DIVERSITY AND EFFICACY OF *TUTA ABSOLUTA* (Meyrick) ASSOCIATED ENTOMOPATHOGENIC FUNGI UNDER DIFFERENT TOMATO AGRO-ECOLOGICAL ZONES OF TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2023

EXTENDED ABSTRACT

Tomato crop is one of the important horticultural vegetables which is used both in consumption and commercially worldwide. Insect pests are the most destructive biotic factors facing tomato production almost in the whole world. *Tuta absoluta* is a one of the most destructive insect which decrease tomato production and increases production cost in Tanzania and in the whole world and its economic effects globally. The management of *T. absoluta* by synthetic chemicals is not working properly due to resistance which is developed by of the pest .Many findings by plant researchers from different countries including Africa have been proved that the use of entomopathogenic fungi such as *Beauveria bassiana, Metarhizium anisopliae,* as biocontrols agent against *Tuta absoluta* is more efficient compare to the use of synthetic pesticides. In this study a total of twenty four soil samples were collected from tomato farms infested by *Tuta absoluta*, the soil samples were from different agro ecological zones of Tanzania.

Culturing, Morphological, Sequencing data and results obtained showed that ten fungi were entomopathogenic fungi and fourteen were non entomopathogenic fungi among ten entomopathogenic fungi only two spp were related to *Tuta absoluta* management in tomato production. The entomopathogenic fungi related to *Tuta absoluta* control were *Metarhizium spp* and *Aspergillus spp*. *Aspergillus spp* is not recommended for *Tuta absoluta* management in tomato plants because it contains afflatoxin which is toxic to human being but it has high pathogenicity against *Tuta absoluta.* The pathogenicity of entomopathogenic fungi against *Tuta absoluta* were tested, against *T. absoluta* larvae, pupa, and adult stages under conditions of 19.5°C and 50%RH, 30.4°C and 70% RH, respectively, at different conidial concentrations (1.0×10^6 , 1.0×10^7 , 1.0×10^8 conidial/ml) in the laboratory for twenty nine days. The factorial experiment design was used where by tomato leaflets from screen house (6 × 7 cm area) were used as

experimental units that were dipped into (1.0×10^6 , 1.0×10^7 and 1.0×10^8 conidia/ml) of isolates and control (water containing 0.1% Triton X-100). Treated leaflets were dried at room temperature for elimination of excess water then was placed in Petri dishes (21 cm diameter) lined with wet paper towel and cotton covering the petiole to prevent leaf dehydration. Then, ten (10) *T. absoluta* larvae were inoculated separately into each of three treated leaflets and control. Each concentration was replicated three times with10 larvae. Treated leaflets were used only once at the beginning of the experiment. After 24 h, when treated leaflets are depleted, treated larvae were fed on fresh untreated leaves. Dead larvae were placed on another Petri dish lined with moist paper towel for re-isolation of fungus. Data of the larvae survival duration, number of dead larvae, number of larvae active (larvae treated but persisted), and number of adults emerged from total treated pupae, number of adult emerged was recorded at interval of 24 h and trials were monitored up to the completion of *T. absoluta* life cycle and lifespan.

Results showed that Metarhizium *anisopliae*, at all concentration caused high larvae mortality rate (98.2%) followed by *Aspergillus spp* (71%), larvae mortality rate increased with incubation time and concentration and consequently inhibited pupation and adult emergence within a short time before the completion of the life cycle of *Tuta absoluta* which is 29 days. *Metarhizium anisopliae* reduced the life span *T. absoluta* adult within 5 days of post inoculation at all conidia/ml, other entomopathogenic spp (*Penicillium spp*, and *Cladosporium spp*,) were also tested against *Tuta absoluta* larvae, pupa and adult stages the results recoded lower pathogenicity. *Penicillium* spp recorded 80% of adult survival at all conidial concentration within the whole incubation time.

Geotrichum candidum recorded 100% of adult survived at all conidial concentration after the incubation time. Whereas in control *T. absoluta* adult survived recorded 100%. This study found that *Metarhizium anisopliae* has the potential to be used as effective biological control against *Tuta absoluta* in tomato production especially at larvae stage which is the most destructive stage.

DECLARATION

I, Mwamu Magwe Magwe, declare to the senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has not been submitted nor being concurrently submitted in another institution.

_____ _____

Mwamu Magwe Magwe Date
(MSc. Crop Science Candidate)

The above declaration is confirmed by;

_____ _____

Dr. Luseko Amos Chilagane. Date
(Supervisor)

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ACKNOWLEDGEMENTS

First of all, I thank God for guidance, help and blessings throughout my life, I would like to express my sincere appreciation and thanks to Late Professor Loth Sikwese Mulungu, Doctor Luseko Amos Chilagane and late Professor Delphina Mamiro of SUA and Doctor Fathiya Khamis of *icipe* Nairobi Kenya who supervised this work and made me grow as a scientist.

I would also like to thank the following:

Mr. Jackson Nashon (SUA), who supported me in laboratory technical aspects,

The Ministry of Agriculture for funding my MSc studies and research work.

International Centre of Insect Physiology and Ecology (*icipe*) for finding my research work.

My parents, Veronica Magwe and Magwe Magwe for all their prayers for me which sustained me this far.

DEDICATION

I dedicate this work to Almighty God who guided me in my study, without Him nothing could happen. Thanks to my family especially my husband Paulo Mapunda and my four children Desideria, Brilliant, Bright and my beautiful baby late Dehateh for their encouragement throughout the study period.

ORGANIZATION OF THE DISSERTATION

This dissertation is developed in the format of publishable manuscripts comprising of four main chapters. Chapter one is a general introduction, chapter two and three consist of manuscripts in the form of publishable papers. Chapter four is the general conclusion and recommendations of the study.

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ABBREVIATIONS AND SYMBOLS

ACT	Actin Gene
ANOVA	Analysis of Variance
DNA	Deoxyribonucleic Acid
EPF	Entomopathogenic Fungi
EPPO	European and Mediterranean Plant Protection Organization
FAO	Food and Agriculture Organization of the United Nations
ITS	Internal Transcribed Spacer
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PIC	Polymorphic Information Content
RH	Relative Humidity
TES	Tris/EDTA/Sucrose
USA	United States of America

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

Tomato (*Solanum lycopersicum*: family Solanaceae) is one of the most important vegetables in the world. It is grown for its diverse use, both for the fresh market and processing industries (Wachira *et al*., 2014). Tomato is the major horticultural crop in Tanzania mostly produced by small and medium scale growers for home consumption and as a source of income. The area under tomato production is approximated to be more than 40 820 hectares (ha) with a production of 627788 tonnes (FAOSTAT, 2019). The average tomato yield under smallholder farming ranges from 2.2t/ha to 17.5 t/ha and the tomato yield average produced by all stakeholders is estimated to be 15.4t/ha (Chidege *et al*., 2016; Msogoya *et al*., 2016).

Morogoro region is the largest producer of tomato in Tanzania with the area of 6 519 ha, followed by Singida, Kilimanjaro and under tomato cultivation (Mutayoba *et al*., 2017). Other regions include, Tanga, Mbeya, Iringa, Arusha, Musoma and Mwanza. About 20% of farmers in Tanzania are preferentially growing F1 hybrid tomato cultivars such as Monica, Anna, Assila, Shanty and Eden for their high yield. Other varieties grown include Tengeru 97, Tengeru 2010, and Tanya, Cal-J, Rio Grande and Money maker.

Despite the potentiality of tomato, its production in Tanzania is challenged by several biotic and abiotic factors (Goftishu *et al.,* 2014). Biotic factors affecting tomato production include weeds, insect pests and diseases (Mahmoud *et al*., 2015). The most serious insect pest of tomato is Leaf miner (*Tuta absoluta*).This pest can cause yield losses of up to 100% (Brevault *et al*., 2014; Chidege *et al*., 2016).

1.2 Origin and Distribution of *Tuta absoluta*

Tomato leaf miner, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) originated from Peru in South America. It is also known as the South American tomato pinworm (Tonnang *et al*., 2015; Chidege *et al.*, 2016; Biondi *et al*., 2018). It is predominantly found in areas below 1000 m above sea level (Tonnang *et al*., 2015). *Tuta absoluta* was first observed in Europe, particularly Spain in year 2006 from where it has spread to different parts of the world and often it has become a serious threat to tomato production industry. By the year 2007, this pest spread/in much tomato growing areas of Asia, Europe and Africa (Biondi *et al*., 2018). It was first reported in the African continent in year 2007, particularly Algeria, Morocco and Tunisia. Up to now it has invaded 41 of the 54 African countries including Tanzania (Aigbedion-Atalor *et al*., 2019). *Tuta absoluta* was first reported in Tanzania in the year 2014 and at present, it is found in almost all tomato hot spot growing regions (Chidege *et al.*, 2016; Aigbedion-Atalor *et al*., 2019). This pest is suspected to be introduced in Tanzania through exchange of fresh tomato, fruits and planting materials between people from other countries where *Tuta absoluta* was already problem. Hence, lack of insufficient quarantine regulations and screening at the boarders are the factors behind the spread of the pest in Tanzania and in other African countries (Chidege *et al*., 2016). The distribution of *T. absoluta* across various countries around the world is presented in (Figure 1.1).

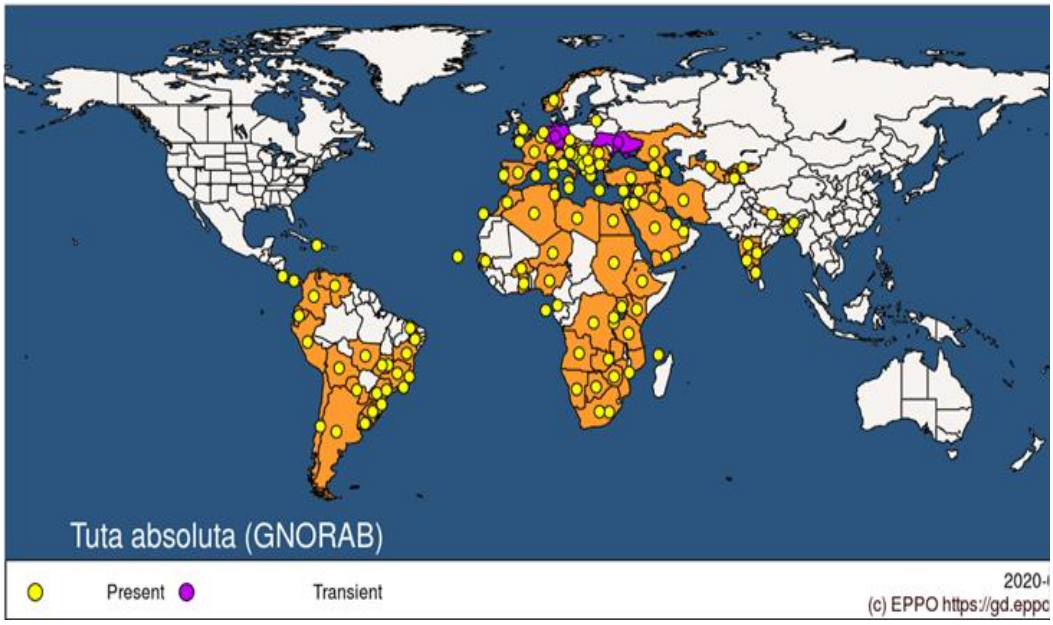

Figure 1.1: Distribution of *Tuta absoluta* across various countries around the world

Source: EPPO (2020)

1.3 Biology of *Tuta absoluta*

Tuta absoluta adults are small, silvery-brown moths, about 3/16 of an inch (4.5 mm) in length. They are most active at dusk and dawn, and rest on leaves and other plant parts during the day (Godfrey *et al*., 2016). *Tuta absoluta* life cycle has four developmental stages namely; egg, larva, pupa and adult. The pest has high reproductive rate between 10 to 12 generations per year (Chidege *et al*., 2016). Female moths lay eggs on different plant parts (i.e. the underside of leaves, petioles, blossom, fruit), either singly or in small batches. Each female can lay up to a maximum of 260-300 eggs during its lifetime. The eggs are deposited on the underneath of tomato leaves or stems and on immature fruits (Chidege *et al*., 2016). The eggs are yellow (Figure 1.2), turning darker about a day before hatching. At 27°C, the eggs hatch in 4 to 7 days (Godfrey *et al*., 2016).

After hatching, the newly emerged larva (caterpillar) penetrates the plant tissue (leaves, fruits or stems) on which the egg was laid, whereby they feed and develop. The larva is

cylindrical in body shape with a distinct head and legs (Figure 1.2). The young larvae are light yellow to green and very small, about 1/64 of an inch (0.5 mm) in length. As the larva grows, it becomes darker green. A larva completes 4 stages of growth (larval instars) in about 12 to 16 days at 27°C (Godfrey *et al*., 2016). The first two instars, larvae feed on mesophyll leaving the epidermis intact, hence creating tunnels on the leaf mines. The third and fourth instars larvae bore into stalks, apical buds and fruits. Pupation takes place in mines, dry leaves or in soil (Chidege *et al*., 2016; Godfrey *et al*., 2016).

