.71[a] (0.00)
LSD (5%)	11.45
CV (%)	7.51

*$\sqrt{(x + 0.5)}$, Where x is the mean number of egg masses

Means followed by the same letters in the same column are not significantly different ($P > 0.05$) but means bearing different letters in the same column are significantly different ($P< 0.05$).

As a result, treatment TECH10 recorded the heaviest shoot weight which was significantly different ($P < 0.05$) from the control but similar to the other *Trichoderma* treatments and

carbofuran. The severely developed galls on the roots of the control treatment influenced the fresh root weight. On the contrary, the limited and less developed galls on treatment P34-5 caused the lightest weight recorded. This shows the potency of *Trichoderma* isolates in inhibiting the growth and development of root-knot nematodes juveniles by reducing the enzymes released by the juveniles into cells of the roots to cause root swelling (Seebold, 2014).

Table 4.6: Effect of *Trichoderma* Isolates on Fresh Weights of Root-Knot Nematode Inoculated Tomato Plants

Treatments	Mean Fresh Shoot Weight (g)	Mean Fresh Root Weight (g)
CONTROL	3.03[a]	18.08[c]
OKK3	11.28[b]	5.78[ab]
MANZ4A	11.44[b]	7.97[b]
P34-5	14.92[bc]	5.68[ab]
WFO6	12.31[b]	7.01[b]
MAM9	13.45[b]	8.85[b]
TECH10	15.61[bc]	6.81[ab]
CARBOFURAN	12.58[b]	7.63[b]
MANZ4B	22.81[c]	2.49[a]
LSD (5%)	7.94	4.32
CV (%)	14.40	8.00

Means followed by the same letters in the same column are not significantly different ($P > 0.05$) but means bearing different letters in the same column are significantly different ($P < 0.05$).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Isolation and Characterization of *Trichoderma* Isolates

Numerous reports have confirmed the occurrence of *Trichoderma* species in almost all agricultural soils and have been reported as the most abundant fungi in soil and decaying plant tissues (Ghazanfar *et al*., 2018; Romika, 2019; Shah and Afiya, 2019). The isolation of nine *Trichoderma* isolates out of the ten soil samples picked for this study adds value to these earlier findings. However, the results obtained from this study revealed that there was complete absence of *Trichoderma* from the rhizosphere of tomato crop from the soil sample picked at Sefwi Essam and coded as ESM1. This is possibly due to the dry environment and inadequate organic matter in the site from which the sample was taken. Previous reports have confirmed similar observations when low *Trichoderma* recovery was retrieved from sandy soils of Egypt and beaches in Brazil where the soil contained little organic matter (Montoya-Gonzalez *et al*., 2016). The observed morphological features produced were similar to the description of Gams and Bisset (2002) confirming the isolates to be *Trichoderma harzianum*, *T. virens*, *T. viride* and *T. asperellum* (Table 4.1).

Among the macrocospic characters considered, colony diameter, colony colour, colony reverse colour did not establish much variation among the isolates hence did not give much realistic information in distinguishing the isolates. However, the green colour possessed by the isolates assisted in identifying and differentiating the isolates as *Trichoderma* species easily when isolated and cultured (Naher *et al*., 2018). Notwithstanding, these recorded similarities are consistent with earlier research findings by Gams and Bisett (2002) who

recorded dark green colony colour and colourless colony reverse colour for isolates identified as *Trichoderma harzianum, Trichoderma virens* and *Trichoderma viride*. Sharma and Singh (2014) found the colony colour to be dark green for *Trichoderma* isolates morphologically identified as *Trichoderma harzianum* and *Trichoderma virens*. Green, dark green and yellow to green were observed as the colony colours for isolates identified as *Trichoderma harzianum* and *Trichoderma viride*. The reverse colony colour was found to be colourless, light yellow and deep yellow (Kumar and Sharma, 2016). In the work of Naher *et al*. (2018), they observed dark green colony colour for isolates identified as *Trichoderma asperellum* while Shah and Afiya (2019) also observed dark green and dull green colours as colony colours and amber, colourless and pale yellow as reverse colony colours for *Trichoderma* sp.

