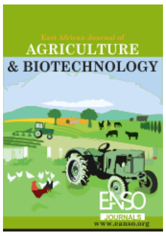

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Original Article

Evaluation of Selected Bio-insecticides on *Tuta absoluta* and *Liriomyza trifolii* on Tomato in Open Fields in Tharaka Nithi County, Kenya

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ABSTRACT

A field experiment to determine the effect of *Azadirachtin*, *Bacillus thuringiensis*, Pyrethrin + Garlic extract, and Petroleum mineral oil in managing the tomato leaf miner (*Tuta absoluta*) and serpentine leaf miner (*Liriomyza trifolii*) was conducted for two seasons between December 2016 and November 2017 at Chuka, Tharaka-Nithi County. Tomato variety, Kilele F1, was planted in the field to evaluate the effect of selected commercial products on *T. absoluta* and *L. trifolii*. The product included *Azadirachtin* 0.03%, Pyrethrin + Garlic extract, *Bacillus thuringiensis* var Kurstaki 5% w/w), and Petroleum mineral oil 98.8%. There were five treatments in a randomized complete block design (RCBD) in three replicates. There was an application of sterile water in the control experiment. There was a significant difference ($P < 0.05$) between the biocontrol products and the control. The *Azadirachtin* at 0.03% treatments had the least number of leaves damaged by *T. absoluta* (0.43; 1.67) in the control treatment (1.7; 5.27) in both seasons. In both seasons, there were significant differences ($P < 0.05$) between treatments in the number of leaflets damaged by *L. trifolii*. In season 1, the least damaged fruits per plant (1.33) were recorded in *Azadirachtin* at 0.03%, compared to the highest damage (5.0) in the untreated control. In the second season, the low fruit damage (0.67) was recorded in Petroleum mineral oil, and the highest (2.33) was in the untreated control. The study concluded that *Azadirachtin* 0.03%, *Bacillus thuringiensis* var Kurstaki 5% w/w, and Petroleum mineral oil were efficacious in managing *T. absoluta* and *L. trifolii* and be integrated into the existing tomato pest management strategies.

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INTRODUCTION

Tomato (*Solanum lycopersicon* L.) is the second most important exotic vegetable crop in Kenya. The total area under production is 29,294 ha with a production of 509,475 MT valued at KES 15.2 billion (HCD, 2017). The crop is attacked by a wide range of arthropod pests including the tomato leaf miner (*Tuta absoluta*), African bollworm (*Helicoverpa armigera*), whiteflies (*Bemisia tabaci*), thrips (*Frankiliniella* spp.), and serpentine leaf miner (*Liriomyza trifolii*) (Kasina & Lusike, 2014; Balabag et al., 2019; Oso, 2020; Ngugi *et al*., 2021). The tomato leaf miner (*T. absoluta)* has recently emerged as a serious threat to tomato production in many parts of the world including Kenya. The pest has been reported to cause considerable yield losses in the open field and greenhouse tomato production systems in the Country. Poudel and Kafle (2021) and Chhetri (2018) reported that *T. absoluta* larvae reduced tomato yield and fruit quality with losses ranging from 80 to 100% by attacking leaves, flowers, burrowing stalks, apical buds, and green and ripe fruits.

Serpentine leaf miner (*L. trifolii)* may cause tomato seedling loss of 46-70%, tomato foliage of 90% (Johnson *et al.,* 1983), and 70% loss in tomato fruit yield (Zoebisch *et al.,* 1984; Pohronezny *et al.,* 1986; Khaliq & Shankar, 2020). Pest management in Kenya is mainly by indiscriminate use of foliar applications of synthetic pesticides up to 24 times in a season. Nevertheless, only a few active ingredients are effective against *T. absoluta* and are selective to beneficial arthropods at the same time. Use of integrated pest management (IPM) strategies become necessary as continued use of synthetic insecticides may be detrimental to the environment, non-target beneficial organisms, and users and consumers of tomato products (Weisenbuger, 1993; Desneux *et al.,* 2007; Landgren *et al.*, 2009). In addition, prolonged use of synthetic pesticides could lead to pesticide resistance (Devonshire & Field, 1991; Ngugi *et al*., 2021). In Kenya, there are several environmentally friendly pest control products registered for the management of major crop pests (PCPB, 2019).