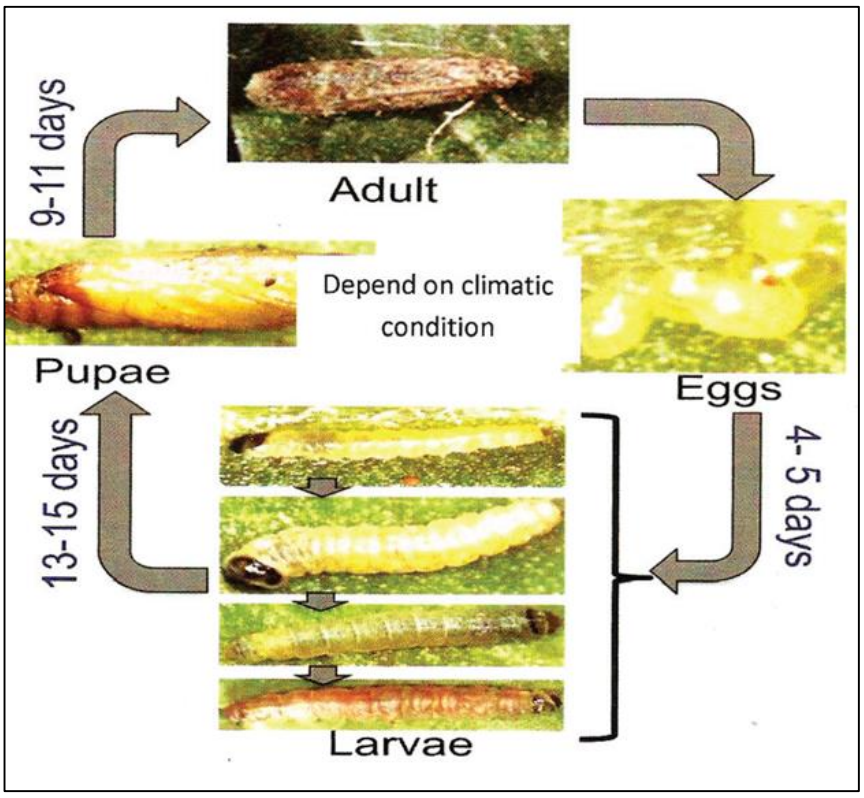

Figure 1.2: Life cycle of tomato *Tuta absoluta*

Source: Mahmoud (2018)

Damage

Tuta absoluta damages tomato plants by mining the leaves (Figure 1.3a) and boring into the apical and flower buds and fruits (Figure 1.3b). The newly hatched larvae penetrate

the plant tissue and begin to feed, resulting in irregularly shaped mines that increase in size as the larva grows (Godfrey *et al*., 2016; Chidege *et al*., 2016). Eventually the larva may leave the original mine and move to another part of the leaf or plant to feed. Sometimes the larger larvae can be found hanging from silken threads as they move around the tomato plant (Godfrey *et al*., 2016). Larvae feeding inside a developing shoot causes the bud death, resulting in poor plant architecture and low fruit set. Young larvae burrowing into the fruit form large enclosed holes, or galleries, where they feed from. The leaf mines and the galleries in the fruit may be invaded by secondary pathogens, resulting in necrotic areas on the leaf and rotting fruit (Godfrey *et al*., 2016).

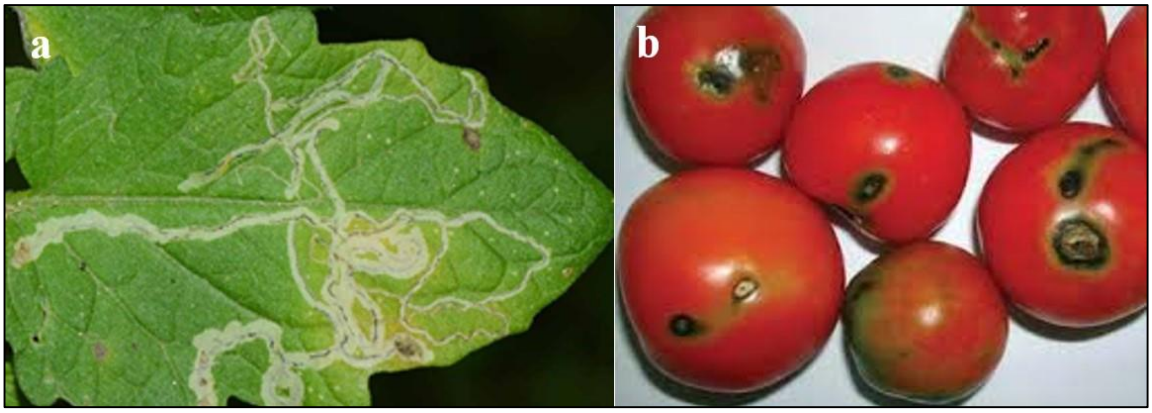

Figure 1.3: Characteristic patterns of damage made by the larvae of *T. absoluta* (a) feeding under the surface layer of the leaves. (b) Fruit damage caused by *T. absoluta* larvae

Source: Godfrey *et al*. (2016); Jackson (2017)

1.4 Management of *Tuta absoluta*

1.4.1 Chemical control

In Tanzania insecticides with active ingredients such as Imidacloprid, Indoxacarb, Spinosad or Deltamethrin have been recommended for *T. absoluta* management (Santos *et al.*, 2011). Nevertheless, *T. absoluta* is a very challenging pest to control due to its

resistance to pesticides, feeding habit and high reproduction capacity, and gene mutation. Synthetic pesticides were previously used as a sole control method, but the efficacy of synthetic pesticides has been declining with time due to resistance created by the pest, probably due to miss use of pesticides by farmers. Farmers tend to use multiple combinations of active ingredients. The pest was reported to develop resistance to dozens of, mamectinbenzoate, bamectin, pesticides (Illakwahhi and Srivastava, 2017). Common chemicals that tomato leaf miner has been reported to develop resistance are cypermethrins, pyrethroids, organophosphates, spinosad chloride channel activators, benzoylure and diamide (Santos *et al.*, 2011).

1.4.2 Use of botanical extracts

Botanical pesticides are naturally occurring chemicals extracted from plants. They have been reported to play a great role in controlling pests as they are relatively safe, easily biodegradable and eco-friendly. Geraldin (2016) reported that plant extracts from turmeric (*Curcuma longa*), lemon (*Citrus lemon*), garlic (*Allium sativum*) and ginger (*Zingiber officinale*) reduced populations of *T. absoluta* under field conditions.

Several groups of phyto-chemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported for their insecticidal activities against the pest (Shaalan *et al.*, 2005). Phytochemicals differ according to plant family and thus exhibit varied modes of action. Neem (*Azadrachtca indica*) contains alkaloids that affect the reproductive and digestive system of *T. absoluta* (Yankova*et al*., 2014). *Moringa oleifera* contains flavonoid which delays digestion and disturb the molting of *Tuta absolut*a (Shah *et al*., 2017). Turmeric and ginger contain aromatic compounds (curcuminoids, turmerones and zingerone) that affect growth and development of

T.absoluta while garlic contains compounds that affect the neuro-system of insects (Tabassum *et al*., 2013).

The efficacy of phytochemicals against insect pests can vary significantly depending on plant species, plant parts used, age of the plant part (young, mature or senescent), solvent used during extraction as well as method of extraction (Wongkaew *et al*., 2014). Reports shows that water extracts are more effective than organic extracts (Dabur *et al*., 2007). However, the use of water in extraction increases the amount of impurities and this could impair the quality of extracts (Bandor *et al*., 2013). Solvents such as methanol and ethanol are used to extract hydrophilic compounds while dichloromethane is used for lipophilic compounds (Sasidharan *et al*., 2011). Natural chemicals from plants are cheap, readily available and cost-effective in developing countries were synthetic insecticides are expensive especially for poor farmers.

1.4.3 Microbial agents for management of *Tuta absoluta*

Biological control method is potentially very beneficial tactic to develop. It has been used against crop insect pests belonging to the orders Homoptera, Diptera, Hymenoptera, Coleoptera and Lepidoptera, among others. Biological control agents (living antagonists natural enemies: predators, parasitoids and pathogens) are considered as one possible solution of the *T. absoluta* crisis. This strategy offers a more sustainable and less expensive alternative to chemical use once developed (Savino *et al*., 2013).

EPF infect insects by making a gap and break through the host cuticle. It is believed that for the penetration of fungus to the haemocoel of the insect both mechanical force and enzymatic action are involved. The cuticle is composed of chitin fibrils embedded in a matrix of proteins, lipids, pigments and N-acylcatecholamines (Richard *et al*., 2010). EPF

secrete extracellular enzymes like proteases, chitinases and lipases to degrade the major constituents of the cuticle and thus allow hyphal penetration (Wang *et al*., 2005). The potential of EPF to control insect pests has been recognized since the late 19th century when *Metarhizium anisopliae* was tested against the wheat cockchafer *Anisopliae austriaca* and sugar beet curculionid *Cleonuspunctuventris* (Gillispie *et al*., 1998).The study done in Ethiopia revealed that *Metarhizium* strains from local environment soils has high pathogenicity against second instar larvae of *Tuta absoluta* by maximum and minimum mortality of 95% and 93% respectively (Ayele *et al.,* 2020) .Another good performance of EPF against *Tuta absoluta* was proved by the study done in Iran whereby *Beauveria bassiana* showed high pathogenicity(70%) against *Tuta absoluta* especially at adult stage by degrading its cuticle and caused it death immediately after its application Seyed (2010).

1.5 Problem Statement and Justification

Tomato production is a major source of employment and income to both rural and urban dwellers in Tanzania (Materu *et al.,* 2016). Recently tomato yield dropped by 50% of the total production due to various constraints which include insect pests and disease. The average tomato yield under smallholder farming ranges from 2.2 to 3.3 t/ha which is very low compared to the world average of 37 t/ha (Minja *et al.,* 2011; FAO, 2016).

Tuta absoluta is one of the invasive and highly destructive tomato pests. The pest attacks tomato plants at all developmental stages and can cause crop losses of up to100% in both greenhouses and open field if not controlled (Chidege *et al.,* 2016). *Tuta absoluta* larva is the most destructive stage that consumes leaves, stems and fruits of the tomato plant by hiding within mesophyll of the plant tissues (Ramzi *et al*., 2018). The pest has high demographical potential, which is due to a short generation time, wide host range, a good

thermal adaptability and ability to develop insecticide resistance (Ramzi *et al*., 2018; Biondi *et al*., 2018).

Chemical control is the main management strategy implemented by growers to cope with insect pests (Santos *et al.,* 2011). Despite the use of many and high cost insecticides in tomato production to control *Tuta absoluta,* none of them has given a full control of *Tuta absoluta*. (Yalcin *et al*., 2015). However, extensive and inappropriate use of synthetic insecticides to control *Tuta absoluta* increases production costs and negatively impacts food safety, human health and environment, especially biodiversity of beneficial arthropods (Biondi *et al.,* 2012). This problem calls an urgent search for eco-friendly alternatives to control *T.absoluta*. Recent investigations have focused on different environmental friendly approaches that are inclusively termed as IPM strategies. The strategies include a combination of cultural practices, bio pesticides, and biological using parasitoids, predators, microbial cells, microbial products, RNAi, inheritance sterility development in insect and pest resistant plant cultivar production.

Biological control agents (living antagonists natural enemies: predators, parasitoids and pathogens) are considered as one possible solution of *T. absoluta* crisis. The use of natural enemies such as predators, parasitoids and entomopathogenic fungi (EPF) in South America and other continents including Africa have been proven to manage *T.absoluta* (Bacci *et al*., 2018). This strategy offers a more sustainable and less expensive alternative to pesticides and insecticides use (Savino *et al*., 2013).

Entomopathogenic fungi were among the first organisms to be used for the biological control of *T. absoluta*. *Metarhizium spp* and *Beauveria bassiana* have been reported to manage *T. absoluta* for more than 90% efficiency (Rodriguez *et al.*, 2006a, 2006b). EPF

are different in pathogenicity than bacteria and viruses (Richard *et al*., 2010). EPF secrete extracellular enzymes like proteases, chitinases and lipases which degrade the insect cuticle and allow hyphal penetration (Wang *et al*., 2005). EPF can be identified, assessed for their role and procedure can be developed for mass rearing of the most promising ones to be introduced/ applied as biocontrol agents (Mahmoud *et al*., 2014). The first report of entomopathogenic fungi in Tanzania against *T. absoluta* was on *Aspergillus* sp. The fungus caused 70% larval mortality 3 days post inoculation and consequently inhibited pupation by 84.5% and adult emergence by 74.4% (Zekeya *et al*., 2019). However, little information is known on diversity and efficacy, of native EPF isolates from different hot-spot areas under varying tomato agro-ecological zones of Tanzania and their use to control *T. absoluta*. This study will provide more scientific information and documentation for the establishment of suitable, ecological friendly**,** and sustainable biocontrol strategy against *T.absoluta* hence, improve productivity of tomato crop.

1.6 Objectives

1.6.1 Overall objective

Improve tomato productivity through ecological friendly biocontrol strategy against *Tuta absoluta*.

1.6.2 Specific objectives

i. To identify entomopathogenic fungi associated with *Tuta absoluta.*
ii. To determine the efficacy of entomopathogenic fungi against *Tuta absoluta* in tomato plants.

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CHAPTER TWO

2.0 IDENTIFICATION OF ENTOMOPATHOGENIC FUNGI ASSOCIATED WITH TUTA ABSOLUTA

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2.1 Abstract

Tomato is a very important vegetable in Tanzania and in the whole world, *Tuta absoluta* is a big challenge facing tomato production in many countries including Tanzania. This insect pest destroys tomato plant at all stages and it has built resistance to many pesticides. Understanding and identification entomopathogenic fungi associated with *Tuta absoluta* management and their efficacy is one of the methods which improves tomato production, food safety and negative impacts caused by the use of pesticides. Also the use of EPF reduces the use pesticides in terms of costs and they are environment friendly. The entomopathogenic fungi are currently used as efficient biological control agents against different insects including *Tuta absoluta*. The use of EPF tend to maintain natural enemies at their natural environment. At this study a total of twenty four soil samples (24) were collected from different tomato farms infested by *Tuta absoluta* and cultured. Among the twenty four (24) only two farms farmers were not using pesticides at that time. Morphological, Sequencing data and results obtained showed that 80% of *Metarhizium spp* were in Morogoro whereas 90% of *Aspergillus spp* were detected in Arusha region than in Morogoro. In case of *Aspergillus spp*, the species contains afflatoxin, therefore this species is not recommended for *Tuta absoluta* management, despite of its high efficacy of killing *Tuta absoluta* especially at second stage larvae instar. The two entomopathogenic fungi associated with *Tuta absoluta* were isolated from soil samples collected from two tomato farms which were not frequently sprayed with pesticides, the remaining soil samples collected from frequently sprayed farms the results turned to negative (the EPF found were not highly associated with *Tuta absoluta*) This implies that entomopathogenic fungi cannot survive in soils which are frequently sprayed with pesticides rather they prefer natural soil. In this study we also discovered that most EPF related to *Tuta absoluta* management prefer hot spot areas than cold spot areas.

