

Biological and eradication parameters of the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) affected by two biopesticides

A. M. A. REDA, A. E. HATEM

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) was treated as 1st and 3rd instars larvae with the biopesticides; *Bacillus thuringiensis* var. *kurstaki*, *Beauveria bassiana* (Balsamo) or both to evaluate their toxicity against the pest. The biological and eradication parameters of the tomato leafminer were affected when treated as newly hatched larvae by LC₅₀s of the biopesticides. The study results obtained determined that the biopesticide *B. thuringiensis* var. *kurstaki* shows greater efficiency than *B. bassiana*. While, *B. thuringiensis* var. *kurstaki* + *B. bassiana* gave antagonistic effect against the tested pest than *B. thuringiensis* var. *kurstaki* or *B. bassiana*. The biopesticide compound, *B. thuringiensis* var. *kurstaki* proved the most potent against newly hatched and 3rd instar larvae of *T. absoluta* followed by *B. bassiana* and *B. thuringiensis* var. *kurstaki* + *B. bassiana*. Newly hatched larvae were more susceptible than 3rd instars. Larval and pupal phase duration was increased and so were the adult phase durations, post oviposition period and life cycle of *T. absoluta* except for with *B. thuringiensis* var. *kurstaki* + *B. bassiana* treatment, the values lower than control. In all treatments both larval mortality and sterility percentages were increased. Moreover, the same biopesticides caused decreases in the oviposition period, egg laying rate, percentages of adult eclosion, pupation, egg hatchability and fecundity compared with the control values.

On the other hand, the prediction (life table) parameters of the tomato leafminer were affected by biopesticide treatments. The female progeny/female (Mx) as well as survival rate (Lx) of *T. absoluta* was decreased in biopesticide treatments, especially in *B. thuringiensis* var. *kurstaki* treatment. The biopesticide treatments decreased the net reproductive rate (R₀), increase rate (intrinsic rate of natural increase (r_m)) and finit rate of increase (e^m) compared with the control. On the other hand, *B. thuringiensis* var. *kurstaki*, followed by *B. bassiana* had increased the times of generation (T) and doubling (DT).

A. M. A. REDA. Plant Protection Research Institute, Agriculture Research Center, 7 Nadi El-Said St., (12311) Dokki, Giza, Egypt.

A. E. HATEM. Entomología Agroforestal. Departamento de Ciencias y Recursos Agrícolas y Forestales. Universidad de Córdoba, Campus Rabanales, Edificio Celestino Mutis (C4). 14071 Córdoba, España, e-mail: cr2sayed@uco.es.

Keywords: Larval duration, pupal duration, life table, Adult longevity.

INTRODUCTION

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae) first described in Peru in 1917, is

now found throughout South America, where it is considered one of the most devastating pests for tomato crops (BARRIENTOS *et al.*, 1998; ESTAY 2000; EPPO 2006). *T. absoluta*

is considered to be a serious threat to tomato production in Mediterranean region (DESNEUX *et al.*, 2010). This pest is crossing borders and devastating tomato production both protected and in open fields. The infestation by *T. absoluta* has also been reported on potato, aubergine and *Phaseolus vulgaris*. *T. absoluta* is a very challenging pest to control. Effectiveness of chemical control is limited due to insect's natural resistance to damage as well as its rapid capability for developing the insecticide resistant strains (LIETTI *et al.*, 2005; IRAC, 2009c; STRATEN *et al.*, 2011). The efficacy of insecticides based on different subspecies of *Bacillus thuringiensis* Berliner, sprayed four times at weekly intervals, varied widely from around 35 to 70% (NANNINI *et al.*, 2011).

Biopesticides are very effective in agricultural pest control without causing serious damage to ecological chain or aggravating environmental pollution. Development of practical applications in the field of biopesticides greatly mitigates environmental pollution caused by chemical pesticide residues and promotes sustainable development of agriculture (LENG *et al.*, 2011). The development of biopesticides stimulates modernization of agriculture and will, undoubtedly, gradually replace chemical pesticides. Many biopesticides are ideal substitutes for their traditional chemical counterparts in pollution-free agricultural production, but some of them display certain toxicity; this should be taken into consideration by the researchers in the field (LENG *et al.*, 2011).

