FIELD EFFICACY AND ECONOMIC VIABILITY OF ENTOMOPATHOGENIC FUNGAL PRODUCTS FOR MANAGING THE TOMATO LEAFMINER (*TUTA ABSOLUTA*) IN UGANDA

BY

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DECLARATION

I, **Kabaale Fred Peter**, do hereby declare that this is my original work and has never been presented for any award in other University or higher institution of learning.

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APPROVAL

This is to certify that this work was conducted under our supervision and is now ready for submission.

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ABSTRACT

Management of the invasive tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) has primarily relied on increased application of hazardous synthetic chemical pesticides with limited success. The use of entomopathogens has been advanced among safer and more sustainable management options. The field efficacy of candidate fungal isolates, *Metarhizium anisopliae* ICIPE 20, *M. anisopliae* ICIPE 69 (Campaign®) and Dudu Acelamectin (positive check) was evaluated against *T. absoluta* on tomato through inundative application. Experiments with treatments laid in Randomised Complete Block Design and replicated thrice, were conducted during cropping seasons April – July, 2019 (first season) and December, 2019 – March, 2020 (second season) at Mukono Zonal Agricultural Research & Development Institute, Mukono district in Uganda. *Tuta absoluta* incidence, injury severity on leaves and fruits, fruit yield loss, and economic viability of test treatments were assessed. Results showed generally reduced injury severity and significantly lower fruit yield loss in treated plots compared to untreated plot in both seasons. Tomato fruit yield was higher in treated plots than the untreated plot. The marketable fruit yield gain for Dudu Acelamectin, *M. anisopliae* ICIPE 20 and *M. anisopliae* ICIPE 69 treatments was respectively 130.15, 72.14 and 55.3% during first season, and 41.21, 22.01 and 15.85% during second season. The three respective treatments had Benefit cost ratio (BCR) of 8.92, 4.31 and 3.43 during first season, and 6.30, 2.84 and 2.14 during second season. The treatments showed a degree of effectiveness and economic viability in controlling *T. absoluta* on tomato in the field. However, the efficacy of *M. anisopliae* ICIPE 20 and ICIPE 69 should be assessed further at different agro-ecological zones, dosages, formulations, large scale, and their compatibility with the pesticides commonly used in tomato production systems.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

Tomato (*Solanum lycopersicum* L.) is a widely grown and consumed vegetable fruit both in its raw and processed forms. A lot of nutritional and health benefits to humans are associated with tomato consumption, being rich in pectin, lycopene, copper, calcium, vitamin C and E, among others (Luna-Guevara *et al*., 2014; Rodrigues *et al*., 2021). Annually, it is estimated that 5 million hectares (ha) are planted worldwide and 170 million tons produced (Biondi *et al*., 2018). The leading tomato-producing countries (China, Mexico, and the United States) account for 25% of the world's tomato area cropped and 42% of tomatoes produced (Biondi *et al*., 2018). In Africa, annual production is estimated at 37.8 million tons and the main producers are Egypt, Nigeria, Tunisia and Morocco (Rwomushana *et al*., 2019). In Uganda, tomato is grown throughout the country with average yield of 6.0 tons per hectare (Dijkxhoorn *et al*., 2019). The crop is socioeconomically a source of livelihood to many rural, peri-urban and urban farmers. The total area under tomato production is estimated at 6671 hectares and the major tomato growing districts include Luweero, Mpigi, Masaka, Kayunga, Nakaseke, Mbale, Kapchwora, Kabale and Kasese (Dijkxhoorn *et al*., 2019).

In Africa, tomato production is constrained by several biotic and abiotic factors. Currently, yield loss due to biotic stress has been worsened by the invasive tomato leafminer (*Tuta absoluta (*Meyrick) (Lepidoptera: Gelechiidae)) (Rwomushana *et al*., 2019). Tomato is the primary host of this pest (Sridhar *et al*., 2015; Younes *et al*., 2018) and the larvae instars utilize all aerial parts during cryptic feeding. The larvae cause severe crop injury at all stages of crop growth cycle in form of galleries on leaves, young shoots, flowers and exit holes with frass on fruits, among others (Sridhar *et al*., 2014; Tumuhaise *et al*., 2016; Simmons *et al*., 2018). This pest is a major biotic hazard to tomato sub-sector in Uganda (Dijkxhoorn *et al*., 2019) and has enormously threatened the sustainable livelihood of tomato farmers all over eastern Africa (Aigbedion-atalor *et al*., 2019).

The menace of *T. absoluta* on tomato is global right from the native South America in Peruvian central highlands (Desneux *et al*., 2010; Biondi *et al*., 2018; Mansour *et al*., 2018). In fact, overwhelming tomato yield loss as high as 100% due to *T. absoluta* has been reported in both greenhouses and open fields (Desneux *et al*., 2010; Mansour *et al*., 2018). In addition, it is a quarantine pest which leads to tomato trade restrictions (Rwomushana *et al*., 2019). Annually, economic loss by *T. absoluta* to smallholder tomato producers in Sub-Saharan Africa and east Africa, respectively, has been estimated at US$ 791.5 million (Rwomushana *et al*., 2019) and US$ 101.1 million (Pratt *et al*., 2017).

Consequently, prevention of the losses has primarily prompted increased synthetic chemical pesticide application by farmers in Africa (Aigbedion-atalor *et al*., 2019; Rwomushana *et al*., 2019). Sadly, mostly high-risk pesticides are used in Africa due to their availability and low-cost (Rwomushana *et al*., 2019).

Besides, the efficacy of these synthetic pesticides is challenged by rapid development of insecticide resistance (Guedes *et al*., 2019) and the cryptic feeding behaviour of *T. absoluta* larvae (Retta & Berhe, 2015). More still, synthetic pesticides are associated with several untenable hazards to humans and the environment. For instance; suppression of non-target beneficial organisms, environmental pollution due to unbiodegradable constituent compounds, toxicity and poisoning to human leading to chronic health problems such as asthma, hypertension, reproductive complications and cancer (Lengai & Muthomi, 2018). Thus, to circumvent the hazards, exploring of safer non-synthetic chemical methods to design an integrated pest management (IPM) approach is being promoted against the tomato leafminer (Aynalem, 2018).

Among the safer options is the use of microbial pesticides based on strains of bacteria, viruses, nematodes, protozoa and fungi that have demonstrated potential pathogenicity and virulence to particular pests (Koul, 2011). However, there is general shortage of locally produced and registered microbial pesticides in Africa (Rwomushana *et al*., 2019). Thus, research to locally identify, evaluate, develop and commercialize strains of such entomopathogens as biopesticides become eminent.

There are candidate strains of entomopathogens that have been identified and evaluated against *T. absoluta* mainly under laboratory conditions. For instance; entomopathogenic bacteria (Gonzalez-Cabrera *et al*., 2011; Alsaedi *et al*., 2017), entomopathogenic nematodes (Batalla-carrera *et al.,* 2010; Garcia-del-pino *et al*., 2013; Shamseldean *et al*., 2014; Damme *et al*., 2016; Kamali *et al*., 2018;

Ndereyimana *et al*., 2019b;), and entomopathogenic fungi (EPF) (Shalaby *et al*., 2013; Contreras *et al*., 2014; El-Aassar et al., 2015; Lakhdari *et al*., 2016; Tadele & Emana, 2017b; Shiberu & Getu, 2018a; Alikhani *et al*., 2019; Ndereyimana *et al*., 2019a; Zekeya *et al*., 2019; Akutse *et al*., 2020a). Nevertheless, field conditions cannot be duplicated exactly in the laboratory and screenhouses, yet effectiveness of candidate microbial pesticides dependent on location specific biotic and abiotic factors (Jaronski, 2010). Accordingly, the efficacy shown by the candidate entomopathogens require validation under field conditions before development into commercial products and adoption into the IPM package for any pest. Thus, this study was based on the need to evaluate the efficacy of candidate fungal microbial biopesticides, *Metarhizium anisopliae* ICIPE 20 and *M. anisopliae* ICIPE 69 against *T. absoluta* in the field.

1.2 Statement of the problem

The use of entomopathogens such as EPF is ranked high among the safer and sustainable pest management packages (Koul, 2011; Kachhawa, 2017). Accordingly, *Metarhizium anisopliae*, whose safety aspects are well documented (Zimmermann, 2007b) is among the candidate EPF for *T. absoluta* control. In fact, studies have shown that candidate strains of *M. anisopliae* evaluated mainly under laboratory conditions demonstrated potential pathogenicity and virulence against *T. absoluta* developmental stages. For instance; (i) larva mortality caused by *M. anisopliae* PPRC-2 (Tadele & Emana, 2017b), *M. anisopliae* FCM Ar 23B3 (Ndereyimana *et al*., 2019a), *M. anisopliae* DEMI 001 (Alikhani *et al*., 2019), and *M. anisopliae* ICIPE 18, ICIPE 20 and

ICIPE 665 (Akutse *et al*., 2020a); (ii) pupa mortality caused by *M. anisopliae* (Contreras *et al*., 2014); and (iii) adult mortality caused by *M. anisopliae* ICIPE 18, ICIPE 20 and ICIPE 665 (Akutse *et al*., 2020a). Unfortunately, the laboratory conditions cannot exactly duplicate field conditions to account for the complex interactions of the biotic and abiotic factors that determine the ecological host-range and virulence of the potent EPF (Hajek & Goettel, 2007; Wraight *et al*., 2007; Jaronski, 2010). Ultimately, adequate field efficacy studies become paramount to validate the outcomes of laboratory experiments.

In practice, Rwomushana *et al*. (2019) reported the use of *M. anisopliae* ICIPE 69 to control *T. absoluta* in some African countries. However, field efficacy data is scanty as this commercial product was not specifically registered for *T. absoluta* control (Akutse *et al*., 2020b). On the other hand, Akutse *et al*. (2020a) evaluated efficacy of *M. anisopliae* ICIPE 20 and reported 100% mortality of 4th instar larvae as well as 87.5% mortality of adults under laboratory conditions. Whereas the isolate was earmarked for development into a microbial pesticide, the suggested field efficacy trials had not been performed. Therefore, there is general scarcity of field efficacy data on the effectiveness of the entomopathogenic fungal products; *M. anisopliae* ICIPE 20 and ICIPE 69 against *T. absoluta* on tomato under natural field infestation.

1.3 Justification of the study

Safer alternative pest control measures need to be explored to mitigate the risks of synthetic chemical pesticides to human and the environment (Lengai &

Muthomi, 2018; Mansour *et al*., 2018). Accordingly, the use of entomopathogens such as entomopathogenic fungi is among the options being promoted. Potential pathogenic isolates of EPF are fronted for development into microbial pesticide for sustainable *T. absoluta* control, at International Centre of Insect Physiology and Ecology (*icipe*) (Akutse *et al*., 2020b). Among the candidate isolates is *Metarhizium anisopliae* strains, of which laboratory efficacy of *M. anisopliae* ICIPE 20 is already documented (Akutse *et al*., 2020a). However, results from laboratory studies must be adequately validated in the field before candidate EPF strains are developed into commercial products, deployed, adopted and integrated into the IPM package for any pest.

It was therefore, envisaged that the findings of this study would provide reliable information on field efficacy and economic viability of *M. anisopliae* ICIPE 20 for managing *T. absoluta* on tomato in Uganda. Such information can contribute a step towards development and commercialisation of *M. anisopliae* ICIPE 20 as a potential microbial pesticide for adoption and integration into the IPM package for *T. absoluta* management.

1.4 Objectives

1.4.1 General objective

To evaluate the field efficacy and economic viability of entomopathogenic fungal-based biopesticides *M. anisopliae* ICIPE 20 and ICIPE 69 for managing *T. absoluta* on tomato.

1.4.2 Specific objectives

The specific objectives of the study were:

i. To assess the effect of *M. anisopliae* ICIPE 20 and ICIPE 69 on incidence and injury severity of *T. absoluta* on tomato under field conditions.

ii. To evaluate the effect of *M. anisopliae* ICIPE 20 and ICIPE 69 on tomato fruit yield under field conditions.

iii. To determine the economic viability of adopting *M. anisopliae* ICIPE 20 and ICIPE 69 for managing *T. absoluta* under field conditions.

1.5 Hypotheses

The study was undertaken on the following premises:

i. *Metarhizium anisopliae* ICIPE 20 and ICIPE 69 do not significantly reduce incidence and injury severity of *T. absoluta* on tomato under field conditions.

ii. Application of *M. anisopliae* ICIPE 20 and ICIPE 69 does not improve tomato fruit yield under field conditions.

iii. *Metarhizium anisopliae* ICIPE 20 and ICIPE 69 are not economically viable for managing *T. absoluta* on tomato in the field.

1.6 Significance of the study

i. The findings of this study provide the first field efficacy data to validate the laboratory efficacy of *M. anisopliae* ICIPE 20 for managing *T. absoluta* in Uganda

ii. The results also provide a baseline for future research on the field efficacy of *M. anisopliae* strains for managing *T. absoluta* in Uganda

iii. The findings also provide justification for allocating more resources to develop and commercialise *M. anisopliae* ICIPE 20 as microbial bio-pesticide for managing *T. absoluta*.

iv. The results when disseminated improve farmers' knowledge on EPF as a source of safer microbial bio-pesticides for managing pests.

1.7 Scope of the study

The study was conducted in two cropping seasons [first season (April – July, 2019) and second season (December, 2019 – March, 2020)] at Mukono Zonal Agricultural Research & Development Institute (MUZARDI). The study evaluated the efficacy and economic viability of oil formulations of entomopathogenic fungal products; *M. anisopliae* ICIPE 69 and ICIPE 20 for managing *T. absoluta* under natural infestation in the field on Rambo F1 tomato variety. The synthetic insecticide Dudu Acelamectin was used for comparison.

1.8 Operational definition of terms

Benefit cost ratio: The ratio of the monetary value of the treatment yield above the untreated plot yield to the total treatment application cost.

Crop injury: The visible or measurable symptoms and/ or signs caused by *T. absoluta*.

Marketable yield: The quantity of tomato fruits harvested per hectare, that lack *T. absoluta* injury symptoms.

Pathogenicity: The potential of the entomopathogenic fungal strain to infect and cause disease to the pest.

Virulence: The disease-causing power of the entomopathogenic fungal strain (degree of pathogenicity of entomopathogenic fungal strains)

Yield gain: The difference between fruit yield in treatment plot and the yield in untreated plot, expressed as a percentage of fruit yield in the untreated plot.

Yield loss: A proportion of the quantity of tomato fruit yield with injury by *T. absoluta*, hence rendered unfit for consumption or sale.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and geographical distribution of *Tuta absoluta*

The tomato leafminer alias South American tomato pinworm, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is reported to have originated from South America in Peruvian central highlands (Biondi *et al*., 2018). The mechanism of spread of *T. absoluta* is through egg or larvae-infested seedlings, fruits, leaves and stems being moved, packaging materials with eggs and pupae, in addition to adult moths flying to new fields and wind currents *(*Karadjova *et al.*, 2013). *Tuta absoluta* was first detected outside the native range in 2006 in eastern Spain (Desneux *et al*., 2010; Guedes *et al*., 2019). Since then, the leafminer has spread rapidly throughout Europe, Africa, Central America, and parts of Asia (https://www.cabi.org/isc/datasheet/49260).

In Africa, *T. absoluta* was first reported in 2008 in North Africa in Algeria, Morocco, Tunisia, and then Libya and Egypt in 2009 (Mansour *et al*., 2018). From North Africa, *T. absoluta* is assumed to have migrated to East Africa through Sudan via Ethiopia to Kenya in 2014 (Mansour *et al*., 2018). In Uganda, *T. absoluta* was first reported in 2015 (Tumuhaise *et al*., 2016). Currently, *T. absoluta* is throughout the country (Aigbedion-atalor *et al*., 2019). This fast widespread of the pest is a great risk to the livelihood of several tomato farming communities.