Keywords: Tomato, *Tuta absoluta*, Biological control, entomopathogenic fungi.

2.2 Introduction

Tomato (*Solanum lycopersicum*: family Solanaceae) is among the most important fruit vegetables cultivated worldwide. Tomato is grown for its diverse use, both for the fresh market and processing industries (Wachira *et al*., 2014; Aigbedion-Atalor *et al*., 2019). The estimated annual global production of tomato is 182 million tones ranking the sixth most valuable crop, worth US$ 87.9 billion. In Africa, total annual production of tomato is 37.8 million tons (Rwomushana *et al*., 2019). Tomato is the most consumed fruit vegetable by people with diverse religions, ethnicity and economic status across the African continent constitute most diets (Rwomushana *et al*., 2019). The average tomato yield under smallholder farming ranges from 2.2 to 3.3 t/ha which is very low compared to the world average of 37 t/ha (Minja *et al.,* 2011; FAO, 2016). Nevertheless, tomato cultivation throughout the world suffers huge losses due to diseases and insect pests attack, resulting into the reduction of fruit quality (Biondi *et al*., 2018).

Like in other parts of the world, tomato production is a major source of employment and income to both rural and urban dwellers in Tanzania (Materu *et al.,* 2016). However since the arrival of *Tuta absoluta*, tomato yield dropped by 50% of the total production due to various constraints which include insect pests and diseases (Materu *et al.,* 2016). Leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae) is one of the main insect pests of tomato and other Solanaceae plants in Tanzania. This pest can cause yield losses of up to 100 % (Brevault *et al*., 2014; Chidege *et al*., 2016). Larvae of this moth are leaf-miners, damaging leaves, stems and terminal buds (Biondi *et al*., 2018). The mining behavior makes this pest very difficult to control and even systemic insecticides result in low levels of pest control, causing contamination of the fruits, environmental poisoning and human health problems (Guedes *et al*., 2019).

Tuta absoluta management using several types of synthetic chemicals/insecticides has been attempted with high prevalence worldwide. However, it has now been less promising due to less response and high resistance of *T. absoluta* to synthetic insecticide, are unaffordable and have been reported to pose hazardous health effects (Silva *et al*., 2011; Guedes and Picanço, 2012; Roditakis *et al*., 2015). The increased magnitude of insecticide resistance and environmental safety concerns have inspired for search of more sustainable pest control measures (Bacci *et al*., 2019). In addition, Desneux *et al*. (2010) reported that failure of most chemical pesticides with contact toxicity may be attribute to the feeding behavior of larvae by mining between plant tissues resulting to search for alternative systemic pest control strategies.

Biological control stands out as an alternative strategy to synthetic insecticides (Ayele *et al*., 2020). The application of bio-control agents like insect pathogens such as entomopathogenic fungi (EPF) have been proposed as a highly promising alternative strategy for the protection of crops against insect pests (Maina *et al*., 2018). EPF penetrates the integument, followed by multiplication in the haemocoel and relatively infect/colonize the tissues, causing insect's death (Silva *et al*., 2020). Parasitoids like *Trichogramma evanesens* (Polaszek*et al*., 2012; Sabbour, 2014) and microbial control including fungi *Beauveria bassiana* and *Metarhizium anisopliae* (Contreras *et al*., 2014; Tadele and Emana, 2017; Ndereyimana *et al*., 2019) and bacteria such as *Bacillus thuriengiensis* (Sabbour, 2014) are sought to be efficient for management of *T. absoluta*. *Metarhizium anisopliae* has been reported to affect both egg and first larval stage of *T. absoluta* (Santi *et al*., 2011; Inanli *et al*., 2012) (Pires *et al*., 2009).

EPF can be identified, assessed for their role and procedure can be developed for mass rearing of the most promising ones to be introduced/applied as bio-control agent

(Mahmoud *et al*., 2014). The first report of EPF in Tanzania against *T. absoluta* was on *Aspergillus* sp. The fungus caused 70% larval mortality 3 days post inoculation and consequently inhibited pupation by 84.5% and adult emergence by 74.4% (Zekeya *et al*., 2019). Nevertheless, information on the diversity and abundance of native EPF isolates from different *T. absoluta* hot-spot areas in Tanzania and their usage against the pest are missing. The present study, therefore, aimed to identify the native EPF isolates from different *T. absoluta* hot-spot areas under varying tomato agro-ecological zones of Tanzania and characterize by their morphological and molecular features so as to determine their abundance and diversity.

2.3 Materials and Methods

Description of the study site

Field surveys were conducted in Mvomero district located in Morogoro region and in Arumeru districts at Arusha region, Tanzania.

Arusha: The location lies between latitude 29° 52' S and longitude 98° 66' E. The districts are located in a tropical semi-arid environment with a bimodal type of rainfall and an average temperature of 25-33°C. Short rains are received from October through to December and the long rains from mid-February through to May. The climatic conditions of the study area vary depending on the agro-ecological zone (Plate 2.1 and Figure 2.1).

Morogoro: Doma and Kauzeni location lies between latitude 7˚ 14´ S and longitude 37˚ 13´ E and have the height of 1 200 and 1 000 m respectively above sea level getting annual rainfall range from 1 000 to 1 600 mm (Chidege *et al*., 2014).

Table 2.1: EPF isolates from different agro-ecological zones and their source

S/N	Isolate	Region
1.	M_1	Morogoro-Doma
2	M_2	Morogoro- Doma
3	M_3	Morogoro- Doma
4	M_4	Morogoro- Doma
5	M_5	Morogoro-Kauzeni
6	M_6	Morogoro- Kauzeni
7	M_7	Morogoro- Kauzeni
8	M_8	Morogoro- Kauzeni
9	M_9	Morogoro- Kauzeni
10	M_{10}	Morogoro-Nanenane
11	M_{11}	Morogoro-Nanenane
12	M_{12}	Morogoro-Nanenane
13	A_1	Arusha-PTC
14	A_2	Arusha-Dolly estate
15	A_3	Arusha-SADEC
16	A_4	Arusha-Singisi
17	A_5	Arusha-Kikwe
18	A_6	Arusha -Nambala
19	A_7	Arusha -Shangarai
20	A_8	Arusha- Shangarai
21	A_9	Arusha- Shangarai
22	A_{10}	Arusha-Kwa pole
23	A_{11}	Arusha- Karangai
24	A_{12}	Arusha-Karangai

Key:

A=Isolates from soil samples collected from Arusha farms

M =Isolates from soil samples collected from morogoro farms

Plate 2.1: Soil sampling

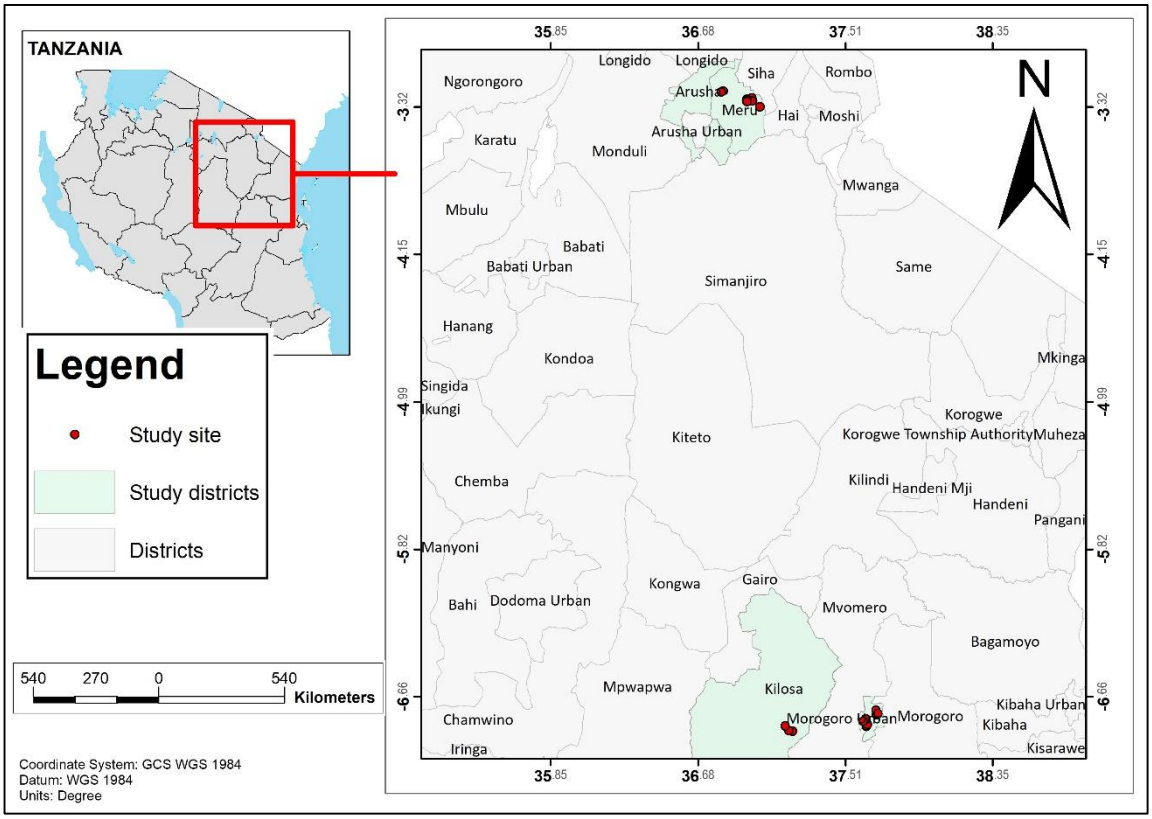

Figure 2.1: Map showing sample collection sites in Arusha and Morogoro regions

2.4 Isolation

EPF were isolated from soil samples collected from twenty four (24) tomato farms infested by *T. absoluta* in Arusha and Morogoro. The soil samples were collected around the root zone of the affected plants Stratified sampling method was used during soil sample collection. At each sampling location, a total number of soil samples were sampled and mixed to form one sample then 10grams of the mixture were taken to represent the five samples. The soil was taken from 0-15 cm deep using soil auger 5cm diameter. Plastic containers were used to keep collected soil samples. EPF were isolated from soil samples using semi-selective medium as per Meyling and Eilenberg (2006). Cultures were maintained at 25^{o}C in dark and examined after four to seven days the growths of fungi were confirmed and conidia characteristic of EPF was transferred into PDA, where purification was done. All isolates were subsequently propagated from a single conidium. A suspension (1µl) containing a low density of conidia in distilled water spread onto PDA. A single germinated conidium was separated from other conidia and identified by compound microscope, using a piece of medium encompassing only the target conidium were removed and transferred to PDA, and the culture maintained at room temperature.

2.5 Morphological and Molecular Characterization

After 5 days, morphological characteristics of EPF were identified according to (Zhang *et al*., 2015) using compound microscope.Structure appearance of mycelia and spore under compound microscope were used to distinguish one fungus from another (Zhang *et al*., 2015). Conidia was collected and stored in sterile 30% glycerol at 8^{0}C for further propagation.

Plate 2.2: Morphological identification of the fungi

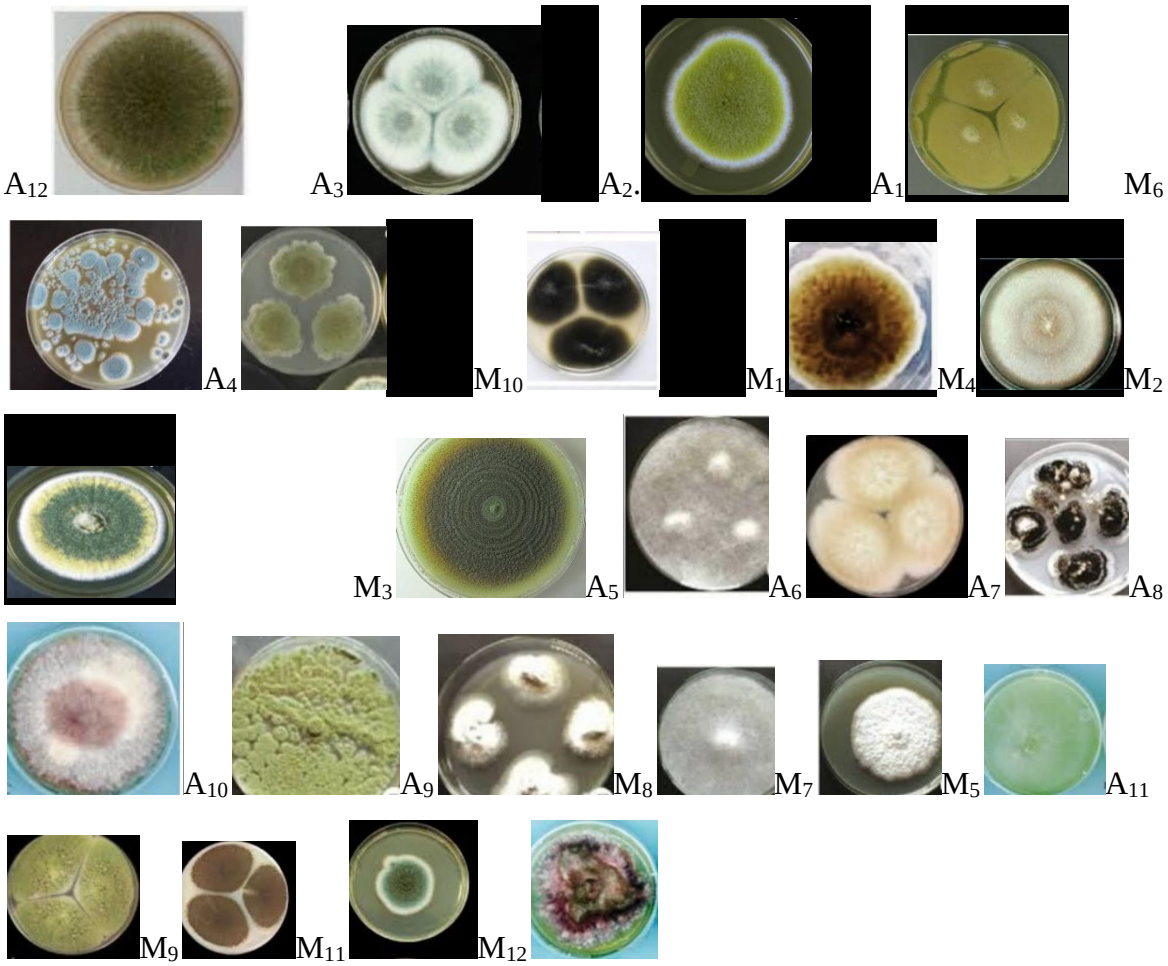

Plate 2.3: Five days old colonies of cultured fungi on PDA

The comparison of isolates using compound microscope showed that some isolates had dark color-olive/brown colour, covered with wooly tufts and smooth surface, where as some showed brighter bright-yellow/green colour, covered with thick short pile like cotton on side and rough surface.