B. thuringiensis var. *kurstaki* (Btk) is a member of the genus *Bacillus*, a diverse group of spore forming bacteria that consists of more than 20 species. The species of *B. thuringiensis* is common in terrestrial habitats, including soil, dead insects granaries and on plants (American Academy of Microbiology, 2002). *B. thuringiensis* must be eaten by a susceptible insect in order to be effective. The microorganism produces both spores (resting stage) and crystalline protein (an endotoxin). When eaten by the insect, this endotoxin be-

comes activated and binds to the insect gut creating a pore through which gut contents can enter to the body cavity and bloodstream of the insect. The insect ceases to feed and dies within a few days (TABASHNIK, *et al.*, 2003, SALVO and VALLADARES, 2007). Btk may not be effective once *T. absoluta* larvae enter plant parts (SIXSMITH, 2009).

Beauveria bassiana (Balsamo), is a pathogenic fungus with a large host range and it is used for biological insect control. *B. bassiana* infects and kills the pest when it comes in to contact with the fungal spores. Once the fungal spores attach to the insect's cuticle, they germinate sending out structures (hyphae) that penetrate and proliferate in the insect's body. It may take 3-5 days for insects to die, but infected cadavers may serve as a source of spores for secondary spreading of the fungus. Insects can also spread the fungus through mating (LONG *et al.*, 2000).

B. bassiana (strain GHA 1991) was tested alone or in combination with *B. thuringiensis* for control of *T. absoluta* in open tomato fields in Ibiza, Spain (TORRES GREGORIO *et al.*, 2009). Both treatments reduced the number and severity of fruit damage when compared to the control.

This study aims to evaluate the toxicity of the two biopesticides Protecto (*B. thuringiensis* var. *kurstaki*) and Biover (*B. bassiana*) (Balsamo) alone and mixture against the tomato leafminer, *T. absoluta* (Meyrick). Also, the biological and eradication parameters of the tested pest as a result of treatments with LC₅₀'s of the biopesticide used were studied.

MATERIALS AND METHODS

Biopesticides

1. Protecto is a commercial formulation of *Bacillus thuringiensis* var. *kurstaki* and it is a product of Special Unit for Producing Bioinsecticides, Plant Protection Research Institute (PPRI), Agriculture Research Center (ARC), Egypt, with 32000 international toxicity units (spores and protein

crystals) per mg. The active ingredient is 6.4% W.P. and the application rate is 300 g/Feddan.

2. Biover a commercial formulation of *Beauveria bassiana* and it is a product of Special Unit of Producing Bioinsecticides, PPRI, ARC, Egypt. The international unit was 32,000 viable spores per mg. The active ingredient was 10% W.P. and the recommended application rate was 200 g /100 liter water/Faddan.

The Pest

The tomato leaf miner, *Tuta absoluta* samples from tomato growers at Wadi El-Natron, Al-Alamein road, Cairo-Alexandria desert road, Egypt in 2012. The pest was reared in the laboratory on tomato seedlings. The larvae were added with tomato seedlings in glass jars which were tightly closed with muslin. If needed more tomato seedlings were added until pupation. When at least 5 pair of adults had emerged, they were put in glass cages (17 cm height and 7-12 cm in diameter) prepared with tomato seedlings as deposit of eggs and a piece of cotton saturated in a sugar solution 10% to feed the moths and tightly closed with muslin. The eggs on the tomato seedling were collected and put in jars until hatching.

Insecticidal activity of *B. thuringiensis* var. *kurstaki* and *B. bassiana* against *T. absoluta*

Four concentrations (1, 2, 3 and 4 g) of *B. thuringiensis* var. *kurstaki* or *B. bassiana* were used. In case combination of *B. thuringiensis* var. *kurstaki* + *B. bassiana* mixed at ratio of 1:1. Three replicates/each concentration were used. Tomato seedlings were embedded in each concentration. It was transferred on a clean white paper until water evaporated. Tomato seedlings treated with biopesticides compounds were put in clean glass jars with 20 newly hatched or 3rd instar larvae field strain of *T. absoluta* / replicate (60 larvae/ concentration). Tomato seedlings embedded in water only were used as control. The jars were closed tightly with muslin and rubber

and kept at 27±2 °C. Mortality was noted after 72 hours of treatments.

Mortality percentages were corrected with the ABBOTT formula (1925).

LC₅₀ and LC₉₀ values were obtained by a software computer probane according to FINNEY (1971). The efficiency of both insecticides could be measured using SUN'S equation (1950) as follows:

Toxicity index = $\frac{[LC_{50} (LC_{90}) \text{ of the compound A} / LC_{50} (LC_{90}) \text{ of the compound B}]}{100}$

Where: A: is the most effective compound. B: is the other tested compound.