2.2 Biology and ecology of *Tuta absoluta*

The micro lepidopteron moths are most active at dusk and dawn, and rest on leaves and other plant parts during the day (Illakwahhi & Srivastava, 2017). *Tuta absoluta* is sexually active by the first day of emergence and mating pairs may take a few minutes up to six hours to uncouple (Lee *et al*., 2014). In addition, the female ability to reproduce parthenogenetically has also been reported (Megido *et al*., 2012).

2.2.1 Life cycle of *Tuta absoluta*

The leafminers undergo complete metamorphosis comprising of egg, larva, pupa and adult (Figure 1) (Desneux *et al*., 2010: Bajracharya & Bhat, 2018).

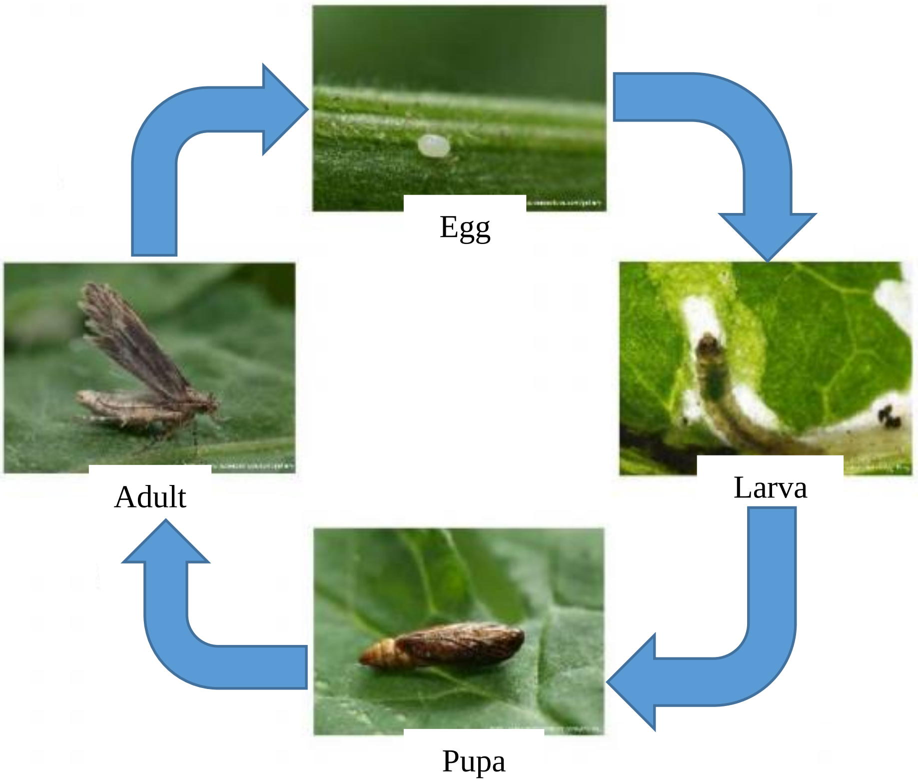

Figure 1: The generalized life cycle of *Tuta absoluta*.

Tuta absoluta is multivoltine and 10 to12 generations can be completed in a year (Desneux *et al*., 2010). The length of time for completion of developmental stages in the life-cycle is shorter in warm conditions (Cuthbertson *et al*., 2013; Rasheed *et al*., 2018a). Therefore, rapid *T. absoluta* population upsurge is expected under apposite temperature of tropical climate.

Eggs are laid on tomato leaves both on the dorsal and ventral surfaces, sometime on buds and green fruits (Toševski *et al*., 2011). They are laid singly or in small groups predominantly on young leaves, stems, sepals and least on green fruits (Garzia *et al*., 2012). *Tuta absoluta* mated females exhibit a preference to lay eggs on leaves of the plant apex compared to other parts of the tomato plant (Cherif *et al*., 2013). Desneux *et al*. (2010) reported fecundity of 250-300 eggs per female lifetime and 7 days after first mating as the most prolific oviposition period. *Tuta absoluta* eggs are cylindrical, 0.43 mm long with breadth of 0.21 mm, creamy white in colour (Toševski *et al*., 2011). The colour of eggs turns into light yellowish and eventually darkens towards hatching, around 4-6 days after laying (Bajracharya & Bhat, 2018).

The larvae that hatch out are cream in colour with characteristic dark head (Figure 1). There are four larval instars. The first larval instar is creamy white in colour but turns greenish to pinkish colour as it develops through the second instar to fourth instar (Bajracharya & Bhat, 2018). Toševski *et al*. (2011) observed whitish-gray larvae with a black head in early instars, which became pinkish-green to green with brown head in the later developmental stages. The average length of the first, second, third and fourth instar larvae is 0.63 mm, 1.59

mm, 3.86 mm and 7.19 mm, respectively (Bajracharya & Bhat, 2018). The larvae mine the leaf mesophyll, buds and fruits to feed (Desneux *et al*., 2010). The duration of development of this most damaging larval stage of *T. absoluta* has been reported to exceed 8 days (Bajracharya & Bhat, 2018). In fact, a study by Rasheed *et al*. (2018a) documented the mean development period for the first, second, third and fourth larvae instars to be 2.02 ± 0.28 days, 2.75 ± 0.65 days, 3.56 ± 0.56 days and 3.86 ± 0.64 days, respectively.

Pupation generally take place in the soil, on the leaf surface or within mines and also in sheltered stems and fruits. If larvae leave the mines pre-pupation, they build silk cocoons on the leaflets or in the soil (Garzia *et al*., 2012). The average size of the pupa is 4.88 mm long and 1.45 mm wide. The pupae are green in colour but later changes to brown colour before adult emergence. According to Bajracharya & Bhat (2018), the average duration of development of pupal stage range from 6 to 9 days.

Adult moths have silverfish-grey scales, alternating light or dark segments, distinctive black spots in anterior wing, filiform antennae and recurved labial palps. Adults on average are 5.49 mm long irrespective of sex, with an average wing span of 9.6 mm (https://www.cabi.org/isc/datasheet/49260). The longevity of adults is reported to be shorter in males compared to female *T. absoluta*. For example, the male and female *T. absoluta* lived for 6.5 and 14.8 days, respectively at 32°C (Tadele & Emana, 2017a). Similarly, adult longevity varied from 5 to 9 days and 12 to 16 days, for the males and females, respectively at 25 ± 2°C (Rasheed *et al*., 2018a).

2.2.2 Symptoms of *Tuta absoluta* incidence on tomato

Tuta absoluta attacks the tomato plant at all development stages injuring stems, leaves, flowers and fruits. Accordingly, the high number of mines, galleries, dark frass and skeletonized leaf are conspicuous indicators of *T. absoluta* infestation (Simmons *et al*., 2018). Sridhar *et al*. (2014) reported that leaf mines are visible from both sides of the leaf with dark frass inside, mined areas turned brown and dried over time, and also pinhole sized holes on fruits covered with frass especially from the stalk end of the fruits. In addition, Tumuhaise *et al*. (2016) observed tiny larvae coloured green and cream with black heads on tomato plant parts, thread-like material produced by the larvae, and the injured leaves appeared burnt. Furthermore, the folding together of leaves, death of plants, and flying of adult moths near to ground surface when plants were shaken was reported (Bajracharya *et al.*, 2016). Other indicators of *T. absoluta* infestation included shedding of inflorescence, premature fruit drop, abnormal fruit shape and reduced fruit size (https://www.cabi.org/isc/datasheet/49260).

2.2.3 Host range of *Tuta absoluta*

Although tomato (*Solanum lycopersicum* L.) is the most preferred host of *T. absoluta* (Sridhar *et al*., 2015; Younes *et al*., 2018), *T. absoluta* has been found to oviposit, develop and feed on a wide host of crops and weeds as alternative hosts (Table 1) (Cherif & Verheggen, 2019; Silva *et al*., 2021). The wider host range seem to partially explain *T. absoluta*'s survival, rapid dispersion and the control difficulties experienced within agroecosystems.

Table 1: Plant species that are hosts for *Tuta absoluta*

Family	Plant species	
	Common name	Botanical name
Cultivated		
Solanaceae	Tomato	*Solanum lycopersicum* L.
	Eggplant	*Solanum melongena* L.
	Potato	*Solanum tuberosum* L.
	Pepper	*Capsicum annuum* L.
	Tobacco	*Nicotiana tabacum* L.
	Cape gooseberry	*Physalis peruviana* L
Amaranthaceae	Beetroot	*Beta vulgaris L.*
	Spinach	*Spinacia oleracea L.*
Cucurbitaceae	Pumpkin	*Cucurbita pepo* L.
Fabaceae	Alfalfa/Lucerne	*Medicago sativa* L.,
	Common bean	*Phaseolus vulgaris* L.
	Broad bean	*Vicia faba* L.
Non-cultivated		
Solanaceae	Deadly nightshade	*Atropa belladonna* L.
	Fierce thornapple	*Datura ferox* L.
	Thornapple	*Datura stramonium* L.
	Tree tobacco	*Nicotiana glauca* Graham
	Black nightshade	*Solanum nigram* L
Amaranthaceae	Spiny amaranth	*Amaranthus spinosus* L.
	Fat hen	*Chenopodium album* L.
	Good King Henry	*Chenopodium bonus-henricus* L.
	Red goosefoot	*Chenopodium rubrum* L.
Convolvulaceae	Hedge bindweed	*Calystegia sepium* (L.) R.Br.
	Bindweed	*Convolvulus arvensis* L.
Euphorbiaceae	Jatrapha	*Jatropha curcas* L.
Geraniaceae	Herb-Robert	*Geranium robertianum* L.
Malvaceae	Common mallow	*Malva sylvestris* L.
Asteraceae	Rough Cocklebur	*Xanthium strumarium* L

Source: Cherif &Verheggen (2019)

2.2.4 Natural fluctuation of *Tuta absoluta* population

Populations of insect pests generally fluctuate depending on the balance of both naturally occurring and human induced abiotic and biotic factors that influence survival versus mortality. Cornell & Hawkins (1995) categorised the determinants of seasonal variation in pest population density into; (1) natural enemies, (2) weather, (3) inter- and intraspecific competition, (4) intrinsic development failure, (5) host plant effects, and (6) human induced factors. In fact, the mentioned factors interfere with the equilibrium of reproduction, mortality, immigration, and emigration of the pest (Naranjo & Ellsworth, 2005).

Among the abiotic factors, the seasonal weather conditions particularly rainfall and temperature exert more pronounced effect on *T. absoluta* population across regions. High rainfall has been reported to decrease *T. absoluta* population. For instance, Sylla *et al*. (2018) reported a drastic reduction from 60 to <10 adults caught per pheromone-baited trap during the rainy season. In addition, Bacci *et al*. (2018) reported a 10 fold increase of *T. absoluta* mortality during summer when the rainfall was highest compared to other seasons. Higher mortality due to rainfall was also reported for other pests such as guava psyllid (*Triozoida limbata*) (Hemiptera: Sternorrhyncha: Triozidae) (Semeão *et al*., 2012), coffee leafminer (*Leucoptera coffeella*) (Lepidoptera: Lyonetiidae) (Pereira *et al*., 2007) and *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) (Naranjo & Ellsworth, 2005). On the other hand, low temperature was reported to impact *T. absoluta* populations by lengthening the development time. For instance, the development from egg to adult emergence at 35^{o}C and 15^{o}C took 16.72 days and

58.92 days, respectively (Negi *et al*.,2020). Similarly, Cuthbertson *et al*. (2013) earlier reported 58 days, 37 days and 23 days, at 13°C, 19°C and 25°C, respectively. Moreover, studies have shown higher *T. absoluta* adult life expectancy at relatively low temperature. For instance, Krechemer & Foerster (2015) reported adult longevity at 35 days and 10 days when the temperature was 15°C and 30°C, respectively. Cuthbertson *et al*. (2013) had earlier reported adult longevity of 40 days at 10°C and 16 days at 19°C.

The environmental biotic factors particularly the natural enemies tremendously impact *T. absoluta* population by causing mortality. In fact, the decrease in population of *T. absoluta* related to an increase in population of natural enemies had been earlier reported (Medeiros *et al*., 2009). The natural enemies include Azoophytophagous mirid bug, *N. tenuis* which was reported as a predator of eggs and early larval instars of *T. absoluta* under field conditions (Sridhar *et al*., 2014). In addition, a study by Bacci *et al*. (2018) found egg parasitoid *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae); larval parasitoids *Bracon* sp. (Hymenoptera: Chalcididae) and *Pseudapanteles* sp. (Hymenoptera: Braconidae) associated to *T. absoluta* population fluctuation. In the same study, egg predators of the thrip family *Phaleothripidae*, the pirate bugs (*Blaptostethus pallescens* Poppius, *Orius tristicolor* White, and *Amphiareus constrictus* (Stål) (Heteroptera: Anthocoridae), the larvae of *Episyrphus* sp. (Diptera: Syrphidae), the adults of Acanthinus sp. (Coleoptera: Anthicidae), the ladybirds *Cycloneda sanguinea* (Linnaeus), *Psyllobora lenta* Mulsant, *Scymnus* sp. and *Hyperaspis* sp. (Coleoptera: Coccinellidae) were reported. In addition, the wasps; *Brachygastra lecheguana* (Latreille), *Polybia scutellaris* (White) and

Protonectarina sylveirae (Saussure) (Hymenoptera: Vespidae) were the larval predators reported. Therefore, understanding the natural biotic and abiotic causes of *T. absoluta* mortality can be very key in development of environmentally friendly and sustainable management packages for *T. absoluta.*

2.3 Economic importance of *Tuta absoluta* in tomato production

The damage by *T. absoluta* onto tomato plants occur throughout the entire crop cycle as the larvae penetrate the leaves, stems or fruits to feed. This pest if not efficiently managed could be responsible for up to 100% crop loss (Desneux *et al*., 2010). The effects of *T. absoluta* do not only reduce quantity but also the quality of tomato yield. The damage to the leaves reduces the photosynthetic area of the plant and consequently lower tomato yield. The injury to the stems and growing points indirectly lower yield by interfering with plant development whereas, the damage to flowers and fruits directly reduces the economic yield. In addition, drying and death of plants under heavy infestation leads to total loss (Bajracharya *et al*., 2016). Notably, the open wounds created as the larvae bore into plant tissues provide entry of pathogens that lead to fruit rot. Also, the boring into fruits leaving exit holes with frass, presence of larvae in the fruits, and abnormal fruit shape does not only make fruits less appealing to consumers but also lead to rejection of the produce in the market. Economically, the effect of *T. absoluta* does not only lower the market value of tomato fruits, but also efforts to avert the situation lead to increased tomato production costs and hence an increase in tomato price (Retta & Berhe, 2015; Rwomushana *et al*., 2019). Moreover, *T. absoluta* being a quarantine pest in European Union countries attracts trade restrictions and increase in costs for exporters to meet the mandatory phytosanitary procedures (Rwomushana *et al*., 2019).

Generally, an estimation of annual economic loss (5–10 year timescale after 2016) caused by *T. absoluta* to smallholder tomato producers in Eastern Africa for the countries already invaded by *T. absoluta* was US$ 101.1 million (Pratt *et al*., 2017), while for Sub Saharan Africa was at US$ 791.5 million (Rwomushana *et al*., 2019). Therefore, such a pest requires developing a comprehensive sustainable management approach.

