Efficacy of *Bacillus thuringiensis* Against the Tomato Leaf Miner, *Tuta absoluta* (Meyrick)

Etsegenet Hailemichael[1] and Gashawbeza Ayalew[2]
[1] *Debre berhan University, College of Agriculture and Natural Resource Science, POB 445, Debre berhan, Ethiopia. Email: etsegenethailemichael@gmail.com; [2]Ethiopian Institute of Agricultural Research, Melkassa center,*
POB 436, Adama, Ethiopia

Abstract

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is an introduced serious insect pest of tomato in Ethiopia. Research on the management of this pest has so far been limited to screening of synthetic insecticides. Owing to the pest's ability to develop resistance to insecticides quickly, biological based approach in its management is a viable option. The study was conducted at Melkassa Agricultural Research Center in 2014. The objectives of the study were to assess the efficacy of the microbial insecticide *Bacillus thuringiensis* (Bt) and the optimum rate and frequency of its application. The tomato variety 'Miya' was used for this experiment. The experiment was laid out in a factorial RCBD with three replications. The treatments consisted of four doses of Bt, 0.5, 1, 1.5 and 2kg per ha applied at 7, 14 and 21 days intervals. Lambda cyhalothrin 5% EC (Karate®) and Chlorantraniliprole 240 SC (Coragen ®) at 320 and 250 ml per ha, respectively were applied biweekly as standard checks along with the untreated control. Egg density, leaf damage score, proportion of damaged fruit by number and weight, and marketable yield were used to assess performance of the treatments. Differences for egg density were insignificant ($P<0.05$) among treatments. Plots treated with the highest rate of Bt (2 kg per ha) at 7 days interval suffered less damage and resulted in higher marketable yield than the rest of Bt treatments. The lowest fruit damage level of 18.7 % was recorded from Coragen treatment and the highest (81.8%) was recorded from the untreated control. The highest marketable yield of 28.39 tonnes per ha and the lowest of 3.25 tonnes per ha were recorded from Coragen and lowest Bt rate applied triweekly, respectively. This corresponds to a yield loss of 88.5%. The study demonstrated the potential of Bt in reducing damage and yield loss due to *T. absoluta* on tomato when applied with high dose (2 kg per ha) weekly. It is suggested that future studies need to look into assessing performance of higher doses and shorter application intervals than considered in this study along with economics of their use in tomato production.

Keywords: Biocontrol, *Bacillus thuringiensis*, Tomato leaf miner**,** Frequency, Rate

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Introduction

Tomato (*Solanum lycopersicum* L.) is a popular and economically important vegetable globally. It is the second largest vegetable both in terms of production and consumption [Food and Agriculture Organization (FAO), 2016]. In Ethiopia, tomato ranks third after red pepper and Ethiopian cabbage with acreage and production share of 4.53% and 10.82%, respectively [Central Statistical Agency (CSA), 2012]. Large scale production of tomato takes place in the upper awash valley under irrigated and rain fed conditions whereas small scale production for fresh market is a common practice around Koka, Ziway, WondoGenet, Guder, Bako and many other areas (Lemma, 2002).

Productivity of tomato in Ethiopia is much lower than the world average due to several biotic and abiotic constraints (Gemechis *et al*., 2012). Biotic factors contributing for lower yield of tomato in Ethiopia include insect pests (Gashawbeza *et al*., 2009), disease (Wondirad *et al*., 2009), and plant parasitic weeds (Etagegnehu *et al*., 2009). Drought, heat, and poor cultural practices constitute abiotic factors for lower productivity of tomato (Lemma, 2002; Lemma *et al*., 2008). *Tuta absoluta* is thought to be originated from Chile has spread to regions of South America, Southeast Asia, and Mediterranean shores into Africa on different solanaceous crops (Siqueira, 2001a; Lietti *et al*., 2005; Ghoneim, 2014). Occurrence of *T. absoluta* in Ethiopia was confirmed following heavy infestation of tomato fields during February 2013 in major tomato production belt of the Central Rift Valley region (Gashawbeza and Abiy, 2013). *T. absoluta* can cause a yield loss of 80-100% in protected and unprotected tomato fields in its native and introduced area (Desneux *et al*., 2010). Fruit quality loss is considerably impacted by direct feeding of the insect and hosting secondary pathogens through wounds made by the insect (Kaoud, 2014).