However, it is important to note that, the macro-morphological characters of *Trichoderma* isolates which served as unique and essential morphological keys and provided information for characterization in this study were culture smell, colour pigmentation and formation of concentric rings (Table 4.1). This result is in consonance with the results obtained by previous researchers. Gams and Bisset (2002) have established that, the production of distinctly aromatic coconut or camphor smell as a result of volatile compounds (6-penthl-α-pyrone) produced by the colonies of *Trichoderma viride* is a distinct characteristic feature of the species while indistinct odour was typical of *Trichoderma virens*.

Meanwhile, *Trichoderma harzianum* produced faintly earthy smell like malt. Also, the culture smell or odour for isolates identified as *Trichoderma harzianum* was malt and coconut for *Trichoderma viride* (Kumar and Sharma, 2016). The production of yellow

diffusible pigments and the formation of three concentric rings by isolates in ST2 and ST1 provided useful information in identifying the isolates. Though more than one *Trichoderma* species can produce yellow pigments, *Trichoderma virens* produces these yellow pigments called sorbicillinoids at 28^{o}C while other species such as *Trichoderma atroviride*, *Trichoderma reesei*, *Trichoderma longibrachiatum* produce these pigments on Potato dextrose agar at 10^{o}C and at the edges of the colony or at 35^{o}C (Gams and Bisset, 2002; Hui, 2013; Oadi, 2018). Also, concentric ring formation is more pronounced in *Trichoderma harrzianum* at 28^{o}C on PDA media than other *Trichoderma* species such as *Trichoderma asperellum* and *Trichoderma pseudokoningii* while the rings are usually in the form of 1-2 and formed near the colony margins (Hui, 2013; Oadi, 2018).

The micro-morphological characters that were useful in identifying the *Trichoderma* isolates included shape and sizes of conidia, the branching patterns of conidiophores and phialide characters. Though conidia colours obtained were in harmony with several findings like Hui (2013) who recorded green conidia for *Trichoderma virens, Trichoderma strigosum, Trichoderma asperellum* and *Trichoderma harzianum.* The conidia colour was not useful in identifying the *Trichoderma* isolates to the species level. This is because the green conidia run through all the isolates. However, conidia shapes and sizes were useful in species identification as the predominant globose shape and the relatively smaller conidia sizes were used to distinguish isolates in cluster ST1 from the other isolates and identified as *Trichoderma harzianum*. This conforms to the work of Hui (2013) who recorded globose conidia shape and relatively small conidia sizes for *Trichoderma harzianum*. *Trichoderma asperellum* had more obvoidal conidia shape than *Trichoderma viride* while globose and

smaller conidia sizes were typical of *Trichoderma harzianum*. The conidia were globose to obvoid in shape for *Trichoderma virens* (Naher *et al*, 2018). In another research, Sekhar *et al* (2017) reported globose conidia shape for *Trichoderma harzianum* and globose to ellipsoidal for *Trichoderma viride*. Conidiophores and phialide characters were crucial to identifying isolates to the species level as they made the distinction among the isolates. Naher *et al*. (2018) reported that *Trichoderma harzianum* possessed frequently branching conidiophres with phialides that were mostly ampulliform to lageniform at the dense area. They noted that, conidiophores of *Trichoderma virens* had many branches with aggregate phialides at the end of the conidiophores and the phialides were lageniform to subulate in shape while *Trichoderma viride* showed long, straight, solitary and fertile apices.

In other situation, Sharma and Singh (2014) observed highly divergent conidiophores with flask-shaped and enlarged in the middle phialides for *Trichoderma harzianum*. On the other hand, *Trichoderma virens* recorded clustered conidiophores and closely appressed and divergently branched phialides. It has been established that, there was phylogenetic relatedness among isolates in each cluster though the soil samples were taken from different locations. It can therefore be inferred that, the same *Trichoderma* species can be isolated from the rhizosphere soils of different crops of different farming districts in Ghana and also the rhizosphere soils of different vegetable crops can host the same species of *Trichoderma*.