2.4 Incidence and crop injury severity due to *Tuta absoluta* natural infestation of tomato in open-fields

The variation in biotic and abiotic factors that influence *T. absoluta* population across locations and seasons has resulted in variance in incidence, injury severity and crop damage (yield loss) reports. In Egypt, Moussa *et al*. (2013) reported variation in *T. absoluta* incidence across sampled fields in different seasons, where plant infestation ranged from 21 to 48% and 50 to 100% in the cropping seasons 2010 and 2011, respectively. In India, Sridhar *et al*. (2014) reported fields with up to 87% of the tomato plants infested. Meanwhile, Rasheed *et al*. (2018b) observed plant infestation ranging from 59.5 to 77.75% during the cropping season 2016-17 and 39.75 to 54.5% during the cropping season 2017-18 in the different villages. From a survey across villages in Nigeria, Ndor (2018) reported *T. absoluta* incidence ranging from 7.91 to 9.49%.

The severity of leaf damage due to *T. absoluta* reported varied for different field studies. For instance, Ndor (2018) leaf infestation ranging from 5.23 to 7.6% in the villages in Nigeria. On the other hand, Rasheed *et al*. (2018b) observed leaflet infestation in villages of India ranging from 30.68 to 45.66% during the cropping season 2016-17 and 15.92 to 22.85% during the season 2017-18.

The level of fruit damage was also reported to be different. For instance, Sridhar *et al*. (2014) observed 3.5% tomato fruits infested by *T. absoluta* in fields in India. In the same country, Rasheed *et al*. (2018b) observed fruit infestation ranging from 32.89 to 50.09% during the cropping season 2016-17 and 14.87 to 26.37% during the cropping season 2017-18 in the different villages. In Nigeria, Ndor (2018) observed tomato fruit infestation by *T. absoluta* ranging from 4.55 to 8.74%. Meanwhile in Iran, Ghaderi *et al*. (2019) reported fruit infestation due to *T. absoluta* natural infestation ranging from 24.96 to 58.2%.

The magnitude of tomato fruit yield loss (crop damage) due to *T. absoluta* varies from place to place. For instance, Mohamed *et al*. (2012) reported crop damage ranging from 80 and 100% in open fields in Sudan. In Ethiopia, Shiberu & Getu (2017) observed crop damage in unprotected farmers' fields ranging from 43.47 to 58.49% whereas, Shiberu & Getu (2018a) observed crop damage in the untreated plots ranging from 59.16 to 70.12%. Meanwhile, Ghaderi *et al*. (2019) observed crop damage level ranging from 26.33 to 59.23% across cultivars in Iran.

However, information on *T. absoluta* incidence, severity of leaf and fruit damage and crop damage (yield loss) due to *T. absoluta* on tomato in Uganda is scanty.

2.5 Management of *Tuta absoluta*

The economic loss caused by *T. absoluta* necessitates timely adoption of appropriate management strategies (Desneux *et al*., 2010; Rwomushana *et al*., 2019). The management strategies begin with timely detection of presence and monitoring density of *T. absoluta* so as to elicit appropriate control measures. Detection can be achieved using *T. absoluta* sex pheromone in traps (lures), field scouting for *T. absoluta* adults, eggs and larvae and the injury symptoms on plants (Simmons *et al*., 2018). Management of *T. absoluta* involves several control methods which are categorized as physical, cultural, chemical, botanical pesticides, semiochemical, and biological approaches. However, due to the concealed feeding habit of *T. absoluta* larvae, none of the methods is individually very effective and therefore integrating as many compatible methods as possible is preferred (Aynalem, 2018). Nonetheless, the physical control methods are impractical in an open-field (Retta & Berhe, 2015).

2.5.1 Cultural control methods for *Tuta absoluta*

There are a number of cultural practices recommended to reduce *T. absoluta* population both in the field and greenhouses. According to Simmons *et al*. (2018), field sanitation is primary in preventing initial infestation and spread of *T. absoluta*. Field sanitation involves selective removal and destruction of any infested plant material, destruction of crop residues after harvest, sanitizing all equipment used in infested fields, and destruction of all possible host plants growing within 50 m from infested fields. In addition, the alternation with nonhost plants in crop rotations, use of healthy transplants, ploughing to bury the pupa deeper, soil solarisation to kill pupae on the ground, and removal of host weeds help to control *T. absoluta* (Illakwahhi & Srivastava, 2017). Besides, allowing at least six weeks period between successive host crops and use of irrigation methods that distract eggs, larvae, and pupae have been reported to increase mortality in the field (Simmons *et al.*, 2018). Also, the use of companion crop planting or intercropping to control *T. absoluta* has been reported. For instance, a study by Medeiros *et al*. (2009) showed lower abundance of *T. absoluta* when tomato was intercropped with Coriander and Gallant soldier. In addition, reduced *T. absoluta* infestation was reported when tomato is intercropped with aromatic plants (Khafagy, 2015).

2.5.2 Chemical control method for *Tuta absoluta*

The use of synthetic pesticides is the primary control method for *T. absoluta* among tomato farmers, not only in Africa but also in other regions of the world (Guedes *et al*., 2019; Rwomushana *et al*., 2019). For example, in east Africa, 100% of the respondents reported using synthetic pesticides as the main control tactic for *T. absoluta* (Aigbedion-atalor *et al*., 2019). According to Guedes *et al*. (2019), there are several examples of synthetic chemical pesticides used in the control of *T. absoluta* including organophosphates, oxadiazines, spinosyns, avermectins, pyrroles, benzoylureas, diamides, diacylhydrazines, semicarbazones and nereistoxin analogues. Accordingly in Africa, the authorised synthetic insecticides used in some countries include neonicotinoids (acetamiprid and imidacloprid), pyrethroids (lambda-cyhalothrin, cypermethrin and deltamethrin), organophosphates (profenofos, chlorpyrifos-ethyl, chlorpyrifos-methyl), carbamate (methomyl), and avermectins (abamectin and emamectin benzoate) (Mansour *et al*., 2018). In fact, the active ingredients of these commercial synthetic insecticides are applied individually or in combinations (Mansour *et al*., 2018).

Nonetheless, efficacy of these synthetic chemicals against the endophytic feeding *T. absoluta* larvae has been unfortunate due to the protection offered by the leaf mesophyll or fruit tissues against applied insecticides (Retta & Berhe, 2015). Furthermore, Biondi *et al.* (2018) highlighted studies demonstrating rapid development of insecticide resistance by *T. absoluta*. For instance, resistance to abamectin, cartap, and methamidophos was observed in South America, resistance to indoxacarb in Europe, and resistance to diamide in Brazil and Europe. To deter the development of insecticide resistance, judicious use by combining insecticides with other *T. absoluta* control methods, and rotation with other active ingredients that differ in modes of action is necessary (Simmons *et al*., 2018).

Unfortunately, reliance on synthetic pesticides to manage *T. absoluta* is associated with several untenable hazards such as toxicity and poisoning to human due to build-up of insecticide residues on tomato fruits, killing of non-target beneficial organisms thereby disrupting biodiversity, among others (Lengai & Muthomi, 2018; Mansour *et al*., 2018). Moreover, synthetic chemical pesticides cause environmental pollution due to constituent compounds, human consumption of accumulated toxic chemical residues in crops poses chronic health problems such as asthma, hypertension, reproductive complications and cancer (Lengai & Muthomi, 2018). The use of synthetic pesticides may also lead to trade restrictions due to use of banned pesticides (Mansour *et al*., 2018) or failure to comply with tolerable levels of pesticide residues in agricultural produce for a particular market. For instance, the use of pesticides containing Dimethoate on vegetables was banned in European Union, whereas adhering to the acceptable levels of residues of restricted chemicals and Maximum Residue Levels (MRLs) of unknown pesticides proves problematic to many farmers in local communities (Lengai & Muthomi, 2018). In fact, it is very unfortunate that in Africa, mostly high-risk pesticides are used due to their availability and low-cost (Rwomushana *et al*., 2019). Therefore, the need to explore safer control tactics for incorporation in the IPM package for *T. absoluta* is underpinned.

2.5.3 Semiochemical control method for *Tuta absoluta*

This involves use of synthetic *T. absoluta* female sex pheromones not only for pest detection and population monitoring, but also for mass trapping, mass annihilation and mating disruption (Megido *et al*., 2013). The pheromone is mainly used in combination with other techniques such as pheromone lure with traps of different colours containing insecticides, or with adhesive coated surfaces or with a water bath. To improve efficiency of the tactic, recommended adjustments in terms of trap height, position with respect to vegetation and trap density per hectarage must be observed (Megido *et al*., 2013). In addition, Braham (2014) reported improved efficacy when using dark coloured traps compared to light coloured ones, and also fresh lure compared to weathered lures. However, the use of these pheromones is inadequate because only male *T. absoluta* are attracted and trapped. Interestingly, the report on the ability of *T. absoluta* females to reproduce parthenogenetically implies that pest population can still build up without necessarily mating (Megido *et al*., 2012). Fortunately, the recent efficacy results of *T. absoluta* control through horizontal transmission of *Metarhizium anisopliae* inoculum when used in combination with the Tuta pheromone (TUA-Optima®), for mass trapping and auto-dissemination is a promising technique to exploit (Akutse *et al*., 2020a).

2.5.4 Use of botanical pesticides for control of *Tuta absoluta*

The use of plant extracts and essential oils derived from fresh or dried plant parts such as leaves, barks, flowers, roots, rhizomes, bulbs, seeds, cloves or fruits to manage pests is a common practice (Lengai & Muthomi, 2018). Isman (2006) described a number of plant species with the respective biochemical compounds that are used for pest control, though at varying scales in different regions. For instance, Pyrethrum daisy (*Tanacetum cinerariaefolium*), Indian neem tree (*Azadirachta indica*), Rosemary (*Rosmarinus officinale*), Eucalyptus (*Eucalyptus globus*), Garden thyme (*Thymus vulgaris*), South American lily (*Schoenocaulon officinale*), Tobacco (*Nicotiana tabacum*), Sweetsop (*Annona squamosa*), Soursop (*Annona muricata*), wild tobacco (*Nicotiana gossei*), Chinaberry tree (*Melia azedarach*) among others.

Studies have reported promising efficacy results from the evaluation of extracts from some plant species against *T. absoluta*. For instance, Durmusoglu *et al*.

(2011) reported effectiveness of plant extracts such as Anonin, Azadirachtin, and their mixtures in controlling *T. absoluta* under laboratory and greenhouses conditions. Similarly, Nilahyane *et al*. (2012) reported effectiveness of extracts from *Thymus vulgaris* leaves and the seeds of *Ricinus communis* agains*t T. absoluta* larvae in the laboratory whereas, Yankova *et al*. (2014) demonstrated the efficacy of phytopesticide Neem Azal T/S® 0.3% against *T. absoluta* larvae under greenhouse conditions. Additionally, Ghanim & Ghani, (2014) reported effectiveness of extracts from Chinaberry, Geranium, Onion and Garlic on *T. absoluta* second instar larvae. Other effective plant extracts reported include; crude hexane extract of *Acmella oleracea* (Asteracea) (Moreno *et al*., 2012), ethanolic leaf extracts from species of genus Piper (*P. amalago* var. medium, *P. glabratum*, *P. mikanianum*, and *P. mollicomum*) (Brito *et al*., 2015) and Simmondsin extracted from Jojoba seeds (Abdel-Baky & Al-Soqeer, 2017). These botanical pesticides are also proved to be compatible with other techniques. For instance, combined use of Azadirachtin with *B. thuringiensis* or *B. bassiana* provided the highest level of *T. absoluta* control in screenhouse (Jallow *et al*., 2018). However, sustainability of the botanical resource, standardization of chemically complex extracts, and regulatory approval process are barricades to the commercialization of botanical insecticides (Isman, 2006).

2.5.5 Use of host plant resistance

The use of host plant resistance an economical, safer and sustainable way of managing insect pests including lepidopterans like the leafminers (Stout, 2014). In fact, plant resistance to pests is related to the inherited resistance-related traits that influence the biochemical and morphological characteristics crop cultivars (Stout, 2014). Specifically, in management of *T. absoluta*, it is suggested that density of glandular trichomes play an important role in tomato cultivars susceptibilities. In a study by Oliveira *et al*. (2012), it was found that the genotypes with higher densities of glandular trichomes and rich in 2-tridecanone, zingiberene and acyl sugars had greater resistance to *T. absoluta*. In fact, the oviposition rate, plant damage severity, injuries to the leaflets and percentage of leaflets attacked by *T. absoluta* were reduced by the presence of the allelochemicals in the genotypes. Notably, other studies also link the resistance of tomato cultivars against *T. absoluta* to the physical or biochemical characteristics of the different tomato host plants studies (Salem *et al*., 2016; Ghaderi *et al*., 2017; Sohrabi *et al*., 2016a). Accordingly, cultivars have exhibited better resistance to *T. absoluta* in the open-field including Shams and Chebli (Cherif *et al*., 2013), Berlina, Golsar, Poolad, and Zaman (Sohrabi *et al*., 2016a), Raha, Quintini and ES9090F1(Sohrabi *et al.,* 2016b) and Logain (Salem *et al*., 2016). Other cultivars that have been studied under protected field and laboratory conditions include; HGB-674, HGB 497, Rio Grande, King ston, Korral, CH Falat, Primo Early and Early Urbana among others (Cherif & Verheggen, 2019). However, the technology using *T. absoluta* resistant varieties of tomato in Africa has not been well utilised (Zekeya *et al*., 2017).

2.5.6 Biological control methods for *Tuta absoluta*

There are living natural enemies of *T. absoluta* that affect eggs, larvae, pupae and adults. These natural enemies are used individually or in combination since there is evidence of improved efficacy or performance when combined. For instance, Kortam *et al*. (2014) in Egypt reported highly significant efficacy in reduction of *T. absoluta* mines in greenhouse tomato by combining the egg-parasitoid (*Trichogramma achaeae*), the predator (*Macrolophus caliginosus*) and the microbial pesticide (*Bacillus thuringiensis kurstaki*), compared to individual bioagent treatments. With regard to how bioagents may be used in control of *T. absoluta*, Mansour *et al*. (2018) suggested; mass-production for regular releases in *T. absoluta* affected fields (augmentative biological control), adaptation of specific cultural practices that boost action of bioagents (conservation biological control), and introduction of exotic bioagents to permanently establish in new areas (classical biological control).

2.5.6.1 Use of predators for controlling *Tuta absoluta*

The predators of *T. absoluta* have been found to prey on eggs, larvae, pupae and adults. Also, some species are found to prey on a combination of development stages of the pest (Biondi *et al*., 2018; Tarusikirwa *et al*., 2020). The predators of *T. absoluta* belong to the orders Mesostigmata, Hemiptera and Hymenoptera (Zappala *et al*., 2013), Dermaptera, Coleoptera, Neuroptera and Thysanoptera (Desneux *et al*., 2010), and Araneae (Ghoneim, 2014). Nonetheless, the majority of the predator species are Hemipterans of the families Miridae, Anthocoridae and Nabidae (Zappala *et al*., 2013). However, others belonging to the families Pentatomidae and Geocoridae have also recorded degrees of success against *T. absoluta* eggs, larvae, pupae or adults in different regions (Biondi *et al*., 2018).

In a review by Tarusikirwa *et al*. (2020), a number of predator species that have been identified to control the various stages of *T. absoluta* (Table 2).