Biological parameters

The tomato leaf miner, *T. absoluta* treated as newly hatched larvae with LC₅₀ of *B. thuringiensis* var. *kurstaki*, *B. bassiana* and *B. thuringiensis* var. *kurstaki* + *B. bassiana* used.

The following biological parameters were investigated as follows:

- Larval development, pupal duration and adult longevity (in days).

- Pupation and moths emergency percentages.

% Pupation = (N° produced pupae / Total tested larvae)* 100

% Moths emergency = (N° emerged moth / Total tested larvae)* 100

- Larval and adult moth mortality percentages: Were corrected according to Abbott's formula (1925).

Mortality % = (N° of dead larvae / Total N° of larvae)* 100

Corrected mortality% = $(P - P_0 / 100 - P_0) * 100$

P = Percent mortality of treated larvae or moths.

P₀ = Percent mortality of untreated larvae or moths.

- Pre-oviposition, oviposition and post-oviposition periods:

2-5 pairs of emerged moths were placed in clean glass jars (17 cm height and 7-12 cm in

diameter) to determine the periods from adult female emergence until death.

- **Egg laying and egg hatchability percentage:**
The total number of eggs per female was calculated from daily counts of deposited eggs on the tomato seedlings. Each treatment yielded data on the daily egg production and on the differential survival of females. The egg hatchability percentage was counted as follows:
% egg hatchability = (N° hatched eggs / N° deposited eggs) * 100
- **Control of hatchability percentage:**
Was calculated according to ZIDAN and ABDEL-MEGEED (1987) as follows:
Hatchability percentage = (N° egg hatchability in check - N° egg hatchability in treatment / N° egg hatchability in check) * 100
- **Fecundity percentage:**
Was calculated according to CRYSTAL and LACHANCE (1963) as follows:
Fecundity percentage = (N° eggs/ treated female / N° eggs/ untreated female) * 100
- **Sterility observed and corrected percentages:**
Were calculated according to ZIDAN and ABDEL-MEGEED (1987) as follows:
% Sterility observed = 100 - Egg hatchability percentage
% Corrected sterility = (% Sterility observed - Check / 100 - Check) * 100
- **Life cycle:**
This period extended from egg deposited until adult emergence (in days).

Eradication parameters

The data of eradication (life table) study were analyzed by a computer program developed by (ABOU-SETTA *et al.*, 1986). The input data for the program includes: insect name, temperature used, number of observation, time interval between observations, development time from egg to adult female, initial number of females, fraction of eggs laid reaching maturity, sex ratio as females per total, egg laying rate of *T. absoluta*. The program's

output data includes information for each interval of adult female age: total progeny per interval (egg laying rate) (M), number of females alive at age x (L), mean female age at each interval mid-point (X), female progeny per female produced during the day x (Mx), rate of survival (Lx), the product of [(Mx)(Lx)] as (MxLx), and the final values of RML (the product of (Mx)(Lx) is then divided by the value e (the base of natural logarithm to the power of (r_m))

Finally, the program prints out the precise life table sheet parameters of that study as the sum of RML, the generation time (T) was calculated by $[\sum ((X)(Lx)(Mx))/Ro]$, the net reproductive rate (Ro) was calculated by $[\sum((Lx)(Mx))]$, the doubling time (DT) was resulted from dividing the normal logarithm on r_m , the intrinsic rate of natural increase (r_m) that was calculated by $[\ln (Ro)/T]$ and the finite rate of increase (e^{r_m}) is the natural antilogarithm of the intrinsic rate of increase and gives the number of times which the population multiplies in a unit time (doubling time, DT). Also, the sex ratio was calculated.

Statistical analysis

All biological parameters of *T. absoluta* were analyzed using Costat 1990 statistical program software, and Duncan's multiple range test (DUNCAN, 1955) at 5% probability level to compare the differences among time means.