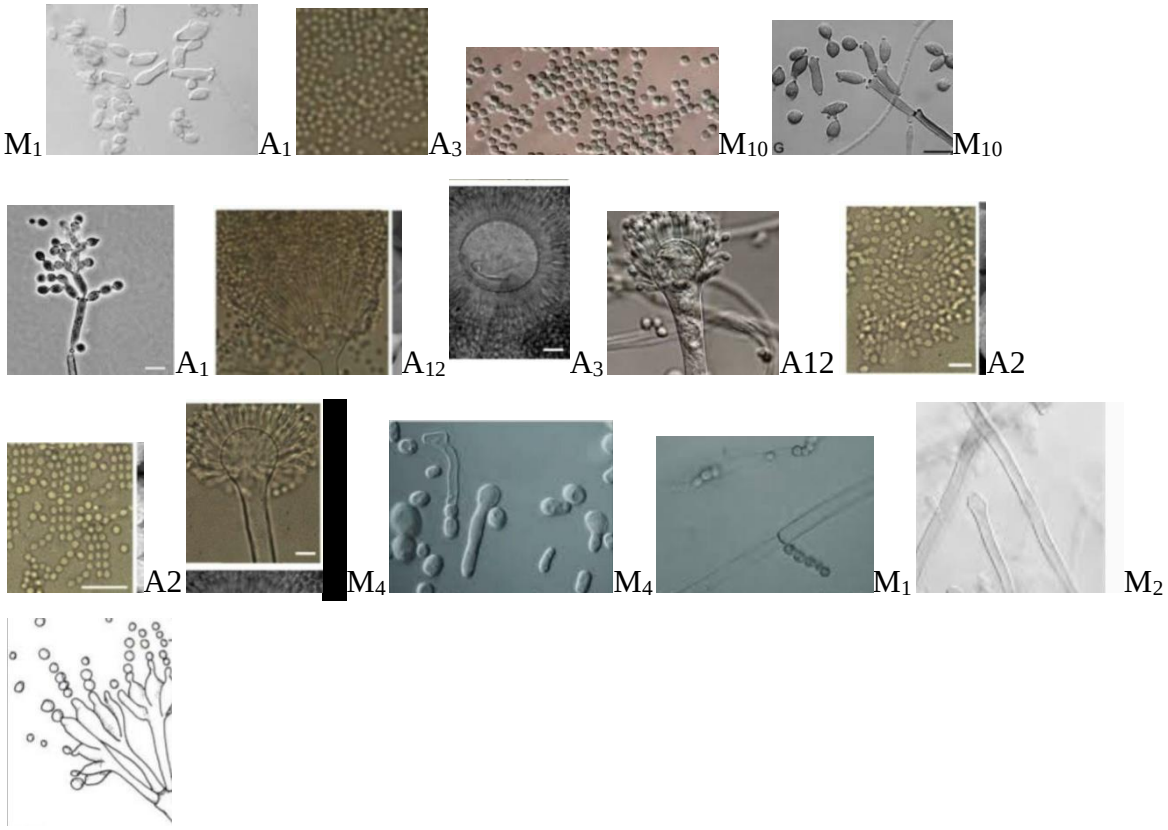

Plate 2.4: Conidia and conidiophores

2.6 Molecular Identification

2.6.1 DNA extraction and PCR

DNA from the EPF mycelia was extracted using TES extraction buffer by the method described by Mahuku (2004) with some modifications. Fungal PCR amplification were completed using two primer sets, the ITS 1 and ITS 4 , to amplify the Internal Transcribed spacer (ITS) region (White *et al*., 1990). The PCR conditions which was used to amplify the ITS region and the Actin gene were 94°C for 4 min for initial denaturation followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C forITS4 and 60^0C for

ITS1 for 30 s, and elongation at 72°C for 1 min followed by another cycle of final elongation at 72°C for 4 min.

Genomic DNA analysis

Agarose gel electrophoresis was conducted to analyze the quality of the obtained genomic DNA. From this analysis it shows that extraction was successfully since the gel shows genomic DNA with good quality. As shown below.

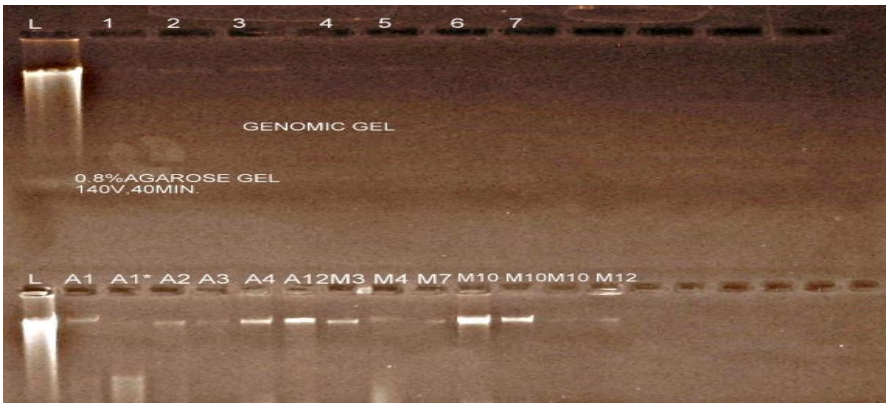

Figure 2.2: Genomic DNA

Polymerase chain reaction (PCR)

PCR was performed with ITS1&ITS4 marker in 19 total reactions.pcr reaction carried out with 25µlreactions containing 1µl of both reverse and forward primer,12.5µl of 2xTaq mastermix, 9µl of pcr water and 1.5µl of DNA template. The expected sizes were 580to 750bp amplifying internal transcribed region gene. The PCR total volume reaction was 20 with the following components.

Gel electrophoresis for PCR product

Then agarose gel electrophoresis was conducted (1.5% agarose gel, 100v for 1hour). Below is the gel picture.

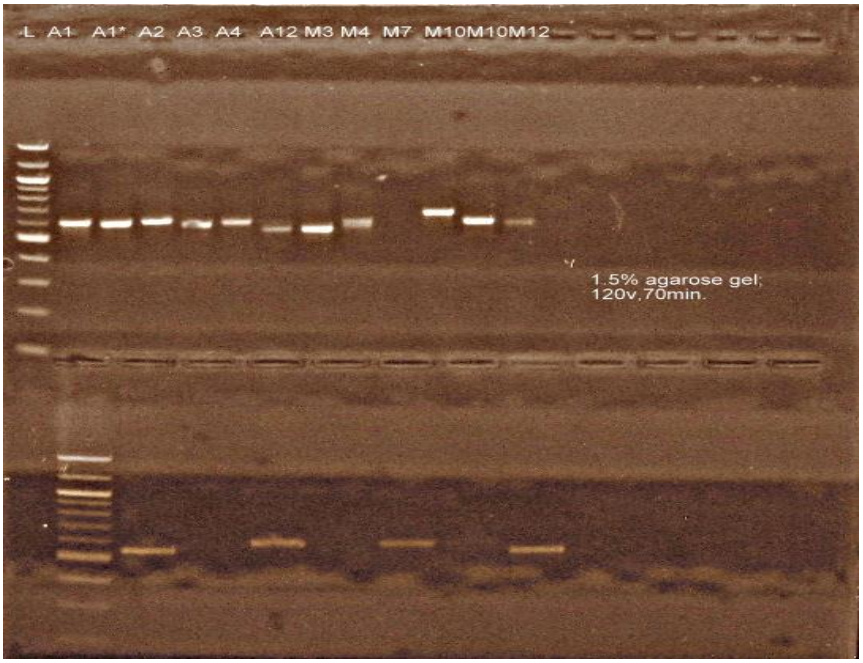

Figure 2.3: Gel electrophoresis for PCR

Results

From 24 samples screened the following are the results scored depends on the presence and absence of the bands in the expected size range of 580 upto 750bp.

Positive samples; A_1, A_1*, A2, A_3, A_4, A_{12}, M_3, M_4, M_{10}, M_{10}, M_{12}, $M_{1,2}$, M_4 and M_6.

Negative samples; M_7, M_3, M_5

2.6.2 Electrophoresis and gel documentation

PCR products from EPF samples was separated by electrophoresis (110 V for one hour and 30 min) and 3 µL of PCR product were run on a 1% agarose gel (Fisher Scientific, NJ) using 1X TBE buffer and 10 µL of gel red (Biotum ®, Hayward, CA). PCR products were visualized using UV–Trans illuminator and photographed using UVP® (Program version 6.5.2a 2007 program) (Upland, CA). PCR products from isolates were separated with electrophoresis at 120 V for 3 h, post-stained with ethidium bromide, and using 1X TAE as the running buffer. The gel visualization and documentation were completed using UV – Tran's illuminator.

2.6.3 Sequencing

DNA sequencing for the ITS region was done using the ITS 1 and ITS 4 primers with the same concentrations were used to search for their similarity index by using BLAST program.

2.7 Data Analysis

Power Marker program (Liu and Muse, 2005) was used to calculate the number of alleles per EPF, gene diversity (He) and the polymorphic information content (PIC) for allelic profile.

2.7.1 Results

Table 2.2: EPF identified

Isolate	EPF
A_I	*Aspergillus terreus*
A_2	*Aspergillus flavus*
A_3	*Aspergillus fimeti*
A_4	*Penicillium pinophilum*
A_{12}	*Aspergillus oryzae*
M_I	*Cladosporium oxysporum*
M_2	*Metarhizium anisopliae*
M_3	*Metarhizium anisopliae*
M_4	*Geotrichum candidum*
M_6	*Penicillium polonicum*
M_{10}	*Cladosporium cladosporioides*

2.7.2 Discussion

Based on morphological and molecular identification, we confirmed that nine species of entomopathogenic fungi was obtained in different location. Morphological features including green/pale green/ yellow green and oval shape revealed that isolates M_2 and M_3 are *Metarhizium anisopliae* (Ayele *et al*., 2020). Although morphology and physiology are traditionally used for the identification of fungi, such characteristics were not quite sufficient thus it was complemented with the molecular identification by the use of ITS1and ITS 4. This study revealed that different entomopathogenic fungus are found in the soil but very few are associated with *T. absoluta,* for example, in this study *Metarhizium anisopliae* and *Aspergillus spp* are the only EPF which showed high ability to manage *Tuta absoluta* at high percentage (Inanli *et al.,* 2012; Biondi *et al.,* 2018) compare to other Entomopathogenic fungi we identified and these was identified from the soil samples collected from tomato farm where little pesticides are used in Morogoro and Arusha. Twenty four soil samples collected from tomato farms In this study we observed that most entomopathogenic fungi related to *T.absoluta* control are mostly found around the root zone of tomato plant this is make the management of *Tuta absoluta* easy especially at pupa stage of the pest because it takes place on the soil (Tadele and Emana, 2017).

2.8 Conclusion and Recommendation

In conclusion of our findings in this research demonstrates high occurrence and abundance of entomopathogenic fungi in soils. This suggests that most entomopathogenic fungi can colonize better in organic soils or environment where pesticides are not frequently used. Field researches which consider environmental friendly factors of entomopathogenic and their inoculation assays will help to improve our understanding of ecological features of many entomopathogenic fungi which related to management of *Tuta absoluta*.