Tomato growers in the affected areas of Ethiopia reacted to the pest damage by applying conventional insecticides locally available in the market. These include organophosphates, such as Profenofos and Pyrethroids, such as Lambda cyhalothrin with no success or reduction of infestation resulting huge financial loss. As it is a recently introduced pest of Ethiopia, no single insecticide based on local efficacy data was registered for the management of the pest in Ethiopia until the third quarter of 2013 (Gashawbeza and Abiy, 2013). Nearly a dozen of insecticides from different insecticide classes are currently registered for use against the pest in Ethiopia.

Several methods including pest monitoring, modifying agricultural practices, chemical control, mating disruption, mass trapping, biological control using *Bacillus thuringiensis*, the egg parasitoid *Trichogramma pretiosum*, and the predators *Nesidiocoris tenuis* and *Podisus* *nigrispinus* are being used to manage the pest elsewhere (Molla *et al*., 2011). Using synthetic insecticides is the primary method to manage *T. absoluta*, but it has serious drawbacks, including destruction of natural enemy populations (Campbell *et al*., 1991) and build-up of insecticide residues on tomato fruits (Walgenbach *et al*., 1991). Consequently, resistance to several insecticides has been reported in several countries. Examples include resistance to organophosphates and pyrethroids in Chile, abamectin, cartap, methamidophos and permethrin in Brazil (Siqueira *et al*., 2001a, 2001b).

Sustainable pest management requires among others replacing broad spectrum insecticides with selective and safer insecticides compatible to Integrated Pest Management (IPM) program. Bio-pesticides are very effective in agricultural pest control without causing serious damage to ecological chain or aggravating environmental pollution. *Bacillus thuringiensis* based insecticides have no toxicity to mammals (Siegel, 2001), and even toxic volatile agents are not released during or after a spraying operation (Van Netten *et al*., 2000). Giustolin et al. (2001) found that *B. thuringiensis* var Kurstaki (Btk) can cause mortality in all *T. absoluta* instars. *B. thuringiensis* has been reported to be effective against lepidopteran pests of vegetables in Ethiopia (Lidet *et al*., 2009; Alemu *et al*., 2011). Few studies have evaluated the efficacy of *B. thuringiensis* on *T. absoluta* (Gonzalez-Cabrera *et al*., 2011; Mantzoukas *et al*., 2019; Akinyelure *et al*., 2021; Shahini *et al*., 2021), although 3000 species, belonging to 16 orders of insects, have been reported as susceptible to *Bt* (Huang *et al*., 2004).

This study was therefore conducted to assess the efficacy of *B. thuringiensis* against *T. absoluta* and its optimum rate and frequency of application.

Materials and Methods

The experiment was conducted during the off season of 2014 at Melkassa Agricultural Research Center (MARC) ($8^0$24' to $8^0$26' N, longitude of $39^0$19' to $39^0$19' E, 1550 meter above sea level). The climate of the area is characterized as semi-arid with uni-modal low and erratic rainfall pattern. The mean maximum temperature varies from 26.3 to 31.0 ^{0}C while the mean minimum temperature varies from 10.4 to 16.4 ^{0}C, with a mean of 21 (Ketema and Abraham, 2022).

