EFFICACY OF SELECTED BIOPESTICIDES AGAINST THE TOMATO LEAF MINER, *TUTA ABSOLUTA* (MEYRICK) (LEPIDOPTERA: GELECHIIDAE) IN ZAMBIA

By

EMMA MAZIMBA

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DECLARATION

I, EMMA MAZIMBA, hereby declare that this dissertation represents my own work and that it has not been previously submitted for a degree at this or any other university.

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This dissertation of EMMA MAZIMBA is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Entomology by the University of Zambia.

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ABSTRACT

The tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a major invasive insect pest attacking tomatoes in Zambia. *Tuta absoluta* was recently introduced into Zambia and was first reported in 2016. The pest is known to cause losses of about 80-100% if not managed. Control strategies of *T. absoluta* include the use of biopesticides. The efficacy of four commercially available biopesticide formulations, *Azadirachtin indica* (Nimbecidine®), *Beauveria bassiana* (Bio-power®), *Metarhizium anisopliae* (Bio-magic®) and *Verticillium lecanii* (Bio-catch®) were compared with an untreated control for the management of *T. absoluta* under laboratory and field conditions. The selected biopesticides are fungal entomopathogens with the exception of *A. indica*, which is a botanical insecticide. This study assessed the most effective biopesticide on larvae mortality and oviposition and assessed effects of the selected biopesticides on leaf damage caused by *T. absoluta*. In laboratory studies, the efficacy of different concentrations of the selected biopesticides was tested on second instar larvae of *T. absoluta*. In the field studies, tomato plants were grown at two locations (Lusaka and Chongwe Districts), and the fields were laid out in a randomised block design with three replications. The selected biopesticides were applied to tomato plants on a weekly basis and efficacy was determined by the density of *T. absoluta* eggs on leaves and from leaf damage using a scoring scale of 1-5. The efficacy of the treatments were analysed using analysis of variance (ANOVA) and means that were statistically different were separated by Fishers least significant difference (LSD) test with $\alpha= 0.05$. Probit analysis was used to determine the estimated median lethal dose (LC_{50}). All the analyses were performed using Genstat statistical software. The selected biopesticides significantly (P<0001) reduced *T. absoluta* egg count compared with the untreated control. All the biopesticides exhibited a higher performance than the control in the following ascending order; *A. indica* (2.8±1.1 and 10.7±1.2), *B. bassiana* (3.0±0.6 and 10.7±1.9) *V. lecanii* (4.3±1.2 and 11.7±1.4) and *M. anisopliae* (6.8±1.4 and 13.0±1.6) for the first and second egg count, respectively. All the biopesticides significantly (P<0001) reduced leaf damage compared to the control. The severity of the leaf damage for all the biopesticides was 25-50% and 50-75% damage in the control. The estimated LC_{50} for *A. indica* was 30.4 ± 0.4 µL, while LC_{50} for *B. bassiana*, *M. anisopliae*, *V. lecanii* were 107.1 ± 0.4µL, 193± 0.4 µL and 118.7 ± 0.4µL, respectively. Thus, *A. indica* was the most effective biopesticide followed by *B. bassiana* then *V. lecanii*, while *M. anisopliae* was the least effective on larval mortality. The greatest percentage corrected mortality was obtained from *A. indica* (69.8±8.1% to 88.4±41.9%), followed by *B. bassiana* (32.6±4.0% to 60.5±16.3%) and *V. lecanii* (30.2±1.7 to 55.8%±14.6%) and lastly *M. anisopliae* (32.6±4.0% to 53.5±5.4%). The findings of this study showed that selected biopesticides were effective and should be used by farmers as an important component of Integrated Pest management (IPM) in the control of *T. absoluta* in Zambia. Further research should evaluate the effectiveness of the selected biopesticides in other agroecological zones of Zambia.

Key words: *Tuta absoluta*, *Beauveria bassiana*, *Metarhizuim anisopliae*, *Verticillium lecanii*, *Azadirachtin indica*.

DEDICATION

This dissertation is dedicated to my loving husband; Frank Sikazwe, to my children; Nataizya, Chazipa, Alinane, Taila and Tupilwe, Happy Nakazwe; my mother and Levison Mazimba; my father.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
CABI	Centre for Agriculture and Biosciences International
DAFF	Department of Agriculture, Forestry and Fisheries
EPF	Entomopathogenic fungi
EPPO	European Plant Protection Organisation
FAO	Food and Agriculture Organisation
IPM	Integrated Pest Management
IPPC	International Plant Protection Convention
NRDC	Natural Resources Development College
PIPs	Plant Incorporated Protectants
RCBD	Randomised Complete Block Design
RCD	Randomised Complete Design
TLM	Tomato leaf miner
UNZA	University of Zambia
ZARI	Zambia Agricultural Research Institute

CHAPTER 1: INTRODUCTION

1.1 Background Information

Tomato (*Solanum lycopersicum* L.) (Solanales: Solanaceae) is an important vegetable grown worldwide for fresh market and processing (Nicola et al., 2009). It is an excellent source of many nutrients such as folate, potassium, vitamin C, vitamin E, beta-carotene and lycopene which are essential elements in human health (Srinivasan, 2010). Global tomato production is currently about 130 million tonnes, of which 88 million tonnes is produced for fresh market and 42 million tonnes is processed in different edible products (FAO, 2015).

Some of the challenges in tomato production include temperature, humidity, diseases and insect pests (Tumuhaise *et al*., 2016). In addition, several insect pests feed on tomato and these include whiteflies, thrips, aphids, mites and African fruit borer (Walgenbach, 2018). *T. absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is a newly introduced and major pest of tomatoes and other solanaceous crops worldwide (Tumuhaise *et al*., 2016) causing serious damage and crop losses (Bawin *et al*., 2014). *T. absoluta* is native to South America (Urbaneja *et al*., 2012; Biondi *et al*., 2018) but it poses a serious threat for tomato production in its original location and throughout the world (Guedes and Picanco 2012; Campos *et al*., 2017).

T. absoluta is present in 41 African countries (Mansour *et al*., 2018), although many of these countries have not officially reported its presence (Campos *et al*., 2017). Regardless, *T. absoluta* remains a serious threat to the uninfected countries (Campos *et al*., 2017). Presence of this pest in Zambia was first reported in 2016 (Luangala *et al*., 2016). The pest is known to cause significant damage by feeding on all aerial parts of tomato plants and causes economic losses of 80 - 100% if not properly managed (Korycinska and Moran, 2009). The feeding behaviour of the larvae results in the loss of photosynthetic capacity and consequently, reduced growth and yields (Boyorni *et al*., 2003). The quality of tomatoes produced is affected by wounds caused by feeding larvae which in turn facilitate entry of secondary pathogens (Kaoud, 2014).

Management of *T. absoluta* is challenging because the larvae are concealed inside plant parts (leaves, stems and fruits) were they feed and are protected from applied insecticides (Biondi *et al*., 2018). Besides, *T. absoluta* has a high reproductive potential and quickly develops resistance to synthetic insecticides (Lietti *et al*., 2005). The management control strategies of *T. absoluta* include biological control (Biondi *et al*., 2013), pheromone traps (Cocco et al., 2013), chemical insecticides (Tome *et al*., 2013; Biondi *et al*., 2018), microbial antagonists (Contreras *et al*., 2014) and biopesticides (Braham *et al*., 2012; Allegrucci *et al*., 2017). Biopesticides are naturally occurring substances which are derived from insects, microorganisms, nematodes, and plants and their by-products (Mazid *et al*., 2011; Glare *et al*., 2012).

Currently, there is an increasing global interest in the use of biopesticides as a safer strategy for pest management (Kumar and Singh, 2015; Dhakal and Singh, 2019). They are ecofriendly, effective in small quantities for pest management and have a low risk of resistance development (Czaja *et al*., 2015; Dubovskiy *et al*., 2013; Khater, 2012). Entomopathogenic fungi and botanical biopesticides provide good alternatives for control of *T. absoluta* (Jallow *et al*., 2018). Many studies have demonstrated that entomopathogenic fungi are effective in reducing populations of *T. absoluta*, notably entomopathogens such as *B. bassiana*, *M. anisopliae* and *V. lecanii* (Braham *et al.,* 2012; Abd El- Ghany *et al*., 2016; Abd El- Ghany *et al*., 2018). Likewise, botanical biopesticides such as *A. indica* have shown great potential for control of *T. absoluta* (Tome *et al*., 2013).

In view of the potential management of *T. absoluta* by biopesticides, the current study evaluated the efficacy of four commercial biopesticides available on the Zambian market; *B. bassiana* (Bio-power®), *M. anisopliae* (Bio-magic®), *V. lecanii* (Bio-catch®) and *A. indica* (Nimbecidine®) for the control of *T. absoluta* under laboratory and field conditions.

1.2 General Objective

To compare the efficacy of selected biopesticides, *A. indica* (Nimbecidine®) *B. bassiana* (Bio-power®), *M. anisopliae* (Bio-magic®) and *V. lecanii* (Bio-catch®) for the control of *T. absoluta* under laboratory and field conditions for the Zambian/ecological conditions specifically Agro- ecological Zone II.

1.3 Specific objectives

i. Evaluate the effects of *A. indica, B. bassiana, M. anisopliae* and *V. lecanii* on larvae mortality.
ii. Evaluate the effects of *A. indica, B. bassiana, M. anisopliae* and *V. lecanii* on *T. absoluta* oviposition; and
iii. Assess the effects of *A. indica, B. bassiana, M. anisopliae* and *V. lecanii* on leaf damage caused by *T. absoluta*.

1.4 Hypotheses

i. There are no significant differences in the efficacy of *A. indica, B. bassiana, M. anisopliae* and *V. lecanii* on larvae mortality.
ii. There are no significant differences in the effects of *A. indica, B. bassiana, M. anisopliae* and *V. lecanii* on *T. absoluta* oviposition.
iii. There are no significant differences in leaf damage by *T. absoluta* treated with *A. indica, B. bassiana, M. anisopliae* and *V. lecanii*.