5.2 Effects of *Trichoderma* Isolates on the Growth of Tomato Crop

The results of *Trichoderma* treatments showed statistically significant effects of *Trichoderma* treatments on tomato growth parameters. This current finding is in agreement with the report

of Halifu *et al.* (2019) who recorded a significant increase in plant height of *Pinus sylvestris* seedlings after *Trichoderma harzianum* and *Trichoderma virens* treatment. Heflish *et al*. (2021) observed a significant increase in plant height compared to the control when *Trichoderma asperelloides* was inoculated to tomato plant. Sundaramoorthy and Balaskar (2013) also observed that tomato plants treated with *Trichoderma harzianum* stimulated plant height. Uddin *et al.* (2016) established that *Trichoderma* species were associated with increased plant height through vitamin production or conversion of materials to a form that could be utilized by plant, increased uptake and translocation of minerals, produced auxins that stimulated plant growth and root development. These hormones affect plant growth by promoting abundant and healthy plant roots (Romika, 2019).

Trichoderma is known to stimulating plant growth and development by degrading and making available soil nutrients to enhance photosynthesis resulting in improving plant growth. As plant growth promoter, *Trichoderma* species produce volatile and non-volatile secondary metabolites such as 6-n-pentyl -6-H-pyran-2-one, viridin, gliotoxin, harziandione, harzianopyridone and peptaibols which enhance plant growth through cell elongation (Heflish, 2021). Studies have shown that *Trichoderma* species can produce Indole Acetic Acid which promotes plant growth in crops such as tomato, cucumber and bitter gourd (Halifu *et al*., 2019). Tricalcium phosphate, Indole acetic acid and siderophore production by *Trichoderma* isolates have been confirmed by Chao and Wen-ying (2019) to promoting plant growth. *Trichoderma harzianum* increased water uptake and translocation of nutrient resulting in increased plant growth (Uddin *et al*., 2018). These findings explain why though it was critically observed during the study that *Trichoderma* isolate inoculated on tomato had ability to generate and revive near dead plants. In related research, the maximum number of

leaves per plant was recorded in *Trichoderma* treated tomato plants compared to the untreated control while the true leaves, fresh weights of tomato plants increased (Sundarannarthy and Balakar, 2013; Romika, 2019).

5.3 Effects of *Trichoderma* isolates on Root-knot Nematodes

Trichoderma species are important candidates for biocontrol of plant pathogens (Soesanto *et al*., 2014) and several authors have reviewed and reported of its potential against different pathogens including root-knot nematodes (Kumar and Sharma, 2016). Results from this study showed that all the *Trichoderma* isolates used in this study exhibited potential antagonism against root-knot nematodes. The results of *Trichoderma* treatments showed statistically significant effects of *Trichoderma* treatments on reproduction and damage of root-knot nematodes. This outcome is in line with the finding by Herrera-Parra *et al* (2017) who reported a 22 to 35% reductions in galling indices in pots treated with *Trichoderma virens, Trichoderma harzianum* and *Trichoderma atroviride* in pepper. Also, *Trichoderma asperellum* has been proven to reduce galling by 82% (Kiriga *et al*., 2018). Plant-derived sucrose was known to nourish *Trichoderma* cells which enhanced root colonization (Uddin *et al* 2016) which prevented root-knot nematodes performance both locally and systematically at different stages of nematodes attack such as invasion, gall formation and reproduction (Marti nez-Medina *et al*., 2017).