Azadirachtin is a tetranortriterpenoid extracted from the seeds of the Neem tree *Azadirachta indica*. The product acts as an antifeedant and growth inhibitor in the development of most insects (Meisner *et al.*, 1981; Raffa, 1987; Chaudhary, 2017). *Bacillus thuringiensis* is a naturally occurring soil bacterium that causes diseases to insect pests. The *B. thuringiensis* is accepted and widely used in organic farming and is considered ideal for pest management due to its low cost, ease of application, high virulence, and narrow host specificity. Thus, *B. thuringiensis* is regarded as environmentally friendly and has no toxic effects on non-target organisms. Gonzalez-Cabrera *et al.* (2011) reported that the application of *B. thuringiensis* greatly reduced the impact of *T. absoluta* resulting in reduced residues on the fruits. Petroleum-derived horticultural mineral oils (HMOs) have been used for pest control for well over a hundred years. Initially, the HMOs were used as dormant oil sprays for deciduous tree crops. In recent years, their use as foliar sprays has increased as improvements in their purity and surfactants have improved efficacy and reduced risks of phytotoxicity (Davidson *et al*., 1991). Mineral oils are thought to control insects by

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blocking the spiracles and causing suffocation (Stansly *et al*., 1996; Helmy *et al.*, 2012). Plant extracts, on the other hand, have been used widely in the management of crop pests. A laboratory and greenhouse study by Ghanim and Ghani (2014) showed that garlic extract was more effective on *T. absoluta* second instar larvae as compared to basil leaf extract which exhibited the least effect. On the other hand, essential aromatic oils have been used to control many pests on various crops. Based on their complex mixtures of constituents of plant extracts are likely to delay the development of pesticide resistance. Pyrethrins are pesticides found naturally in some chrysanthemum flowers. They have been used as models to produce longer-lasting chemicals called pyrethroids, which are man-made. Assessing the insecticidal activity of pyrethrum extract in a laboratory bioassay. Moreno *et al.* (2017) found that the cis isomer of the extract was the most toxic since it caused *T. absoluta* mortality of up to 100% in less than 12 h. The sole control method for the two pests was 12- and 24-times foliar sprays per season with synthetic insecticides. The study aimed to establish the comparative effect of *Azadirachtin* 0.03% (Nimbecidine EC), *Bacillus thuringiensis* (Halt 5WP), *Pyrethrin+ Garlic extract* (Pyegar 35.7EC) and Petroleum mineral oil *98.8%* (DC-Tron Plus) on tomato leaf miner (*T. absoluta*), and serpentine leaf miner (*T. trifolii*).

MATERIALS AND METHODS

Experimental Site

The experiment was conducted at Chuka (Lat. 0º19'39.8"S; Long. 37º 43' 39.2" E.; 1056 metres above sea level), a major tomato growing area in Tharaka Nithi County, for two seasons. The first season started in November 2016 and ended in February 2017, while the second season was in August 2017 and ended in November 2017.

Establishment of the Tomato Crop

In both seasons, the farmer preferred the tomato variety, Kilele F1 was planted. The seedlings were raised in the germination trays with coco peat, placed on raised benches, and covered with insect-proof netting. Watering was done on alternate days using a can for three weeks when seedlings were ready for transplanting. In the field, holes were made at a spacing of 45 cm between plants, 60 cm between rows with a 1 m path between the 5 x 5 m plots, and 1.5 m between blocks. Cattle manure and Diammonium phosphate (DAP) fertilizer was applied at the rate of 5 kg (manure) + 10g (DAP) and mixed with soil before planting. In both seasons, the fields were irrigated twice a week from transplanting to the first harvesting. After the first harvest, the fields were irrigated once a week. Other crop management practices including pruning, staking, and management of fungal diseases, were done by the farmers.