1.5 Significance of the study

Some studies have demonstrated that the use of biopesticides does not only promote food safety but also enhances the survival and effectiveness of natural enemies (Mazid *et al*., 2011). However, little is known about the efficacy of biopesticides that are currently available on the Zambian market. This study will provide baseline information on the most effective biopesticide against *T. absoluta*. Consequently, this will help tomato growers in making informed choices on the use of biopesticides against *T. absoluta*

CHAPTER 2: LITERATURE REVIEW

2.1 Taxonomy of *Tuta absoluta* (Meyrick, 1917*)*

The genus *Tuta* (Kiefffer and Jorgensen, 1910) belongs to the family Gelechiidae, subfamily Gelechiinae and the tribe Gnorimoschemini. It was first described by Kiefffer and Jorgensen (1910) as a type species. *T. absoluta* was originally described by Meyrick in 1917 as *Phthorimaea absoluta*. Later, the taxonomic status went through a lot of changes as the genus changed to *Gnorimoschema* (1962), *Scrobipalpula* (1964) and *Scrobipalpuloides* (1987). Currently, *T. absoluta* is the accepted name (EPPO 2005).

2.2 Origin and geographical distribution of *T. absoluta*

T. absoluta is native to South America and was reported outside its native range in Europe in 2006 (Desneux *et al.*, 2010). Many of the sub-Saharan countries have also been invaded by *T. absoluta* (Guimapi *et al.*, 2016). *T. absoluta* is present in 41 African countries (Mansour et al., 2018) (Figure 1.0). It was first reported in the North African countries of Algeria, Morocco, and Tunisia in 2008 (Harbi *et al.*, 2012). *T. absoluta* then spread to Sudan and Ethiopia in East Africa (Goftishu *et al.*, 2014). This pest was later reported in other East African countries that included Kenya and Tanzania in 2014 (IPPC 2014; Retta and Berhe, 2015; Chidege *et al.*, 2016).This pest was also reported in West Africa, where it was first detected in 2012 (Pfeiffer *et al.*, 2013). *T. absoluta* was later reported in central Africa and southern Africa (FAO 2015; FAO 2016; DAFF 2016; Mutamiswa *et al.*, 2017).

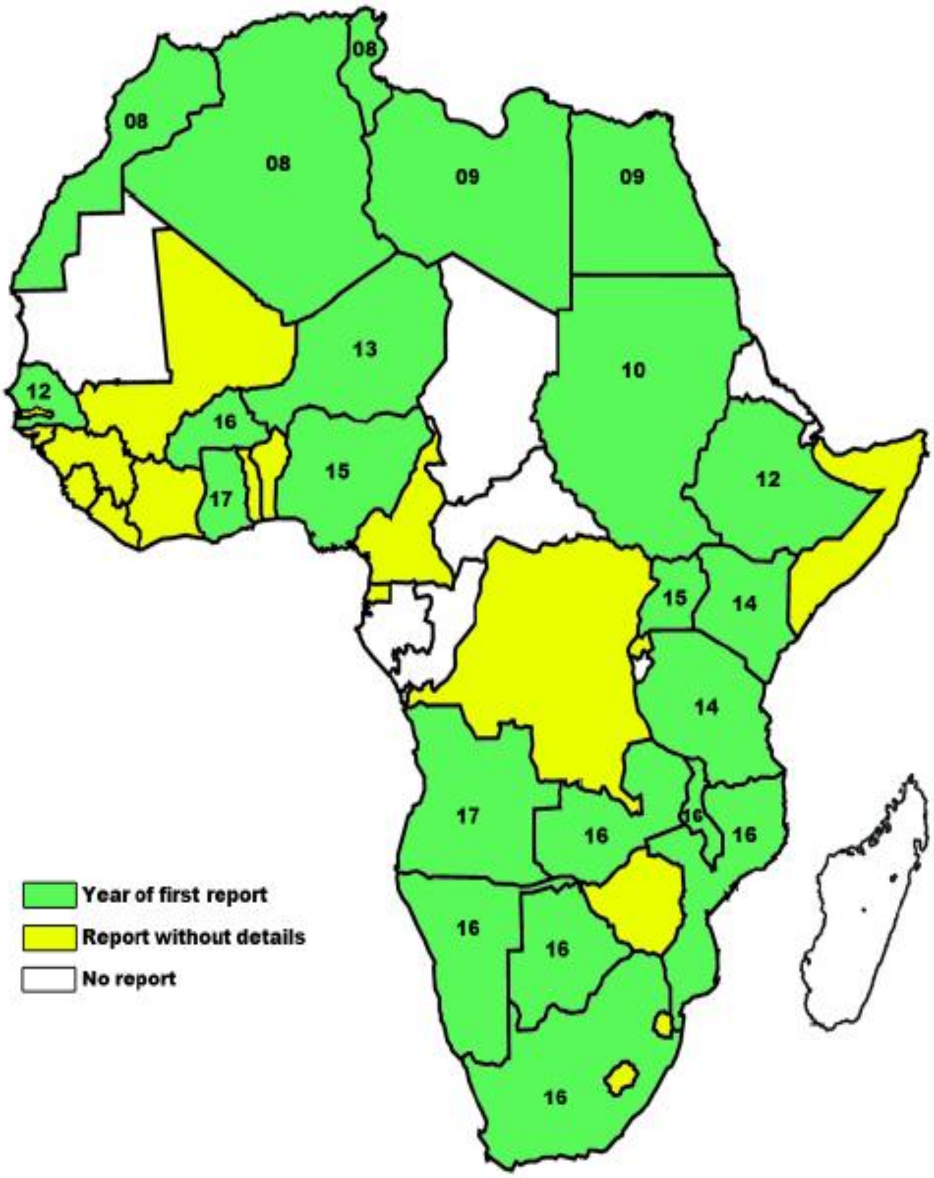


Figure 1: Map showing distribution of *T. absoluta* in Africa. (After Mansour *et al*., 2018).

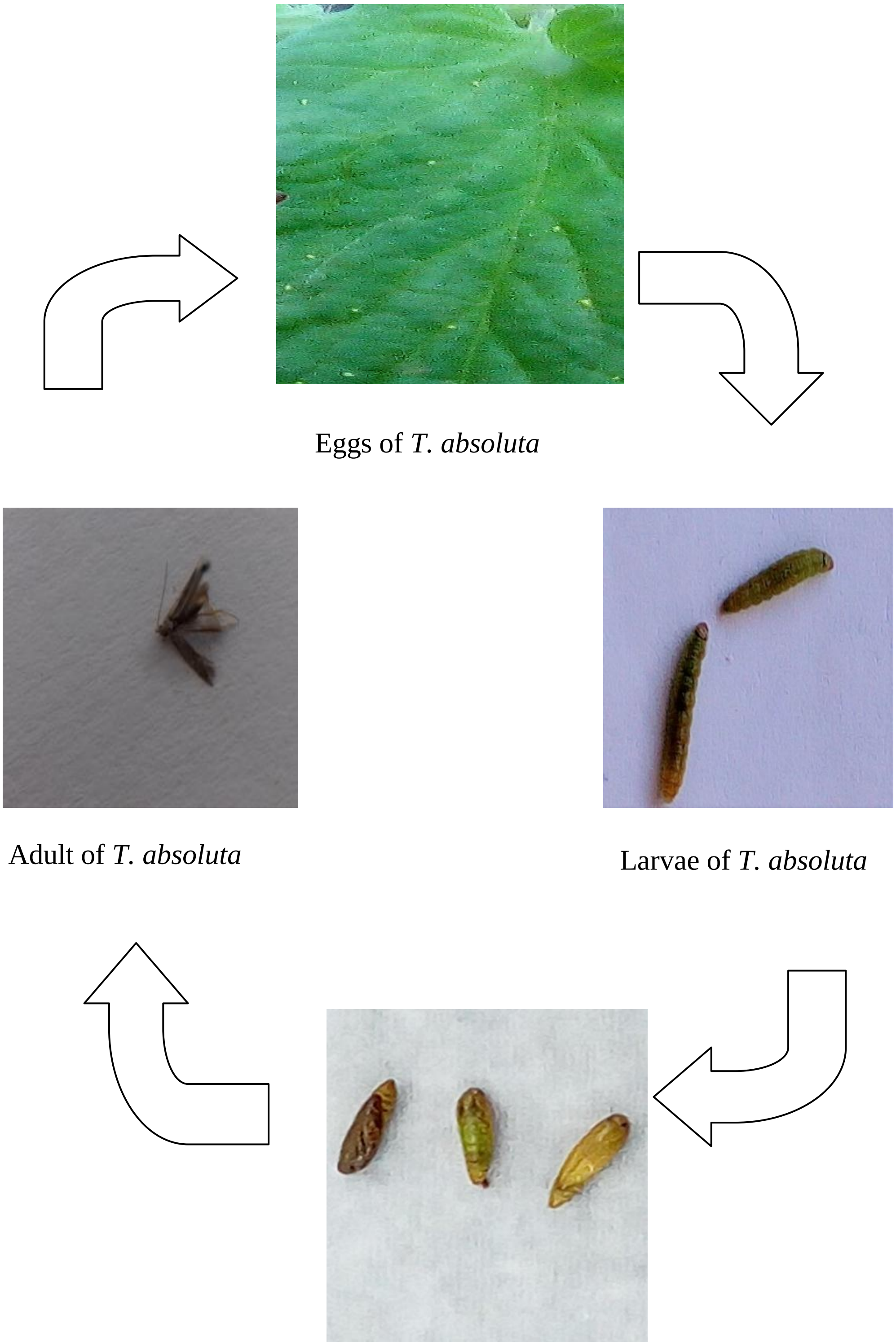
***The numbers in each country represent the year *Tuta absoluta* was introduced between 2008 and 2017.**

2.3 Entry and geographic spread / dispersal of *T. absoluta*

Studies on *T. absoluta* invasion of Europe, Asia and Africa showed that this insect has several pathways of entry (Desneux *et al*., 2010). Xian *et al*. (2017) described its potential pathways of introduction, host plant species and climatic suitability. The most prominent pathways include the imports of fresh tomato and seedlings from affected countries as well as the use of infested packaging materials such as crates which are used for exporting tomatoes, eggplant, potatoes and pepper (Retta and Berhe 2015). Packaging materials and crates can harbour all stages of the pest, with adults escaping upon unpacking, while the larvae and pupae continue their development and escape as adults when packaging materials are discarded (Karadjova *et al*., 2013). Natural factors such as wind and water as well as the flight of adult insects, also aid in the short distance dispersal of *T. absoluta* (Galdino *et al*., 2015).