Table 2: Predator species identified to control *Tuta absoluta*

Host stage	Predator species
Egg	*Cycloneda sanguinea, Doru lineare, Engytatus varians, Eriopsis conexa,* and *Nesidiocoris tenuis,*
Egg and Larvae	*Amblyseius cucumeris, Amblyseius swirskii, Coleomegilla maculate, Dicyphus errans, Dicyphus maroccanus, Dicyphus. tamaninii, Macrolophus pygmaeus*, and *Orius insidiosus,*
Larvae	*Brachygastra lecheguana, Franklinothrips vespiformis, Nabis ibericus, Podisus nigrispinus, Polistes carnifex, Polistes melanosome, Polistes versicolor, Polybia ignobilis, Polybia scutellaris, Protonectarina sylveirae, Protopolybia exigua, Synoeca cyanea,* and *Scolothrips sexmaculatus*
Larvae and Pupa	*Calosoma granulatum, Lebia concina, Solenopsis geminate,* and *Solenopsis saevissima*
Pupa	*Labidura riparia*

Source: Tarusikirwa *et al*., 2020

2.5.6.2 Use of parasitoids for controlling *Tuta absoluta*

Several species of parasitoids belonging to the order Hymenoptera have been recognized parasitizing *T. absoluta* particularly eggs and larvae in different regions. The egg parasitoids belong to the families; Trichogrammatidae (Zappala *et al*., 2013), Encyrtidae and Eupelmidae (Desneux *et al*., 2010). The larval parasitoids belong to the families; Ichneumonidae, Braconidae, Eulophidae (Zappala *et al*., 2013), Bethylidae, Braconidae and Tachinidae, as well as the pupal parasitoids of the family Chalcididae (Desneux *et al*., 2010). In a review by Tarusikirwa *et al*. (2020), a number of parasitoid species have been identified to control *T. absoluta* including; *Capidosoma desantis*, *Encarsia porter, Trichogramma achaeae*, *Trichogramma pretiosum* and *Trichogramma telengai* (against eggs); *Agathis fuscipennis*, *Baryscapus bruchophagi*, *Bracon lucileae, Campoplex haywardi*, *Habrobracon osculator* and *Pteromalus intermedius* (against larvae) and *Horismenus* sp, (against pupae). The species *Apanteles dignus* was found to be hosted by both the larvae and pupae of *T. absoluta* (Tarusikirwa *et al*., 2020)

2.5.6.3 Use of microbial pesticides for controlling *Tuta absoluta*

Microbial pesticides based on entomopathogenic organisms such as bacteria, fungi, viruses, protozoa or nematodes as active ingredient are employed in management of several pests (Lacey *et al*., 2001; Koul, 2011). These entomopathogens have attracted attention due to their host-specificity, improved public perception of the hazards accompanying synthetic pesticides and pest development of resistance to orthodox synthetic pesticides among others (Koul, 2011). Although there might be no commercial products as yet in a number of instances, potential entomopathogenic species of mainly fungi, nematodes, bacteria and Baculoviruses have been employed to control *T. absoluta* (Tarusikirwa *et al*. (2020).

2.5.6.3.1 Entomopathogenic bacteria for controlling *Tuta absoluta*

Bacillus thuringiensis (Bt) is the most widely used bacterial entomopathogen for controlling of insect pests in crops. For example, with several isolates pathogenic to Lepidoptera, Coleoptera, and Diptera are commercially available (Lacey *et al*., 2001; Urbaneja *et al*., 2012). *Bacillus thuringiensis* is known to produce virulent crystal toxins, vegetative insecticidal proteins, phospholipase, immune inhibitors, antibiotics, including delta-endotoxins to the susceptible hosts (Kumari *et al*., 2014). In fact, a number of studies have reported efficacy of the strains of *Bacillus thuringiensis* either individually or in combination with other techniques against the larvae of this invasive pest. In Spain, Gonzalez-Cabrera *et al*. (2011) reported that *B. thuringiensis* was highly efficient in reducing the *T. absoluta* larvae damage on tomato in the laboratory, greenhouse, and open-field experiments. Also in Iran, the highest mortality of all *T. absoluta* larva instars was recorded in the laboratory following treatment with *B. thuringiensis var*. *kurstaki* at a concentration of 10^6 cell/ml (Alsaedi *et al*., 2017). In fact, combination with other techniques produced a positive synergistic effect for instance when *B. bassiana* was combined with Btk (Costar®) against *T. absoluta* larval instars (Tsoulnara & Port, 2016).

2.5.6.3.2 Entomopathogenic nematodes for controlling *Tuta absoluta*

Nematode species of the families, Mermithidae, Tetradonematidae, Allantonematidae, Phaenopsitylenchidae, Sphaerulariidae, Steinernematidae, and Heterorhabditidae, have shown potential as biocontrol agents of specific insects (Lacey *et al*., 2001). The nematodes exhibit qualities of parasitoids and entomopathogens in pest management (Lacey *et al*., 2001). Studies have revealed the potential of species of nematodes in controlling *T. absoluta*. For instance, Batalla-carrera *et al*. (2010) evaluated three species of entomopathogenic nematodes (*Steinernema carpocapsae*, *Steinernema feltiae* and *Heterorhabditis bacteriophora*) and reported *T. absoluta* larval mortality ranging from 78.6 to 100% and larval parasitism in leaf galleries ranging from 77.1 to 91.7%. Meanwhile, soil treatments by *Steinernema carpocapsae, Steinernema feltiae* and *Heterorhabditis bacteriophora* against *T. absoluta* resulted into larvae mortality of 100% for *S. carpocapsae*, 52.3% for *S. feltiae* and 96.7% for *H. bacteriophora* (Garcia-del-pino *et al*., 2013). In Egypt, Shamseldean *et al*. (2014) reported *T. absoluta* larval mortality of 67% caused by *Steinernema monticolum* and 80% by *Heterorhabditis bacteriophora* (HP88). In Turkey, Gozel & Kasap (2015) reported that *S. feltiae* (isolate 879) caused *T. absoluta* larval mortality ranging from 90.7 to 94.3%. Whereas in Belgium, Damme *et al*. (2016) reported that *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* caused mortality of *T. absoluta* larvae ranging from 77.1to 97.4% under laboratory conditions. In Iran, Kamali *et al*. (2018) reported that *S. carpocapsae* and *H. bacteriophora* caused mortality of *T. absoluta* larvae ranging from 89 to 93% under laboratory conditions. Also, local species of *Steinernema* and *Heterorhabditis* evaluated in laboratory in Rwanda were found to cause *T. absoluta* larval mortality ranging from 53.3 – 96.7% (Ndereyimana *et al*., 2019b).

2. 5.6.3.3 Entomopathogenic fungi for controlling *Tuta absoluta*

2.5.6.3.3.1 Overview of the entomopathogenic fungal use in Africa

Fungal species exceeding 750 are generally known to be entomopathogenic to arthropod pests (Ramanujam *et al*., 2014). However, majority of the species used in pest control belong to the order Entomophthorales in the class Zygomycota, and the classes; Hyphomycetes and Deuteromycota (Maina *et al*., 2018). Nonetheless, efficacious utilization of fungal based biopesticides necessitates enhanced virulence, desiccation tolerance, thermal tolerance, fast germination and infection, environmental stability and reproduction, and UV radiation tolerance by the conidia (Jackson *et al*., 2010). Currently, entomopathogenic fungal products mainly based on *Beauveria bassiana*, *Metarhizium anisopliae, Isaria fumosorosea*, and *Beauveria brongniartii* are found to be prevalent for host insects in the orders Hemiptera, Coleoptera, Lepidoptera, Thysanoptera, and Orthoptera (Faria & Wraight, 2007). However, the entomopathogenic fungal based pesticides registered in African countries are mainly imported (Rwomushana *et al*., 2019). Meanwhile, there are some locally produced entomopathogenic fungal products based mainly on *B. bassiana* and *M. anisopliae* for some pests to address the general shortage in African (Akutse *et al*., 2020b). Unfortunately, none of the EPF products was specially developed for *T. absoluta* control (Rwomushana *et al*., 2019). This therefore, leaves a gap for research to locally identify, evaluate, develop and commercialize such biopesticides to improve access to these safer pest control options for the African farmers.

2.5.6.3.3.2 Entomopathogenic fungal strains evaluated against *Tuta absoluta*

There have been studies on strains of *Beauveria* that yielded promising results against *T. absoluta*. For instance, Shalaby *et al.* (2013) reported that *Beauveria bassiana* treatment at the concentration of 10^{10} conidia/ml reduced hatchability of *T. absoluta* eggs to 0% compared to 86.7% in control. In the same study *B. bassiana* also caused 100% mortality of neonate larvae by the 5th day post-treatment under laboratory conditions. Meanwhile, a field study by El-Aassar *et al*. (2015) showed that *B. bassiana* (Biovar®) treatment resulted into 29.8% infested leaf area compared to 72.3% in the control, and 34.7% fruit infestation compared to 54.3% in the control. Furthermore, Tadele & Emana (2017b) evaluated *B. bassiana* (PPRC-56) and reported 95.83% larval mortality at 7 days after treatment in laboratory. When *B. bassiana* (PPRC-56) was applied under glasshouse conditions, 84.04% larval mortality at 10 days post-treatment was observed. On the other hand, Shiberu & Getu (2018) reported larval mortality of 74.14% in fields in Ethiopia due to application of *B. bassiana*. From the laboratory evaluation of pathogenicity in Rwanda, Ndereyimana *et al*. (2019a) reported *T. absoluta* larvae mortality of 60.8% and 48,8% for Beauvitech® WP (*B. bassiana* Strain J25) and Botanigard ES® (*B. bassiana* Strain GHA), respectively, 6 days after treatment.

In other developments, laboratory studies have shown pathogenicity of *Fusarium* sp. leading to 100% larval mortality 7 days after treatment (Lakhdari *et al*., 2016). Furthermore, Zekeya *et al*. (2019) reported pathogenicity of

Aspegillus oryzae A-Tz1 and *A. oryzae* A-Tz2 against *T. absoluta*. *Aspegillus oryzae* A-Tz1 and A-Tz2 not only caused 70% larval mortality, but also inhibited pupation and adult emergence by 84.5% and 74.4%, respectively, in addition to shortening adult lifespan to 5 days compared to 25 days in control.

Furthermore, the efficacy of strains of *Metarhizium* against *T. absoluta* has been studied. For example, Shalaby *et al.* (2013) reported reduction of hatchability of *T. absoluta* eggs to 0% compared to 80.0% in control, and 100% mortality of neonate larvae by 5th day post treatment with *M. anisopliae* at the concentrations of 10^9 and 10^{10} conidia/ml under laboratory conditions. In addition, Contreras *et al*. (2014) in a laboratory study reported 100% mortality of *T. absoluta* pupae when treated with *M. anisopliae* at 5.58 X 10^9 viable conidia per litre. In a field study, El-Aassar *et al*. (2015) reported 26.8% infested leaf area compared to 72.3% in the control, and 43.4% fruit infestation compared to 54.3% in the control following treatment of *M. anisopliae* (Bioranza®). Tadele & Emana (2017b) also evaluated *M. anisopliae* (PPRC-2) and reported 87.5% larval mortality at 7 days after treatment in laboratory and correspondingly 76.31% at 10 days after treatment under glasshouse. Also, results from laboratory study by Alikhani *et al*. (2019) showed pathogenic effects of *M. anisopliae* (isolate DEMI 001) on third-instar larvae of *T. absoluta*. In addition, laboratory evaluation of pathogenicity of Metatech® WP (*M. anisopliae* Strain FCM Ar 23B3) against *T. absoluta* larvae showed mortality of 82.8% at 6 days after treatment (Ndereyimana *et al*., 2019a). Furthermore, in a laboratory study by Akutse *et al*. (2020a), dry conidia of *M. anisopliae* ICIPE 18, ICIPE 20, ICIPE 665, ICIPE 41, GZP and ICIPE 41 caused *T. absoluta* adult mortality of 95.0%, 87.5%, 86.3%, 68.8%, 67.5% and 62.5%, respectively. In the same study, the soil treated with *M. anisopliae* ICIPE 18, ICIPE 20 and ICIPE 665 also resulted into 100% death of all the fourth instar larvae and consequently, no pupa or adult emerged.

Nonetheless, the efficacy of candidate *M. anisopliae* entomopathogens demonstrated in laboratory and greenhouse trials require validation under field conditions before development into commercial EPF products for *T. absoluta* control. The necessity for field evaluation is based on the fact that laboratories and greenhouses cannot exactly duplicate the field complex biotic and abiotic interactions that determine the EPF ecological host-range and virulence (Hajek & Goettel, 2007).

In other developments to address EPF efficacy constraints notably related to the cryptic feeding habit of *T. absoluta* larvae, endophytic colonization and insecticidal properties of some isolates has been evaluated. For instance, Agbessenou *et al*. (2020) reported endophytic colonization by *Trichoderma asperellum* M2RT4, *B. bassiana* ICIPE 706, and *Hypocrea lixii* F3ST1, which eventually reduced significantly *T. absoluta* eggs laid, mines developed, pupae formed and adults that emerged. In the same study, *Trichoderma* sp. F2L41, and *B. bassiana* isolates ICIPE 35(4) and ICIPE 35(15) significantly reduced survival of exposed adults and F1 progeny. In addition, Akutse *et al.* (2020b) reported the effectiveness of *Trichoderma harzianum* ICIPE 709, *Clonostachys rosea* ICIPE 707 and *Trichoderma atroviride* ICIPE 710 as endophytes against *T. absoluta*. These findings are a tremendous milestone in developing endophytic fungal-based biopesticide for control of this cryptic pest.

2. 5.6.3.3.3 Infection process of entomopathogenic fungi

The pathway of host insect infection by EPF involves chronological steps viz; (i) adhesion of fungal propagules, (ii) spore germination, (iii) penetration through the cuticle, integument, wounds, or trachea, (iv) overcoming the host response, (v) hyphal or blastospores formation, and (vi) outgrowing the dead host and production of new conidia (Zimmermann, 2007b). Maina *et al*. (2018) described the infection process as follows; when the fungal conidia encounter the host, they attach themselves to the cuticle through hydrophobic mechanisms and germinate to form germ tubes in favourable conditions. In the process, the fungus produces numerous specialized infection structures like penetration pegs and /or appressoria to enable the growing hyphae to penetrate into the host integuments and cuticle, facilitated by enzymes that include metalloid proteases and amino peptidases. When inside the host, fungal hyphal bodies develop and spread into the haemocoel, invading various cells and tissues, eventually causing death of the host 3 to 14 days after infection. Subsequently, the fungus breaks open the integument, forms aerial mycelia and sporulate on the cadavers. The death of susceptible hosts is believed to be a consequence of depletion of nutrients, physical obstruction of organs and toxicosis (Inglis *et al*., 2001).

2. 5.6.3.3.4 Formulations of entomopathogenic fungi

The variation in entomopathogen strain characteristics and application technique to be employed make some formulations more ideal/effective than the others. As a result, there exist several formulations of EPF for agricultural use.

According to Faria & Wraight (2007), the following formulations are common; (i) Wettable powder (WP), (ii) Granule (GR), (iii) Bait (ready for use) (RB), (iv) Water dispersible granule (WG), (v) Contact powder (CP), (vi) Suspension concentrate (flowable concentrate) (SC), (vii) Oil miscible flowable concentrate (oil miscible suspension) (OF), (viii) Ultra-low volume suspension (SU) and (ix) Oil dispersion (OD). Nonetheless, formulation of EPF based biopesticides is not only paramount for convenient handling and optimal delivery of the entomopathogen to the target environment or pest but also ensuring spore viability for an acceptable shelf-life, in addition to maintaining the capability to infect and kill the targeted pest under prevailing environmental conditions (Jackson *et al*., 2010). Thus, the suitable formulation will depend on the specific strain of the EPF. For example, the hydrophobic cell walls of *Beauveria* and *Metarhizium* propagules challenge suspension in water-based formulations but readily suspended in oils (Wraight *et al.*, 2007). However, the use of surfactants, sonication, mechanical agitation, or a combination of these techniques facilitate the suspension of hydrophobic propagules (Wraight *et al*., 2007).

2. 5.6.3.3.5 Factors affecting efficacy of entomopathogenic fungi

Entomopathogenic fungi face numerous stresses from biotic and abiotic factors in a field which eventually affect their efficacy as biological control agents (Jaronski, 2010). These factors and their interactions impact survival, spore propagation and germination, vegetative growth, and virulence of EPF, and ultimately influence the development of epizootics triggered by EPF in an environment (Wraight *et al*., 2007). Thus, there exist complex interactions between the host insect, the entomopathogen and the environment over time that influence the ultimate efficacy of EPF (Inglis *et al*., 2001).