Seeds of the tomato (*Solanum lycopersicum* L.) variety 'Miya' obtained from MARC was used for the experiment. Seeds were seeded on seedbed on January 27, 2014. Seedlings were raised in nursery beds measuring 2 x 1m, raised 5 cm from the soil surface to provide good drainage for the removal of surplus irrigation water. The seeds were sown in rows spaced 15 cm apart and covered lightly with fine soil before irrigation. The beds were irrigated daily until germination and three times a week between germination and transplanting. Seedlings were transplanted to the main field on March 02, 2014. Each plot had 6 rows of 4 m long each with a row and plant spacing of 1 m and 30 cm, respectively. The experiment was laid out in a Randomized Complete Block Design (RCBD) in factorial arrangement with three replications. Spacing between plots and blocks were 1.5 m and 2 m, respectively. The fields were fertilized with diammonium phosphate (DAP) and urea at the rate of 200 and 150 kg/ha, respectively. The whole amount of DAP was applied just before transplanting, while urea was applied by splitting the total amount in to two. Half of the amount was applied one week after transplanting and the remaining half at the beginning of flowering stage. Other field management practices like weeding, cultivation and maintenance of ridges were carried out as needed.

Treatments

The treatments were combinations of four levels (0.5, 1, 1.5 and 2 kg per ha) and three application frequencies (7, 14, and 21, days interval) of *Bacillus thuringiensis* var. kurstaki (Berliner) (Bacillales: Bacillaceae). Coragen 240 SC (Chlorantraniliprole) and Karate 5 EC (Lambda cyhalothrin) which is the commonly used insecticides for the control of insect pests in the vegetable production system of Central Rift Valley (CRV) were included as standard checks together with the untreated control.

Treatment application started three weeks after transplanting when infestation was observed. Treatment application dates for weekly application frequency were March 27, April 04, 11, 18, 25, May 02, 09, 16 and 23; for biweekly application March 27, April 11, 25, May 9 and 23; and for tri-weekly March 27, April 18 and May 9 in 2014. Sprays were made for a total of 9, 5 and 3 times for weekly, biweekly and triweekly application frequencies. Spray was made using manually operated knapsack sprayer of 15 liters capacity using flat fan nozzle.

Data collection and analysis

Data on severity of leaf damage was recorded on a plot basis on a scale of 1 to 5 (1= no infestation; 2= 1- 25% slight infestation; 3= 26-50% moderate infestation; 4= 51-75% heavy infestation; 5= 76-100% very heavy infestation) (Gashawbeza, 2011). Egg density, number and weight of damaged fruits and yield loss data were collected from the central four rows of each plot. Twelve leaflets of tomato were picked randomly from four randomly selected plants per plot at weekly interval to assess egg density. Leaflets were collected by taking one leaflet each from the top, middle and lower portion of randomly sampled plants. Samples were kept in plastic bags and transported to MARC entomology laboratory. Egg counting was made with the help of a stereoscopic binocular microscope.

At harvest, tomato fruits from the central four rows of each plot were sorted into healthy or marketable and damaged based on presence of *T. absoluta* damage symptoms and others. Damaged fruits were further grouped into *T. absoluta* damaged and damage caused by other factors. Percentages of fruit damages caused by *T. absoluta* and other factors were calculated as follows:

$$\text{Number or Weight of damaged fruit by other factors (\%)} = \frac{\text{Number or Weight of damaged fruit by other factors}}{\text{Total number or weight of harvested fruits}} \text{ X } 100$$

$$\text{Number or Weight of } \mathit{Tuta\ absoluta} \text{ damaged fruit (\%)} = \frac{\text{Number or Weight of } \mathit{Tuta\ absoluta} \text{ damaged fruit}}{\text{Total number or Weight of harvested fruits}} \text{ X } 100$$

Relative yield loss was calculated according to the following equation (Sabbour and Soliman, 2014):

$$\text{Relative yield loss (\%)} = \frac{\text{Marketable Yield (MY) from best performing treatment} - \text{MY from a treatment}}{\text{MY from best performing treatment}} \text{ X } 100$$

All the collected data were analyzed using the SAS statistical version 9.2 Software (SAS, 2009). The egg density count data were square-root transformed to stabilize variance before subjecting them to ANOVA. Significance means were separated using Student-Newman-Keuls test.