2.4 Biology and lifecycle of *T. absoluta*

The life cycle of *T. absoluta* has four stages namely, egg, larvae, pupa and adult (Abbes *et al*., 2012). This life cycle is completed in 30-35 days (Harizanova *et al*. 2009) (figure 2). Mating, which lasts up to maximum of six hours, begins after the females produce sexual pheromones which attract male moths (Lee *et al*., 2014). A female moth has a high reproductive potential, laying about 250 to 300 eggs before completing its life cycle and has 10 to 12 generations per year (Desneux *et al*., 2010). *T. absoluta* females have ovipositional preferences with most eggs being deposited on the adaxial side of the tomato leaves than on the abaxial side, the least preferred areas being the stems, fruits, and flowers (Shiberu and Getu, 2017). The choice of the ovipositional site by the females of *T. absoluta* is influenced by larval density in the plants and more eggs are deposited on uninfested plants compared to larvae-infested plants (Bawin *et al*., 2014). Hatching of eggs takes four to six days (Cuthbertson *et al*., 2013). After hatching, the neonate larvae penetrate tomato fruits, stems and leaves and create conspicuous mines and galleries (Younes *et al*., 2018). Larval development takes 12-15 days (Nayana and Kalleshwaraswamy, 2015) and the mature larvae pupates in the soil, on the leaf or other plant parts where they produce a thin silken cocoon (Genc, 2016).The pupal stage takes 6 to 12 days before emerging as adults and the male adults have an average longevity of 7.55±1.60 days while female adults live for 12.65±1.97 days (Nayana and Kalleshwaraswamy, 2015).



Eggs of *T. absoluta*

Adult of *T. absoluta*

Larvae of *T. absoluta*

Pupae of *T. absoluta*

Figure 2 : Life cycle of *T. absoluta*

2.5 Description and identification of *T. absoluta*

The eggs have a cylindrical shape and are cream to white in colour when oviposited but turn yellow when they are about to hatch (CABI, 2015; Nayana and Kalleshwaraswamy, 2015). The larvae have four instars, with the first and second instars having a greyish to whitish colour, while third instar is green and the fourth instar is greenish pink (EPPO, 2005; Nayana and Kalleshwaraswamy, 2015). The pupae are cylindrical in shape and are initially green-yellow with colour becoming darker as pupae approaches adult emergence (Desneux *et al*., 2010). The average length of pupae is about 4.28± 0.43 mm, and the average width is 0.85± 0.11 mm (Nayana and Kalleshwaraswamy, 2015). Adults are tiny moths measuring approximately 5- 7 mm in size and are covered with silverish grey scales with black spots on anterior wing (Desneux *et al*., 2010). The females have a wider abdomen while the male's abdomen is narrow and pointed (Nayana and Kalleshwaraswamy, 2015).

2.6 Feeding behaviour and damage by *T. absoluta*

Tomato is a major host plant for *T. absoluta* oviposition and growth (Desneux *et al*., 2010; Abbes *et al*., 2016). The larvae are the most destructive stage of *T. absoluta* (Aynalem, 2018). The larvae feed on all parts of tomato plants, including leaves, fruits and stems (Desneux *et al*., 2010; Biondi *et al*., 2018). The damaged tissue dries up and turns yellow, while the fruits are destroyed (Oliveira *et al*., 2012). Damaged leaves have lesions (Figure 3a), while shoots become distorted and wilt (Mutamiswa *et al*., 2017) and consequently, the entire plant dries up (Chidege *et al*., 2016) (Figure 3b). Larvae also feed on the fruit leading to total crop failure (Desneux *et al*., 2010). Damaged fruits are characterised by distinct exit holes found on the fruit (Figure 4), with immature fruits showing symptoms of reduced fruit size and distorted shapes (Mutamiswa *et al*., 2017). However, mature damaged fruits show signs of secondary infection and decomposition (Mutamiswa *et al.,* 2017). In addition, tissue damage predisposes the plant to secondary infections especially from bacterial and fungal diseases leading to fruit rot (Desneux *et al*., 2010; Kaoud 2014). The feeding behaviour of the larvae results in the loss of the photosynthetic area and consequently, reduced growth and yields (Boyorni *et al*., 2003; Desneux *et al*., 2010).


Figure 3a: Leaf damage caused by *T. absoluta* larvae

Figure 3b: Plant damage by *T. absoluta*

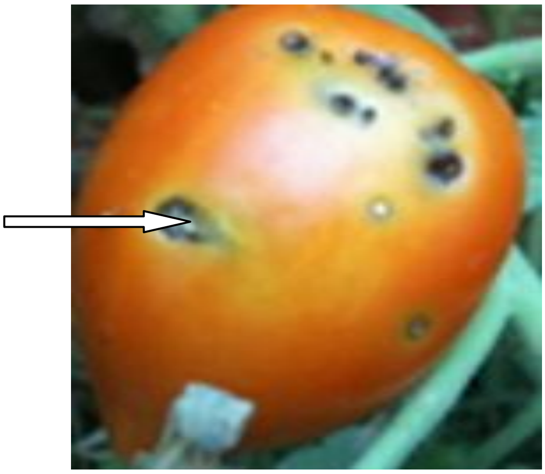


Figure 4: Fruit damage by *T. absoluta* larvae

2.7 Host range

Although tomato is a major host plant of *T. absoluta*, it also attacks other Solanaceae crops such as tobacco (*Nicotiana tabacum* L.), sweet pepper (*Solanum muricatum* L.), eggplants (*Solanum melongena* L.), and Irish potato (*Solanum tuberosum* L.)

(Abbes *et al*., 2016). Wild species include Jimson weed/thorn apple (*Datura stramonium* L.) and black night shade (*Solanum nigrum* L) (Desneux *et al*., 2010; Cocco *et al*., 2013).

2.8 Management of *T. absoluta*

The current management strategies of *T. absoluta* include sex pheromone traps and the use of chemical insecticides (Tome *et al*., 2013; Biondi *et al*., 2018), biopesticides derived from different organisms (Braham *et al*., 2012; Allegrucci *et al*., 2017), biological control (Biondi *et al*., 2013), microbial antagonists (Contreras *et al*., 2014) and a combination of different strategies (Molla *et al*., 2011). However, *T. absoluta* is a difficult insect to control because the larvae are found inside the mines, adults have a high reproductive potential and the pest easily develops tolerance to a wide range of insecticides (Lietti *et al*., 2005).

2.8.1 Cultural control

Cultural control include habitat management aimed at conserving natural enemies (Jonsson *et al*., 2010), crop rotation and polyculture practices (Markovi 2013). Management practices such as intercropping, application of manure, environment sanitation and destruction of alternative hosts of *T. absoluta* and infested plant can also be effective (CABI 2015).

2.8.2 Biological control

Natural enemies such as pathogens, parasitoids and predators have been considered as possible management options for *T. absoluta* infestation (Zappala *et al*., 2004). Luna *et al*. (2012) showed that there were about 50 species of natural enemies of *T. absoluta* and these were grouped as parasitoids, predators and entomopathogens. Some species belonging to the family Braconidae were reported as natural enemies of *T. absoluta* in the Mediterranean and South America (Desneux *et al*., 2010). Studies to select potential candidates of natural enemies of *T. absoluta* in Argentina showed that considerable mortality of *T. absoluta* could be achieved by the larval parasitoids, *Dineulophus phthorimaeae* (de Santis) (Hymenoptera: Eulophidae) and *Pseudapanteles dignus* (Muesebeck) (Hymenoptera: Braconidae) (Luna *et al*., 2015). Parasitoids from the family Eulophidae were the most abundant and the species *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae) was reported to be present in France, Spain, Algeria and Italy (Noyes, 2013). *Bracon nigricans* (Szépligeti) (Hymenoptera: Braconidae) and *Dolichogenidea appellator* (Telenga)

(Hymenoptera: Braconidae) were reported to be natural enemies of *T. absoluta* in Sudan (Idriss *et al*., 2018). Two larval parasitoids, *Bracon* sp. (Hymenoptera: Braconidae) and *Necremnus* sp. nr *artynes* (Hymenoptera: Eulophidae) with the later being more abundant, were also found to be natural enemies of *T. absoluta* in Tunisia (Abbes *et al*., 2013). Other natural enemies that showed great potential for management of *T. absoluta* were mirid predators (Zappala *et al*., 2013). Bondi *et al*. (2013) reported that *T. absoluta* was successfully managed by mirid predators using conservation and augmentation methods. Studies on natural enemies of *T. absoluta* are currently ongoing in countries with *T. absoluta* infestation (Chailleux *et al*., 2013)

2.8.3 Pheromones

Pheromones can be used as the first line of defence against *T. absoluta* (Desneux *et al*., 2010; Cocco *et al*., 2013). In addition, pheromone traps are an important tool used for determining the presence and abundance of insects which helps in decision making on chemical control measures (Cocco *et al*., 2013). The decision to use insecticides to control *T. absoluta* is dependent on the number of adults caught on the traps since the number of adult insects caught correlates with larval damage and yield losses (Benvenga *et al*., 2007). Benvenga *et al*. (2007) further reported an action threshold level of 45 males per hectare of *T. absoluta* caught daily using pheromone traps. Mass trapping has the potential to reduce male populations of *T. absoluta*, although it is more effective when used together with recommended pesticides (Witzgall *et al*., 2010). This technique has been successfully used in the control of *T. absoluta* in both green house and open field studies conducted in Tunisia, Italy and Egypt (Cocco *et al*., 2013; El- asasaretal *et al*., 2015; El-Heneidy *et al*., 2015).

2.8.4 Chemical control

Application of insecticides is the main control strategy for *T. absoluta* (Biondi *et al*., 2018). However, chemicals have a number of drawbacks such as the high cost resulting in reduced profit, negative effect on natural enemies and build-up of residues on tomatoes (Desneux *et al*., 2007). Furthermore, most chemical insecticides kill insects by direct contact but *T. absoluta* is found inside the leaves, fruits and stems and is therefore, protected from contacting chemicals (Ayalew, 2015). Another challenge posed is that *T. absoluta* has a short generation time and high reproductive potential, and this attribute can increases mutations which cause resistance development to pesticides (Desneux *et al*., 2010). Resistance has been reported to develop in Brazil and Argentina against the insecticides cartap, abamectine, deltamethrin, permethrin and methamidophos (Siqueira *et al*., 2000; Lietti *et al*., 2005). Consequently, resistance development has led to an increase of chemical applications in many countries (Campos *et al*., 2017; Roditakis *et al*., 2018). Continuous and inappropriate use of chemicals has also led to the build-up of residues and in some instances; this has led to human health problems (Abdel-Raheem *et al*., 2015). In order to overcome such problems, biopesticides are an effective potential alternative that can be used for achieving crop protection against *T. absoluta* in integrated pest management systems (BPIA, 2017).