The severely developed galls on the roots of the control treatment influenced the fresh root weight which recorded the heaviest weight of 18.08 g. Jaiteh *et al*. (2012) had similar results for the control plants when he inoculated tomato with root-knot nematodes juveniles and reported that, there was greater increase in the fresh root weight of plants with higher nematode inocula density due to higher infection incidence and reproduction rates which

induced greater root galling making it heavier to weigh. Also, the heaviest fresh shoot weight was as a result of increased water uptake which was retained in the shoot and translocation of nutrients which is associated with healthy roots (Uddin *et al*., 2018) due to the suppressive capacity of *Trichoderma* on root-knot nematodes (Kariuki *et al*., 2020). It has also been confirmed that *Trichoderma* species can significantly improve plant and root lateral growth which facilitate greater uptake of water into the shoot due to Indole Acetic Acid production (Heflish *et al*., 2021). They reported that *Trichoderma asperelloides* improved fresh shoot weight by recording 16.4 g compared to the control which recorded 7.6g. Furthermore, *Trichoderma viride* and *Trichoderma harzianum* have been reported to improve shoot weight when 1g/kg soil was applied (Mumpi and Sobita, 2018).

Tomato plants treated with *Trichoderma* isolates produced less number of egg masses deposited on the roots by root-knot nematodes. This result confirms the earlier finding by (Kiriga *et al*., 2018) who reported that *Trichoderma asperellum* reduced egg masses and egg number of root-knot nematodes by 78% and 88%, respectively in pineapple. According to Nkechi and Abiri (2015) *Trichoderma* species reduced egg population laid by root-knot nematodes in soyabean in *Trichoderma* treated fields. *Trichoderma* spp. may also reduce root- knot nematode infections by triggering host defense by inducing resistance in root-knot nematodes. The induced resistance facilitates the poor development of root-knot nematode eggs though the eggs have been reported to survive in the soil under favourable conditions for at least one year (Mitkowski and Abawi, 2011).

Among the *Trichoderma* treatments, isolate P34-5 significantly reduced final population of second stage juveniles' recovered compared to other treatments. It is worthy to note that, although *Trichoderma* treatments MANZ4A, WF06 and MAM9 recorded higher numbers of

juveniles, the values did not exceed that of the control indicating the suppressive ability of *Trichoderma*. However, what is accounting for the variation in the suppressive ability of *Trichoderma* isolates MANZ4A, WFO6 and WFO6 compared to OKK3 though identified as *Trichoderma virens* has been confirmed by previous studies and reported by Kanngara *et al*. (2014) that the biological control abilities of different isolates that belong to the same assigned species vary hence their inability to inhibit the growth and reproduction of root-knot nematodes as observed in OKK3. Mumpi and Sobita (2018) have reported that the treatment with *Trichoderma* isolates improved root-knot nematode control as the isolates significantly reduced their population. *Trichoderma was* antagonistic against root- knot nematodes (Izuogu *et al*., 2019). They trap, coil around and kill them at different life stages in the soil or root system of crop plants (Forgahani and Hajihassan, 2020).

The production of secondary metabolites like anti-biotics such as Trichodermol A has nematicidal effect which had strong lethal effect on *Meloidogyne incognita* in tomato when second stage juveniles were exposed to *Trichoderma longibrachiatum* (Zhang *et al*., 2015). Le *et al*. (2016) has also confirmed this current outcome by stating that out of colonization of plants roots, *Trichoderma* served as repellant by reducing second stage juvenile attraction to roots of infected plants. *Trichoderma* was associated with the production of diffusible antimicrobial compounds such as lytic enzyme or water-soluble metabolites (Kannangara *et al*., 2016) which degraded the cuticle of the of the root-knot nematodes (Okendi *et al*, 2014) rendering them incapable of causing damage to plant and prevented them from reproducing. *Trichoderma* application caused the delay development and reduced fecundity of adult female root-knot nematodes Martinuz *et al*. (2013). This is accounting for the lower reproductive factors and number of juveniles recorded by treatments P34-5, OKK3 and TECH10.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The nine *Trichoderma* isolates were morphologically characterized into four as *Trichoderma harzianum, Trichoderma virens, Trichoderma viride* and *Trichoderma asperrellum. Trichoderma* species have proven to have the potential to increase plant height, stem girth, and number of leaves of tomato plant in this study. It is also worthy to note that root galls, the number of juveniles and egg masses of root knot-nematodes in surrounding soil and roots of tomato plants were greatly reduced leading to improved plant health. The fresh root weight of tomato roots was significantly reduced by Trichoderma treatments while the fresh shoot weight of the *Trichoderma* treated plants increased tremendously. The experiment also revealed that *Trichodema viride* (P34-5), *Trichoderma virens* (OKK3) and *Trichoderma harzianum* (TECH10) were the most promising species with biological control potential against root-knot nematodes on tomato crop