2. 5.6.3.3.5.1 Host insect

The host insect susceptibility to entomopathogens is linked to nutritional stress, immature developmental stage of the insect, higher insect densities, and insect behaviour (Inglis *et al*., 2001). The authors underlined exploitation of any stress factors that can impair the physiology and morphology of the pest to enhance efficacy of EPF. It is also noted that the insect cuticular structure which determines success of conidial binding in addition to the fungistatic compounds possessed by some insects influence infection and ultimate efficacy of EPF (Jaronski, 2010).

2. 5.6.3.3.5.2 Entomopathogenic fungi

The ultimate degree of efficacy of EPF based pesticide products is reported to dependent on EPF strain virulence, propagule density, persistence in environment and efficiency of dispersal of the entomopathogen (Inglis *et al*., 2001). In addition, the variations in pest host plant cuticular compounds that are lethal to the EPF spore, and the difference in plant leaf surface topography which determine the infective spore quantity and retention by the phylloplane, ultimately impact field efficacy of EPF based pesticides (Jaronski, 2010). This therefore imply that, the use of satisfactory spore density to increase the chance of the pest acquiring adequate inoculum, applying the most virulent EPF strains, using strains that can persist in an environment and using strains with efficient dispersal mechanisms enhances efficacy of EPF in pest control.

2. 5.6.3.3.5.3 Environmental conditions

The efficacy of EPF in the field is dependent on a number of factor namely, the environmental conditions especially weather (Wraight *et al*., 2007) and pest host plant canopy characteristics (Jaronski, 2010). Temperature affects spore germination, vegetative growth and viability of EPF both in the laboratory and the field. For example, in the field, the phylloplane temperature is adjusted by the plant canopy characteristics to impact efficacy of EPF (Jaronski, 2010). The optimum temperature for germination and growth of most fungal isolates ranges from 25 to 30^{o}C, although decreasing storage temperature is better for dry conidia viability. Moreover, the relative humidity affects efficacy and survival of entomopathogens (Zimmermann, 2007b). However, the relative humidity within the crop canopy is adjusted by wind velocity and evapotranspiration rate (Jaronski, 2010). Although there is variability in adaptation to relative humidity across fungal species and strains (Wraight *et al*., 2007), increasing relative humidity generally improves conidial germination and success of EPF infection (Zimmermann, 2007b). On the other hand, the solar ultraviolet radiation UV-B (280-320 nm) and UV-A (320-400 nm) reduce survival and germination of conidia (Zimmermann, 2007b). Notably, the different entomopathogenic fungal species and strains show significant variation in vulnerability to solar irradiation, though formulations with UV radiation protectants are better in the field (Inglis *et al*., 2001; Wraight *et al*., 2007). More so, the rainfall soon after EPF treatment may wash off spores from host insect integuments. In addition, windblown rain may remove significant quantity of inoculum sources from crop canopy (Inglis *et al*., 2001; Wraight *et al*., 2007). Nonetheless, oil or emulsifiable oil formulations persist longer on foliage exposed to rain (Wraight *et al*., 2007).

In practice, Lacey *et al*. (2001) underlined prerequisites for successful use of EPF as microbial control agents including the use of the right propagule, appropriate formulation and application of propagule at appropriate dosages, susceptible host developmental stages, timing application and aligning application schedules with other agricultural practices.

2.6 Cost benefit analysis of using entomopathogens in pest control

Understanding economic and social aspects provide valuable input to the justification, development, decision making and evaluation of improved pest-management techniques from planning to implementation (Cameron, 2007). Therefore, economic criteria must be satisfied for a new pest-management technique to be judged a feasible alternative to current practices (Reichelderfer & Bottrell, 1985). In this study, cost benefit analysis was computed as benefit cost ratio (BCR) and evaluated using the rule for BCR, where only BCR > 1 denoted cost effectiveness (economic viability) of the control option (Gayi *et al*., 2016).

As much as several studies have been done to document the economic viability of pest control measures, few have handled the use of entomopathogens on tomato. Nonetheless, the available reports indicate varying degrees of economic viability (BCR >1) of the entomopathogens in the control of specific pests. For instance, field evaluation of EPF on Groundnut pests by Sahayaraj & Namachivayam (2011) revealed that all the entomopathogens including *B. bassiana*, *Paecilomyces fumosoroseus*, and *Verticillium lecanii* exhibited cost effectiveness in control (BCR > 1). In addition, a field study on long duration pigeon pea by Narasimhamurthy & Keval (2013) showed BCR > 1 for the entomopathogen *B. bassiana* against tur pod bug, *Clavigralla gibbosa* (Spinola) (Hemiptera: Coreidae). Meanwhile, Sujatha & Bharpoda (2017) also reported that *B. bassiana*, and *Lecanicillium lecanii* were cost effective against sucking pests of Green gram. Furthermore, all doses of *Bacillus thuringiensis kurstaki* (*Btk*), *B. bassiana* and *Helicoverpa armigera* nuclear polyhedrosis virus (*Ha*NPV) against 2nd instar larvae of *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) on chickpea in the field exhibited BCR >1 (Ojha *et al*., 2017). On the other hand, Sathish *et al*. (2018) observed BCR >1 for *B. thuringiensis kurstaki* (*Btk*), *M. anisopliae*, and *Ha*NPV against tomato fruit borer, *H. armigera* Hubner on tomato in the field. In fact, scanty information is existent on cost benefit analysis of using EPF in control of tomato pests, particularly the use *M. anisopliae* isolates that have been identified potent in controlling *T. absoluta*.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site

Field experiments were conducted at Mukono Zonal Agricultural Research & Development Institute (MUZARDI), Mukono district, Uganda (0°23'02.3"N 32°44'03.4"E). The site is located at 20km east of Kampala city centre along the Kampala - Jinja highway. Mukono Zonal Agricultural Research & Development Institute lies in the central agro-ecological zone of Uganda with a tropical climate characterised by a bi-modal rainfall pattern ranging from 915 – 1,800mm per annum with peaks normally in March – May and September – November. The temperature ranges between 16 and 28^{o}C throughout the year (www.slm.go.ug).

3.2 Establishment of experimental field, nursery, and management

The experimental field was prepared by slashing, ploughing and harrowing to obtain a fine seedbed. The field was divided into twelve experimental plots, each measuring 4 x 5m, and constituted an experimental unit. Inter-plot spaces measuring 1m wide were maintained. Tomato seedlings (variety: Rambo F1) were raised from the screenhouse. The tomato seeds were first sown into seedtrays and managed until germination. The potting soil was from a mixture of sieved forest soil (2 parts) and coarse sand (1 part). The seedlings were pricked-out into polypots and managed up to four weeks at which transplanting into experimental units was done. In each experimental unit, tomatoes were planted at a spacing of 0.75m x 0.60m. The seedlings were transplanted late evening and watered by means of a watering can, using water obtained from a fishpond at MUZARDI. *Ad libitum* watering continued once a day in late evening, up to four days when seedlings were considered to have fully established. Weeding was done whenever necessary, using a hand hoe and by hand-pulling. Mulching was done using dry grass mowed from the compound at MUZARDI. Staking was done using bamboo stems. No fungicides and other pesticides were applied to the experiment during the trials. Harvesting was done weekly by picking mature fruits that had reached the pink stage of ripening.

3.3 Experimental design and treatments

The treatments were laid in Randomised Complete Block Design (RCBD) with each replicated three times. The experiment involved four treatments, namely (i) *Metarhizium anisopliae* isolate ICIPE 20 (MA20), (ii) *Metarhizium anisopliae* isolate ICIPE 69 (MA69), (iii) Synthetic pesticide, Dudu Acelamectin - positive control (ACEL), and (iv) Untreated plot – negative control (CONT). Randomisation of treatments was done using GenStat computer software (12th Edition for Windows) as shown in the Figure 2 below.

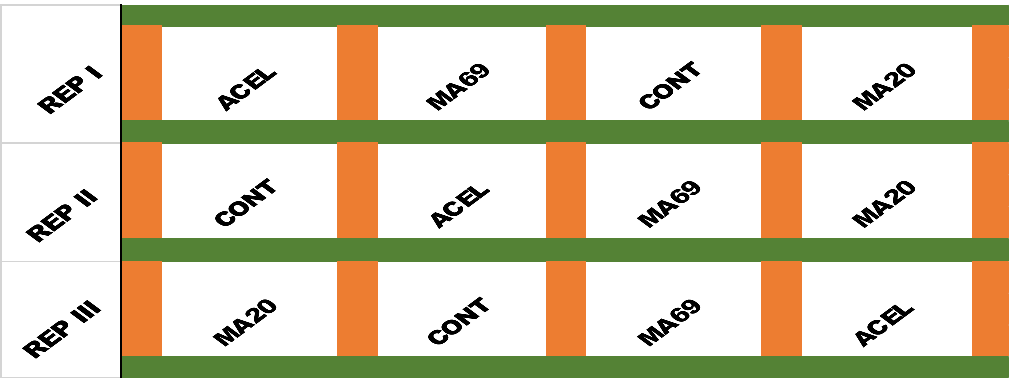

Figure 2: Experimental layout

3.4 Confirmation of *Tuta absoluta* incidence, leaf and leaflet damage before treatment application in the experimental plots

Prior to application of entomopathogenic fungal products, plots were assessed to confirm *T. absoluta* incidence and determine whether there were any significant differences in incidence, leaf damage and leaflet damage. Scouting of experimental field was done to ascertain presence of *T. absoluta*, based on visual observation of characteristic injury symptoms such as mines and frass on stems and leaves (Sridhar *et al*., 2014; Tumuhaise *et al*., 2016; Simmons *et al*., 2018). To assess *T. absoluta* incidence level, total number of plants per plot and the number of plants with symptoms per plot was recorded. For damage level, the ten innermost plants from each plot were assessed. On each plant, total number of leaves and the number of injured leaves were recorded. In addition, the number of injured leaflets and total number of leaflets on affected leaves per plant was recorded. *Tuta absoluta* incidence, percentage leaf and leaflet damage were computed as described by Rasheed *et al*., (2018b) as follows:

$$Tuta\ absoluta\ \text{incidence} = \frac{\text{Total number of injured plants in a plot}}{\text{Total number of plants in the plot}} \text{X } 100 \quad \text{……... (i)}$$

$$\text{Percentage leaf damage} = \frac{\text{Total number of injured leaves on plant}}{\text{Total number of leaves on the plant}} \text{X } 100 \quad \text{…….. (ii)}$$

$$\text{Percentage leaflet damage} = \frac{\text{Total number of injured leaflets}}{\text{Total number of leaflets on injured leaves}} \text{X } 100 \quad \text{….. (iii)}$$

3.5 Preparation and formulation of treatment materials

3.5.1 *Metarhizium anisopliae* ICIPE 20 suspension and oil formulation

The entomopathogenic fungal isolate, *M. anisopliae* ICIPE 20 was obtained from the International Centre of Insect Physiology and Ecology (*icipe*), Nairobi, Kenya, as dry conidia produced on grain rice. The freshly produced dry conidia of *M. anisopliae* ICIPE 20 had 95% viability. The concentration of conidia per gram of the isolate was determined following the haemocytometer quantification method described by Inglis *et al*. (2012). Accordingly, a conidial suspension was prepared by adding 0.01g of *M. anisopliae* ICIPE 20 dry conidia to 100mls of sterile distilled water mixed with Triton X-100 (0.05%) in a conical flask, and vortexed for 5 minutes at ~ 700rpm. Thereafter, 1 ml of the suspension was pipetted into the Improved Neubauer haemocytometer, and conidia count was conducted under a light microscope. The average number of propagules per 'cell' was multiplied by the volume conversion factor (2.5×10^5) to obtain the number of propagules per ml of suspension. The quantity of dry conidia of *M. anisopliae* ICIPE 20 required to provide a concentration of 1.0×10^9 viable conidia/ml (an equivalent to field application rate of the commercial product *M. anisopliae* ICIPE 69) was computed. In fact, Canola oil (at 1% v/v) and Triton X-100 (at 1% v/v) were used to facilitate the suspension of the hydrophobic propagules of *M. anisopliae* ICIPE 20 during the preparation (Wraight *et al*., 2007). Eventually, an oil-in-water formulation of *M. anisopliae* ICIPE 20 was prepared following the procedure described by Ummidi & Vadlamani (2014) and the mixture was then vortexed to get a homogenized stable formulation.

3.5.2 *Metarhizium anisopliae* ICIPE 69 suspension

Metarhizium anisopliae ICIPE 69, commercially registered as Campaign® was obtained from Real IPM (U) Ltd. Mazao Campaign® is an oil dispersion containing *M. anisopliae* ICIPE 69 at a concentration of 1.0 x 10^9 cfu/ml to be mixed at a rate of 10ml in 20L of water, and applied at 200ml/Ha. It is recommended to be applied once a week as a foliar spray, preferably late in the afternoon after 3 PM (East African Time) and pre-harvest interval (PHI) is 0 day (https://realipm.com). The product is registered in South Africa for control of mealybugs, thrips, and leafminers (Akutse *et al*., 2020b). Mazao Campaign® is reported to kill the host insect in 7 to 21 days by contact through a process that starts with viable spores attaching to the cuticle of the insect, germinating and producing a penetrating germ tube and establishing a systemic infection which finally kills the host (https://realipm.com).

3.5.3 Dudu Acelamectin (Synthetic pesticide)

Dudu Acelamectin 5% EC was obtained from an agro-input shop; AFRICA ONE FARMER’S SHOP, Container Village, Kampala, Uganda. Though not registered as the standard *T. absoluta* pesticide in Uganda, this pesticide was the most used synthetic chemical by farmers to control *T. absoluta* and had registered better effectiveness than other pesticides at MUZARDI. Dudu Acelamectin is a combined insecticide/miticide for effective control of leafminers, thrips, mites, beetles, fruit flies, plant bugs, fire ants and so many more insect pests on all crops (http://bukoolachemicals.com). The active ingredients of Dudu Acelamectin are Abamectin 20g/L + Acetamiprid 3%, recommended to be sprayed at an interval of 7-14 days. The recommended mixing of Dudu Acelamectin is 20-30ml of Dudu Acelamectin in 20L of water, a rate equivalent to 400-500ml/Ha. In addition, the PHI for Dudu Acelamectin is 7 days on most crops (http://bukoolachemicals.com).

3.6 Treatment application

The oil-in-water formulations of *Metarhizium anisopliae* ICIPE 69 and *Metarhizium anisopliae* ICIPE 20 containing approximately $1.0x10^9$ conidia/ml were each mixed at a rate of 10ml in 20L of water and applied as a foliar spray. Dudu Acelamectin, considered as positive control, was mixed at a rate of 20 ml in 20L of water and also applied as a foliar spray. The treatments were administered using separate hand operated backpack knapsack sprayers (Farmate Knapsack Sprayer 20L) for the entomopathogenic fungal products and synthetic chemical pesticide to avoid cross contaminations. The negative control plots were sprayed with water. All treatments were applied at weekly interval in the evening between 4 – 6 PM (East African Time).

3.7 Assessing *Tuta absoluta* incidence and severity of damage on tomato treated with entomopathogenic fungal products

3.7.1 *Tuta absoluta* incidence

Scouting of plants was done weekly to identify *T. absoluta* infested plants, based on presence of mines and frass on stems, leaves, and approximately pinhole sized holes on fruits (Sridhar *et al*., 2014; Simmons *et al*., 2018). On each sampling occasion, the total number of plants in each plot and the number of plants with *T. absoluta* injury symptoms were recorded. Thereafter, *T. absoluta* incidence was computed using the formular of Rasheed *et al*. (2018b) (Equation (i)).