2.8.5 Biopesticides

Biopesticides are naturally occurring substances which are derived from microorganisms, nematodes and plants (Mazid *et al*., 2011; Glare *et al*., 2012). Currently, there is an increasing global interest in the use of biopesticides as a safer strategy for pest management (Kumar and Singh, 2015). This has necessitated the use of biopesticides such as *B. bassiana*, *A. indica*, *V. lecanii* and *M. anisopliae* in the management of *T. absoluta* (Braham *et al*., 2012; Shiberu and Getu, 2017).

2.8.5.1 Advantages of biopesticides

Biopesticides have a number of advantages over synthetic pesticides because they are biodegradable and do not cause environmental pollution (Khater, 2012). In addition, biopesticides have a short pre-harvest interval and are considered safe to use on vegetables and fruits (Khater, 2012). Biopesticides are often effective in small quantities for pest management and they may not adversely impact biodiversity (Czaja *et al*., 2015). Furthermore, biopesticides have a low risk of resistance development in the targeted pests (Dubovskiy *et al*., 2013).

2.8.5.2 Mode of action of the selected biopesticides

Entomopathogenic fungi are microorganisms that develop and complete their life cycle on insect hosts (Mora *et al*., 2015) and are capable of keeping insect populations below economic injury levels (Tanzini *et al*., 2001). The development of fungal infections in insect hosts is complex and involves several steps which in turn make it difficult for pests to develop resistance against these fungi (Khani *et al*., 2012; Mora *et al*., 2015). Firstly, asexual spores or conidia attach and adhere to the insect cuticle through a process mediated by chemical components on the outer layers of the spore (Gabarty *et al*., 2014). Then the spores germinate and produce appressoria (Putcheta *et al*., 2006). Cuticle penetration is achieved by a combination of pressure from appressoria and by cuticle degrading enzymes such as melloproteases, trypsin and aminopeptidases (Bidocka and Small, 2005). Upon entering the insect's body and circulatory system (haemolymph), the fungus causes the death of the insect by defeating the insect's immune system (Inglis *et al*., 2001).

Azadirachtin is currently one of the most important plant extract because of its secondary metabolites produced (Regnault *et al*., 2004). These secondary metabolites act as repellents or antifeedants and cause sublethal effects on the insects (Isman, 2006). Azadirachtin has been successful in pest control of *T. absoluta* worldwide (Oliveira *et al.,* 2012). The high effectiveness of azadirachtin is evidenced by its mode of action where it acts as a systemic and contact insecticide against larval stages of *T. absoluta* (Goncalves-Gerrasio and Vendramin, 2007). Abd-El-Ghany *et al*. (2016) recorded high mortality of larval stages of *T. absoluta* inside and outside the mines by using azadirachtin.

2.8.6 Integrated Pest Management (IPM)

Integrated pest management (IPM) is an ecosystem based strategy that focuses on the long term prevention of crop damage as a result of pests (Flint, 2012). In order to control pests effectively, IPM focuses on the use of ecologically friendly options such as biological control, host plant resistance and other cultural options, with chemical control as a last option (Huesing *et al*., 2018). IPM has a number of advantages such as reduced chemical insecticide input costs, sustainable and effective pest management and reduced environmental pollution (Urbaneja *et al*., 2012). Biopesticides are an important component of IPM (Kumar and Singh, 2015).

2.9 Previous efficacy studies conducted against *T. absoluta*

Jallow *et al*. (2018) evaluated the efficacy of azadirachtin, *B. thuringiensis, Steinerma feltiae* and *B. bassiana* used in combination or individually against second instar larvae of *T. absoluta* in laboratory and green house conditions. Laboratory results obtained showed that all biopesticides used had great potential with the exception of *S. feltiae* which resulted in 26-42% mortality.

Field studies demonstrated that *B. beauveria* and *M. anisopliae* had no effect on larval mortality three days after treatment, but mortality was seen to gradually increase after ten days (Shiberu and Getu, 2018). Additionally, both *B. beauveria* and *M. anisopliae* caused significant larval mortality when compared to untreated control and standard check (Coragen 200SC) within ten days of application (Shiberu and Getu, 2018). It was also shown that mortality was high by direct contact of larvae with conidia of *B. bassiana* as well as indirect contact via ingestion of leaves inoculated with *B. bassiana* (Allegrucci *et al*., 2017).

The efficacy of five different biorational insecticides namely spinosad (*Saccharopolyspora spinosa*), *Bacillus thuringiensis* subsp. k*urstaki* (BtK), azadirachtin, *M. anisopliae* and *B. bassiana* against *T. absoluta* in greenhouse trials showed that spinosad was the most effective and the entomopathogens were least effective (Abd El Ghany *et al.*, 2016). *Beauveria bassiana* and *M. anisoplia* caused high mortality on eggs and neonates stages while BtK had a moderate effect on neonate and third instar larvae (Shalaby *et al.*, 2013).

Gonçalves-Gervásio and Vendramim (2007) reported that aqueous neem seed extracts on *T. absoluta* in green house and laboratory studies led to larval mortality of 56%-100% when aqueous neem seed extracts were applied to foliar adaxil surfaces and 52.4%- 95.4% for contact action on larvae.

Barakat *et al*. (2015) reported that Nimbecidine® showed effectiveness after two and five days of application against the egg stage (65.92% and 64.27%) and against the larval stage (55.25% and 61.57%). In addition, the entomopathogenic fungus, *M. anisopliae* was found to be effective against pupae of *T. absoluta* at the recommended label rate (Contreras *et al*., 2014).

The longevity of *T. absoluta* larvae was significantly lower after feeding on epiphytic *B. bassiana* treated leaves compared to endophytic treated leaves (Klieber *et al*., 2015). Younes *et al*. (2018) using *B. thuringiensis* and *B. bassiana* showed that the development, survival and reproduction on moths resulting from treated larvae were lower compared to that of untreated larvae of *T. absoluta*. Tsoulnara and Port (2016) demonstrated that *B. bassiana* and *B. thuringiensis* were effective in controlling first and second instar larvae compared to third instar. However, the recommended dose for *B. bassiana* was not as effective as the higher doses and the combination of treatments showed a high percent mortality against all larval stages. Inanl and Oldargc (2012) showed that *B. bassiana* and *M. anisopliae* were effective against the egg and larval stage of *T. absoluta*. Egg mortality due to *B. bassiana* was 41.7% and 66.7% on the 7th and 9th day, while larval mortality was 4.2% and 12.5%, respectively. Mortality of eggs due to *M. anisopliae* was 91.7% and 100%, while larval mortality was 91.7%.

Berxolli and Shahini (2017) demonstrated that azadirachtin was effective against first and second instars but less effective in third and fourth instars. However, the effectiveness of azadirachtin was observed to have increased with time (Berxolli and Shahini, 2017). Tome et al. (2013) showed that azadirachtin caused high larval mortality using a recommended dose. Furthermore, egg deposition was avoided by adult females on plants treated with the lowest concentration of azadirachtin (Tome *et al*. 2013).

Kichaoui *et al.* (2016) conducted green house studies and the effect of *B. bassiana* (2.5×10^7 spores/ml) was compared with chemical insecticides chlorofenapyr and thiocyclam hydrogen oxalate against *T. absoluta* (stage was not specified). *Beauveria bassiana* was most effective with 95% mortality of *T. absoluta* while chemical treatment resulted in 88% mortality.

2.10 Summary of knowledge gaps to be achieved by current study

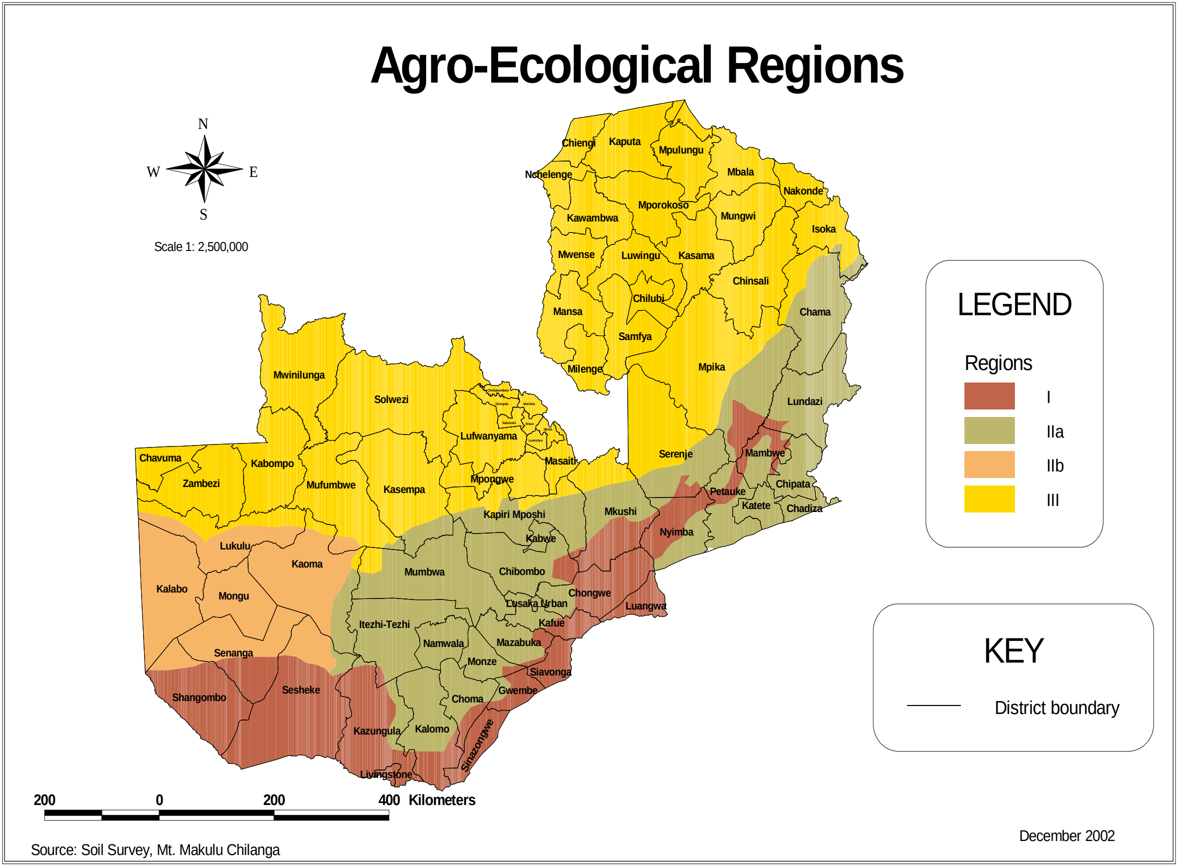
Although many studies using biopesticides have been conducted in other countries, no similar studies have been conducted in Zambia on the efficacy of *A. indica*, *B. bassiana*, *M. anisopliae* and *V. lecanii* against *T. absoluta*. There is therefore a need to test how these biopesticides will perform in Zambia which happens to have different environmental and biodiversity conditions from places were similar studies have been conducted as this may affect the efficacy of the biopesticides under study. Among the studies conducted in other countries, very few have used *V. lecanii* for controlling *T. absoluta* (Gindin *et al.*, 2000; Abdel-Raheem & Al-Keridis, 2017). However, most of the studies have used only one or two of the aforementioned biopesticides. For instance, *B. bassiana* and *M. anisopliae* were compared for their effects against egg and larval stages of *T. absoluta* (Inanli and Oldargc, 2012;