Results

Effect on egg number

Due to total absence of eggs on the leaf with the aging of the crop, data on egg density were collected weekly four times after the first spray. Mean number of *T.absoluta* eggs per leaf before and after application ranged from 2 to 9 and 0 to 4.8 per 12 leaflets, respectively with a post spray mean ranging between 0 to 1.8 (Table 1). Treatments did not show significant difference for egg number ($p>0.05$) in all occasions or sampling weeks (Table 1).

Table 1. Egg density of *T. absoluta* per twelve leaflets of tomato on plots treated with different rates and application frequencies of *Bt*

Treatments	Pre spray	Post spray (sampling weeks)				
		1	2	3	4	mean
Bt @ 0.5 kg/ ha 7 days	3.7a	0.7a	0.3a	0.3a	0.0a	0.3a
Bt @ 0.5 kg/ ha14 days	9.0 a	2.0a	0.0a	0.0a	0.0a	0.5a
Bt @ 0.5 kg/ ha 21 days	2.3a	4.0a	0.0a	0.0a	0.0a	1.0a
Bt @ 1 kg/ ha 7 days	5.7a	1.3a	0.0a	0.0a	0.0a	0.3a
Bt @ 1 kg/ha 14 days	5.0a	3.7a	0.0a	0.0a	0.0a	0.9a
Bt @ 1 kg/ ha 21 days	6.3 a	4.7a	0.0a	0.0a	0.0a	1.2a
Bt @ 1.5 kg/ha 7 days	4.7a	0.7a	0.3a	0.0a	0.0a	0.3a
Bt @ 1.5 kg/ha 14 days	7.0a	2.0a	0.0a	0.0a	0.0a	0.5a
Bt @ 1.5 kg/ha 21 days	3.7a	1.3a	0.0a	1.0a	0.0a	0.6a
Bt @ 2 kg/ha 7 days	4.7a	0.0a	0.0a	0.0a	0.0a	0.0a
Bt @ 2 kg/ha 14 days	3.3a	1.3a	0.7a	0.7a	0.3a	0.8a
Bt @ 2 kg/ha 21 days	3.3a	0.3a	0.0a	0.0a	0.3a	0.2a
Coragen 250 ml/ha 14 days	4.0a	0.3a	0.0a	0.0a	0.0a	0.1a
Karate 320 ml/ha 14 days	4.67a	2.0a	0.0a	0.3a	0.7a	0.8a
Untreated check	2.0a	4.3a	0.7a	1.0a	1.0a	1.8a

Means in a column followed by the same letter are not significantly different from each other at 5% significance level.

Effect on leaf damage

No leaf damage due to *T.absoluta* was observed in any of the plot until fruiting started in the first week of May. Hence leaf damage score was recorded on May 1st as presented in Fig. 1. Leaf damage score on this date ranged between 1.7 and 3.0 (Fig. 1). Significantly lowest and highest damage level was recorded from coragen treatment and untreated control (Fig. 1). Performance of *Bt* treatments and Karate was intermediate between coragen and untreated plots with insignificant difference among them (Fig. 1).