6.2 Recommendations

The experiment should be tried in pots outside the screen house or in the open field to ascertain the effects of *Trichoderma* isolates on tomato crop. Different concentrations of the *Trichoderma* isolates can also be assessed on tomato crop.

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APPENDICES

APPENDIX A

Taylor and Sasser (1978) Scoring Scale (0 – 5)

GRADE	**DESCRIPTION**
0	No Galls/Root System
1	1 -2 Galls/Root System
2	3 – 10 Galls/Root System
3	11 - 30 Galls/Root System
4	31 - 100 Galls/Root System
5	> 100 Galls/Root System

APPENDIX B

Summary Analysis of Variance for Colony Diameter of *Trichoderma* Isolates Cultured on Potato Dextrose Agar at 28^{o}C for Three Days

Variate: COLONY_DIAMETER_OF_*TRICHODERMA*_ISOLATES

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TREATMENT	8	9.82	1.23	0.12	0.998
Residual	18	186.30	10.35		
Total	26	196.13			

APPENDIX C

Summary Analysis of Variance for Effect of *Trichoderma* Isolates on Galling of Root-knot nematode-Inoculated Tomato Plants

Variate: GALLS

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK stratum	2	0.0741	0.0370	0.05	
BLOCK.*Units* stratum					
TRT	8	49.6296	6.2037	9.08	<.001
Residual	16	10.9259	0.6829		
Total	26	60.6296			

APPENDIX D

Summary Analysis of Variance for Effect of *Trichoderma* Isolates on Number of Juveniles of Root-knot nematode-Inoculated Tomato Plants

Variate: NUMBER_ OF_ JUVENILES

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK stratum	2	635.1	317.6	1.58	
BLOCK.*Units* stratum					
TRT	8	17122.1	2140.3	10.64	<.001
Residual	16	3217.3	201.1		
Total	26	20974.5			

APPENDIX E

Summary Analysis of Variance for Effect of *Trichoderma* Isolates on Fresh Shoot Weight of Root-knot nematode-Inoculated Tomato Plants

Variate: FRESH_SHOOT_WEIGHT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK stratum	2	67.60	33.80	1.61	
BLOCK.*Units* stratum					
TRT	8	637.67	79.71	3.79	0.011
Residual	16	336.77	21.05		
Total	26	1042.04			

APPENDIX F

Summary Analysis of Variance for Effect of *Trichoderma* Isolates on Fresh Root Weight of Root-knot nematode-Inoculated Tomato Plants

Variate: FRESH_ROOT_WEIGHT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK stratum	2	7.095	3.548	0.57	
BLOCK.*Units* stratum					
TRT	8	435.393	54.424	8.75	<.001
Residual	16	99.510	6.219		
Total	26	541.998			

APPENDIX G

Summary Analysis of Variance for Effect of *Trichoderma* Isolates on Egg Masses of Root-knot nematode-Inoculated Tomato Plants

Variate: EGG_MASSES

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK stratum	2	71.00	35.50	3.41	
BLOCK.*Units* stratum					
TRT	8	448.67	56.08	5.39	0.002
Residual	16	166.39	10.40		
Total	26	686.06			

APPENDIX H

Summary Analysis of Variance for Effect of *Trichoderma*Isolates on Plant Height of Root-knot nematode-Inoculated Tomato Plants

Variate: PLANT_HEIGHT

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK stratum	2	323.49	161.74	1.64	
BLOCK.*Units* stratum					
TRT	8	1178.62	147.33	1.49	0.236
Residual	16	1580.72	98.80		
Total	26	3082.83			

