Efficacy of selected biopesticides against the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), in Lusaka, Zambia

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ARTICLE INFO

Article history:
Received: 23 April 2021
Received in revised form: 15 September 2021
Accepted: 27 September 2021
Published: 25 February 2022
Edited by KC Chinsembu

Keywords:
Tuta absoluta
Biopesticide
Nimbecidine
Bio-magic
Bio-power
Bio-catch

ABSTRACT

The aim of this research was to evaluate the efficacy of four commercially available biopesticide formulations, *Azadirachtin indica, Beauveria bassiana, Metarhizium anisopliae* and *Verticillium lecanii* in the management of *Tuta Absoluta* (Meyrick)(Lepidoptera: Gelechiidae) under laboratory and field conditions. Analysis of variance (ANOVA) using Fisher's least significant difference test ($\alpha = 0.05$) showed that the mean percent mortality ranged from $41.3 \pm 6.8\%$ to $77.9 \pm 14.3\%$. *A. indica* caused the highest mortality of $77.9 \pm 14.3\%$ while mortality due to *B. bassiana, V. lecanii*, and *M. anisopliae* was $48.3 \pm 2.8\%$, $44.8 \pm 4.8\%$ and $41.3 \pm 6.8\%$, respectively. The estimated LC_{50} for *A. indica* was $30.4\,\mu L \pm 0.4$, while LC_{50} for *B. bassiana, M. anisopliae, V. lecanii* were $107.1\,\mu L \pm 0.4$, $193\,\mu L \pm 0.4$ and $118.7\,\mu L \pm 0.4$, respectively. Biopesticide efficacy on larval mortality from highest to least was *A. indica, B. bassiana, V. lecanii* and *M. anisopliae* respectively. Percentage corrected mortality ranged from *A. indica* (69.8 ± 8.1 to $88.4 \pm 41.9\%$), *B. bassiana* ($32.6 \pm 4.0\%$ to $60.5 \pm 16.3\%$), *V. lecanii* ($30.2 \pm 1.7\%$ to $55.8 \pm 14.6\%$) and *M. anisopliae* ($32.6 \pm 4.0\%$ to $53.5 \pm 5.4\%$) respectively. The selected biopesticides significantly ($P < 0.001$) reduced *T. absoluta* egg count compared with the untreated control. Performance ranged from *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 egg mortality, respectively. The study showed that selected biopesticides were effective and should be used by farmers as an integral 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.

1 Introduction

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 (Luthria *et al*, 2006). 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 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). *Tuta 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

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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*, 2019). 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) where 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; Dhaka and Singh, 2019) because biopesticides 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, 2014). 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 *Beauveria bassiana*, *Metarhizium anisopliae* and *Verticillium lecanii* (Braham *et al*, 2012; Abd El-Ghanny *et al*, 2016, 2018). Likewise, botanical biopesticides such as *Azadirachtin 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®) (T. Stanes and Company Limited Coimbatore, India) for the control of *T. absoluta* under laboratory and field conditions.

2 Materials and methods

2.1 Location and study site

The bioassay was conducted at the Entomology Laboratory in the Department of Plant Science, School of Agricultural Sciences at the University of Zambia. Field studies were conducted at 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 1).