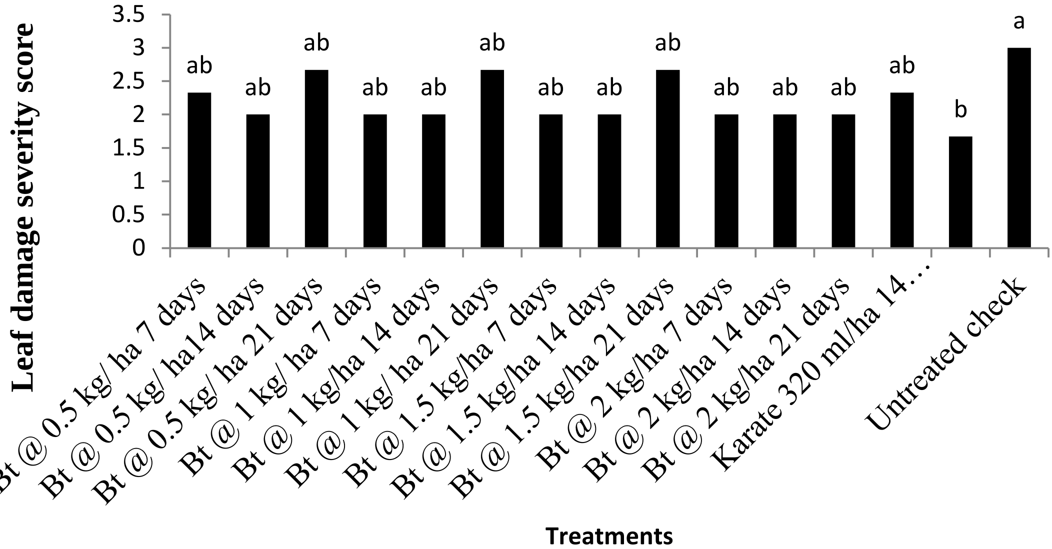

Fig. 1. Leaf damage score (1 to 5 scale) of *T. absoluta* on plots treated with different rates and application frequencies of *B*t.

Effect on total yield

Total fruit number ranged between 532,292 and 726,667 fruits per ha, and total yield ranged between 27.42 and 46.48 tons per ha (Table 2). The lowest fruit number and total yield were recorded from *Bt* applied at 0.5 kg per ha biweekly and tri-weekly, respectively. Highest total yield was recorded from *Bt* applied weekly at a rate of 1.5 kg per ha (Table 2). However, difference between treatments both for total fruit number and yield were insignificant.

Table 2. Total fruit number and yield of tomatoes treated with different rates and frequencies of *Bt* against *T. absoluta*

Treatments	Fruit number per ha	Total yield (tons/ha)
Bt @ 0.5 kg/ ha 7 days	643958a	43.46a
Bt @ 0.5 kg/ ha14 days	532292a	34.75a
Bt @ 0.5 kg/ ha 21 days	542917a	27.42a
Bt @ 1 kg/ ha 7 days	630625a	38.94a
Bt @ 1 kg/ha 14 days	650000a	42.02a
Bt @ 1 kg/ ha 21 days	592083a	38.71a
Bt @ 1.5 kg/ha 7 days	686458a	46.48a
Bt @ 1.5 kg/ha 14 days	595208a	32.73a
Bt @ 1.5 kg/ha 21 days	612708a	34.44a
Bt @ 2 kg/ha 7 days	726667a	45.71a
Bt @ 2 kg/ha 14 days	601875a	38.67a
Bt @ 2 kg/ha 21 days	678542a	40.5a
Karate 320 ml/ha 14 days	553542a	36.6a
Coragen 250 ml/ha 14 days	714792a	42.19a
Untreated check	597083a	36.31a

Means in a column followed by the same letter are not significantly different from each other at 5% significance level

Effect on marketable yield

Marketable fruit number ranged between 55,417 and 451,042 and marketable yield between 3.25 and 28.39 tons per ha (Table 3). Both marketable fruit number and yield were lower in the *Bt* applied tri-weekly at 0.5 kg per ha and higher in the Coragen treatment. Coragen treated plots resulted in significantly higher marketable fruit number and yield than the rest of the treatments. All *Bt* treatments except for the high rates (1.5 kg and 2 kg per ha) applied weekly did not show significant difference from the untreated check for marketable fruit number. The performance of Karate was intermediate between the Coragen and *Bt* treatments. Karate resulted in the highest marketable fruit weight next to Coragen. Performance of *Bt* applied weekly at 1.5 and 2 kg per ha was on par with the Karate. Yield losses ranged between 41.9 and 88.5% based on marketable yield difference of each treatment with the best performing treatment (Coragen). Yield losses were higher in the untreated control and low rate of *Bt* treatments applied biweekly and triweekly (Table 3).