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CHAPTER THREE

3.0 EFFICACY OF THE KEY ISOLATED ENTOMOPATHOGENIC FUNGI FOR THE BIOCONTROL AGAINST *TUTA ABSOLUTA* IN TOMATO PLANTS

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3.1 Abstract

Tomato is an important vegetable crop both domestically and commercially. Recently the crop is facing the problem of insect pest destruction and this causes its production to drop up to 50% if not controlled. Tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is one of the most destructive insect of tomato. Entomopathogenic fungi (EPF) are among the microorganisms used in biological control against *Tuta absoluta*. The entomopathogenic fungus *Beauveria bassiana* and *Metarhizium anisopliae* currently used as an efficient biological control agent against different insects including *Tuta absoluta*. In this study the efficacy of entomopathogenic fungi were tested from EPF obtained from twenty four (24) soil samples collected from tomato farms infested by *Tuta absoluta*. Culturing was done under recommended laboratory conditions. Morphological identification, DNA extraction and Sequencing of PCR products were carried out. Tuta absoluta cohort was established for the purpose of collecting all required *Tuta absoluta* stages (eggs, larvae, pupa and adults) this was done by collecting tomato leaves, fruits and other parts infected by *T.absoluta*. The infected parts were incubated at 25^0C, relative humidity (RH) 75% and photoperiod of 12:12 hours. The emerged *T. absoluta* collected and reared onto tomato plants in the green house. Then twenty adult pairs were introduced to the rearing cages containing tomato seedlings of 40-45 days from sowing date. At this age the plants were suitable for egg laying. After 24hrs all adults of *T. absoluta* were removed and thereafter, the four larvae instars from the infested plant leaves were collected and be used to conduct EPF efficacy experiment. An EPF concentration was made from ten identified EPF, by separating a single germinated conidium from other conidia and identified by compound microscope, using a piece of medium encompassing only the target conidium were removed and transferred to PDA, and the culture maintained at room temperature. After 10 - 15 days isolates of conidial matured on PDA in Petri dishes, spores were gently scrapped from the media by suspending into 10 ml of

distilled water with 0.1% Triton X-100 to make a stock suspension. From each stock suspension of isolates, concentrations were assessed by counting spores using haemocytometer neubaeur (Manfield, German). After mixing suspension in one beaker the stock solution concentration at 1×10^{10} conidial/ml. In which working concentrations was prepared by the addition of water followed by counting spore using haemocytometer neubaeur at temperature range of 25.5°C under stereo microscope to obtain 1.0×10^{8}, 1.0×10^{7} and 1.0×10^{6} conidia/ml for EPF test against stages of *T. absoluta*.The efficacy of EPF were tested against *Tuta absoluta* larvae instars, pupa, and adults at different conidial concentration and incubation time. The results showed that among all EPF, *Metarhizium anisopliae* had high pathogenicity and recorded high percentage of *Tuta absoluta* larvae mortality rate (98.2%), followed by *Aspergillus spp* which recorded 71%.Other EPF recoded lower percentages which implies lower pathogenicity. Despite of *Aspergillus spp* being an entomopathogenic fungi which had high pathogenicity on *Tuta absoluta* but is not recommended for *Tuta absoluta* management in tomato production due to afflatoxin which is very toxic to human. Based on this observations *Metarhizium anisopliae* have the potential to be used as effective biological control of Tuta absoluta in tomato production.

Keywords: Biological control, Entomopathogenic fungi, *Tuta absoluta*, Tomato

3.2 Introduction

Tomato leaf miner, *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) is one of the invasive and highly destructive pests attacking tomato and other solanaceae plants (Smith *et al*., 2018). Depending on the degree of the infestation, *T. absoluta* attacks tomato plants at all developmental stages and can cause considerable crop losses of up to100% in both greenhouses and open field if not controlled timely (Chidege *et al.,* 2016). The damage

caused by *T. absoluta* is approximately 80% significantly when exposed tomato plants to secondary infection by facilitating the entry of pathogens (Rwomushana *et al*., 2019).

Tuta absoluta lifecycle has four developmental stages namely; egg, larva, pupa and adult. The pest has high reproductive rate between 10 to 12 generations per year (Chidege *et al*., 2016; Ramzi *et al*., 2018). Female moths lay eggs on different plant parts (i.e. the underside of leaves, petioles, blossom, fruit), either singly or in small batches. Each female can lay up to a maximum of 250-300 eggs during its lifetime. Female insects usually deposit eggs on the underneath of tomato leaves or stems and on immature fruits. *Tuta absoluta* larva is the most destructive stage that consumes leaves, stems and fruits of the tomato plant by hiding within mesophyll of the plant tissues (Ramzi *et al*., 2018). The pest has high demographical potential, which is due to a short generation time, wide host range, a good thermal adaptability and ability to develop insecticide resistance (Biondi *et al*., 2018; Ramzi *et al*., 2018).

Several management strategies including biological control as well as application of synthetic and botanical pesticides have been attempted against *T. absoluta*. Geraldin *et al*. (2016) reported that plant extracts from turmeric (*Curcuma longa*), lemon (*Citrus lemon*), garlic (*Allium sativum*) and ginger (*Zingiberofficinale*) have insecticidal activities due to phytochemicals that exhibit varied modes of action hence reduced populations of *T. absoluta*under field conditions. Turmeric and ginger contain aromatic compounds (curcuminoids, turmerones and zingerone) that affect growth and development of *T.absoluta* while garlic contains compounds that affect the neuro-system of insects (Tabassum *et al*., 2013). Neem (*Azadrachtcaindica*) contains alkaloids that affect the reproductive and digestive system of *T. absoluta* (Yankova*et al*., 2014). *Moringaoleifera* contains flavonoid which is potential by delaying digestion and disturbing the molting of

the *T. absolut*a (Shah *et al*., 2017). Natural chemicals from plants are cheap, readily available and cost-effective in developing countries where synthetic insecticides are expensive especially for poor farmers.

Insecticides with active ingredients such as Imidacloprid, Indoxacarb, Spinosad or Deltamethrin have been recommended for *T. absoluta*managementin Tanzania (Santos *et al*., 2011; MOA, 2020). Synthetic pesticides were previously used as a sole control method, but this method has been declining with time due to resistance created by the pest and farmers tend to use multiple combinations of active ingredients. *T. absoluta* has become less responsive and has highly developedresistance to dozens of synthetic insecticides and difficulties have been experienced when botanical pesticides and cultural practices are utilized alone (Roditakis *et al*., 2015; Illakwahhi and Srivastava, 2017). Common chemicals that tomato leaf miner has been reported to develop resistance are cypermethrins, pyrethroids, organophosphates, spinosad, mamectinbenzoate, bamectin, chloride channel activators, benzoylure and diamide.

Previous studies have shown that biocontrol stands out as an alternative strategy to synthetic insecticides and has more promising impact against *T. absoluta* (Tadele and Emana, 2017; Ayele *et al*., 2020). The application of bio-control agents like insect pathogens such as entomopathogenic fungi (EPF) have been proposed as a highly promising alternative strategy for the protection of cropsagainst insect pests (Maina *et al*., 2018). EPFpenetratesthe integument, followed by multiplication in the hemocoel and relatively infect/colonize the tissues, causinginsect'sdeath (Silva *et al*., 2020). Parasitoids like *Trichogrammaevanesens* (Polaszek *et al*., 2012; Sabbour, 2014) and microbial control including fungi *Beauveriabassiana*, and *Metarhizium anisopliae* (Pires*et al*., 2009; Contreras *et al*., 2014; Ndereyimana *et al*., 2019) and bacteria such as *Bacillus*

thuriengiensis (Sabbour, 2014) are sought to be efficient for management of *T. absoluta*. *M. anisopliae* has been reported to affect both egg and first larval stage of *T. absoluta* (Santi *et al*., 2011; Inanli *et al*., 2012) and has been reported to parasitize eggs of *T. absoluta* (Pires *et al*., 2009).

Attempts have been made for the search of more effective EPF species to control *T. absoluta* in Tanzania (Zekeya *et al*., 2019). However, only two fungal isolates (A-Tz1 and A-Tz2) of *Aspergillus oryzae* have been evaluated against *T. absoluta* in Tanzania so far.The fungus caused 70% larval mortality 3 days post inoculation and consequently inhibited pupation by 84.5% and adult emergence by 74.4%. Nevertheless, information on the pathogenic effects of native EPF isolates from different *T. absoluta* hot-spot areas in Tanzania and their usage against the pest is missing. Therefore, in the present study, the key isolated EPF *Beauveriaspp* and *Metarhizium spp* that were discovered from different soil types are indexed for their biological efficiency and evaluated for their pathogenicity against *T. absoluta*.

3.3 Materials and Methods

3.3.1 Description of isolates

Twenty four (24) soil samples collected from different agro ecological zones of Tanzania were taken to Sokoine University of Agriculture for culturing, isolation of fungi, incubation, DNA extraction and PCR analysis. EPF were isolated from soil samples collected from twenty four (24) tomato farms infested by *T. absoluta* in Arusha and Morogoro. The soil samples were collected around the root zone of the affected plants Stratified sampling method was used during soil sample collection. At each sampling location, a total number of soil samples were sampled and mixed to form one sample then 10grams of the mixture were taken to represent the five samples. The soil was taken from

0-15 cm deep using soil auger 5cm diameter. Plastic containers were used to keep collected soil samples. EPF were isolated from soil samples using semi-selective medium as per Meyling and Eilenberg (2006). Cultures were maintained at 25ºC in dark and examined after four to seven days the growths of fungi were confirmed and conidia characteristic of EPF was transferred into PDA, where purification was done. All isolates were subsequently propagated from a single conidium. A suspension (1µl) containing a low density of conidia in distilled water spread onto PDA. A single germinated conidium was separated from other conidia and identified by compound microscope, using a piece of medium encompassing only the target conidium were removed and transferred to PDA, and the culture maintained at room temperature.

DNA from the EPF mycelia was extracted using TES extraction buffer by the method described by Mahuku (2004) with some modifications. Fungal PCR amplification were completed using two primer sets, the ITS 1 and ITS 4 , to amplify the Internal Transcribed spacer (ITS) region (White *et al*., 1990). The PCR conditions which was used to amplify the ITS region and the Actin gene were 94°C for 4 min for initial denaturation followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C forITS4 and 60^0C for ITS1 for 30 s, and elongation at 72°C for 1 min followed by another cycle of final elongation at 72°C for 4 min.

PCR was performed with ITS1&ITS4 marker in 19 total reactions. PCR reaction carried out with 25µlreactions containing 1µl of both reverse and forward primer,12.5µl of 2xTaq mastermix,9µl of PCR water and 1.5µl of DNA template. The expected sizes were 580to 750bp amplifying internal transcribed region gene. . The PCR total volume reaction was 20 with the following components.

Then PCR products were transferred to South Africa at Inquaba laboratory. Out of sixteen isolates which were sent to laboratory for sequencing only ten specie turned to be identified as entomopathogenic fungi.

3.3.2 Establishment of *Tuta absoluta* cohort

Culture of *T. absoluta* was initiated from soil and leaves collected from farmer's fields, infected leaves then incubated at 25±2°C, relative humidity (RH) 75±5% and photoperiod of 12:12 (L: D) hr(Biondi *et al*., 2012), The emerged *T. absoluta* collected and reared onto tomato plants in the green house. Then twenty adult pairs were introduced to the rearing cages containing tomato seedlings 40-45 days from sowing date. At this age the plants are suitable for egg laying. After 24hrs all adults of *T. absoluta* were removed and thereafter, the four larva instars from the infested plant leaves were collected and be used to conduct various experiments.

3.3.3 Preparation of entomopathogenic fungi concentrations

Culturing and isolation were made from twenty four (24) soil samples collected around root zones of tomato plants from tomato farms infested by *T. absoluta* in Arusha and Morogoro. Cultures were maintained at 25^{o}C in dark and examined after four to seven days the growths of fungi were confirmed and conidia characteristic of EPF was transferred into PDA, where purification was done. All isolates were subsequently propagated from a single conidium. A suspension (1µl) containing a low density of conidia in distilled water spread onto PDA. A single germinated conidium was separated from other conidia and identified by compound microscope, using a piece of medium encompassing only the target conidium were removed and transferred to PDA, and the culture maintained at room temperature.After 10 - 15 days isolates of conidial matured on PDA in Petri dishes, spores were gently scrapped from the media by suspending into 10

ml of distilled water with 0.1% Triton X-100 to make a stock suspension. From each stock suspension of isolates, concentrations were assessed by counting spores using haemocytometer neubaeur (Manfield, German). After mixing suspension in one beaker the stock solution concentration at 1×10^{10} conidial/ml. in which working concentrations was prepared by the addition of water followed by counting spore using haemocytometer neubaeur at temperature range of 25.5°C under stereo microscope to obtain 1.0×10^{8}, 1.0×10^{7} and 1.0×10^{6} conidia/ml for EPF test against stages of *T. absoluta*.

3.3.4 Pathogenicity of isolates against *T. absoluta* larva

Pathogenic effect of isolates against second instar larvae was conducted in the laboratory at 19.5°C and 50% RH, 30.4°C and 70% RH, (the lowest and optimal, respectively). The factorial experiment design was used were by tomato leaflets from screen house (6 × 7 cm area) were used as experimental units that were dipped into (1.0×10^{6} , 1.0×10^{7} and 1.0×10^{8} conidia/ml) of isolates and control (water containing 0.1% Triton X-100). Treated leaflets were blotted at room temperature for elimination of excess water then was placed in Petri dishes (21 cm diameter) lined with wet paper towel and cotton covering the petiole to prevent leaf dehydration. Then, ten (10) *T. absoluta* larvae were inoculated separately into each of three treated leaflets and control. Each concentration was replicated three times with10 larvae. Treated leaflets was used only once at the beginning of the experiment. After 24 hrs, when treated leaflets are depleted, treated larvae were fed on fresh untreated leaves. Dead larvae were placed on another Petri dish lined with moist paper towel for re-isolation of fungus. Data from the study was recorded at interval of 24 h and trials were monitored up to the completion of *T. absoluta* life cycle and lifespan.

3.4 Data to Collection

The following data were collected from the study:

Larval survival duration, number of dead larvae, number of larvae active (larvae treated but persisted), and number of adults emerged from total treated pupae, number of adult emerged.

3.5 Data Analysis

Data were analysed with the General Linear Model procedure of SAS, version 9.1 (SAS Institute, Cary, NC, USA) and tested for normality and homogeneity of variance. Mortality rate and adult life span were transformed and subjected to analysis of variance (ANOVA) whereas larval mortality and adult life span were subjected to Kruskal Wallis.