APPENDIX I

Summary Analysis of Variance for Effect of *Trichoderma* Isolates on Stem Girth of Root-knot nematode-Inoculated Tomato Plants

Variate: STEM_GIRTH

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK stratum	2	0.07389	0.03694	3.11	
BLOCK.*Units* stratum					
TRT	8	0.20080	0.02510	2.11	0.096
Residual	16	0.18998	0.01187		
Total	26	0.46467			

APPENDIX J

Summary Analysis of Variance for Effect of *Trichoderma* Isolates on Number of Leaves of Root-knot nematode-Inoculated Tomato Plants

Variate: NUMBER_OF_LEAVES

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
BLOCK stratum	2	5041.4	2520.7	9.75	
BLOCK.*Units* stratum					
TRT	8	7424.0	928.0	3.59	0.014
Residual	16	4138.2	258.6		
Total	26	16603.5			

APPENDIX K

Pictures of the Experimental Process

Plate 1: Soil Sampling for *Trichoderma*

Plate 2: Isolated and Cultured *Trichoderma*

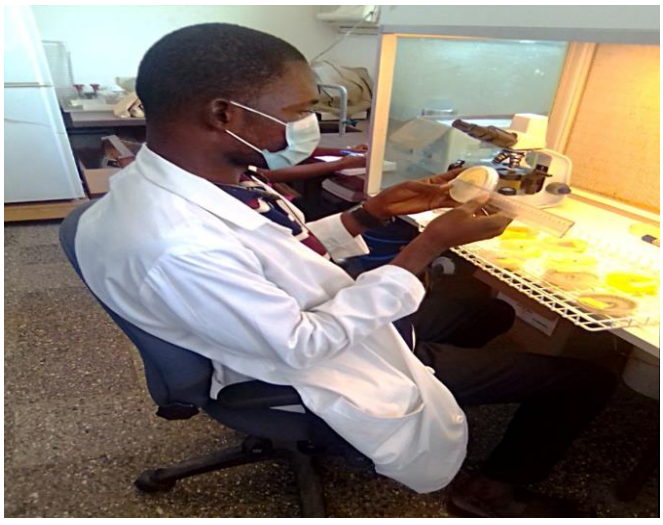

Plate 3: Measuring Colony Diameter of *Trichoderma*

Plate 4: Soil Sterilization

Plate 5: Sterilized Soil

Plate 6: Isolating Root-knot Nematode Juveniles

Plate 7: Transplanted Tomato Seedlings

Plate 8: Experimental Set-up in the Screen house

Plate 9: Root-knot Nematode Inoculum

Plate 10: Root-knot Nematode Inoculation

Plate 11: *Trichoderma* Suspension

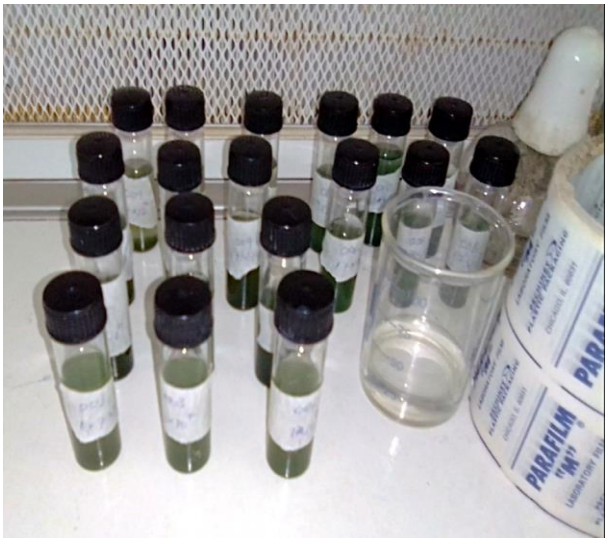

Plate 12: *Trichoderma* Inoculum

Plate 13: *Trichoderma* Inoculation

Plate 14: Collecting Data on Growth Parameters