Experimental Design and the Treatments

This study used a complete block design (RCBD) with three replications. The products to be tested were from local traders. There were five (5) treatments; *Azadirachtin* 0.03% (Nimbecidine EC), *Bacillus thuringiensis* (Halt 5WP), Pyrethrin+ Garlic extract (Pyegar 35.7EC), Petroleum mineral oil *98.8%* (DC-Tron Plus, and untreated control (*Table 1*). The treatment application started four weeks after transplanting when the pest populations had fully established and were repeated every two weeks up to the start of crop harvesting, using a 15-litre, Knapsack sprayer fitted with a flat fan nozzle at the recommended dosages. Sterile water was applied to the untreated control plots.

Table 1: Description of treatments used against *T. absoluta* and *L. trifolli* during Seasons 1 and 2

Treatments	Active Ingredient (a.i.)	Dosage
Azadirachtin 0.03%,	*Azadirachtin* 0.03% EC	3 ml per L of water
Bacillus thuringiensis	*Bacillus thuringiensis* var Kurstaki 5% w/w	0.6 g per L of water
Pyrethrin + Garlic extract	Pyrethrin +Garlic extract 35.7 EC	3.0 ml per L of water
Petroleum mineral oil 98.8%	Petroleum mineral oil 98.8 %	5.0 ml per L of water
Untreated control	Water	1.0 L of water

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Data Collection Procedure

Four weeks after transplanting, when the first pest damage was observed, the number of tomato leaflets per plant damaged by the tomato leaf miner (*T. absoluta*) and the serpentine leaf miner individually or both was taken using the method described by Gonzalez-Cabrera *et al.* (2011). Three plants were randomly selected from the two central rows of each plot. The number of infested leaflets (mines, galleries, blotches, and frass) per plant was recorded every two weeks up to the end of the season. The mean number of damaged leaves per season was calculated. The maturity (a sign of reddening at the fruit tip), fruits were harvested from the same two middle rows and sorted for marketable (with no pest damage-no frass, holes) and unmarketable (damaged fruits), the mean numbers per season were calculated.

Data Analysis

All data was subjected to analysis of variance (ANOVA) using the Generalized Linear Model (GLM) procedure of the statistical analysis system (SAS, 2019). Means were separated using the student- Newman- Keuls (SNK) test.

RESULTS

Effects of *Azadirachtin, Bacillus thuringiensis*, Pyrethrin +Garlic extract, Petroleum mineral oil on leaf injury by *T. absoluta* and *L. trifolii*

Tuta absoluta

There were significant differences (P <0.05)) between treatments in the number of leaflets damaged by *Tuta absoluta* during Season 1. The untreated control was significantly different from other treatments. The untreated control had the highest mean number of damaged leaflets (1.70) by *T. absoluta*, while the *Azadirachtin* 0.03% treatments had the least mean number of damaged leaflets (0.43). Similarly, in season 2 there were significant differences (P< 0.05) between treatments (*Table 2*). Plots treated with *Azadirachtin* 0.03%, Pyrethrin + Garlic extract), *Bacillus thuringiensis var Kurstaki 5% w/w)* and Petroleum mineral oil 98.8% recorded low mean (<5.00) *T. absoluta* damage compared to the untreated control. The least number of damaged leaflets (0.43;1.67) was recorded in *Azadirachtin* 0.03% treatments, while the highest mean number of leaflets (1.70;5.27) damaged by *T. absoluta* was recorded in the untreated control. In seasons 1 and 2, plots treated with *Azadirachtin 0.03%* and *B. thuringiensis* consistently recorded the least mean number of leaflets damaged by *T. absoluta* (*Table 2*).