Effect on fruit damage

Proportion of damaged fruit by the pest in number and weight ranged between 21.8 to 83.8 and 18.7 and 81.8%, respectively (Table 4). Plots treated with coragen resulted in significantly lower damage both by number and weight than the rest of the treatments (Table 4). On the other hand, damage was highest in the untreated check. *Bacillus thuringiensis* at rates of 1.5 kg and 2 kg per ha applied weekly resulted in significantly lower *T. absoluta* damaged fruit number than the untreated plot. Karate treated plots resulted in significantly higher fruit damage by number than coragen treatment but significantly lower than the rest of the treatments. Similar trend was observed in proportion of *T. absoluta* damaged fruit by weight. Damaged fruit by other factors did not show significant difference both in number and weight.

Table 3. Marketable fruit number, yield and yield loss of tomatoes with different rates and frequencies of *Bt* and synthetic insecticides

Treatments	Marketable fruit number per ha	Marketable yield (tons/ha)	Yield loss (%)
Bt @ 0.5 kg/ ha 7 days	137500de	10.46bcd	63.16
Bt @ 0.5 kg/ ha14 days	71042e	5.37d	81.08
Bt @ 0.5 kg/ ha 21 days	55417e	3.25d	88.55
Bt @ 1 kg/ ha 7 days	148750cde	10.56bcd	62.8
Bt @ 1 kg/ha 14 days	119167de	9.25bcd	67.42
Bt @ 1 kg/ ha 21 days	110625de	7.81bcd	72.49
Bt @ 1.5 kg/ha 7 days	202917bcd	15.02bc	47.09
Bt @ 1.5 kg/ha 14 days	98333e	7.19cd	74.67
Bt @ 1.5 kg/ha 21 days	93333e	6.27cd	77.91
Bt @ 2 kg/ha 7 days	240000b	16.42b	42.16
Bt @ 2 kg/ha 14 days	123542de	8.81bcd	68.97
Bt @ 2 kg/ha 21 days	124167de	8.85bcd	68.83
Karate 320 ml/ha 14 days	224167bc	16.48b	41.95
Coragen 250 ml/ha 14 days	451042a	28.39a	---
Untreated check	58542e	3.89d	86.29

Means in a column followed by the same letter are not significantly different from each other at 5% significance level

Table 4. Proportion of number and weight of tomato fruits damaged by *T. absoluta* and other factors treated with different rates and frequency of *Bt*

Treatments	Fruit damaged by *T. absoluta*		Fruits damaged by other factors	
	Number (%)	Weight (%)	Number (%)	Weight (%)
Bt @ 0.5 kg/ ha 7 days	70.5ab	68.1ab	8.2a	8.5a
Bt @ 0.5 kg/ ha14 days	80.6a	78.5a	6.1a	6.1a
Bt @ 0.5 kg/ ha 21 days	82.8a	81.4a	7.9a	6.6a
Bt @ 1 kg/ ha 7 days	68.5ab	64.3ab	8.2a	8.3a
Bt @ 1 kg/ha 14 days	76.2ab	71.8ab	5.5a	5.3a
Bt @ 1 kg/ ha 21 days	73.7ab	71.9ab	7.2a	7.93a
Bt @ 1.5 kg/ha 7 days	59.4bc	56.3b	10.9a	11.2a
Bt @ 1.5 kg/ha 14 days	76.4ab	70.5ab	7.1a	7.4a
Bt @ 1.5 kg/ha 21 days	75.1ab	74.6a	9.7a	7.7a
Bt @ 2 kg/ha 7 days	57.6bc	54.8b	9.7a	9.9a
Bt @ 2 kg/ha 14 days	70.7ab	68.1ab	8.8a	9.4a
Bt @ 2 kg/ha 21 days	74.5ab	71.7ab	7.4a	7.2a
Karate 320 ml/ha 14 days	47.7c	43.2c	11.8a	12.7a
Coragen 250 ml/ha 14 days	21.8d	18.7d	14.9a	14.3a
Untreated check	83.8a	81.9a	6.3a	7.1a