Shalaby *et al*., 2013; Siberu and Getu 2017). Other studies only evaluated one of the biopesticides, for example, *B. bassiana* was evaluated against larval stage (Klieber & Reineke, 2015), *M. anisopliae* was tested against egg, adult and pupal stages (Pires et al., 2009; Contreras *et al*., 2014) and azadirachtin against the larval stages (Berxolli and Shahini, 2017). This study will evaluate the efficacy of four biopesticides, *A. indica, B. bassiana*, *M. anisopliae* and *V. lecanii* on larval mortality, *T. absoluta* oviposition and leaf damage under laboratory and field conditions.

CHAPTER 3: MATERIALS AND METHODS

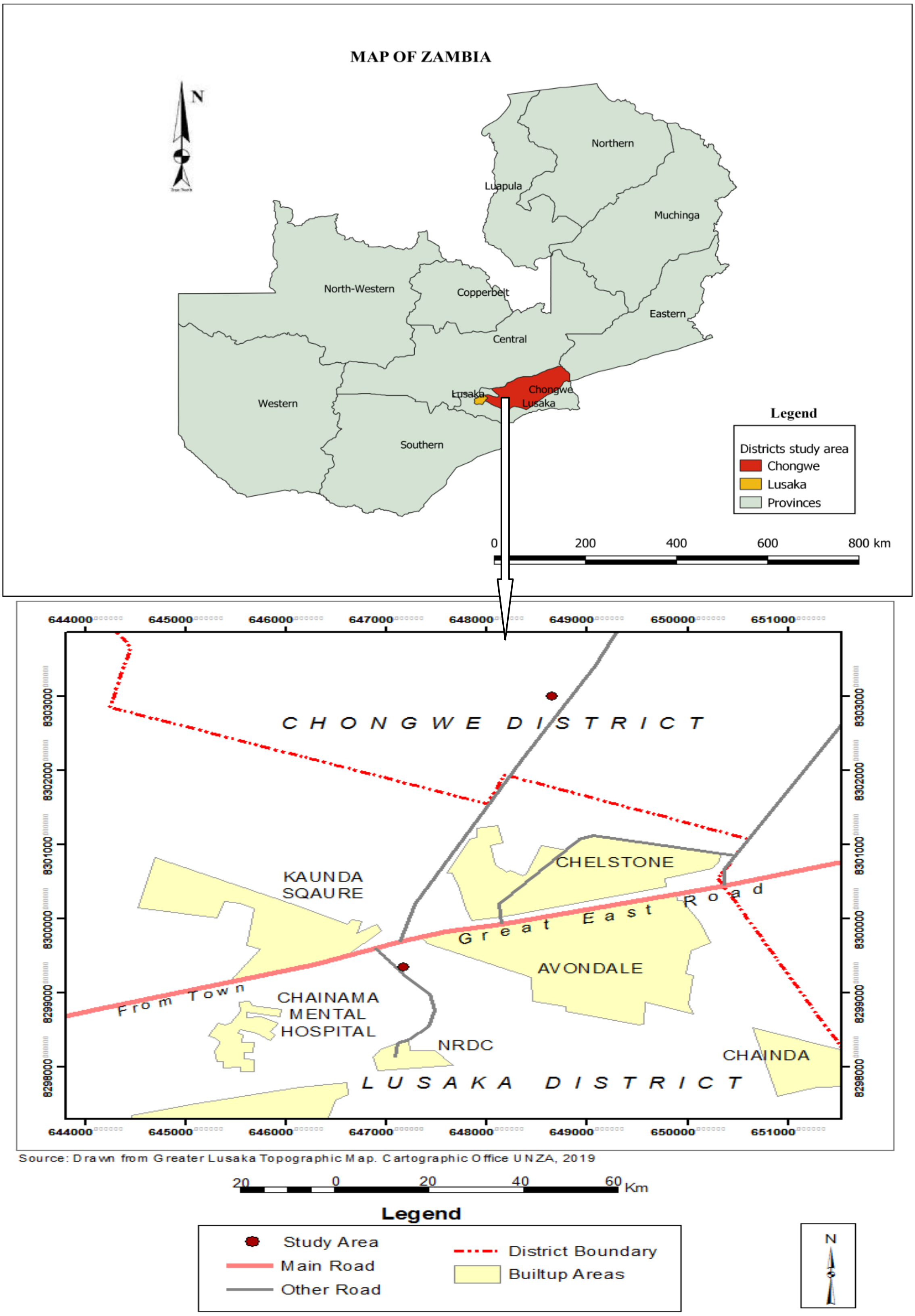
3.1 Location and study site

The study was conducted in agro-ecological zone II (Figure 5). The study involved a laboratory bioassay conducted at Entomology Laboratory (Department of Plant Science) at the University of Zambia (UNZA). Field studies were conducted in two locations, Natural Resources Development College (NRDC) in Lusaka (latitude 15°22'43"S and longitude 28°22'16"E) and a private farm in Chongwe district (latitude 15°20'38"S and longitude 28°23'4"E) (Figure 6).



Source: Soil Survey, Mt. Makulu Chilanga

Figure 5: Map showing agro ecological zones in Zambia



Source: Drawn from Greater Lusaka Topographic Map. Cartographic Office UNZA, 2019

Figure 6: General map showing location of field study sites. (Source: Geography department, UNZA)

3.2 Climatic and weather conditions

Agro-region II experiences medium rainfall ranging from 800 to 1000mm/annum. During the study conducted from July, 2018 to November, 2018, mean temperature ranged between 15.8°C to 24.3°C while the mean relative humidity ranged between 37.75% to 65.3% (Table 1).

Table 1 : General weather conditions for study area during 2018 farming season

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Mean Temperature (°C)	22.9	21.2	21.6	20.5	19.5	16.9	15.8	20.7	24.3	23.5	24.3	22.6
Mean minimum Temperature (°C)	14.5	16.7	17.2	10.3	11.2	7.4	8.6	12.1	13.9	13.1	16.3	16.5
Maximum Temperature (°C)	32.3	29.2	29.1	28.2	29.3	27.2	26.3	30.9	33.6	34.9	35.5	33.8
Rain Days (mm)	27.4	343.2	143.6	8.0	32.2	0.0	0.0	0.0	0.0	0.4	47.2	142.2
Mean Relative humidity (%RH)	69.9	90.4	86.4	78.3	73.2	61.3	65.3	43.4	37.7	42.5	54.1	79.1
Minimum Relative humidity(%RH)	17.7	48.7	50.4	36.8	31.3	25.7	25.1	16.2	10.7	11.6	13.8	28.2
Maximum Relative Humidity (%RH)	100	100	100	100	100	94.4	97.5	84.8	71.6	80.6	100	100

Source: Metrological Department of Zambia

3.3 Soil type

Soil texture analysis conducted for NRDC indicated that the soils under consideration were classified as silty clay loam and clay in the 0 - 15cm and 15 - 30cm depths, respectively. Additionally, the soils showed increasing clay content with depth. Soil texture analysis conducted for private farm indicated that the soils were classified as loamy sand and sandy clay loam in the 0 - 15cm and 15 - 30cm depths, respectively. The soils showed increasing clay content with depth. Loamy sands and sandy clay loams are lighter textured soils with desirable infiltration rates (Kopec, 1995).

3.4 Vegetation type

The vegetation in the study area was predominately Munga woodland. The one story deciduous woodland is open or pack like and composed of tree species such as *Combretum spp.*, *Terminalia* spp. and *Acacia.* spp. These trees are one or two storey and are found in clusters or scattered (Siampale, 2008). The undergrowth is characterised by tall and dense grass belonging to the genera *Hyparrhenia, Seteria, Digitaria and Brachiaria* (Everisto and Kitalyi, 2002).

3.5 Study Design

3.6.1 Laboratory Experimental Design and Treatments

Randomised complete design (RCD) was used in the laboratory studies. Four treatments were used in the study and these included *Azadirachta indica* (Nimbecidine®) (Osho Chemicals Zambia Limited, Lusaka, Zambia), *Beauveria bassiana* (Bio-power®) (Osho Chemicals Zambia Limited, Lusaka, Zambia), *Metarhizium anisopliae* (Bio-magic®) (Osho Chemicals Zambia Limited, Lusaka, Zambia), *Verticillium lecanii* (Bio-catch®) (Osho Chemicals Zambia Limited, Lusaka, Zambia) and an untreated control. Four concentrations for each treatment consisted of the recommended dose, a dose lower than the recommended dose and two doses higher than the recommended dose. The aforementioned concentrations were measured using a micropipette. All treatments were replicated three times. Details of the concentrations used are listed in (Table 2).