3.5.1 Results

Table 3.1: Effect of entomopathogenic fungi, conidial concentration and incubation duration on *T. absoluta* survival and mortality

	Dead larvae	Active pupa	Active adults
	Percentage (%)	**Percentage (%)**	**Percentage (%)**
Factor A (Entomopathogenic fungi)			
Metarhizium anisopliae	45.9±2.39h	54.1±2.39a	54.1±2.39a
Aspergillus flavus	31.7±1.68g	68.3±1.68b	68.3±1.68b
Cladosporium oxysporum	29.3±1.52f	70.7±1.52c	70.7±1.52c
Aspergillus oryzae	28.6±1.48f	71.4±1.48c	71.4±1.48c
Aspergillus fimeti	23.8±1.34e	76.2±1.34d	76.2±1.34d
Aspergillus terrius	20.4±1.24d	79.6±1.24e	79.6±1.24e
Penicillium polonicum	19.8±1.20d	80.2±1.20e	80.2±1.20e
Cladosporium cladosporioides	14.1±1.04c	86.0±1.04f	86.0±1.04f
Penicillium pinophilum	8.5±0.73b	91.5±0.73g	91.5±0.73g
Geotrichum candidum	1.8±0.30a	98.2±0.30h	98.2±0.30h
Mean	22.39	77.61	77.61
P-value	<0.001	<0.001	<0.001
Factor B (Conidial concentration - ml)			
1.0×10^{6}	47.7±1.04d	52.3±1.04a	52.3±1.04a
1.0×10^{7}	28.1±0.80c	71.9±0.80b	71.9±0.80b
1.0×10^{8}	13.8±0.56b	86.2±0.56c	86.2±0.56c
Control (0.1 Tween solution)	0.0±0.00a	100.0±0.00d	100.0±0.00d
Mean	22.39	77.61	77.61
P-value	<0.001	<0.001	<0.001
Factor C (Incubation duration)			
Day 1	9.4±0.85	90.6±0.85d	90.6±0.85d
Day 2	16.3±1.17b	83.7±1.17c	83.7±1.17c
Day 3	22.9±1.34c	77.1±1.34b	77.1±1.34b
Day 4	26.4±1.38d	73.6±1.38a	73.6±1.38a
Day 5	27.1±1.38d	72.9±1.38a	72.9±1.38a
Day 6	27.3±1.38d	72.7±1.38a	72.7±1.38a
Day 7	27.3±1.38d	72.7±1.38a	72.7±1.38a
Mean	22.39	77.61	77.61
P-value	<0.001	<0.001	<0.001
Interaction			
A x B	<0.001	<0.001	<0.001
A x C	<0.001	<0.001	<0.001
B x C	<0.001	<0.001	<0.001
A x B x C	<0.001	<0.001	<0.001

Means bearing the same letter(s) within a column are not significantly different at $p = 0.05$

3.5.2 *Tuta absoluta* larvae mortality.

The results (Figure 3.1) highest larvae mortality (53.3%) was recorded on *Metarhizium anisopliae* at 6th and 7th day after incubation, whereas no dead larvae (0%) was recorded on *Geotrichum candidum* at the initial incubation time (day 1), followed by the 2nd day of incubation (0.3%).

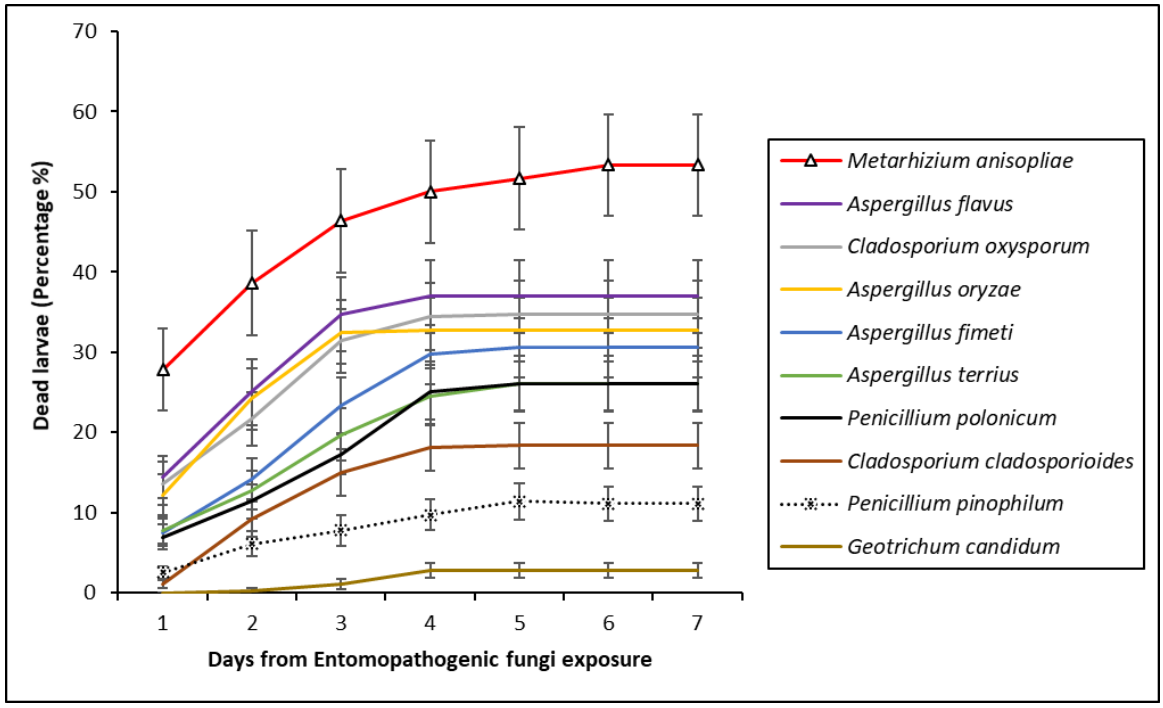

Figure 3.1: *Tuta absoluta* larvae mortality following seven days of entomopathogenic fungi exposure

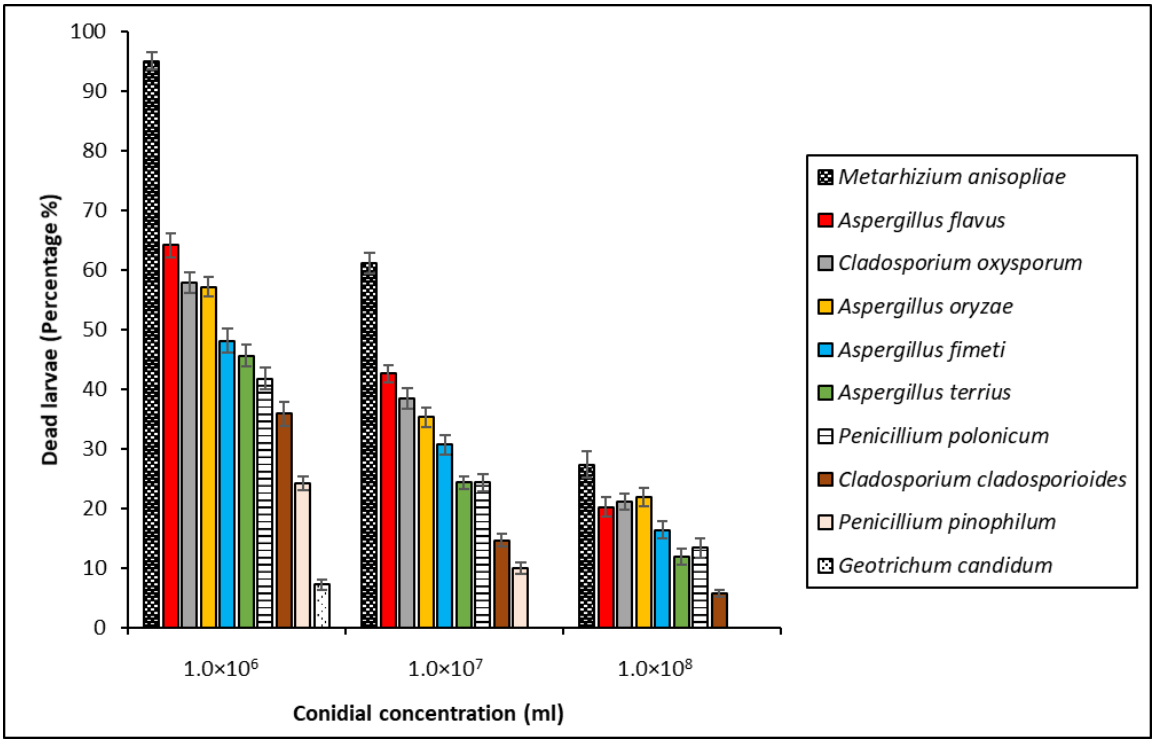

Figure 3.2: **Interaction effect of entomopathogenic fungi and conidial concentration on *T. absoluta* larvae mortality**

Table 3.2: **Interaction effect of entomopathogenic fungi, conidial concentration and days of exposure on *T. absoluta* larvae mortality**

	Concentrations (conidia/ml)	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Metarhizium anisopliae	1.0×10^6	71.1±3.09[a]	94.4±2.94	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
	1.0×10^7	38.9±3.09	52.2±2.78	61.1±3.09	66.7±3.33	68.9±3.51	70.0±3.33	70.0±3.33
	1.0×10^8	1.1±1.11	7.8±3.24	24.4±1.76	33.3±1.67	37.8±2.78	43.3±3.33	43.3±3.33
Aspergillus flavus	1.0×10^6	33.3±3.73	55.6±2.42	71.1±2.61	72.2±2.22	72.2±2.22	72.2±2.22	72.2±2.22
	1.0×10^7	23.3±2.36	37.8±4.65	45.6±2.94	47.8±2.22	47.8±2.22	47.8±2.22	47.8±2.22
	1.0×10^8	1.1±1.11	6.7±2.89	22.2±3.24	27.8±2.22	27.8±2.22	27.8±2.22	27.8±2.22
Cladosporium oxysporum	1.0×10^6	35.6±4.44	47.8±4.34	63.3±2.36	64.4±2.42	64.4±2.42	64.4±2.42	64.4±2.42
	1.0×10^7	14.4±2.94	28.9±2.00	38.9±2.61	46.7±2.89	46.7±2.89	46.7±2.89	46.7±2.89
	1.0×10^8	4.4±1.76	10.0±2.36	23.3±2.36	26.7±1.67	27.8±1.47	27.8±1.47	27.8±1.47
Aspergillus oryzae	1.0×10^6	34.4±3.38	54.4±3.38	62.2±2.78	62.2±2.78	62.2±2.78	62.2±2.78	62.2±2.78
	1.0×10^7	13.3±2.89	33.3±3.73	40.0±2.89	40.0±2.89	40.0±2.89	40.0±2.89	40.0±2.89
	1.0×10^8	1.1±1.11	8.9±2.61	27.8±2.22	28.9±2.00	28.9±2.00	28.9±2.00	28.9±2.00
Aspergillus fimeti	1.0×10^6	21.1±3.09	33.3±3.33	51.1±2.00	57.8±2.22	57.8±2.22	57.8±2.22	57.8±2.22
	1.0×10^7	7.8±2.22	20.0±2.36	31.1±2.00	38.9±2.00	38.9±2.00	38.9±2.00	38.9±2.00
	1.0×10^8	1.1±1.11	3.3±2.36	11.1±3.89	22.2±2.22	25.6±1.76	25.6±1.76	25.6±1.76
Aspergillus terrius	1.0×10^6	21.1±2.00	31.1±3.09	44.4±3.38	55.6±1.76	55.6±1.76	55.6±1.76	55.6±1.76
	1.0×10^7	10.0±2.36	16.7±2.89	27.8±1.47	28.9±1.11	28.9±1.11	28.9±1.11	28.9±1.11

	1.0×10^{8}	0.0±0.00	3.3±3.33	6.7±3.73	13.3±2.36	20.0±1.67	20.0±1.67	20.0±1.67
Penicillium polonicum	1.0×10^{6}	20.0±2.36	27.8±2.78	35.6±2.42	52.2±2.22	52.2±2.22	52.2±2.22	52.2±2.22
	1.0×10^{7}	7.8±2.22	10.0±2.89	23.3±2.36	32.2±1.47	32.2±1.47	32.2±1.47	32.2±1.47
	1.0×10^{8}	0.0±0.00	7.8±3.24	10.0±2.89	15.6±1.76	20.0±5.27	20.0±5.27	20.0±5.27
Cladosporium cladosporioides	1.0×10^{6}	3.3±1.67	27.8±3.64	42.2±2.78	44.4±1.76	44.4±1.76	44.4±1.76	44.4±1.76
	1.0×10^{7}	1.1±1.11	7.8±2.22	13.3±2.36	20.0±1.67	20.0±1.67	20.0±1.67	20.0±1.67
	1.0×10^{8}	0.0±0.00	1.1±1.11	4.4±1.76	7.8±1.47	8.9±1.11	8.9±1.11	8.9±1.11
Penicillium pinophilum	1.0×10^{6}	6.7±2.36	21.1±2.00	25.6±1.76	26.7±2.61	31.1±1.67	28.9±1.11	28.9±1.11
	1.0×10^{7}	3.3±1.67	3.3±1.67	5.6±2.42	12.2±2.22	14.4±1.76	15.6±1.76	15.6±1.76
	1.0×10^{8}	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
Geotrichum candidum	1.0×10^{6}	0.0±0.00	1.1±1.11	4.4±2.42	11.1±2.00	11.1±2.00	11.1±2.00	11.1±2.00
	1.0×10^{7}	0.0±0.00	0.0±0	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
	1.0×10^{8}	0.0±0.0	0.0±0	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
L.S.D	5.442							
p-value	<0.001							

[a] Meanpercentage±S.E; S.E = Standard error of mean; L.S. D = Least significant difference. The difference between mean percentages lower than L.S.D value are considered not significantly different at $p = 0.05$

3.5.3 *Tuta absoluta* active pupa following seven days of entomopathogenic fungi exposure

The results (Figure 3.3) show that, significantly (p<0.001) highest active pupa (100%) was recorded on *Geotrichum candidum* at the initial incubation time (day 1), whereas the lowest active pupa (46.7%) was recorded on *Metarhizium anisopliae* at 6th and 7th day of incubation.