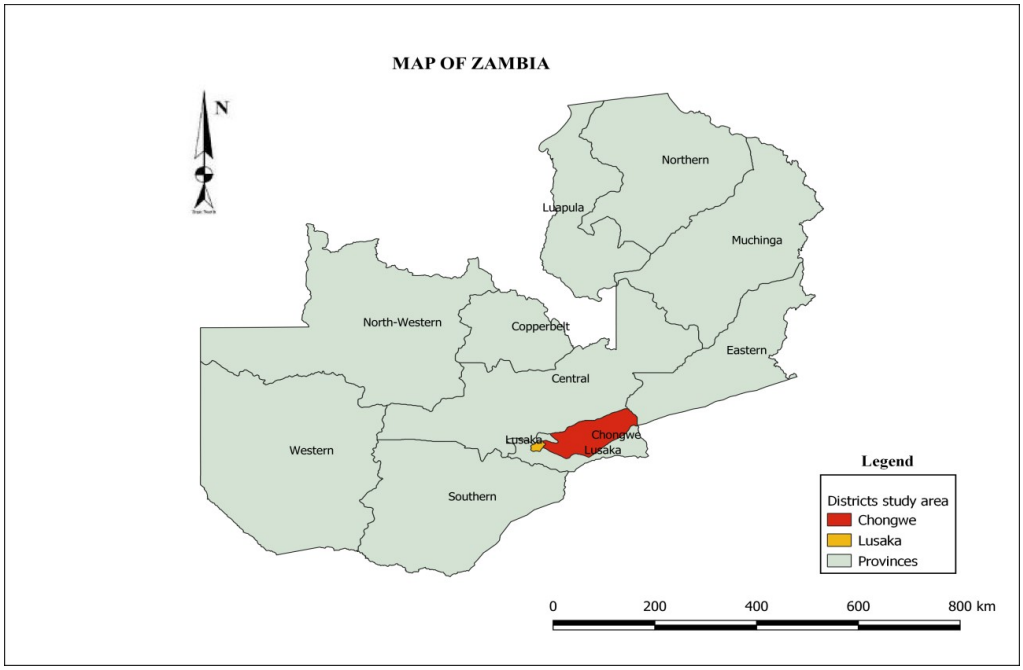

Figure 1: Shows the location of study area. (Source: Department of Geography and Environmental Studies, School of Natural Sciences, University of Zambia)

2.2 Laboratory Experimental Design and Treatments

Four treatments were used in the study and these included *A. indica* (Nimbecidine®) (Osho Chemicals Zambia Limited, Lusaka, Zambia), *B. bassiana* (Bio-power®) (Osho Chemicals Zambia Limited, Lusaka, Zambia), *M. anisopliae* (Bio-magic®) (Osho Chemicals Zambia Limited, Lusaka, Zambia), *V. lecanii* (Bio-catch®) (Osho Chemicals Zambia Limited, Lusaka, Zambia) and an untreated control. Four concentrations for each treatment consisted of the commercially recommended dose, a dose lower than the recommended dose and two doses higher than the recommended dose. Four concentrations of each biopesticide were used in the bioassay. *M. anisopliae* ($100\,\mu$L, $150\,\mu$L, $175\,\mu$L and $200\,\mu$L), *B. bassiana* ($75\,\mu$L, $100\,\mu$L, $125\,\mu$L and $150\,\mu$L), *V. lecanii* ($75\,\mu$L, $100\,\mu$L, $125\,\mu$L and $150\,\mu$L) and *A. indica* ($50\,\mu$L , $75\,\mu$L, $100\,\mu$L and $125\,\mu$L). The aforementioned concentrations were measured using a micropipette. A randomised complete design (RCD) was used in the allocation of different treatments and all treatments were replicated three times.

2.3 Field Experimental design and Treatments

The treatments consisted of *A. indica* (Nimbecidine®), *B. bassiana* (Bio-power®), *M. anisopliae* (Bio-magic®), *V. lecanii* (Bio-catch®) and an untreated control All the treatments were applied to the tomato plants using a knapsack sprayer. The manufacturers recommended rate was used for all treatments. A total of 15 plots with each plot measuring 5 m in length and 4 m in width were laid out in a randomised complete block design (RCBD) and each treatment was replicated three times. 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 m $\times$ 28 m (Figure 2).

REP I				
101 *A. Indica*	102 *B. bassiana*	103 *M. anisopliae*	104 *V. lecanii*	105 Control
REP II				
201 *B. bassiana*	202 *M. anisopliae*	203 *V. lecanii*	204 Control	205 *A. Indica*
REP III				
301 Control	302 *V. lecanii*	303 *A. indica*	304 *M. anisopliae*	305 *B. bassiana*

Figure 2: Shows the field layout and randomisation plan from site 101 (replicate I) to 305 (replicate III) for each biopesticide including the controls.

2.4 Land Preparation and Planting

During land preparations, conventional ploughing was adopted using a tractor drawn plough followed by disc harrowing to achieve a fine tilth. Three planting ridges that were 1.5 m apart were made per plot and drippers were laid on top of each ridge (Figure 3a). 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 (Zambia Seed Company Limited, Lusaka, Zambia). Three weeks old seedlings were planted in the first week of July, 2017 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 3b). Trellising of tomatoes was done at three weeks after planting.