Table 2: Concentrations of selected biopesticides used against 2nd instar larvae of *T. absoluta* in the laboratory studies

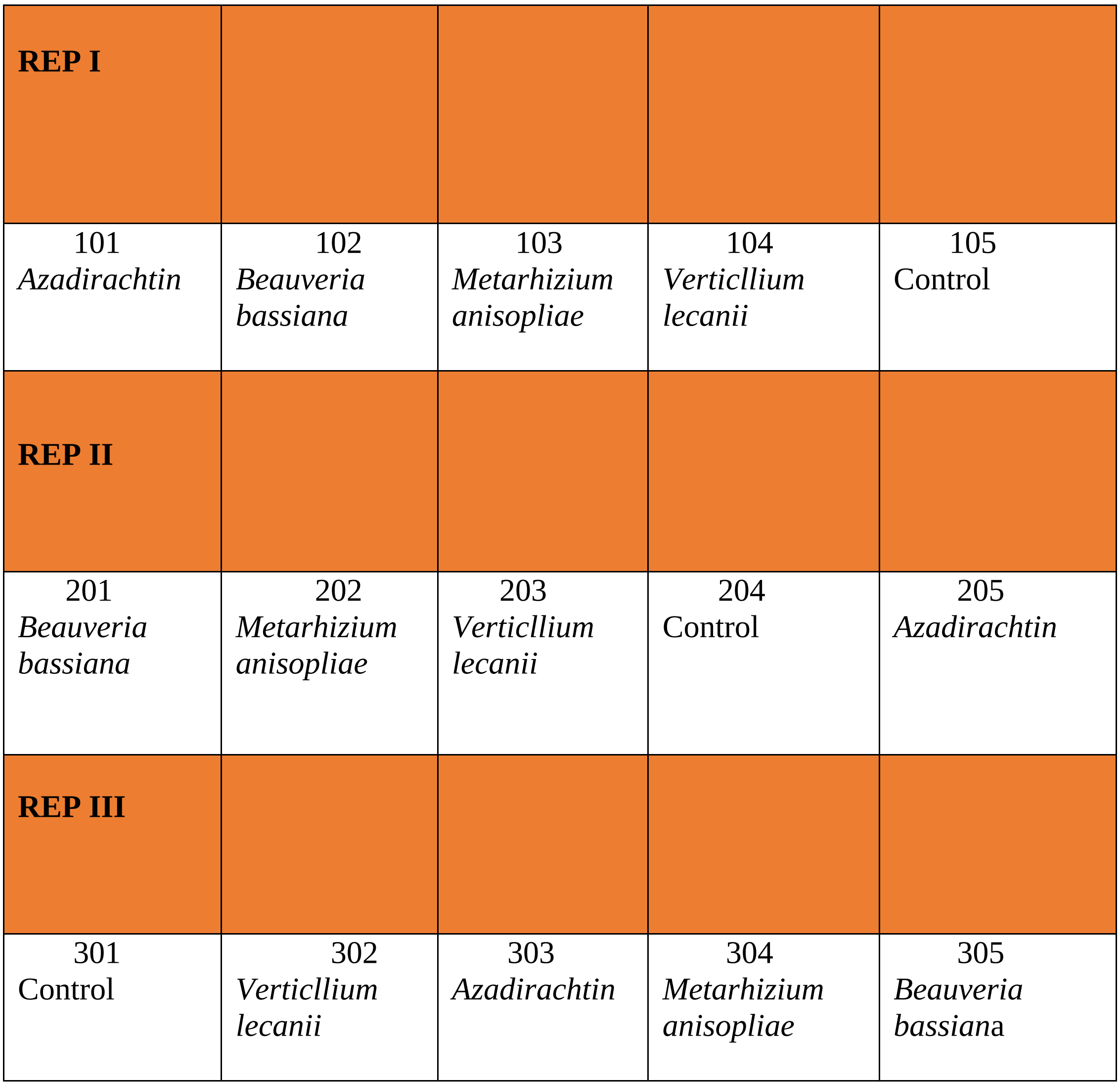
Biopesticides	Recommended dose in 25ml water	Dilute Concentration in 25ml water	Higher Concentration in 25ml water	
Verticillium lecanii (Bio-Catch®)	100 µl	75 µl	125 µl	150 µl
Metarhizium anisopliae (Bio-Magic®)	150 µl	100 µl	175 µl	200 µl
Beauveria bassiana (Bio-Power®)	100 µl	75 µl	125 µl	150 µl
Azadirachta indica (Nimbecidine®)	75 µl	50 µl	100 µl	125 µl

3.6 Field Experimental design and Treatments

The field was prepared in a randomised complete block design (RCBD) with three replicates and four treatments. The treatments consisted of *A. indica* (Nimbecidine®), *B. bassiana* (Bio-power®), *M. anisopliae* (Bio-magic®), *V. lecanii* (Bio-catch®) and an untreated control; which were applied using a knapsack sprayer. The recommended rate was used for all treatments. Details of the biopesticides used are shown below (Table 3). There were a total of fifteen plots with each plot measuring 5 m in length and 4 m in width. There were three rows with eight plants per row and thus, a total of 24 plants per plot. The total field experimental area was 40× 28m (Figure 7).

Table 3: List of biopesticides and recommended application rates

Trade name	Active ingredients	Type of biopesticide	Recommended dose in 20l water
Nimbecidine®	Azadirachtin	Plant extract	80ml
Bio-Catch®	*Verticllium lecanii*	Entomopathogenic fungus	80ml
Bio-Magic®	*Metarhizium anisopliae*	Entomopathogenic fungus	80ml
Bio-Power®	*Beauveria bassiana*	Entomopathogenic fungus	80ml



REP I				
101 *Azadirachtin*	102 *Beauveria bassiana*	103 *Metarhizium anisopliae*	104 *Verticllium lecanii*	105 Control
REP II				
201 *Beauveria bassiana*	202 *Metarhizium anisopliae*	203 *Verticllium lecanii*	204 Control	205 *Azadirachtin*
REP III				
301 Control	302 *Verticllium lecanii*	303 *Azadirachtin*	304 *Metarhizium anisopliae*	305 *Beauveria bassiana*

Figure 7: Diagram showing the field layout and randomisation

3.7 Land Preparation and Planting

During land preparations conventional ploughing was adopted. A tractor drawn plough was used. In order to achieve a fine tilth, ploughing was followed by harrowing using a disc harrow. A total of 15 treatment plots were marked out as shown in figure 7. Three planting ridges that were 1.5 m apart were made per plot and drippers were laid on top of each ridge (figure 8). A space of 1.5 m was maintained between plots in order to reduce the effects of pesticide drift between the treatments. The tomato variety used in the study was Domino which is an indeterminate variety. Seedlings of this variety were obtained from Zamseed (Zambia Seed Company Limited, Lusaka, Zambia). Three weeks old seedlings were transplanted in the first week of July in each of the ridges at 0.6 m spacing between plants and each plot had 24 plants with a total of 360 seedlings. The seedlings were transplanted in the afternoon to avoid sunburn and this was followed by drip irrigation (Figure 9). Trellising of tomatoes was done at three weeks after planting.

3.8 Fertilisation and Spraying

Basal dressing was applied using compound D fertiliser (N=10, P=20 and K=10) (Zambian Fertilizer Ltd, Lusaka, Zambia) at a rate of 1000kg/ha one week after transplanting. Three weeks later, the tomato plants were top dressed using Veg Top 32 (N=21, P=0 and K=32) (Zambian Fertilizer Ltd, Lusaka, Zambia) and this application was repeated every three weeks. Application of the biopesticides began a week after transplanting and continued until harvest. Treatments were applied on a weekly basis in the late afternoon between 05:00PM and 06:30PM hours using a knapsack sprayer. The fungicide Macozeb® (Hygrotech Sustainable Solutions Ltd, Lusaka, Zambia) was applied every two weeks at the rate of 40g/16L to control fungal and bacterial diseases. The fungicides were applied on different days with the biopesticides.

Figure 8: Laying of drippers before transplanting

Figure 9: Transplanting of tomato seedlings.

3.9 Data collection

540 leaves from 180 plants were examined for numbers of eggs deposited by *T. absoluta*. To evaluate the egg density, nine leaves of tomato were picked randomly from three randomly selected plants in each plot. Each leaflet was collected from the top, middle and bottom portion of a plant. The number of eggs from each selected leaves were counted and recorded. The egg counting was done in two separate counts, the first count was made in the second month after transplanting of seedlings (August, 2018) and second count was done a month later (September, 2018). To evaluate the severity of leaf damage, data was collected from each plot using a scoring scale of 1 to 5 where; 1= no damage, 2=25% of plants damaged, 3=25 % to 50 plants damaged, 4=50-75% plants damaged and 5= >75% plants damaged (Ayalew, 2015). Inspection of the plot of residues (data not shown) revealed no violation of ANOVA for leaf damage accounting for the use of this qualitative leaf damage score in the subsequent analysis.

3.10 Part B: Laboratory Studies

Mortality of *T. absoluta* due to treatment by azadirachtin, *B. bassiana*, *M. anisopliae* and *V. lecanii* against *T. absoluta* was carried out in the laboratory using Abbott's formula (1925). Second instar larvae were used in all the bioassays and. This experiment was carried out in a controlled room (temperature 25 ± 2ºC, photoperiod 14:10 (L: D) and relative humidity (RH) 30-40 %.

3.11 Rearing of *T. absoluta* in the laboratory

A starting pure culture of *T. absoluta* was collected from infested tomato fields at Natural Resources Development College (NRDC). Tomato leaves infested with *T. absoluta* larvae were collected and placed into ventilated plastic bags and later transferred to the laboratory within 1-2 hours of collection. Fourth instar larvae were removed from infested leaves and placed in glass jars which were then plugged tightly with cotton wool in the laboratory. Pupation took place after two days and pupae were placed in small rearing cages measuring (45 cm height ×30 cm width×55 cm length) (Figure 10). Adult moths emerged after nine days and these were fed 20% honey solution placed on cotton wool in small plastic cups within the rearing cages.

Tomato seedlings (45 days old) planted in small plastic pots were placed in rearing cages and exposed to *T. absoluta* adults for 24 hrs. The adult *T. absoluta* were removed from cages and the eggs deposited on leaves were left to hatch. Larvae were left to develop on the plants until they reached the second instar. Second instar larvae were used in laboratory studies because this stage has been reported to be the most damaging and yet, most vulnerable stage to insecticides (Cherif *et al*., 2013). The second instar larvae were carefully removed from the leaf mines using a size 1 paint brush. The different stages of larvae were identified using morphological features according to Nayana and Kalleshwaraswamy (2015).