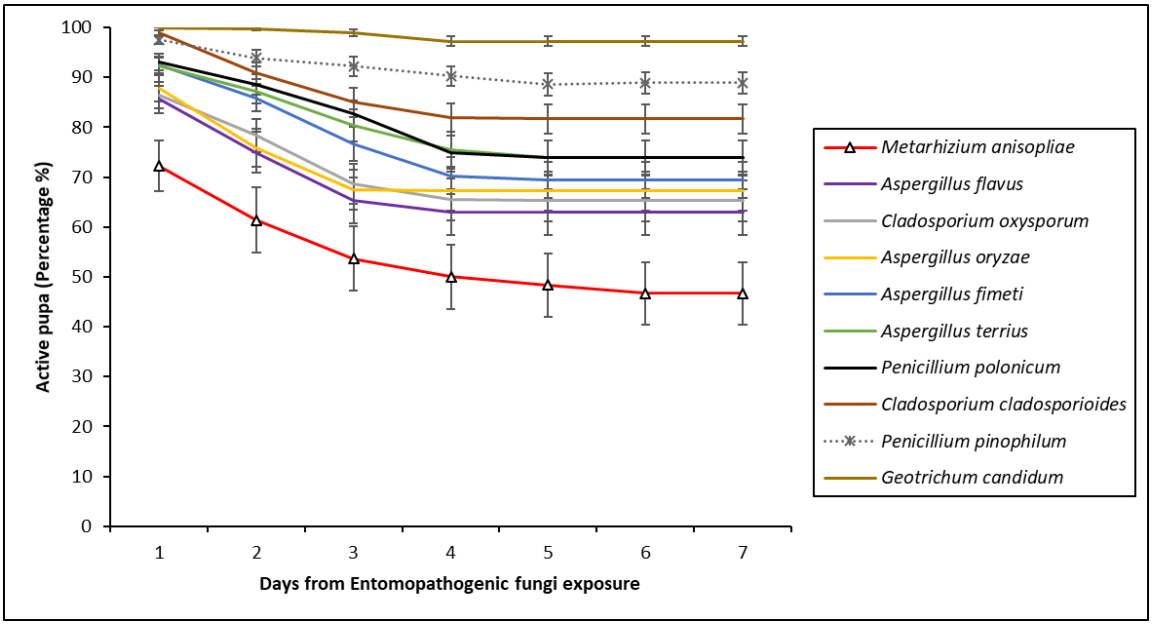

Figure 3.3: ***T. absoluta*** **active pupa following seven days of entomopathogenic fungi exposure**

3.5.4 Interaction effect of entomopathogenic fungi and conidial concentration on *T. absoluta* active pupa

Highest active pupa (94.3%) was recorded on *Cladosporium cladosporioides* sat 1.0×10^{8}, conidial concentrations (Figure 3.4). Whereas, the lowest active pupa (4.9%) was recorded on *Metarhizium anisopliae*at 1.0×10^{6}conidial concentrations.

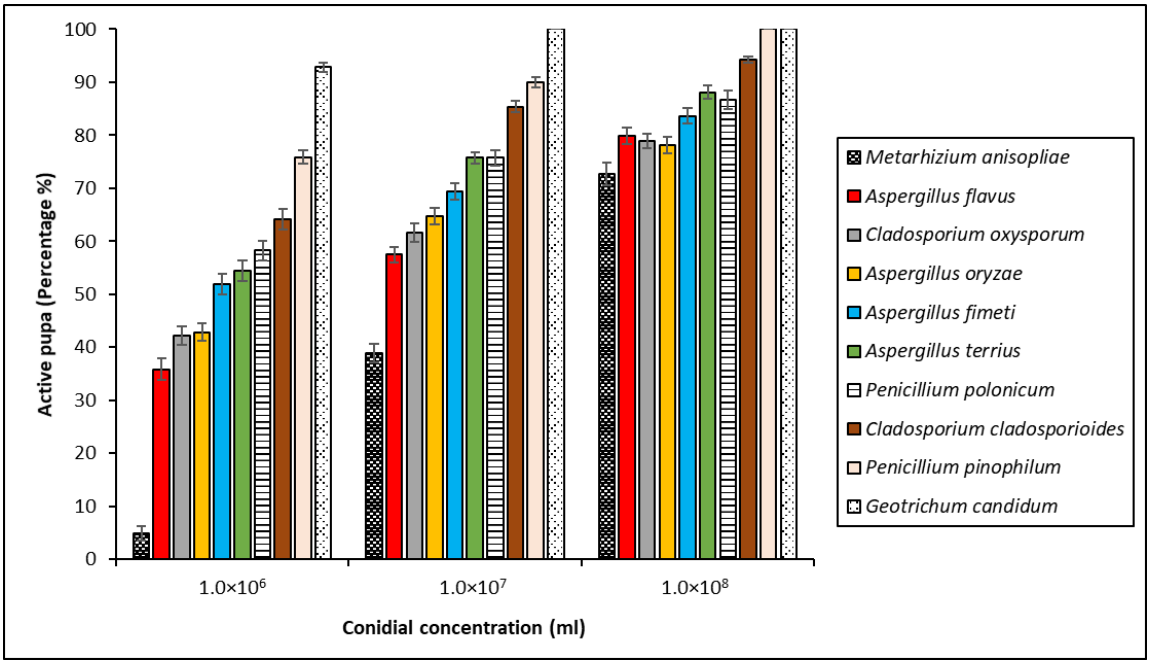

Figure 3.4: **Interaction effect of entomopathogenic fungi and conidial concentration on *T. absoluta* active pupa**

Table 3.3: Interaction effect of entomopathogenic fungi, conidial concentration and days of exposure on *T. absoluta* active pupa

	Concentrations (conidia/ml)	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Metarhizium anisopliae	1.0×10^6	28.9±3.09	5.6±2.94	0.0±0.00	0.00±0.00	0.0±0.00	0.0±0.00	0.0s±0.00
	1.0×10^7	61.1±3.09	47.8±2.78	38.9±3.09	33.3±3.33	31.1±3.51	30.0±3.33	30.0±3.33
	1.0×10^8	98.9±1.11	92.2±3.25	75.6±1.76	66.7±1.67	62.2±2.78	56.67±3.33	56.67±3.33
Aspergillus flavus	1.0×10^6	66.7±3.73	44.4±2.42	28.9±2.61	27.8±2.22	27.8±2.22	27.8±2.22	27.8±2.22
	1.0×10^7	76.7±2.36	62.2±4.65	54.4±2.94	52.2±2.22	52.2±2.22	52.2±2.22	52.2±2.22
	1.0×10^8	98.9±1.11	93.3±2.89	77.8±3.24	72.2±2.22	72.2±2.22	72.2±2.22	72.2±2.22
Cladosporium oxysporum	1.0×10^6	64.4±4.44	52.2±4.34	36.7±2.36	35.6±2.42	35.6±2.42	35.6±2.42	35.6±2.42
	1.0×10^7	85.6±2.94	71.1±2.00	61.1±2.61	53.3±2.89	53.3±2.89	53.3±2.89	53.3±2.89
	1.0×10^8	95.6±1.76	90.0±2.36	76.7±2.36	73.3±1.67	72.2±1.47	72.2±1.47	72.2±1.47
Aspergillus oryzae	1.0×10^6	65.6±3.38	45.6±3.38	37.8±2.78	37.8±2.78	37.8±2.78	37.8±2.78	37.8±2.78
	1.0×10^7	86.7±2.89	66.7±3.73	60.0±2.89	60.0±2.89	60.0±2.89	60.0±2.89	60.0±2.89
	1.0×10^8	98.9±1.11	91.1±2.61	72.2±2.61	71.1±2.00	71.1±2.00	71.1±2.00	71.1±2.00
Aspergillus fimeti	1.0×10^6	78.9±3.09	66.7±3.33	48.9±2.00	42.2±2.22	42.2±2.22	42.2±2.22	42.2±2.22
	1.0×10^7	92.2±2.22	80.0±2.36	68.9±2.00	61.1±2.00	61.1±2.00	61.1±2.00	61.1±2.00
	1.0×10^8	98.9±1.11	96.7±2.36	88.9±3.89	77.8±2.22	74.4±1.76	74.4±1.76	74.4±1.76
Aspergillus terrius	1.0×10^6	78.9±2.00	68.9±3.09	55.6±3.38	44.4±1.76	44.4±1.76	44.4±1.76	44.4±1.76
	1.0×10^7	90.0±2.36	83.3±2.89	72.2±1.47	71.1±1.11	71.1±1.11	71.1±1.11	71.1±1.11
	1.0×10^8	100.0±0.00	96.7±3.33	93.3±3.73	86.7±2.36	80.0±1.67	80.0±1.67	80.0±1.67

Penicillium polonicum	1.0×10^{6}	80.0±2.36	72.2±2.78	64.4±2.42	47.8±2.22	47.8±2.22	47.8±2.22	47.8±2.22
	1.0×10^{7}	92.2±2.22	90.0±2.89	76.7±2.36	67.8±1.47	67.8±1.47	67.8±1.47	67.8±1.47
	1.0×10^{8}	100.0±0.00	92.2±3.24	90.0±2.89	84.4±1.76	80.0±5.27	80.0±5.27	80.0±5.27
Cladosporium cladosporioides	1.0×10^{6}	96.7±1.67	72.2±3.64	57.8±2.78	55.6±1.76	55.6±1.76	55.6±1.76	55.6±1.76
	1.0×10^{7}	98.9±1.11	92.2±2.22	86.7±2.36	80.0±1.67	80.0±1.67	80.0±1.67	80.0±1.67
	1.0×10^{8}	100.0±0.00	98.9±1.11	95.6±1.76	92.2±1.47	91.1±1.11	91.1±1.11	91.1±1.11
Penicillium pinophilum	1.0×10^{6}	93.3±2.36	78.9±2.00	74.4±1.76	73.3±1.67	68.9±2.61	71.1±1.11	71.1±1.11
	1.0×10^{7}	96.7±1.67	96.7±1.67	94.4±2.42	87.8±1.76	85.6±1.76	84.4±1.76	84.4±2.22
	1.0×10^{8}	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
Geotrichum candidum	1.0×10^{6}	100.0±0.00	98.9±1.11	95.6±2.42	88.9±2.00	88.9±2.00	88.9±2.00	88.9±2.00
	1.0×10^{7}	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
	1.0×10^{8}	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
L.S.D	5.442							
p-value	<0.001							

[a] Meanpercentages'; S.E = Standard error of mean; L.S. D = Least significant difference. The difference between mean percentages lower than L.S.D value are considered not significantly different at $p = 0.05$

3.5.5 *Tuta absoluta* adults' survival following seven days of entomopathogenic fungi exposure

High adults' survival (100%) was recorded on *Geotrichum candidum* at the initial incubation time (day 1), whereas low adults' survival (46.7%) was recorded on *Metarhizium anisopliae* at 6th and 7th day of incubation.

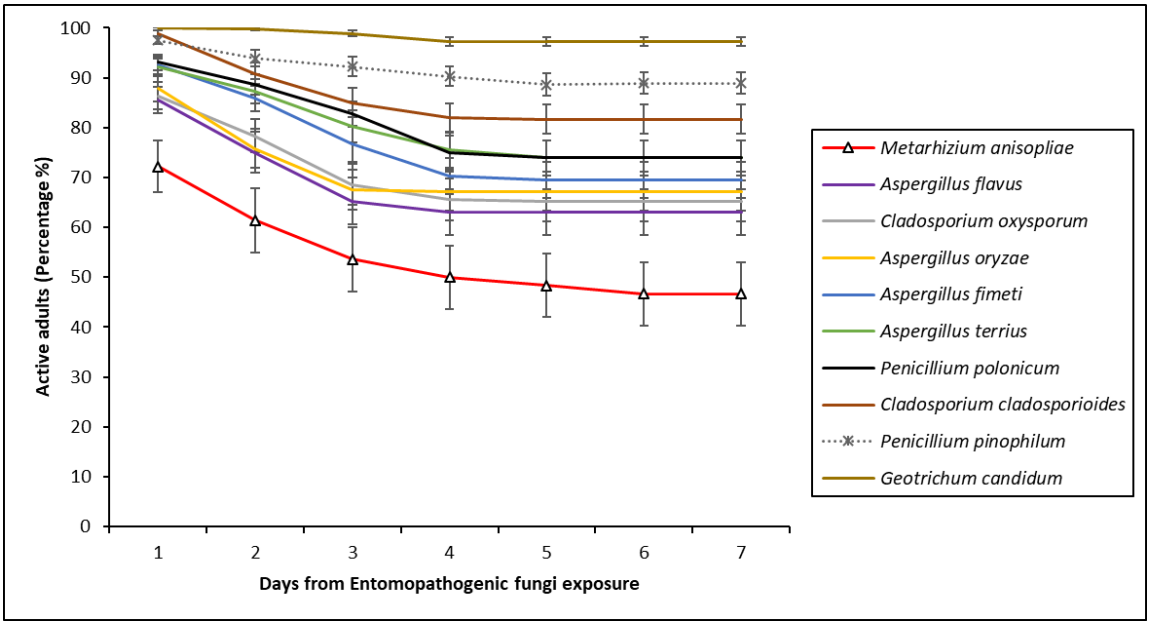

Figure 3.5: ***T. absoluta* adults' survivalfollowing seven days of entomopathogenic fungi exposure**

3.5.6 Interaction effect of entomopathogenic fungi and conidial concentration on *T. absoluta* adults' survival

High adults' survival (94.3%) was recorded on *Cladosporium cladosporioides* sat 1.0×10^{8}, conidial concentrations (Figure 3.6). Whereas, the lowest adults 'survival (4.9%) was recorded on *Metarhizium anisopliae* at 1.0×10^{6}conidial concentrations.