Means in a column followed by the same letter are not significantly different from each other at 5% significance level

Discussion

The level of leaf infestation by *T. absoluta* observed in this study was far below the observation during the period of the pest outbreak in February 2013 in the Central rift Valley of Ethiopia (Gashawbeza and Abiy, 2013). This could be due to location of the experimental field and season of planting among possible reasons. Climate change has also a significant impact on the ecological parameters and the duration of the life cycle of *T. absoluta* pest (Abolmaaty *et al*., 2010). Interaction effect between rate and frequency was not significant for all the parameters considered.

Absence of significant difference among treatments for egg density was expected from *Bt* treatments as the product needs to be ingested only by the insect larvae to be effective. Similarly, absence of significant effect of coragen and karate in reducing egg number of *T. absoluta* suggests lack of ovicidal effect of both insecticides. Hafsi *et al*. (2012) also reported low ovicidal effect of insecticides recommended for *T. absoluta* control. No leaf damage was observed until early fruiting stage. However, the data recorded in the first week of May show variability between treatments for leaf damage with the highest damage level from the untreated plot and the lowest from Coragen treatment. Leaf damage in the *Bt* treatments was intermediate between Coragen and the untreated check without significant difference between them. Derbalah *et al*. (2012) reported intermediate performance of *Bt* between untreated check and synthetic insecticide in terms of reducing leaf damage. They reported up to 30 and 68% reduction in leaf blotch in *Bt* and Imidacloprid treated plots, respectively. Sabbour (2014) and Sabbour and Soliman (2014) also reported reduction of leaf infestation in *Bt* treatment compared to the untreated control. They reported leaf infestation level of 14.5 ± 11.5 in Bt treatment as opposed to 35.4 ± 12.3 in the control. Absence of significant variability among treatments for total yield could be due to low infestation of *T. absouta* during the vegetative growth stage of tomato. Severe infestation during vegetative could lead to total crop failure (Gashawbeza and Abiy, 2013). Treatments showed significant variability for fruit damage by the pest both in number, weight, and marketable yield. Differences for total fruit number and total yield between treatments were insignificant. This indicates infestation level was relatively higher during fruiting stage of the crop with a pronounced effect on the quality of the produce through piercing and rotting of the fruit with little or no effect on total fruit number and yield. Coragen has been reported as the most effective insectiicde affecting *T. absoluta* population (Ghanim and Ghani, 2014) which is in agreement with results of this study. Moussa *et al*. (2013) also reported the effectiveness of Coragen and *Bt* for controlling tomato with high and moderate effect, respectively, in Egypt. The decrease in effectiveness of *Bt* at lower application rate agreed with reports of Gonzale-cabrera et al. (2011) who reported that marketable yield of tomatoes were significantly higher than the untreated control at higher rates of *Bt*. According to Picanco et al. (1998), using *Bt* at low rates for *T. absoluta* control resulted in control failures, which may be minimized by mixing *B. thuringiensis* with mineral oil.

Complete control of *T. absoluta* was not achieved even from the best performing treatment i.e. Coragen. Complete control of *T. absoluta* with insecticides is difficult. Lobue et al. (2012) reported 30% damaged fruit weight due to *T. absoluta* from insecticide treated control plots. This study demonstrated the potential of *Bt* in reducing damage and yield loss due to *T. absoluta* on tomato when applied with high dose (2 kg per ha) at weekly intervals. It is suggested that future studies need to look into assessing performance of higher doses and shorter application intervals than considered in this study along with economics of their use in tomato production.

Conclusion and Recommendation

The study demonstrated the potential of Bt in reducing damage and yield loss due to *T. absoluta* on tomato when applied with high dose (2 kg per ha) weekly. It is suggested that future studies need to look into assessing performance of higher doses and shorter application intervals than considered in this study along with economics of their use in tomato production.

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