Figure 10: Cage used for rearing of *T. absoluta*

3.12 Laboratory bioassays

A total of 900 second instar larvae obtained from insects reared in the laboratory, were used in the laboratory biossays. Four dilutions of each biopesticide were used in the bioassay. *M. anisopliae* (100 µl, 150 µl, 175 µl and 200 µl), *B. bassiana* (75 µl, 100 µl, 125 µl and 150 µl), *V. lecanii* (75 µl, 100 µl, 125 µl and 150 µl) and *A. indica* (50 µl,75 µl 100 µl and 125 µl) (Table 3). Each of which was diluted in 25ml of distiled water. Each of these dilutions were replicated three times. 3 ml of the prepared dilutions was applied onto the 15 larvae placed in petri dishes lined with grade one 9 cm filter paper (GE Healthcare UK Limited, Little Chalfont, UK). The petri dishes were sealed with perforated lids to allow air circulation. 3 ml of distilled water was placed on the filter paper lining the petri dish before placing *T. absoluta* larvae. Fresh tomato leaves (2.5cm length × 1.0cm width) washed with distilled water were supplied on a daily basis as a source of nourishment for *T. absoluta* larvae. The number of dead larvae of each treatment and control were counted daily for seven days. Larvae were considered to be dead when they failed to move back to the ventral position after being placed on their dorsum. The bioassay was conducted for seven days and the median lethal concentration (LC_{50}) values were obtained using Probit analysis, which was performed using Genstat statistical software (VSN International, 2014). The larval mortality was corrected using Abbott's formula (Abbott 1925) as shown below:

$$\text{Corrected mortality} = \frac{X - Y}{X} \times 100$$

Where X= Percent living in the untreated control

Y= Percent living per treatment

X – Y = The percent killed by the treatment

According to Abbott (1925), the control mortality should be less than 20% for the corrected mortality to be reliable.

3.13 Data analysis

3.13.1 Field study

The efficacy of treatments on mean number of eggs and leaf damage was analysed using one-way analysis of variance (ANOVA) and means that were statistically different were separated by Fisher's least significant difference (LSD) test with $\alpha = 0.05$. All the analyses were performed using Genstat statistical software (VSN International, 2014).

3.13.2 Laboratory Bioassay

In laboratory studies, all statistical analysis were performed using Genstat statistical software (VSN international, 2014). Larval percent mortality was corrected using Abbott's formula (1925). Probit analysis was used to determine the estimated median lethal dose (LC_{50}). Analysis of variance (ANOVA) was used for determination of statistical differences and Fisher's least significant difference (LSD) test to separate statistically different means at $\alpha = 0.05$.

CHAPTER 4: RESULTS

4.1 Determination of the most effective biopesticide on larval mortality

The mean percent mortality ranged from 41.3±6.8% to 77.9±14.3% (Table 4). Application of *A. indica* led to the highest mortality of 77.9±14.3% while mortality due to *B. bassiana*, *V. lecanii*, and *M. anisopliae* was 48.3±2.8%, 44.8±4.8% and 41.3±6.8%, respectively. However, there were no significant differences (*P*<0.001) among the entomopathogens (*B. bassiana*, *V. lecanii*, and *M. anisopliae*).

Table 4: Mean percent mortality of *T. absoluta* larvae caused by selected biopesticides

Biopesticide	Mean % Mortality
V. lecanii	44.8±4.8a
M. anisopliae	41.3±6.8a
B.bassiana	48.3±2.8a
A. indica	77.9±14.3b

Means within columns followed by common letters are not significantly different (Fisher's Protected Least Significance test)

Dose mortality due to *M. anisopliae* was significantly higher among the biopesticides (*P*<0.001). *Azadirachtin indica* caused the least mean dose mortality on *T. absoluta* larvae (Table 5).

Table 5: Mean dose of selected biopesticides used on *T. absoluta* larvae.

Biopesticides	Dose (µL)
M. anisopliae	162.5±25.3a
V.lecanii	112.5±3.6b
B. bassiana	112.5±3.6b
A. indica	87.5±18.0b

Means within columns followed by common letters are not significantly different (Fisher's Protected Least Significance test)

4.2 Median lethal doses for selected biopesticides

The estimated median lethal concentration (LC_{50}) values of the selected biopesticides at 95% confidence limits are shown in Table 6. The LC_{50} for *A. indica* (30.4± 0.4 µL) was lower than the field recommended dose (50µL). The LC_{50} for *B. bassiana* and *V. lecanii* were 107.1 ± 0.4 µL and 118.7± 0.4µL, respectively. The LC_{50} for both *B. bassiana* and *V. lecanii* were slightly higher than the recommended dose of 100µl. However, the LC_{50} for *M. anisopliae* (193±0.4µL) falls between the higher concentrations of 175µl and 200µl. Overall, *A. indica* was the most effective followed by *B. bassiana*, then *V. lecanii* and *M. anisopliae* showed the least efficacy.

Table 6: Probit Analysis (LC_{50}) for selected Biopesticides

Group	Lethal Dose (LD)	Estimated Dose (µL)	Lower 95%	Upper 95%
V. lecanii	50	118.7±0.4	106.8	133.1
M. anisopliae	50	193±0.4	173.4	218.7
B. bassiana	50	107.1±0.4	96.0	119.3
A. indica	50	30.4±0.4	23.0	37.1

4.3 Corrected larval mortality

The corrected percent mortalities are shown in Table 7. The mean number of dead larvae due to application of *A. indica* ranged from 10.7±0.3 to 13.3±0.7 and the percent corrected mortality ranged from 69.8±8.1% to 88.4±41.9%. Mean mortality due to *V. lecanii* application ranged from 5.0±0.0 to 8.7±0.9 with corrected mortalities ranging from 30.2±1.7% to 55.8±14.6%. *M. anisopliae* mean mortality ranged from 5.3±0.3 to 8.3±0.3 with corrected mortality of 32.6±4.0% to 53.5±5.4%. Mean mortality due to *B. bassiana* ranged from 5.3±0.3 to 9.3±0.9 and the corrected mortality were 32.6±4.6% to 60.5±16.3%. The mean mortality for the control was 0.7±0.3. *A. indica* had the highest corrected mortality followed by *B. bassiana* and then *V. lecanii*, while *M. anisopliae* had the least percent mortality. The corrected percent mortality in all biopesticides increased with increasing concentrations across all treatments (highest concentrations led to the highest mortalities).

Table 7: Corrected Mortalities (% SE) of selected Biopesticides

Biopesticide	Dose(μL)	M_{av}	$\%M_{av}$	L_{av}	$\%L_{av}$	$\%M_{corr}$
B. bassiana	75	5.3 ± 0.3	35.6 ± 6.3	9.7 ± 0.3	64.4 ± 3.4	32.6 ± 4.0
B. bassiana	100	7.3 ± 0.3	48.9 ± 4.5	7.7 ± 0.3	51.1 ± 4.3	46.5 ± 4.7
B. bassiana	125	8.3 ± 0.3	55.6 ± 4.0	6.7 ± 0.3	44.4 ± 5.0	53.5 ± 5.4
B. bassiana	150	9.3 ± 0.9	62.2 ± 9.4	5.7 ± 0.9	37.8 ± 15.6	60.5 ± 16.3
V. lecanii	75	5.0 ± 0.0	33.3±0.0	10.0 ± 0.0	66.7 ± 0.0	30.2 ± 1.7
V. lecanii	100	7.0 ± 0.6	46.7 ± 8.2	8.0 ± 0.6	53.3 ± 7.2	44.2 ± 7.7
V. lecanii	125	7.7 ± 0.7	51.1 ± 8.7	7.3 ± 0.7	48.9 ± 9.1	48.8 ± 9.6
V. lecanii	150	8.7 ± 0.9	57.8 ± 10.2	6.3 ± 0.9	42.2 ± 13.9	55.8 ± 14.6
M. anisopliae	125	5.3 ± 0.3	35.6 ± 6.3	9.7 ± 0.3	64.4 ± 3.4	32.6 ± 4.0
M. anisopliae	150	6.0 ± 0.6	40.0 ± 9.6	9.0 ± 0.6	60.0 ± 6.4	37.2 ± 6.9

M. anisopliae	175	6.7 ± 0.3	44.4 ± 5.0	8.3 ± 0.3	55.6 ± 4.0	41.9 ± 4.4
M. anisopliae	200	8.3 ± 0.3	55.6 ± 4.0	6.7 ± 0.3	44.4 ± 5.0	53.5 ± 5.4
A. indica	50	10.7 ± 0.3	71.1 ± 3.1	4.3 ± 0.3	28.9 ± 7.7	69.8 ± 8.1
A. indica	75	11.3 ± 0.3	75.6 ± 2.9	3.7 ± 0.3	24.4 ± 9.1	74.4 ± 9.5
A. indica	100	12.0 ± 0.0	80.0 ± 0.0	3.0 ± 0.0	20.0 ± 0.0	79.1 ± 0.5
A. indica	125	13.3 ± 0.7	88.9 ± 5.0	1.7 ± 0.7	11.1 ± 40.0	88.4 ± 41.9
Control	0	0.7±0.3	4.4±50.0	14.3 ± 0.3	95.6 ± 2.3	0

***M_{av} denotes average mortality**
***%M_{av} denotes percent average mortality**
***L_{av} denotes average larvae alive as at day seven**
***%L_{av} denotes percent average larvae alive as at day seven**
%M_{corr} denotes percent corrected mortality

4.4 Evaluation of effects of selected biopesticides on *T. absoluta* oviposition

The average number of eggs per plant ranged from 2.8 ±1.1 to 33.8 ±2.7 across all the treatments in both locations (Table 8). The highest egg counts were found in the untreated control plots and these were 17.0±1.5 and 33.8 ± 2.7 during the first and second count, respectively. There were significant differences among the treatments in the first count ($P < 0.001$). In the first count, *A. indica* significantly reduced the number of eggs deposited, followed by *B. bassiana* then *V. lecanii* and *M. anisopliae* had the highest number of eggs Table 8. In the second count, there were no significant differences in number of eggs deposited on plants treated with biopesticides.