Table 2: Effect of *Azadirachtin*, Pyrethrin +Garlic extract, and *Bacillus thuringiensis* and Petroleum mineral oil on *Tuta absoluta* tomato leaf injury

Treatments	Season 1	Season 2
	Damaged leaflets/plant	Damaged leaflets/plant
	Mean ± Std Error	Mean ± Std Error
Azadirachtin 0.03%,	0.43 ± 0.12b	1.67 ± 0.25b
Bacillus thuringiensis var Kurstaki 5% w/w)	0.63 ± 0.16b	2.27 ± 0.32b
Pyrethrin + Garlic extract	0.70 ± 0.17b	3.47 ± 1.09ab
Petroleum mineral oil 98.8%	0.73 ± 0.15b	2.73 ± 0.56b
Untreated control	1.70 ± 0.26a	5.27 ± 1.06a
LSD	0.499	2.110
P value	<0.0001	0.013

Means in the same column followed by the same letter are not significantly different, SNK test at P= 0.05

Liriomyza trifolii

In seasons 1 and 2, there were significant differences between treatments and untreated control in the number of leaflets damaged by the serpentine leaf miner (*L. trifolii*). There was also a significant difference (P < 0.0001) between *Bacillus thuringiensis* var Kurstaki 5% w/w)

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treatment and all the other biocontrol products and the control (*Table 3*).

Table 3: Effect of *Azadirachtin*, Pyrethrin +Garlic extract, *Bacillus thuringiensis* and Petroleum mineral oil on *Liriomyza trifolii* damage on tomato leaves

Treatments	Season 1	Season 2
	Damaged leaflets/plant	Damaged leaflets/plant
	Mean ± Std Error	Mean ± Std Error
Azadirachtin 0.03%,	5.67 ± 0.54c	3.33 ± 0.64c
Bacillus thuringiensis var Kurstaki 5% w/w)	9.33 ± 0.73b	5.00 ± 0.37b
Pyrethrin + Garlic extract	7.00 ± 0.45c	3.33 ± 0.53c
Petroleum mineral oil 98.8%	7.00 ± 0.70c	4.00 ± 0.65bc
Untreated control	17.67 ± 0.65a	11.33 ± 0.67a
LSD	1.737	1.642
P value	<0.0001	<0.0001

Means in the same column followed by the same letter are not significantly different, SNK test at P= 0.05.

Effects of *Azadirachtin, Bacillus thuringiensis* var Kurstaki, Pyrethrin +Garlic extract and Petroleum Mineral Oil on Tomato Yields

There was no significant difference (p .0.05) in the number of tomato fruits from the different treatments. However, in season 1, *Azadirachtin* 0.03% treatments gave the highest mean number of non-infested fruits (14.27), while the untreated control had the least mean number of fruits (8.87) per plant. Similarly, in Season 2, there was no significant difference (P ≥.0.05) between the treatments. In season 2, *Azadirachtin* 0.03% treatments also had the highest mean number of non-infested fruits (12.25), while the untreated control had the least mean number of fruits (8.42) per plant (*Table 4*).

Table 4: Effect of *Azadirachtin*, Pyrethrin +Garlic extract, *Bacillus thuringiensis* var Kurstaki and Petroleum mineral oil 98.8% on tomato yields

Treatments	Season 1	Season 2
	No. of non-infested fruits/plant	No. of non-infested fruits/plant
	Mean ± Std Error	Mean ± Std Error
Azadirachtin 0.03%,	14.27 ± 1.99	12.25 ± 1.82
Bacillus thuringiensis var Kurstaki 5% w/w)	13.13 ± 2.56	12.42 ± 3.12
Pyrethrin + Garlic extract	11.27 ± 2.76	8.75 ± 2.67
Petroleum mineral oil 98.8%	10.80 ± 2.83	9.50 ± 2.75
Untreated control	8.87 ± 2.12	8.42 ± 2.89
P value	0.581	0.724