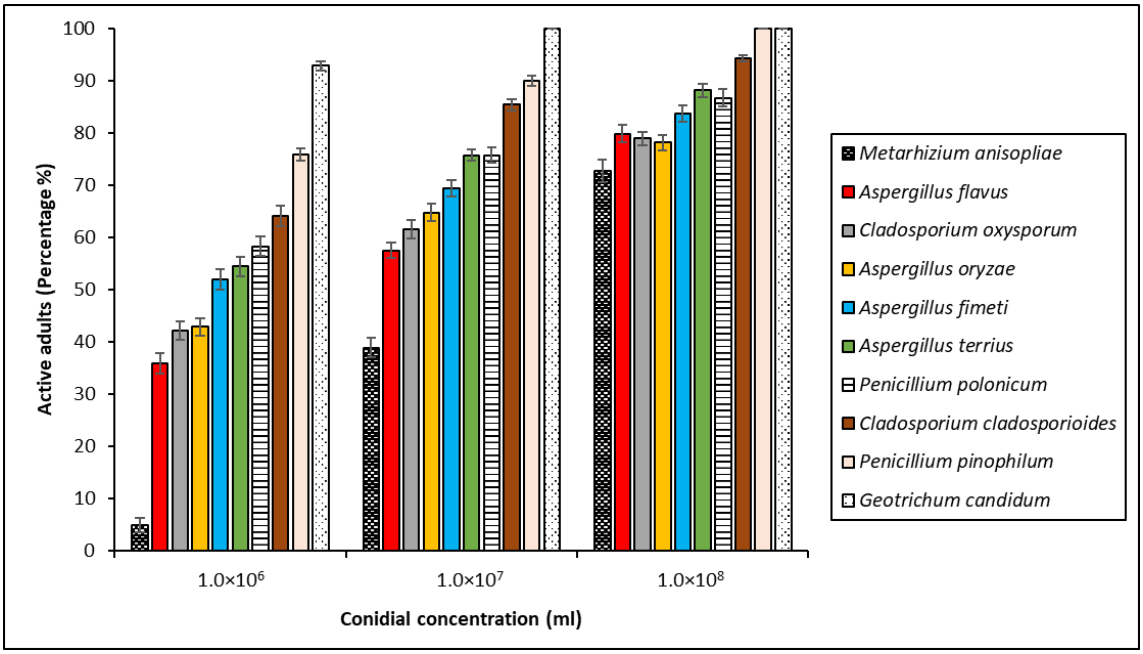

Figure 3.6: **Interaction effect of entomopathogenic fungi and conidial concentration on *T. absoluta* adults' survival.**

Table 3.4: **Interaction effect of entomopathogenic fungi, conidial concentration and days of exposure on *T. absoluta* adults' survival**

	Concentrations (conidia/ml)	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Metarhizium anisopliae	1.0×10^6	28.9±3.09	5.6±2.94	0.0±0.00	0.00±0.00	0.0±0.00	0.0±0.00	0.0±0.00
	1.0×10^7	61.1±3.09	47.8±2.78	38.9±3.09	33.3±3.33	31.1±3.51	30.0±3.33	30.0±3.33
	1.0×10^8	98.9±1.11	92.2±3.25	75.6±1.76	66.7±1.67	62.2±2.78	56.67±3.33	56.67±3.33
Aspergillus flavus	1.0×10^6	66.7±3.73	44.4±2.42	28.9±2.61	27.8±2.22	27.8±2.22	27.8±2.22	27.8±2.22
	1.0×10^7	76.7±2.36	62.2±4.65	54.4±2.94	52.2±2.22	52.2±2.22	52.2±2.22	52.2±2.22
	1.0×10^8	98.9±1.11	93.3±2.89	77.8±3.24	72.2±2.22	72.2±2.22	72.2±2.22	72.2±2.22
Cladosporium oxysporum	1.0×10^6	64.4±4.44	52.2±4.34	36.7±2.36	35.6±2.42	35.6±2.42	35.6±2.42	35.6±2.42
	1.0×10^7	85.6±2.94	71.1±2.00	61.1±2.61	53.3±2.89	53.3±2.89	53.3±2.89	53.3±2.89
	1.0×10^8	95.6±1.76	90.0±2.36	76.7±2.36	73.3±1.67	72.2±1.47	72.2±1.47	72.2±1.47
Aspergillus oryzae	1.0×10^6	65.6±3.38	45.6±3.38	37.8±2.78	37.8±2.78	37.8±2.78	37.8±2.78	37.8±2.78
	1.0×10^7	86.7±2.89	66.7±3.73	60.0±2.89	60.0±2.89	60.0±2.89	60.0±2.89	60.0±2.89
	1.0×10^8	98.9±1.11	91.1±2.61	72.2±2.61	71.1±2.00	71.1±2.00	71.1±2.00	71.1±2.00
Aspergillus fimeti	1.0×10^6	78.9±3.09	66.7±3.33	48.9±2.00	42.2±2.22	42.2±2.22	42.2±2.22	42.2±2.22
	1.0×10^7	92.2±2.22	80.0±2.36	68.9±2.00	61.1±2.00	61.1±2.00	61.1±2.00	61.1±2.00
	1.0×10^8	98.9±1.11	96.7±2.36	88.9±3.89	77.8±2.22	74.4±1.76	74.4±1.76	74.4±1.76
Aspergillus terrius	1.0×10^6	78.9±2.00	68.9±3.09	55.6±3.38	44.4±1.76	44.4±1.76	44.4±1.76	44.4±1.76
	1.0×10^7	90.0±2.36	83.3±2.89	72.2±1.47	71.1±1.11	71.1±1.11	71.1±1.11	71.1±1.11
	1.0×10^8	100.0±0.00	96.7±3.33	93.3±3.73	86.7±2.36	80.0±1.67	80.0±1.67	80.0±1.67

Penicillium polonicum	1.0×10^{6}	80.0±2.36	72.2±2.78	64.4±2.42	47.8±2.22	47.8±2.22	47.8±2.22	47.8±2.22
	1.0×10^{7}	92.2±2.22	90.0±2.89	76.7±2.36	67.8±1.47	67.8±1.47	67.8±1.47	67.8±1.47
	1.0×10^{8}	100.0±0.00	92.2±3.24	90.0±2.89	84.4±1.76	80.0±5.27	80.0±5.27	80.0±5.27
Cladosporium cladosporioides	1.0×10^{6}	96.7±1.67	72.2±3.64	57.8±2.78	55.6±1.76	55.6±1.76	55.6±1.76	55.6±1.76
	1.0×10^{7}	98.9±1.11	92.2±2.22	86.7±2.36	80.0±1.67	80.0±1.67	80.0±1.67	80.0±1.67
	1.0×10^{8}	100.0±0.00	98.9±1.11	95.6±1.76	92.2±1.47	91.1±1.11	91.1±1.11	91.1±1.11
Penicillium pinophilum	1.0×10^{6}	93.3±2.36	78.9±2.00	74.4±1.76	73.3±1.67	68.9±2.61	71.1±1.11	71.1±1.11
	1.0×10^{7}	96.7±1.67	96.7±1.67	94.4±2.42	87.8±1.76	85.6±1.76	84.4±1.76	84.4±2.22
	1.0×10^{8}	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
Geotrichum candidum	1.0×10^{6}	100.0±0.00	98.9±1.11	95.6±2.42	88.9±2.00	88.9±2.00	88.9±2.00	88.9±2.00
	1.0×10^{7}	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
	1.0×10^{8}	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00	100.0±0.00
L.S.D	5.442							
p-value	<0.001							

[a] Meanpercentages'; S.E = Standard error of mean; L.S. D = Least significant difference. The difference between mean percentages lower than L.S.D value are considered not significantly different at $p = 0.05$

3.6 Discussion

3.6.1 Effect of entomopathogenic fungi, conidial concentration and incubation duration on *T. absoluta* survival and mortality

Pathogenicity of the tested entomopathogenic fungi (EPFs) and conidial concentrations against motile stages of *T. absoluta* with respect to incubation time shown in (Table 3.1) revealed significant difference among the tested EPFs, conidial concentrations and incubation time on percentage dead larvae, active pupa and active adults whereby *Metarhizium anisopliae* recorded the highest percentage of dead larvae (45.9%), lowest percentage of active pupa (54.1%) and active adults (54.1%) than other tested EPFs. Whereas, *Geotrichum candidum* had the lowest percentage of dead larvae (1.8%), highest percentage of active pupa (98.2%) and active adults (98.2%) than other tested EPFs (Maina *et al.,* 2018).

Highest percentage of dead larvae (47.7%), lowest percentage of active pupa (52.3%) and active adults (52.3%) was recorded on 1.0×10^{6}conidial concentrations than other tested conidial concentrations. Whereas, control (0.1 Tween solutions) recorded no dead larvae (0%), highest percentage of active pupa (100%) and active adults (100%) than other tested conidial concentrations.

The percentage of dead larvae increased with incubation time, while active pupa and adults decreased with incubation time. The lowest percentage of dead larvae (9.4%), highest percentage of active pupa (90.6) and adults (90.6) were recorded at the initial time (day 1), whereas the highest percentage of dead larvae (27.3%), lowest percentage of active pupa (72.7%) and adults (72.7%) were recorded at the 7^{th} day of incubation. Highest larvae mortality was recorded on *Metarhizium anisopliae* both at 1.0×10^{6}(95.1%), 1.0×10^{7}(61.1%) and 1.0×10^{8}(27.3%) conidial concentrations (Figure. 3.2). Whereas the

lowest larvae mortality was recorded on *Geotrichum candidum* both at 1.0×10^{7} and1.0×10^{8}(0%) conidial concentrations, as well as on *Penicillium pinophilum*at 1.0×10^{8}(0%)conidial concentrations (Ndereyimana *et al.,* 2019).

Tuta absoluta larvae mortality differed significantly with the interaction between EPF, conidial concentration and incubation time (Table 3.3). The highest percentage mortality of *T. absoluta* larvae was recorded on *Metarhizium anisopliae* at day one to seven while the lowest mortality percentage of *T. absoluta* larvae was recorded on Geotrichum *candidum* at day one to seven (Silvia *et al.,* 2020).

Highest active pupa was recorded on *Cladosporium cladosporioides* with conidial concentration of 1.0×10^{8}, (Table 3.4). While the lowest active pupa were recorded on *Metarhizium anisopliae* with conidial concentrations 1.0×10^{6}.

3.6.2 Interaction effect of entomopathogenic fungi, conidial concentration and days of exposure on *T. absoluta* active pupa

*T. absoluta*active pupa differed significantly with the interaction between EPF, conidial concentration and incubation time (Figure 3.4). The highest percentage of *T. absoluta* active pupa was recorded on *Geotrichum candidum* with 1.0×10^{6}conidia/ml, *Cladosporium cladosporioides* with 1.0×10^{8}conidia/ml at the initial time (day 1)as well as *Geotrichum candidum* with 1.0×10^{7}conidia/ml, *Aspergillus terrius*, *Geotrichum candidum*, *Penicillium pinophilum* and *Penicillium polonicum* with 1.0×10^{8} at day 1-7(100%).While, no *T. absoluta* active pupa(0%)was recorded on *Metarhizium anisopliae* with 1.0×10^{6}conidia/ml at 3 - 7 days.

3.6.3 Interaction effect of entomopathogenic fungi and conidial concentration on *T. absoluta*adults' survival

Highest adults' survival percentage (94.3%) was recorded on *Cladosporium cladosporioides* at 1.0×10^8, conidial concentrations (Table 3.4) whereas, the lowest adults 'survival (4.9%) was recorded on *Metarhizium anisopliae* at 1.0×10^6conidial concentrations.

Tuta absoluta adults' survival differed significantly with the interaction between EPF, conidial concentration and incubation time (Figure 3.6). The highest percentage of *T. absoluta* adults survival (100%) was recorded on *Geotrichum candidum*with 1.0×10^6conidia/ml, concentration, *Cladosporium cladosporioides* with 1.0×10^8conidia/ml at the initial time (day 1) as well as *Geotrichum candidum* with 1.0×10^7conidia/ml, *Aspergillus terrius*, *Geotrichum candidum*, *Penicillium pinophilum* and *Penicillium polonicum* with 1.0×10^8 at day 1-7 (100%). While, no *T. absoluta*adult's survival (0%) was recorded due to EPF *Metarhizium anisopliae* with 1.0×10^6conidia/ml at 3 - 7 day. These results suggest that entomopathogenic fungi are not equally distributed in each soil sample and location where samples collected; this is due to lack of environmental conditions favorable to most entomopathogenic fungi. Unfavorable environmental condition is caused by frequent use of pesticides in most tomato farms; the use of different pesticides can kill or harm entomopathogenic fungi or other natural enemies. Most entomopathogenic fungi are found in organic soils or in tomato farms whereby little pesticides in combination of organic pesticides are used.

3.7 References

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CHAPTER FOUR

4.0 GENERAL CONCLUSION AND RECOMMENDATIONS

This study revealed that entomopathogenic fungi related to *T. absoluta* management are more abundant in Morogoro which is a hot spot area compares to Arusha, that means Morogoro weather condition favour EPFs than Arusha weather condition. *Metarhizium anisopliae* strains which were isolated from soils samples collected from tomato farms from Morogoro have higher pathogenicity effects against *T. absoluta* compared to other entomopathogenic species that were identified. Other entomopathogenic species such as *Aspergillus* may be promising species for management of *T. absoluta* at early stages especially at egg stage. Further field researches and evaluation is required to determine the potential of entomopathogenic fungi and possibly their combinations against *T. absoluta*.