Table 8: Mean egg count of *T. absoluta* on tomato plants for selected treatments

Biopesticides	**1st count**	**2nd count**
A. indica	2.8 ±1.1 a	10.7 ±1.2 a
B. bassiana	3.0 ±0.6 a	10.7±1.9 a
V. lecanii	4.3 ±1.2ab	11.7±1.4 a
M. anisopliae	6.8 ±1.4 b	13.0±1.6 a
Control	17.0 ±1.5 c	33.8 ±2.7 b
LSD(α=0.05)	3.7	3.9

Means within columns followed by common letters are not significantly different (Fisher's Protected Least Significance test)

4.5 Assessment of the effects of selected biopesticides on leaf damage caused by *T. absoluta*

Leaf damage ranged from 1.2±0.2 to 4.5±0.3 for all treatments in both locations (Table 9). The lowest leaf damage was observed in the second month in plots treated with *M. anisopliae* (1.2±0.2) and *A. indica* (1.2±0.2) followed by *V. lecanii* (1.3±0.2) and *B. bassiana* (1.3±0.2). No infestation was observed in August, however, there was an increase in leaf damage in September with infestation levels of 25 to 50% (Table 10). All the treatments significantly reduced leaf damage compared with the control ($P<0.001$). The leaf damage in the control plots had higher infestation levels of 50 to 75%. Further, there were significant differences ($P< 0.001$) in leaf damage between NRDC (Location A) and the private farm in Chongwe district (Location B) (Table 10). Leaf damage was higher at the Private farm (25%) in the 2nd month and (25- 50%) in the 3rd month. On the other hand, there was no leaf damage at NRDC in the 2nd month but leaf damage of 25-50% was observed in the 3rd month. Severity of damage was highest in the 3rd month compared to 2nd second month at both locations.

Table 9: Mean leaf damage severity score on a 1-5 scale (1= no damage, 2=25% of plants damaged, 3=25 to 50% plants damaged, 4=50-75% damage and 5= >75% damage) on tomato plants

Biopesticide	**2nd Month**	**3rd Month**
M. anisopliae	1.2 (±0.2) a	3.3 (±0.3) a
A. indica	1.2 (±0.2) a	3.2 (±0.3) a
V.lecanii	1.3 (±0.2) a	3.3 (±0.3) a
B. bassiana	1.3 (±0.2) a	3.2 (±0.3) a
Control	4.3 (±0.3) b	4.5(±0.3) b
LSD(α=0.05)	0.4	0.3

Means within columns followed by common letters are not significantly different (Fisher's Protected Least Significance test)

Table 10: Mean leaf damage score on a 1-5 scale caused by *T. absoluta* for the two different locations

Location	**2nd Month (August)**	**3rd Month (September)**
NRDC (Location A)	1.5 (No infestation)	2.8 (25 % infestation)
Private Farm (Location B)	2.2 (25% infestation)	3.3 (25-50 % infestation)
LSD α= 0.05	0.31	0.32

CHAPTER 5: DISCUSSION

In this study, the efficacy of commercial formulations of *B. bassiana*, *M. anisopliae*, *V. lecanii* and *A. indica* are reported. Results from both the laboratory and field studies demonstrate the possibility of reducing *T. absoluta* populations through the use of the selected biopesticides. The results obtained from laboratory studies demonstrated that there were significant differences on larval mortality among the selected biopesticides. *A. indica* was more effective than the entomopathogenic fungi and caused mortality of 71.45% - 85.7%. This is collaborated by previous studies which showed percent mortality of 52.4% - 95% (Goncalves, 2007) and 70% - 86% when 2^{nd} instar larvae were treated with *A. indica* (Jallow *et al*., 2018). There were no significant differences among the entomopathogens and this can be attributed to a similar mode of action by all entomopathogenic fungi (Inglis *et al*., 2001). However, *B. bassiana* was the most effective among the entomopathogens in the current study. In contrast, Inanl *et al*. (2012) reported that *M. anisopliae* was more effective than *B. bassiana* against the egg and larval stages of *T. absoluta*. The results from the current study are compatible with Tadele and Emana (2017) who demonstrated that *B. bassiana* caused higher mortality than *M. anisopliae* against *T.absoluta*. *Beauveria bassiana* has been widely studied and has been considered as one of the most important biopesticides (Qazzaz *et al*., 2015). The second most effective entomopathogen was *V. lecanii*. *V. lecanii* has been reported to be effective against neonate, 2^{nd} instar and 3^{rd} instar larvae of *T. absoluta* (Abdel-Raheem *et al*., 2015). The least effective biopesticide in the current study was *M. anisopliae.*

The laboratory findings of this study showed that LC_{50} for azadirachtin was 30.4±0.4 µL while the recommended dose is 50µL indicating that it is effective even at doses lower than the recommended.

Effectiveness of the selected entomopathogens used in the laboratory studies demonstrated that the corrected percent mortality began two days after application and increased with time. These results are collaborated by other studies which demonstrated that *M. anisopliae, B. bassiana* and *V. lecanii* were effective two days after application of entomopathogenic fungi (Abdel-Raheem *et al*., 2017; Shiberu and Getu 2018). This delay could be attributed to the nature of fungal infection process, which is complex and involves several stages (Mora *et al*., 2015). On the contrary, there was a quick knockdown effect on larvae treated with *A. indica* and corrected percent mortality was observed from the first day of treatment. Other studies have also shown this quick knock down effect of azadirachtin on *T. absoluta* larvae (Goncalves 2007). In addition, other authors have also reported a very strong contact toxicity by azadirachtin on larvae of *Tirathaba rufivena* Walker (Lepidoptera: Pyralidae) (Baozhu *et al*., 2017). For all the biopesticides used, the corrected percent mortality increased with increased concentration.

Although all the selected biopesticides exhibited a lower egg count compared to the control, *M. anisopliae* had the highest egg count among the biopesticides in the first egg count. However, *M. anisopliae* is not very effective against the adult stage (Pires *et al*., 2009). In the second count *M. anisopliae* did not differ from the rest of the selected biopesticides. The possible explanation would be that plants treated with *M. anisopliae* had the highest number of eggs resulting in having more larvae during the second count. Plants with a high infestation provide a less favourable environment for the pest to deposit eggs when compared to less infested sites, which are unlikely to support the development and growth of newly laid eggs (Bawin *et al*. 2014). Plants treated with azadirachtin recorded the least egg count followed by those treated with *B. bassiana*, then *V. lecanii* and *M. anisopliae*, respectively. These findings are also collaborated by a study that showed a reduction in the number of eggs deposited on leaves that had been treated with azadirachtin (Tomé *et al*., 2013). Tomé *et al*. (2013) also observed avoidance behaviour in *T. absoluta* moths to tomato leaves that had been sprayed with azadirachtin. It is possible that the reduced egg oviposition on treated tomato plants could be a result of the selected biopesticides masking the tomato leaf secondary compounds that are responsible in attracting female moths of *T. absoluta* as previously suggested (Hasan and Ansari, 2011). Plants produce volatile substances that play a role in attracting or deterring females and mediating oviposition (Bawin *et al*., 2014). It was reported that *T. absoluta* upward orientation flight, landing and oviposition are influenced by volatile terpenoid compounds produced by tomato leaves (Proffit *et al*., 2011).

Results for leaf damage demonstrated that biopesticides did not differ significantly from each other but differed significantly from the control. The possible explanation for this could be that the treatments applied could have caused larvae to disperse to untreated plots. Previous studies have suggested that choice avoidance was able to happen in small cultivated fields having untreated plants (Tome *et al*., 2013). There were significant differences between the two locations with regard to leaf damage. Leaf damage at the private farm in Chongwe district was higher than that at NRDC possibly due to its close proximity to another tomato field about 500m away. It is likely that *T. absoluta* could have migrated from the highly infested field to the private farm since *T. absoluta* adults has been reported to move 400m overnight (Salama *et al*., 2015). In addition, the tomato field at NRDC was surrounded by uncultivated land characterised by long grass and shrubs. These areas could possibly harbour natural enemies for *T. absoluta* and contributed to more effective control resulting in less leaf damage. Jonsson *et al*. (2010) reported that the landscape structure and habitat plays a role in conserving natural enemies which in turn enable the effective control of invasive pests. It was also reported that combination of natural enemies and biopesticides enhanced the efficacy against *T. absoluta* (Soares *et al*., 2019). The soil types from the different locations had an impact on leaf damage. The soil at NRDC which were predominantly clay had less leaf damage compared to the soil at private farm which were sandy in nature. Clay soil holds more water and nutrients, in addition clay particles tend to have a variety of minerals than sandy soil Crouse (2018). Miguel et al., (2012) reported that nutrient rich soil results into healthy plants that can better withstand pest pressure.

Furthermore, this study showed that leaf damage was higher in September than August. This could be attributed to increase in temperature as previously demonstrated by Lacordaire and Feuvrier (2010). Balzan and Moonen (2012) also showed that there is an exponential increase of *T. absoluta* with increase in temperature. This is consistent with the current findings that leaf damage was high in September than August which recorded mean temperature of 24.3°C and 20.7°C, respectively.

Results from this study also provide a clear understanding on the integration of biopesticides in the current management practices of *T. absoluta*. Currently, the main control strategy of *T. absoluta* is the application of conventional chemicals (Lietti *et al*., 2005). However, *T. absoluta* has been reported to show resistance to some classes of conventional chemicals (Lietti *et al*., 2005). Therefore, the use of biopesticides would provide a sustainable management tactic for *T. absoluta* in Zambia.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This current study clearly demonstrates the efficacy of the selected biopesticides against the egg and larval stages of *T. absoluta*.

A. indica was the most effective biopesticide against *T. absoluta* larvae and it is effective at 30.4±0.3 µL, a dosage lower than the ones recommended. However, entomopathogens can be more effective at a dose slightly higher than the label recommended doses at 107.1±0.3 µL, 118.7±0.4 µL and 193.0±0.4 µL for *B. bassiana, V. lecanii and M. anisopliae,* respectively.

A. indica B. bassiana and *V. lecanii* had the same effect on egg oviposition. However, *M. anisopliae* performance was similar to *V. lecanii* but was not as effective as *A. indica* and *B. bassiana*. In terms of leaf damage, all the tomato plants used in the study appeared healthier than the untreated plants showing that the biopesticides were all effective.

These biopesticides are affordable, do not affect natural enemies of *T. absoluta*, have no negative effects on the environment and on human health. However, in order to effectively control of *T. absoluta* it is important that all control measures such as cultural control, biological control and judicial use of registered pesticides are used.

6.2 Recommendations

1. Based on the results obtained from this study, the selected biopesticides can be recommended for the control of *T. absoluta*. Farmers are encouraged to use these biopesticides in controlling *T. absoluta* in IPM programme

2. Future studies should assess how these biopesticides might work in other agroecological zones of Zambia.

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APPENDICES

Appendix A: Natural enemies present in fields treated with selected biopesticides at NRDC field

Appendix B. Leaf damage caused by feeding larvae of *T. absoluta*

Appendix C. Larvae of *T. absoluta* subjected to biopesticide treatments in the laboratory

Appendix D. Seedlings used in the field study

Appendix E. Land preparation at NRDC before laying of drippers