(a) Laying of drippers before transplanting **(b)** Transplanting of tomato seedlings

Figure 3

2.5 Fertilisation and Spraying

Compound D fertiliser ($N = 10$, $P = 20$ and $K = 10$) (Zambian Fertilizer Ltd, Lusaka, Zambia) was applied as basal dressing at a rate of 1000 kg/ha one week after transplanting. Three weeks later, Veg Top 32 ($N = 21$, $P = 0$ and $K = 32$) (Zambian Fertilizer Ltd, Lusaka, Zambia) was applied to the tomato plants 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 40 g/16 L to control fungal and bacterial diseases. The fungicides were applied on different days with the biopesticides.

2.6 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 (Ayalew, 2015). 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).

2.7 Laboratory Studies

Mortality of *T. absoluta* due to treatment by *A. indica, 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 details of the bioassay are discussed in this section. This experiment was carried out in a controlled room (temperature $25 \pm 2^{\circ}C$, photoperiod 14:10 (L: D) and relative humidity (RH) $30 - 40\%$.

A starting culture of *T. absoluta* was collected from infested tomato fields at Natural Resources Development College (NRDC), in Lusaka, Zambia. 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 $\times$ 30 cm width $\times$ 55 cm length). Adult moths emerged after nine days and these were feed 20% honey solution placed on cotton wool in small plastic cups within the rearing cages. Potted tomato seedlings (45 days old) were placed in rearing cages and exposed to *T. absoluta* adults for 24 hrs. Later, the adult *T. absoluta* moths were removed from cages and the eggs deposited on leaves were left to hatch. 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 one paint brush. The different stages of larvae were identified using morphological features according to Nayana and Kalleshwaraswamy (2015).

A total of 765 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. Each of these biopesticides was diluted in $25 ml$ of distiled water. Each of these concentrations were replicated three times. Three ml of the prepared concentrations 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. Three ml of distilled water was placed on the filter paper lining the petri dish before placing *T. absoluta* larvae and fresh tomato leaves for the untreated control. 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. Fresh tomato leaves (2.5 cm length $\times$ 1.0 cm width)

washed with distilled water were supplied on a daily basis as a source of nourishment for *T. absoluta* larvae. 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, 2011). The larval mortality was corrected using Abbott's formula (Abbott, 1925) as shown below:

$$\text{Corrected mortality } = \left(\frac{X-Y}{X}\right) \times 100,$$

where

$$\begin{aligned} X &= \text{Percent of larvae living in the untreated control,} \\ Y &= \text{Percent of living larvae per treatment,} \\ X-Y &= \text{The percent of larvae killed by the treatment.} \end{aligned}$$

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

2.8 Data analysis

2.8.1 Laboratory Bioassay

In laboratory studies, all statistical analysis were performed using Genstat statistical software (VSN International, 2011). 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 $\alpha = 0.05$.

2.8.2 Field study

The efficacy of treatments on mean number of eggs 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 14th edition version 14.1 statistical software (VSN International, 2011).

3 Results

3.1 Determination of the most effective biopesticide on larval mortality

The mean percent mortality ranged from $41.3 \pm 6.8\%$ to $77.9 \pm 14.3\%$ (Table 1). There were significant differences ($P < 0.001$) among the biopesticides. Application of *A. indica* led to the highest mortality of $77.9 \pm 14.3\%$ while mortality due to *B. bassiana, V. lecanii*, and *M. anisopliae* was $48.3 \pm 2.8\%$, $44.8 \pm 4.8\%$ and $41.3 \pm 6.8\%$, respectively.

Table 1: Mean percent mortality of *T. absoluta* larvae caused by selected biopesticides.

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

Mean percent mortality of *T. absoluta* larvae during a period of seven days. Means within columns followed by common letters are not significantly different (Fisher's Protected Least Significance test).

3.2 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 2). The LC_{50} for *A. indica* (30.4 ± 0.4 μL) was lower than the field recommended dose (75 μL). The LC_{50} for *B. bassiana* and *V.lecanii* were 107.1 ± 0.4 μL and 118.7 ± 0.4 μL, respectively. The LC_{50} for *B. bassiana* and *V.lecanii* were slightly higher than the recommended dose of 100 μL. However, the LC_{50} for *M. anisopliae* (193 ± 0.4 μL) falls between the higher concentrations of 175 μL and 200 μL. Overall, *A. indica* was the most effective followed by *B. bassiana*, then *V.lecanii* and *M. anisopliae* showed the least efficacy

Table 2: 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

Estimated median lethal concentration (LC_{50}) values of the selected biopesticides at 95% confidence limits calculated using probit analysis.