3.7.2 Severity of crop damage by *Tuta absoluta*

Data on severity of tomato injury by *T. absoluta* on tomato plants was collected weekly after commencing treatment application until harvesting of tomato fruits. Ten innermost plants from each plot were assessed for damage level and the data was recorded as follows:

(i) Severity of leaf damage by *T. absoluta* was based on visual observation and counting of number of injured leaves and leaflets. The total number of leaves and number of injured leaves on each plant were recorded. Also, the total number of leaflets on damaged leaves and number of specific leaflets bearing *T. absoluta* injury symptoms were recorded. All injured leaves and leaflets were left on the plants. The formulae of Rasheed *et al*. (2018b) were used to compute percentage leaf damage (Equation (ii)) and leaflet damage (Equation (iii)).

(ii) Severity of fruit damage by *T. absoluta* was based on visual observation and counting of injured fruits. The total number of fruits on each plant and the number of injured fruits were recorded. All the injured fruits were left on the plants after assessment. The percentage fruit damage was then computed following the formular of Rasheed *et al.* (2018b) as follows:

Percentage fruit damage

$$= \frac{\text{Total number of injured fruits}}{\text{Total number of fruits on the plant}} \text{ X } 100 \quad \text{……… } \mathbf{(iv)}$$

(iii) Tomato fruit yield loss due to *T. absoluta* was assessed according to the procedure described by Ghaderi *et al*. (2019). Mature fruits at pink stage of ripening were harvested from the ten innermost plants of each plot. At each harvest, visual fruit inspection was done to sort out and weigh injured fruits. The weight of non-injured (marketable) fruits was also taken and recorded. The weights were measured using a mechanical Salter kitchen weighing scale. The weights of marketable fruits and the injured fruits were summed up to get the total weight of fruits. Then percentage fruit yield loss was computed as follows:

$$\text{Fruit yield loss} = \frac{\text{Weight of injured fruits}}{\text{Total weight of fruits}} \text{ X } 100 \quad \text{…………… } \mathbf{(v)}$$

3.7.3 Assessing the effect of entomopathogenic fungal products on tomato fruit yield

The total weight of tomato fruits and the weight of marketable fruits obtained in section 3.7.2 (iii) for each treatment and the untreated plot (control) was converted to fruit yield per plant. Then fruit yield per plant was used to compute fruit yield per treatment plot and eventually extrapolated to fruit yield per hectare as described by Shabozoi *et al*. (2011). For each treatment plot, the weights of total fruit yield (TFY) and marketable fruit yield (MFY) above the untreated plot were calculated. The untreated plot was used as a standard for comparison of treatments performance. The percentage of the resulting weight difference was considered as fruit yield gain (FYG). The total fruit yield gain (TFYG) and marketable fruit yield gain (MFYG) were computed following the formulae of Banerjee & Pal (2020) as follows:

$$\text{TFYG}\ (\%) = \frac{\text{TFY in treated plot} - \text{TFY in untretaed plot}}{\text{TFY in untreated plot}} \text{X}\ 100 \text{ .... } \mathbf{(vi)}$$

$$\text{MFYG}\ (\%) = \frac{\text{MFY in treated plot} - \text{MFY in untreated plot}}{\text{MFY in untreated plot}} \text{X}\ 100 \text{ ...... } \mathbf{(vii)}$$

3.8 Assessing economic viability of entomopathogenic fungal products for management of *Tuta absoluta* on tomato

To assess economic viability, the total season crop protection cost for each experimental plot was computed and extrapolated to costs per hectare. The costs for each treatment included expenses on buying each pesticide, pesticide application equipment, labour for pesticide application and the extra labour for harvesting the additional yield above the yield of the control plot. The cost of each unit of *M. anisopliae* ICIPE 20 was equated to the price of each unit of *M. anisopliae* ICIPE 69 (UG Shs.15,000 (USD 4.05)) per 20mls sachet from Real IPM Uganda). The pesticide application equipment was costed at UG Shs. 50,000 (USD 13.5) based on depreciation over an estimated 3-year lifespan. Labour for treatment application per spray per plot was extrapolated to per hectare and fixed at UG Shs.125,000 (USD 33.78) per hectare. The labour for harvest of the tomato yield above the yield from the control plot was fixed at an estimated average of UG Shs.100,000 (USD 27.03) per tonne. As prices of farm produce always vary during seasons, an average farm gate price of tomato was fixed at UG Shs.1,200 (USD 0.32) per kilogram for the calculation of revenue (Dijkxhoorn *et al*., 2019). The revenue per hectare for each treatment plot was obtained by multiplying the price per kilogram of tomato and marketable tomato yield per hectare. The revenue from the untreated plot was deducted from that of each treatment plot to obtain the benefit (value of yield of treatment plot above value of the yield of untreated plot) for the respective treatment plots, following the approach described by Shabozoi *et al*. (2011). Cost benefit analysis (CBA) of the management options was calculated as the benefit cost ratio (BCR) (Shiberu & Getu, 2018b), as follows:

$$\mathrm{BCR} = \frac{\text{Benefit of the treatment}}{\text{Crop protection cost}} \quad \text{.........................} \; \textbf{(viii)}$$

3.9 Data analysis

Analysis of variance (ANOVA) was done for comparison of treatment performance. Differences in means were separated using Fisher's protected least significant difference (LSD) at 5% probability. Repeated Measures Analysis (t-test) was done to compare treatment effect to the levels of *T. absoluta* incidence and severity of leaf damage before treatment. Analyses were done using GenStat computer software (12th Edition for Windows). Productivity was expressed as percentage fruit yield gain whereas, the cost benefit analysis was computed as BCR and evaluated using the rule for BCR (Gayi *et al*., 2016).

CHAPTER FOUR

RESULTS

4.1 Confirmation of *Tuta absoluta* incidence, leaf and leaflet damage before application of entomopathogenic fungal products

At 0 day prior to application of entomopathogenic fungal products, there was no significant difference in *T. absoluta* incidence level among the plot means during first season ($F_{3,6}$ = 0.14, p = 0.932) and second season ($F3,6$ = 2.77, p = 0.133). Even leaf damage was not significantly different during first season ($F_{3,6}$ = 1.59, p = 0.288) and second season ($F3,6$ = 0.65, p = 0.611). Similarly, there was no significant difference in leaflet damage during first season ($F_{3,6}$ = 1.12, p = 0.414) and second season ($F_{3,6}$ = 1.03, p = 0.445) (Table 3).

Table 3: *Tuta absoluta* mean incidence, leaf and leaflet damage (± SE) at the commencement of application of entomopathogenic fungal products

Season / Treatment plot	Mean ± SE (%)		
	Incidence	Leaf damage	Leaflet damage
First season			
Untreated plot	30.01 ± 6.06	4.94 ± 1.79	15.07 ± 1.77
Dudu Acelamectin	29.58 ± 3.24	7.94 ± 2.54	15.50 ± 1.93
M. anisopliae ICIPE 69	31.61 ± 1.25	6.91 ± 1.78	18.78 ± 1.28
M. anisopliae ICIPE 20	30.74 ± 2.29	7.82 ± 1.46	17.06 ± 0.33
Second season			
Untreated plot	33.92 ± 1.17	7.30 ± 1.52	21.16 ± 2.13
Dudu Acelamectin	39.79 ± 6.83	10.46 ± 4.26	34.40 ± 2.39
M. anisopliae ICIPE 69	27.03 ± 7.69	12.84 ± 6.21	19.81 ± 10.27
M. anisopliae ICIPE 20	40.85 ± 3.48	8.12 ± 5.34	22.25 ± 5.66

4.2 Effect of entomopathogenic fungal products on experimental plots

4.2.1 Incidence of *Tuta absoluta*

Tuta absoluta incidence was significantly different among treatments during first season ($F_{3,6} = 9.72$, $p = 0.010$) but not in second season ($F_{3,6} = 3.53$, $p = 0.088$). During the first season, mean incidence was highest (36.88 ± 2.88%) in the untreated plot and lowest (20.05 ± 1.98%) in Dudu Acelamectin treated plot. During the second season also, incidence was highest (30.94 ± 1.74%) in the untreated plot and lowest (18.51 ± 4.50%) in Dudu Acelamectin treated plot (Figure 3).

When *T. absoluta* incidence level was compared to the incidence at commencement of treatment application (at 0 day prior to treatment) (Table 3), significantly lower incidence ($t_2 = 5.01$, $p = 0.038$) was observed only within Dudu Acelamectin treated plots during first season. The incidence level was higher (but not significant) within *M. anisopliae* ICIPE 20 ($t_2 = -0.63$, $p = 0.592$), *M. anisopliae* ICIPE 69 ($t_2 = -0.34$, $p = 0.763$) and the control plot ($t_2 = -1.36$, $p = 0.308$). During second season, mean incidence levels were lower (but not significant) within all treatment plots; Dudu Acelamectin ($t_2 = 3.54$, $p = 0.071$), *M. anisopliae* ICIPE 20 ($t_2 = 3.43$, $p = 0.076$), *M. anisopliae* ICIPE 69 ($t_2 = 1.45$, $p = 0.283$) and the Control ($t_2 = 1.15$, $p = 0.370$) when compared to the mean incidence level at commencement of treatment application.

Moreover, comparison of seasons showed generally lower (but not significant) *T. absoluta* mean incidence during second season compared to first season for all treatment plots; Dudu Acelamectin ($t_4 = 0.31$, $p = 0.770$), *M. anisopliae* ICIPE

20 ($t_4 = 1.33$, $p = 0.254$), *M. anisopliae* ICIPE 69 ($t_4 = 2.13$, $p = 0.100$) and the untreated plot ($t_4 = 1.76$, $p = 0.153$).

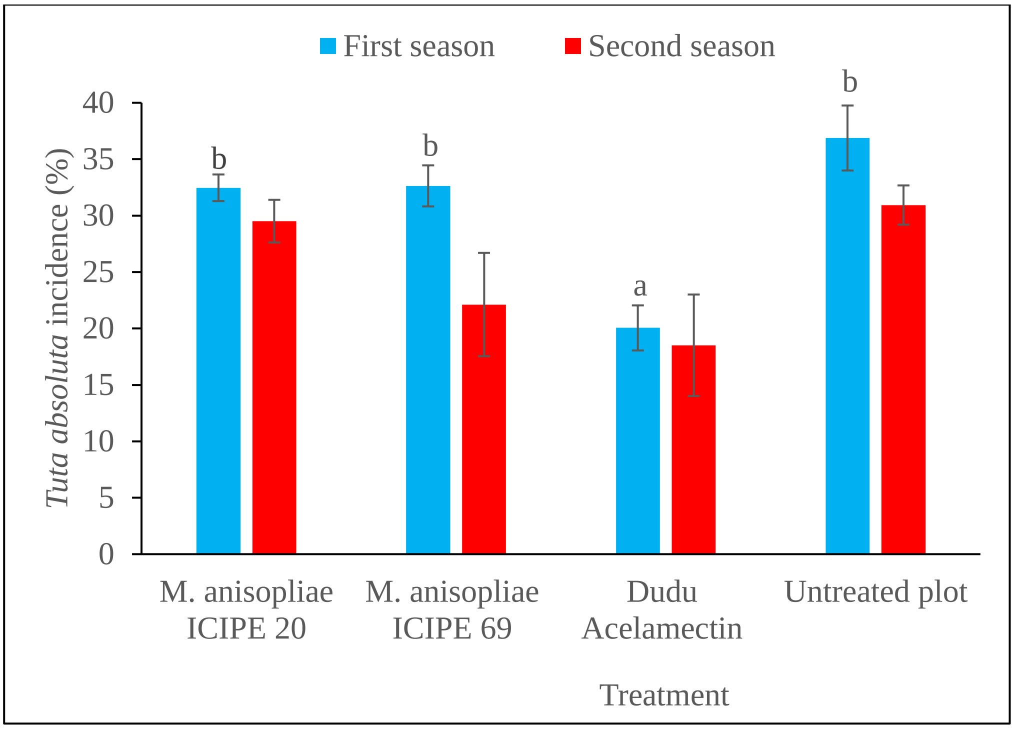

Figure 3: Mean incidence (± SE) of *Tuta absoluta* on tomato treated with entomopathogenic fungal products, at MUZARDI, 2019/2020. In a season, means that share a letter are not significantly different by Fisher's protected LSD test ($p = 0.05$)

4.2.2 Leaf damage by *Tuta absoluta*

Leaf damage level significantly differed among the treatments during first season ($F_{3,6} = 98.60$, $p < 0.001$) but not in second season ($F_{3,6} = 3.70$, $p = 0.081$). The mean leaf damage was lowest (6.06 ± 1.46%) in Dudu Acelamectin and highest (18.58 ± 2.39%) in untreated plots during first season. The leaf damage levels were also lower in entomopathogenic fungal treated plots than in untreated plots. Similarly, during second season, leaf damage was lowest (4.86 ± 1.56%)

in Dudu Acelamectin and highest (11.36 ± 2.68%) in the untreated plots, although no significant differences were observed between the treatments (Figure 4).

When the leaf damage level was compared to the leaf damage at commencement of treatment application (Table 3), significantly high ($t_2 = -12.86$, $p = 0.006$) leaf damage was observed within the untreated plots during first season. The leaf damage was higher (but not significant) in *M. anisopliae* ICIPE 69 ($t_2 = -0.95$, $p = 0.442$), and lower (but not significant) within Dudu Acelamectin ($t_2 = 2.50$, $p = 0.130$) and *M. anisopliae* ICIPE 20 ($t_2 = 1.04$, $p = 0.406$) treated plots. During second season, leaf damage was higher (but not significant) in untreated plot ($t_2 = -1.85$, $p = 0.205$). However, lower (but not significant) leaf damage was observed within Dudu Acelamectin ($t_2 = 1.80$, $p = 0.214$), *M. anisopliae* ICIPE 20 ($t_2 = 0.22$, $p = 0.846$) and *M. anisopliae* ICIPE 69 ($t_2 = 0.79$, $p = 0.513$) treated plots, when compared to the level of leaf damage at commencement of treatment application.

Furthermore, comparison of seasons showed generally lower leaf damage by *T. absoluta* during second season compared to first season. Nonetheless, no significant difference was observed in all the treatments; Dudu Acelamectin ($t_4 = 0.56$, $p = 0.606$), *M. anisopliae* ICIPE 20 ($t_4 = 0.55$, $p = 0.613$), *M. anisopliae* ICIPE 69 ($t_4 = 1.21$, $p = 0.294$) and the untreated plot ($t_4 = 2.01$, $p = 0.115$).