The number of damaged fruits was significantly different (P≤0.05) between the treatments. The untreated control recorded a greater number (5.00; 2.33 of damaged fruits in both seasons. The *Azadirachtin* 0.03% recorded the least number (1.33) of fruits damaged by *T. absoluta* in season 1. *Azadirachtin* 0.03%, *Bacillus thuringiensis var Kurstaki 5% w/w*) and Petroleum mineral oil 98.8% recorded fewer number (>5.00) damaged fruits as compared to the untreated control in season 1. There was a similar trend in the second season with the untreated control recording high numbers (2.33) of fruits damaged by *T. absoluta*. as compared to *Azadirachtin* 0.03%, Pyrethrin +

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Garlic extract), *Bacillus thuringiensis var Kurstaki 5% w/w) and Petroleum* mineral oil *98.8%)*, which recorded 0.83, 0.92,1.04 and 0.67 damaged fruits, respectively (*Table 5*).

Table 5: Effect of *Azadirachtin*, Pyrethrin +Garlic extract, *Bacillus thuringiensis* and Petroleum mineral oil tomato fruit damage by *Tuta absoluta*

Treatments	Season 1	Season 2
	No. of damaged fruits/plant	No. of damaged fruits/plant
	Mean ± Std Error	Mean ± Std Error
Azadirachtin 0.03%,	1.33 ± 0.30c	0.83 ± 0.27b
Bacillus thuringiensis var Kurstaki 5% w/w)	1.67 ± 0.49c	0.92 ± 0.28b
Pyrethrin + Garlic extract	3.27 ± 0.34b	1.04 ± 0.41b
Petroleum mineral oil 98.8%	1.87 ± 0.52c	0.67 ± 0.20b
Untreated control	5.00 ± 0.67a	2.33 ± 0.52a
LSD	1.369	0.990
P value	0.0001	0.008

Means in the same column followed by the same letter are not significantly different, SNK test at P= 0.05.

DISCUSSION

Effect on Tomato Leaf Damage by *T. absoluta*

In seasons 1 and 2, *Azadirachtin* 0.03% and *Bacillus thuringiensis* reduced damage by tomato leaf miner *(T. absoluta)* on leaflets and fruits. *Azadirachtin* 0.03% and *B. thuringiensis* reduced the damage to tomato fruits in both seasons. The reduced damage by *T. absoluta* larvae on tomato leaflets and fruits agree with Braham *et al*. (2012), who reported that the efficacy of Nimbecidine (*Azadirachtin* 0.03%) in the management of tomato leaf miner, *T. absoluta* was comparable to; Tracer® (Spinosad), Tutafort (plant extracts), Voliam Targo (chlorantraniliprole + abamectin), and Ampligo 150ZS (chlorantraniliprole + lambda-cyhalothrin). All these treatments recorded significantly higher larval mortality compared with the untreated control. Similarly, *B. thuringiensis* recorded low leaflet damage by *T. absoluta*. These findings concur with Gonzalez-Cabrela *et al*. (2010), who found that *B. thuringiensis* was highly efficient in controlling *T. absoluta*. The presented results reveal that *Azadirachtin* 0.03% and *B. thuringiensis* reduced Tuta damage on tomato leaflets and fruits. The findings agree with those obtained by Khidr *et al. (*2013), who found that a combination of *B. thuringiensis* and Neem (*Azadirachtin*) were effective in managing *T. absoluta*. The findings conform with Sabbour and Soliman (2014), who found that *B. thuringiensis var kurstaki* was efficacious in managing *T. absoluta*. In addition, Lo Bue *et al*. (2012) and Wafula *et al*. (2018) found that a combination of *Azadirachtin* and *B. thuringiensis* effectively reduced *Tuta absoluta* damage in tomato open-field cultivation. Reduced leaf infestation by *T. absoluta* larvae after treatment with *Azadrachtin 0.03%* agrees with that of Shiberu and Getu (2018), who reported that tomato crops treated with Vayego 200SC (Tetraniliprole), *Beauveria bassiana*, *Azadirachta indica*, *Allium sativum* and *Cymbopogon citratus* reduced fruit infestation by *T. absoluta* larvae. The findings agree with Ghanim and Ghani (2014), who found garlic had the highest effect on *T. absoluta* second instar larvae. Badran *et al*. (2018) reported that chemical pesticides, essential aromatic oils (garlic), and mineral oil alone were effective and caused a gradual reduction of tomato leaf miner *T. absoluta* numbers.