3.3 Corrected larval mortality

The corrected percent mortalities are shown in (Table 3). 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 $69.8 \pm 8.1\%$ to $88.4 \pm 41.9\%$. Mean mortality due to *V. lecanii* application ranged from 5.0 ± 0.0 to 8.7 ± 0.9 with corrected mortalities ranging from $30.2 \pm 1.7\%$ to $55.8 \pm 14.6\%$. *M. anisopliae* mean mortality ranged from 5.3 ± 0.3 to 8.3 ± 0.3 with corrected mortality of $32.6 \pm 4.0\%$ to $53.5 \pm 5.4\%$. Mean mortality due to *B. bassiana* ranged from 5.3 ± 0.3 to 9.3 ± 0.9 and the corrected mortality were $32.6 \pm 4.6\%$ to $60.5 \pm 16.3\%$. 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 3: Corrected mortalities (% SE) of test biopesticides

Biopesticide	Dose(μL)	$\mathbf{M_{av}}$	$\%\mathbf{M_{av}}$	$\mathbf{L_{av}}$	$\%\mathbf{L_{av}}$	$\%\mathbf{M_{corr}}$
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

Percent corrected mortalities calculated based on the Abbot equation $(x - y/x) \times 100$. Errors were expressed as percent standard errors

3.4 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 4). 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 4). In the second count, there were no significant differences in number of eggs deposited on plants treated with biopesticides.

Table 4: Mean egg count of *T. absoluta* on tomato plants for selected treatments.

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

The average number of eggs per tomato plants across both locations. Means with the same alphabet letter are not significantly different (Fisher's protected least significance test)

4 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% (Gonçalves-Gervásio and Vendramim, 2007) and 70% - 86% when second 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 and Oldargc (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 similar to other studies, which demonstrated that *B. bassiana* caused higher mortality than *M. anisopliae* against *T. absoluta* (Shiberu and Getu, 2018). *B. bassiana* has been widely studied and has been considered as one of the most important biopesticides (Qazzaz *et al*, 2015). It has also been reported to be effective against maize stem borer, *Chilo partellus* in field studies (Maniania, 1993). The second most effective entomopathogen was *V. lecanii*. *V. lecanii* has been reported to be effective against neonate, second instar and third 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 50 μL 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*, 2015; 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 *A. indica* on *T. absoluta* larvae (Gonçalves-Gervásio and Vendramim, 2007). In addition, other authors have also reported a very strong contact toxicity by *A. indica* 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). The fungus, *M. anisopliae*, induced 50 - 100% mortalities in all developmental stages of the tick species in Namibia (Hedimbi *et al*, 2011).

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 (Tome *et al*, 2013). Tome *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).

Okech *et al* (1994) working in the ICIPE/PESTNET Project at Mt. Makulu Research Station, Chilanga, Zambia, commonly observed mortality of maize and sorghum stem borers due to naturally occurring *B. bassiana* and *M. anisopliae*. In Zambia, the potential to include entomopathgenic fungi in integrated pest management is still untapped. Results from this study 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, as well as plant-based natural products (Chinsembu *et al*, 2019), may provide a sustainable management tactic for *T. absoluta* in Zambia.

5 Conclusion

The current study clearly demonstrates the efficacy of the selected biopesticides against the larval stages of *T. absoluta*. *A. indica* was the most effective biopesticide against *T. absoluta* larvae and it is effective at $30.4 \pm 0.3\ \mu$L, a dosage lower than the ones commercially recommended. However, entomopathogens were more effective at a dose slightly higher than the commercially recommended doses at $107.1 \pm 0.3\ \mu$L, $118.7 \pm 0.4\ \mu$L and $193 \pm 0.4\ \mu$L 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 order to effectively control *T. absoluta* it is important that all control measures such as cultural control, biological control and judicial use of registered pesticides are used.

Acknowledgements

The authors would like to thank the Department of Biological Sciences, Department of Chemistry in the School of Natural Sciences, and Department of Plant Science in School of Agricultural Sciences at the University of Zambia for providing the facilities to conduct laboratory studies. Special thanks to the Natural Resources Development College (NRDC) and the Julius family for allowing the use of their premises for experimental field plots. Funding for this work was provided by the Ministry of Higher Education of the Government of the Republic of Zambia.

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