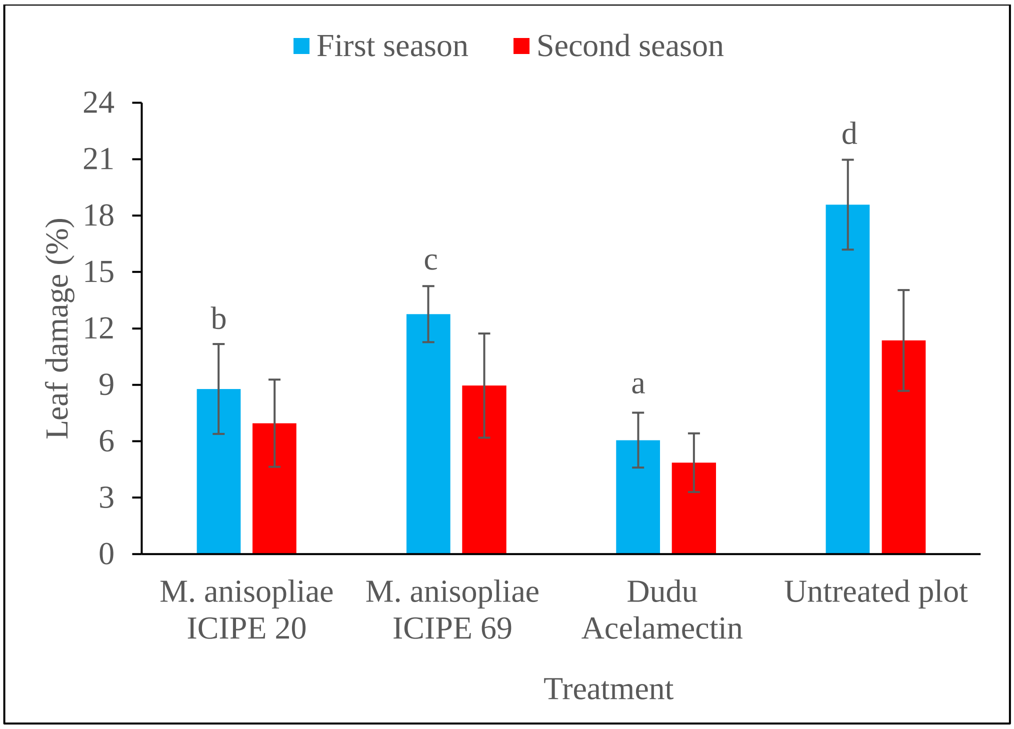

Figure 4: Mean leaf damage (± SE) by *Tuta absoluta* of the tomato treated with entomopathogenic fungal products, at MUZARDI, 2019/2020. In a season, means that share a letter are not significantly different by Fisher's protected LSD test ($p = 0.05$)

4.2.3 Leaflet damage by *Tuta absoluta*

Leaflet damage level significantly differed among treatments during first season ($F_{3,6} = 17.08$, $p = 0.002$) whereas, no significant differences were observed in second season ($F_{3,6} = 1.86$, $p = 0.238$). Dudu Acelamectin had the lowest leaflet damage (15.54 ± 0.65% during first season, and 14.40 ± 2.04% during second season), whereas untreated plots had the highest (28.84 ± 1.62% during first season, and 24.17 ± 5.17% during second season) (Figure 5). Also, during first season where significant differences were observed among the treatments, leaflet damage was low in the entomopathogenic fungal treated plots compared to untreated plot, but similar between *M. anisopliae* ICIPE 69 and *M. anisopliae* ICIPE 20 (Figure 5).

Within each treatment, comparison to the leaflet damage at commencement of treatment application (Table 3) during first season showed higher (but not significant) leaflet damage within untreated plot (t_2 = -3.62, *p* = 0.069), *M. anisopliae* ICIPE 69 (t_2 = -2.35, *p* = 0.143) and *M. anisopliae* ICIPE 20 (t_2 = -3.50, *p* = 0.073) treated plots. However, lower (but not significant) leaflet damage was observed within plots treated with Dudu Acelamectin (t_2 = 0.60, *p* = 0.612). During second season, leaflet damage was higher (but not significant) in untreated plot (t_2 = -0.99, *p* = 0.427). On the other hand, leaflet damage was lower though not significant within *M. anisopliae* ICIPE 20 (t_2 = 2.48, *p* = 0.131) and *M. anisopliae* ICIPE 69 (t_2 = 0.36, *p* = 0.752), and significant within Dudu Acelamectin (t_2 = 6.14, *p* = 0.026) treated plots, when compared to the leaflet damage at commencement of treatment application.

Additionally, comparison of seasons showed generally lower leaflet damage by *T. absoluta* during second season compared to first season. However, no significant difference was observed in all the treatments; Dudu Acelamectin (t_4 = 0.53, *p* = 0.621), *M. anisopliae* ICIPE 20 (t_4 = 1.65, *p* = 0.175), *M. anisopliae* ICIPE 69 (t_4 = 1.79, *p* = 0.148) and untreated plot (t_4 = 0.86, *p* = 0.436).

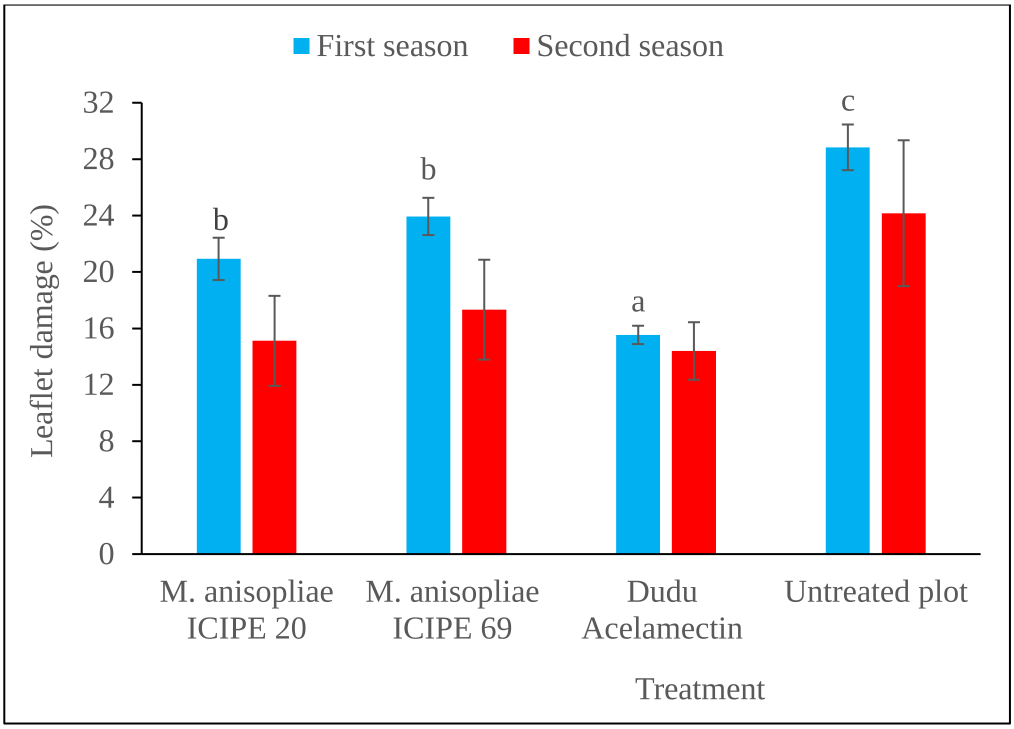

Figure 5: Mean leaflet damage (± SE) by *Tuta absoluta* of the tomato treated with entomopathogenic fungal products, at MUZARDI, 2019/2020. In a season, means that share a letter are not significantly different by Fisher's protected LSD test ($p = 0.05$)

4.2.4 Fruit damage by *Tuta absoluta*

Significant differences were observed among treatment plots during first season ($F_{3,6} = 5.17$, $p = 0.042$), but not in second season ($F_{3,6} = 1.36$, $p = 0.341$). Dudu Acelamectin had the lowest fruit damage (10.87 ± 1.62% during first season, and 2.81 ± 0.61% in second season), whereas untreated plots had the highest (26.48 ± 4.13% during first season, and 6.03 ± 2.21% in second season) (Figure 6). Also, during first season where significant differences were observed, fruit damage was low in *M. anisopliae* ICIPE 20 compared to untreated plot, but similar for Dudu Acelamectin and plots treated with EPF products (Figure 6).

Furthermore, comparison of seasons showed significantly lower fruit damage by *T. absoluta* during second season compared to first season for Dudu Acelamectin ($t_4 = 4.66$, $p = 0.010$), *M. anisopliae* ICIPE 20 ($t_4 = 4.95$, $p = 0.008$), *M. anisopliae* ICIPE 69 ($t_4 = 4.84$, $p = 0.008$) and the untreated plot ($t_4 = 4.37$, $p = 0.012$).

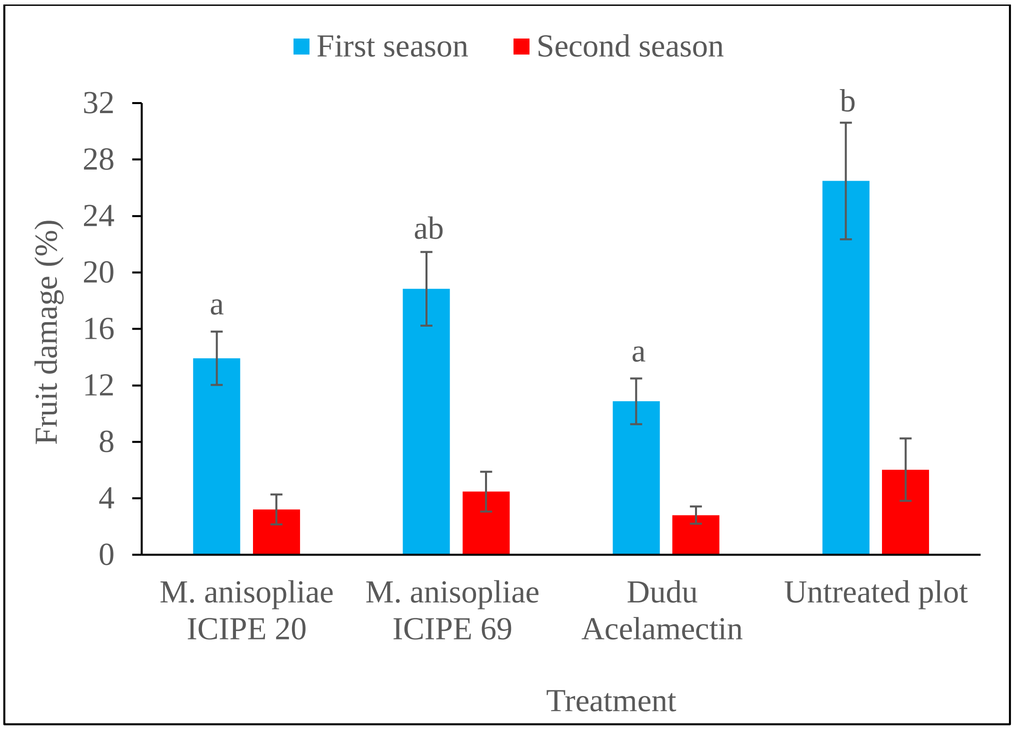

Figure 6: Mean fruit damage (± SE) by *Tuta absoluta* of the tomato treated with entomopathogenic fungal products, at MUZARDI, 2019/2020. In a season, means that share a letter are not significantly different by Fisher's protected LSD test ($p = 0.05$)

4.2.5 Tomato fruit yield loss due to *Tuta absoluta*

There were significant differences among the treatment plots during first season ($F_{3,6} = 22.38$, $p = 0.001$) and second season ($F_{3,6} = 68.81$, $p < 0.001$). Dudu Acelamectin had the lowest fruit yield loss (6.73 ± 3.64% during first season, and 2.82 ± 0.48% during second season), whereas untreated plots had the highest

(43.41± 2.63% during first season, and 13.01 ± 0.47% during second season) (Figure 7). During both seasons, fruit yield loss was significantly low in *M. anisopliae* ICIPE 20 and ICIPE 69 treated plots compared to untreated plot, but similar between *M. anisopliae* ICIPE 20 and ICIPE 69 treated plots (Figure 7).

Additionally, comparison of seasons showed significantly lower fruit yield loss due to *T. absoluta* during second season compared to first season for *M. anisopliae* ICIPE 69 ($t_4 = 3.75$, $p = 0.020$) and the untreated plot ($t_4 = 11.39$, $p < 0.001$). However, the observed low fruit yield loss in second season was not significant for Dudu Acelamectin ($t_4 = 1.07$, $p = 0.395$) and *M. anisopliae* ICIPE 20 ($t_4 = 1.11$, $p = 0.328$) treated plots.

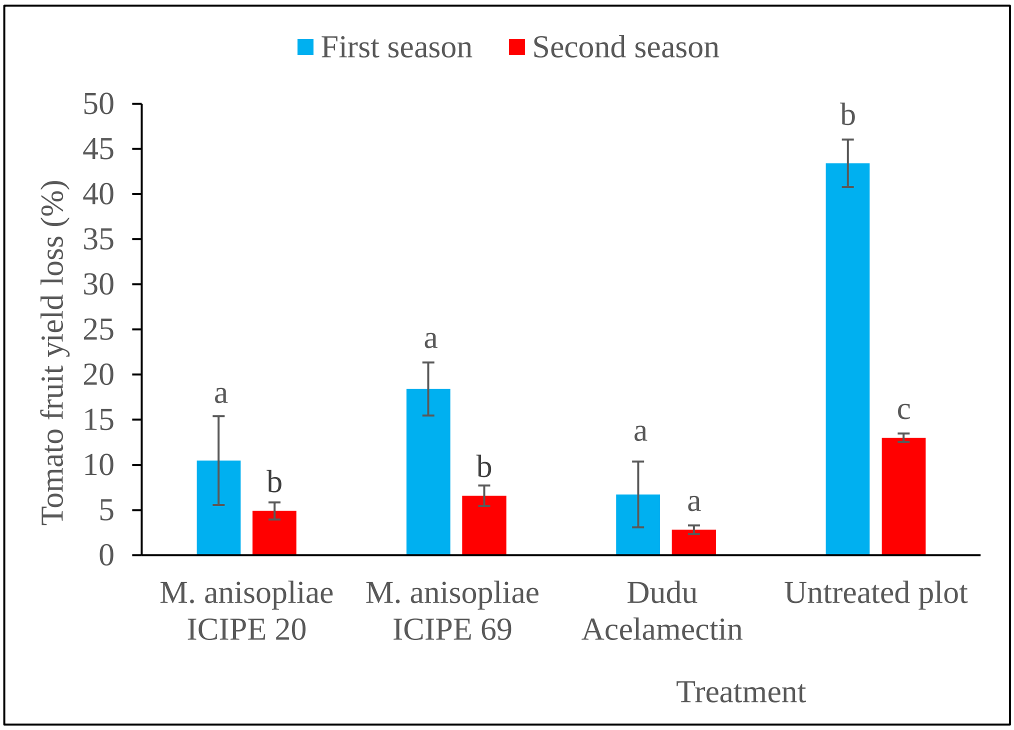

Figure 7: Mean fruit yield loss (± SE) due to *Tuta absoluta* of the tomato treated with entomopathogenic fungal products, at MUZARDI, 2019/2020. In a season, means that share a letter are not significantly different by Fisher's protected LSD test ($p = 0.05$)

4.2.6 Fruit yield of tomato treated with entomopathogenic fungal products

4.2.6.1 Total fruit yield

There were no significant differences in total fruit yield (TFY) among treatments during first season ($F_{3,6} = 0.59$, $p = 0.641$) and second season ($F_{3,6} = 0.39$, $p = 0.768$). Dudu Acelamectin had the highest TFY (11.97 ± 1.56 ton/ha during first season, and 16.43 ± 1.14 ton/ha during second season), whereas untreated plots had the lowest (8.46 ± 1.01 ton/ha during first season, and 12.67 ± 3.22 ton/ha during second season) (Table 4).

During both seasons, TFY was higher in *M. anisopliae* ICIPE 20 and ICIPE 69 treated plots compared to untreated plot. Concomitantly, TFY gain for *M. anisopliae* ICIPE 20 treated plots was 10.64 % in first season and 12.15 % in second season, whereas *M. anisopliae* ICIPE 69 showed 6.38 % and 8.29 %, during first season and second season, respectively (Table 4).

Additionally, although comparison of seasons showed higher TFY during second season compared to first season, no significant differences were observed for all the treatments; Dudu Acelamectin ($t_4 = -2.31$, $p = 0.082$), *M. anisopliae* ICIPE 20 ($t_4 = -1.46$, $p = 0.218$), *M. anisopliae* ICIPE 69 ($t_4 = -1.77$, $p = 0.151$) and the untreated plot ($t_4 = -1.25$, $p = 0.281$).