Effect on Tomato Leaf Damage by Serpentine Leaf Miner *(L. trifolii)*

In season 1, all the treatments; *Azadirachtin* 0.03%, Pyrethrin +Garlic extract) and Petroleum mineral oil *98.8%)* reduced serpentine leaf damage. Among the treatments applied, *Azadirachtin* 0.03% recorded consistently fewer leaflets damaged by the serpentine leaf miner in both seasons. The findings agree with Barde and Shrivastava (2017) who found that neem seed kernel extract (NSKE) and neem oil were effective against the serpentine leaf miner on tomato plants. Trinide *et al.* (2000) reported 82 to 94.7% mortality of egg and larvae of serpentine leaf miners after treatment with neem seed kernel extract (NSKE). Viraktamath *et al.* (1993) reported that neem seed kernel extracts of 4% were effective against *L. trifolii* on tomatoes. The reduction of serpentine leaf miner damage by petroleum mineral oil *98.8% agrees with* Beattie *et al.* (1995) report that petroleum spray oil (1.25-10 ml per Litre of water suppressed oviposition of the citrus leaf miner, *Phyllocnistis citrella*, and also reduced the number of mines per leaf. The least mean number of leaflets damaged by serpentine leaf miners was observed in Pyrethrin + Garlic extract treated plots. The reduction of leaf damage by *L. trifolii* after using Pyrethrin +Garlic extract is reported by Rahardjo *et al.* (2020), who found that pyrethrum leaf extracts reduced damage.

Effect on Fruit Quantity and Quality

There was no significant difference in the cumulative total of the harvested fruits in both seasons. However, the *Azadirachtin* treatments gave more fruit compared to the control treatments. In addition, plots treated with *Azadirachtin* 0.03%, *B. thuringiensis var Kurstaki 5% w/w),* and Petroleum mineral oil *98.8*%) recorded the least number of fruits damaged by the *T. absoluta* larvae as compared to the untreated control. The findings of the current study agree with those of Shiberu and Getu (2018), who reported that tomato crops treated with Vayego 200SC (*Tetraniliprole), Beauveria bassiana, Azadirachta indica, Allium sativum,* and *Cymbopogon citratus* reduced fruit infestation by *T. absoluta* larvae.

CONCLUSION AND RECOMMENDATION

The study concludes that *Azadirachtin* 0.03% and *Bacillus thuringiensis* are effective management options for tomato leaf miners *(Tuta absoluta).* Also, *Azadirachtin* 0.03%; (Pyrethrin +Garlic extract) and Petroleum mineral oil 98.8 were effective against *Liriomyza. trifolii*. The study recommends that the *Azadirachtin* 0.03% and *B. thuringiensis* be included in the tomato integrated pest management (IPM) packages for the management of *T. absoluta. The Azadirachtin 0.03%*, Pyrethrin +Garlic extract, and Petroleum mineral oil 98.8% to be included for the management of *L. trifolii.* There is a need to train farmers on use of these products through farmer field schools (FFSs), on-farm demonstrations, and field days and to include cost-benefit analysis in future research programs to enhance the quick adoption of the IPM technologies by farmers.

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