Table 4: Mean total fruit yield (± SE) and fruit yield gain of the tomato treated with entomopathogenic fungal products, at MUZARDI, 2019/2020

Treatment	Total fruit yield (ton/ha)	Total fruit yield gain[1] (%)
First season		
Untreated plot	8.46 ± 1.01	-
Dudu Acelamectin	11.97 ± 1.56	41.49
M. anisopliae ICIPE 69	9.00 ± 2.12	6.38
M. anisopliae ICIPE 20	9.36 ± 2.22	10.64
Second season		
Untreated plot	12.67 ± 3.22	-
Dudu Acelamectin	16.43 ± 1.14	29.68
M. anisopliae ICIPE 69	13.72 ± 1.62	8.29
M. anisopliae ICIPE 20	14.21 ± 2.40	12.15

[1](Equation (vi))

4.2.6.2 Marketable fruit yield

There were no significant differences in marketable fruit yield (MFY) among treatment during both first season ($F_{3,6}$ = 2.34, p = 0.173) and second season ($F_{3,6}$ = 0.80, p = 0.536). Dudu Acelamectin treated plot had the highest MFY (11.07± 1.18 ton/ha during first season, and 15.59± 1.06 ton/ha during second season), whereas untreated plots had the lowest (4.81± 0.71 ton/ha during first season, and 11.04± 2.86 ton/ha during second season) (Table 5). During both seasons, MFY was higher in *M. anisopliae* ICIPE 20 and ICIPE 69 treated plots compared to untreated plot. Concomitantly, the marketable fruit yield gain (MFYG) for *M. anisopliae* ICIPE 20 treated plots was 72.14% during first season and 22.01% in second season, whereas *M. anisopliae* ICIPE 69 showed 55.3% and 15.85%, during first season and second season, respectively (Table 5).

Comparison of seasons generally showed higher MFY during second season which was significant for Dudu Acelamectin treated plot ($t_4 = -3.09$, $p = 0.037$) but not for *M. anisopliae* ICIPE 20 ($t_4 = -1.83$, $p = 0.141$), *M. anisopliae* ICIPE 69 ($t_4 = -2.24$, $p = 0.089$) and untreated plot ($t_4 = -2.11$, $p = 0.102$).

Table 5: Mean marketable fruit yield (± SE) and marketable fruit yield gain of the tomato treated with entomopathogenic fungal products, at MUZARDI, 2019/2020

Treatment	Marketable fruit yield (ton/ha)	Marketable fruit yield gain[1] (%)
First season		
Untreated plot	4.81± 0.71	-
Dudu Acelamectin	11.07± 1.18	130.15
M. anisopliae ICIPE 69	7.47± 1.94	55.3
M. anisopliae ICIPE 20	8.28± 1.72	72.14
Second season		
Untreated plot	11.04± 2.86	-
Dudu Acelamectin	15.59± 1.06	41.21
M. anisopliae ICIPE 69	12.79± 1.38	15.85
M. anisopliae ICIPE 20	13.47± 2.25	22.01

[1] (Equation (vii))

4.2.7 Cost-benefit analysis of entomopathogenic fungal products in *Tuta absoluta* control

4.2.7.1 Crop protection cost per hectare per season

During the first season, the total crop protection cost (TCPC) was USD 260.79, USD 251.06 and USD 227.56, for *M. anisopliae* ICIPE 20, *M. anisopliae* ICIPE 69 and Dudu Acelamectin treated plots, respectively (Table 6a).

Table 6a: Crop protection cost per hectare per three treatment sprays during the first season, 2019, at MUZARDI

Treatment	Items	Unit cost[1] (USD)	Total cost (USD)
M. anisopliae ICIPE 69	Biopesticide	40.54	121.62
	Labour for application	33.78	101.34
	Knapsack sprayer	13.51	13.51
	Extra harvesting[2]		14.59
	Total		**251.06**
M. anisopliae ICIPE 20	Biopesticide	40.54	121.62
	Labour for application	33.78	101.34
	Knapsack sprayer	13.51	13.51
	Extra harvesting[2]		24.32
	Total		**260.79**
Dudu Acelamectin	Pesticide	5.95	17.85
	Labour for application	33.78	101.34
	Knapsack sprayer	13.51	13.51
	Extra harvesting[2]		94.86
	Total		**227.56**

[1] Cost for a single spray, computed based on per hectare rate of application

[2] Harvesting yield above the untreated plot (Table 4), estimated at USD 27.03 per tonne

During the second season, TCPC was USD 277.82, USD 264.85and USD 234.32, for *M. anisopliae* ICIPE 20, *M. anisopliae* ICIPE 69 and Dudu Acelamectin treated plots, respectively (Table 6b). The TCPC during both first and second season was highest in *M. anisopliae* ICIPE 20 treated plots and lowest in Dudu Acelamectin treated plots.

Table 6b: Crop protection cost per hectare per three treatment sprays during the second season, 2019/20, at MUZARDI

Treatment	Items	Unit cost[1] (USD)	Total cost (USD)
M. anisopliae ICIPE 69	Biopesticide	40.54	121.62
	Labour for application	33.78	101.34
	Knapsack sprayer	13.51	13.51
	Extra harvesting[2]		28.38
	Total		**264.85**
M. anisopliae ICIPE 20	Biopesticide	40.54	121.62
	Labour for application	33.78	101.34
	Knapsack sprayer	13.51	13.51
	Extra harvesting[2]		41.35
	Total		**277.82**
Dudu Acelamectin	Pesticide	5.95	17.85
	Labour for application	33.78	101.34
	Knapsack sprayer	13.51	13.51
	Extra harvesting[2]		101.62
	Total		**234.32**

[1] Cost for a single spray, computed based on per hectare rate of application

[2] Harvesting yield above the untreated plot (Table 4), estimated at USD 27.03 per tonne

4.2.7.2 Revenue, benefit and benefit cost ratio per season

The revenue from sale of marketable tomato fruits per hectare was higher in *M. anisopliae* ICIPE 20 (USD 2,685.41 during first season and USD 4,368.65 during second season) than *M. anisopliae* ICIPE 69 treated plots (USD 2,422.70 during first season and USD 4,148.11 during second season). On the other hand, during both seasons, the highest revenue was got from Dudu Acelamectin treated plot while the untreated plots showed the lowest revenue (Table 7).

Correspondingly, the benefit of treatment application was higher in *M. anisopliae* ICIPE 20 (USD 1,125.41 during first season and USD 788.11 during second season) compared to *M. anisopliae* ICIPE 69 treated plot (USD 862.70 during first season and USD 567.57 during second season). The plots treated with Dudu Acelamectin showed the highest benefit in both first season and second season (Table 7).

Moreover, the computed benefit cost ratio (BCR) of treatment application was higher in *M. anisopliae* ICIPE 20 (4.32 during first season and 2.84 during second season) compared to *M. anisopliae* ICIPE 69 treated plot (3.44 during first season and 2.14 during second season). During both first season and second season, the BCR was highest in Dudu Acelamectin treated plots (Table 7).

Table 7: Revenue, benefit and benefit cost ratio per hectare of treating tomato with entomopathogenic fungal products, at MUZARDI, 2019/2020

Treatment	Revenue[1] (USD)	Benefit[2] (USD)	TCPC[3] (USD)	BCR[4]
First season				
Untreated plot	1,560	-	-	-
Dudu Acelamectin	3,590.27	2,030.27	227.56	8.92
M. anisopliae ICIPE 69	2,422.70	862.70	251.06	3.44
M. anisopliae ICIPE 20	2,685.41	1,125.41	260.79	4.32
Second season				
Untreated plot	3,580.54	-	-	-
Dudu Acelamectin	5,056.22	1,475.68	234.32	6.30
M. anisopliae ICIPE 69	4,148.11	567.57	264.85	2.14
M. anisopliae ICIPE 20	4,368.65	788.11	277.82	2.84

[1] Marketable fruit yield (Table 5) (kg) x Price per kg (Estimated at USD 0.32 per kg)
[2] Treatment revenue – Revenue of untreated plot
[3] (Table 6a and 6b)
[4] Benefit ÷ TCPC (Equation (viii))
TCPC (Total crop protection cost)
BCR (Benefit cost ratio)

CHAPTER FIVE

DISCUSSION

5.1 Effect of treatment with *M. anisopliae* ICIPE 20 and ICIPE 69 on severity of damage by *Tuta absoluta* on tomato

The results showed significantly lower leaf, leaflet and fruit damage levels in the treatments (except *M. anisopliae* ICIPE 69 for fruit damage) compared to the untreated plots during first season. The fruit yield loss was significantly lower in treated plots compared to untreated plots in both first season and second season. The general level of damage severity in both seasons trended as follows; Dudu Acelamectin < *M. anisopliae* ICIPE 20 < *M. anisopliae* ICIPE 69 < untreated plots. These observations seem to imply that the applied treatments have potential to control *T. absoluta* in the field. The observed outperformance trend for Dudu Acelamectin was probably due to the quick action and broad-spectrum nature of this synthetic pesticide (http://bukoolachemicals.com). On the other hand, the performance of *M. anisopliae* ICIPE 20 and ICIPE 69 may probably be attributed to their slow infection mechanism as compared to synthetic pesticide (https://realipm.com**;** Maina *et al*., 2018), the dosage used may not be the most adequate and their vulnerability to field weather conditions (Jaronski, 2010; Agbessenou *et al*., 2021). The performance of *M. anisopliae* ICIPE 20 could be related to its efficacy under laboratory conditions where mortality rates of 87.5 and 100% were recorded among *T. absoluta* adults and fourth instar larvae, respectively (Akutse *et al*., 2020a). In addition, the low performance of *M. anisopliae* ICIPE 69 compared to ICIPE 20 may be attributed to the fact that, the former could be more effective in controlling other leafminers than *T. absoluta* (Akutse *et al*., 2020b). Although there exists scanty information on field efficacy of EPF against *T. absoluta*, findings of this study concur with previous field studies; for example, reduction of tomato leaf area infestation by *M. anisopliae* (Bioranza®) (El-Aassar *et al*., 2015), and reduction of tomato fruit yield loss due to *M. anisopliae* treatment (Shiberu & Getu, 2018).

5.2 Effect of treatment with *M. anisopliae* ICIPE 20 and ICIPE 69 on tomato fruit yield

The results showed higher (although not significant) total fruit yield (TFY) and marketable fruit yield (MFY) in the treatments compared to the untreated plot. In fact, during both first season and second season, the corresponding magnitude of total fruit yield gain (TFYG) and marketable fruit yield gain (MFYG) of the treatments over the untreated plot was in the order of Dudu Acelamectin > *M. anisopliae* ICIPE 20 > *M. anisopliae* ICIPE 69 treated plot. The findings seem to propose the potential of the treatments to suppress *T. absoluta* in the field and protect the crop from severe injury, compared to the untreated plot. As a result, there was better photosynthesis, growth, development, flower and fruit retention hence better yields and less damaged yield in the treated plots compared to the untreated plot. Although no statistically significant difference was observed among the treatments for TFY and MFY, this may not be equated to absence of economic benefit to the growers. Therefore, the fruit yield gain due to application of *M. anisopliae* ICIPE 20 and ICIPE 69 could be a desirable efficacy parameter in their evaluation as *T. absoluta* control products (FAO, 2006). These findings seem to concur with field studies of Shiberu & Getu (2018) and Ndereyimana *et al*. (2020) that reported improved tomato productivity in plots treated with *M. anisopliae* compared to the untreated control.

5.3 Economic viability of adopting *M. anisopliae* ICIPE 20 & ICIPE 69 for managing *Tuta absoluta*

The results showed that all treatments exhibited a BCR > 1 during first season and second season. The magnitude of benefit cost ratio (BCR) of treatment application during both seasons trended in the order; Dudu Acelamectin > *M. anisopliae* ICIPE 20 >*M. anisopliae* ICIPE 69 treated plot. The BCR > 1 denotes that the applied treatments were economically viable. The differences in the levels of economic viability could partly be attributed to the difference in efficacy of the treatments regarding the marketable yield saved, and the cost of the pesticides. In spite of the shortage of information on BCR of EPF against *T. absoluta* on tomato, the findings of this study corroborate with those on other lepidopteran pests where BCR values > 1 were reported; for example, the use of *M. anisopliae* against *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) (Sathish *et al*., 2018) and *B. bassiana* against *H. armigera* (Ojha *et al*., 2017). Our results also corroborate with previous studies on EPF against other non-lepidopteran pests, for instance; *B. bassiana* and *Lecanicillium lecanii* against sucking pests of green gram (Sujatha & Bharpoda, 2017), *B. bassiana* against *Clavigralla gibbosa* Spinola (Hemiptera: Coreidae) (Narasimhamurthy & Keval, 2013), and *B. bassiana*, *Paecilomyces fumosoroseus* and *Verticillium lecanii* against groundnuts pests (Sahayaraj & Namachivayam, 2011).

CHAPTER SIX

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

This study aimed to assess the possibility of efficacy of the entomopathogenic fungal products, *M. anisopliae* ICIPE 20 and *M. anisopliae* ICIPE 69 against *Tuta absoluta* under natural field infestation. In addition, the economic viability of these entomopathogenic fungal products when adopted as control option in the field was investigated.

The results seem to indicate a degree of field efficacy and economic viability of the entomopathogenic fungal products against *Tuta absoluta*. In the plots treated with *M. anisopliae* ICIPE 20 and *M. anisopliae* ICIPE 69, there was significant lower leaf, leaflet and fruit damage levels, and significant lower fruit yield loss compared to untreated plot. The treatments also exhibited fruit yield gain over the untreated plot and concomitant BCR > 1. The results also indicate that the synthetic chemical pesticide (Dudu Acelamectin) outperformed *M. anisopliae* ICIPE 20 and *M. anisopliae* ICIPE 69 in all the measured parameters. Unfortunately, the synthetic chemicals are a great risk to biodiversity as they kill non-target beneficial organisms, cause environmental pollution, including toxicity and poisoning to humans leading to chronic health problems (Lengai & Muthomi, 2018). Nonetheless, in spite of the relatively lower performance of *M. anisopliae* ICIPE 20 and ICIPE 69 compared to Dudu Acelamectin, the EPF products are generally associated with plenty of non-monetary benefits (Zimmermann, 2007a, 2007b; Skinner *et al.*, 2014); for instance, they are not toxic and poisonous to humans, harmless to beneficial organisms like pollinators (Omuse *et al*., 2021), leave no toxins in the environment, leave no toxic residues in the food product, and the pest cannot develop resistance against them (Akutse *et al*., 2020b). These attributes make these entomopathogens more appealing compared to the synthetic pesticides.

6.2 Conclusion

The reduction of crop injury and tomato fruit yield loss due to *T. absoluta* by application of *M. anisopliae* ICIPE 20 and *M. anisopliae* ICIPE 69 imply that these fungal isolates can contribute to suppression of *T. absoluta* population in the field. In addition, the tomato fruit yield gain and BCR > 1 indicate economic benefit to the tomato farmers. These findings seem to be a promising milestone for the candidate entomopathogens to be developed into commercial products and promoted for safer control of *T. absoluta* in tomato production systems after further validation. However, the study efficacy results reported were for one agro-ecological zone, on small scale, using a single dosage and formulation of candidate entomopathogenic fungal products.

6.3 Recommendations

Further research is needed to assess:

- Field efficacy of different dosages of *M. anisopliae* ICIPE 20 and *M. anisopliae* ICIPE 69 to establish the most effective dosage.

- Field efficacy of *M. anisopliae* ICIPE 20 and *M. anisopliae* ICIPE 69 in other agro-ecological zones.

- Field efficacy of other formulations of *M. anisopliae* ICIPE 20 and *M. anisopliae* ICIPE 69 to establish the most effective formulation for adoption into IPM package for *T. absoluta.*

- The non-target effect of *M. anisopliae* ICIPE 20 and *M. anisopliae* ICIPE 69 with *T. absoluta* major associated parasitoids and predators.

- The field efficacy of *M. anisopliae* ICIPE 20 at large scale.

- The interactions between the commonly use synthetic insecticides and these potent fungal isolates to enhance *T. absoluta* population suppression in tomato production systems.

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