RESULTS AND DISCUSSION

Efficacy of *B. thuringiensis* var. *kurstaki* and *B. bassiana* against *T. absoluta*

Table (1) showed that the biopesticide compound, *B. thuringiensis* var. *kurstaki* was the most potent compound against newly hatched larvae of *T. absoluta* (LC₅₀: 0.479 g/l), followed by *B. bassiana* (LC₅₀: 1.047 g/l) and *B. thuringiensis* var. *kurstaki* + *B. bassiana* (LC₅₀: 1.624 g/l). The same trend of the biopesticide efficacy against the newly hatched was found in 3rd instars larvae as illustrated in table 1. *B. thuringiensis* var. *kurstaki*

Table 1: Efficacy of the biopesticide compounds against *T. absoluta*

Biopesticides	LC ₅₀ (gm/L) 95% Confidence limits	LC ₉₀ (gm/L) 95% Confidence limits	Toxicity index	
			LC ₅₀	LC ₉₀
1st instars larvae				
<i>B. thuringiensis</i> var. <i>kurstaki</i>	0.479 0.283±0.680	3.143 1.760 ±13.52	100	100
<i>B. bassiana</i>	1.047 0.649 ±2.615	14.21 4.314±18.69	45.75	22.1
<i>B. thuringiensis</i> var. <i>kurstaki</i> + <i>B. bassiana</i>	1.624 1.241±2.095	6.903 4.552±15.25	24.6	45.53
3rd instars larvae				
<i>B. thuringiensis</i> var. <i>kurstaki</i>	1.158 0.504±1.589	3.189 2.343±6.959	100	100
<i>B. bassiana</i>	1.310 0.230 ±1.945	6.432 3.691±19.85	88.39	49.58
<i>B. thuringiensis</i> var. <i>kurstaki</i> + <i>B. bassiana</i>	2.331 1.825±2.868	5.178 3.897±10.18	49.68	61.58

showed the highest toxicity (LC₅₀: 1.580 g/l), followed by *B. bassiana* (LC₅₀: 1.310 g/l) and *B. thuringiensis* var. *kurstaki* + *B. bassiana* (LC₅₀: 2.331 g/l).

The biopesticide compound, *B. thuringiensis* var. *kurstaki* was the most efficient against newly hatched and 3rd instar larvae (Toxicity index=100) both at levels LC₅₀ and LC₉₀. *B. bassiana* on the other hand, had lower efficacy than *B. thuringiensis* var. *kurstaki* (Toxicity index=45.750 according to LC₅₀) for newly hatched and 88.390 according to LC₅₀ for 3rd instar larvae. Moreover *B. thuringiensis* var. *kurstaki* + *B. bassiana* had antagonistic effect against the pest at two treatment stages. While, at LC₉₀ levels, *B. thuringiensis* var. *kurstaki* + *B. bassiana* gave lower efficacy than treatments of *B. bassiana* only as showed in table (1). The same result has been observed by GONZÁLEZ-CABRERA *et al.*, 2011 they found that 1st instar larvae

were the most susceptible, while susceptibility was lower in 2nd and 3rd instar larvae. On the contrary, AMER and EL-NEMAKY (2008) showed that *B. thuringiensis* var. *kurstaki* + *B. bassiana* gave synergistic effect against the newly hatched larvae of *Pectinophora gossypiella* (Saunders 1844) than *B. thuringiensis* var. *kurstaki* and *B. bassiana* when used singly.

Biological parameters of *T. absoluta* when treated with the biopesticides

Larval development, pupal duration and adult longevity

The larval period duration of the tomato leaf miner, *T. absoluta* increased compared to control when treated tomato leaf miner as newly hatching larvae by LC₅₀'s of *B. thuringiensis* var. *kurstaki*, followed by *B. bassiana*. While, *B. thuringiensis* var. *kurstaki* + *B.*

Table 2: Effect of tested biopesticides on some biological parameters of *T. absoluta* treated as newly hatched larvae

Biopesticides	Larval duration (days)	% Larval Mortality	Pupal duration (days)	% Pupation	Adult duration (days)	% Adult emergency	Female adult longevity (days)			% Adult mortality
							Pre-oviposition period	Ovipos. period	Post-Ovipos. period	
<i>B. thuringiensis</i> var. <i>kurstaki</i>	17 ^a (14-20)	83 ^a (80-86)	19 ^a (17-21)	27 ^c (23-31)	23 ^a (21-25)	20 ^c (18-22)	2 ^a (2-2)	10 ^b (8-12)	11 ^a (10-12)	7 ^a (5-9)
<i>B. bassiana</i>	15 ^b (13-17)	68 ^b (66-70)	12 ^b (10-14)	42 ^b (40-44)	23 ^a (20-26)	40 ^b (36-44)	2 ^a (2-2)	10 ^b (9-11)	11 ^a (10-12)	2 ^b (1-3)
<i>B. thuringiensis</i> var. <i>kurstaki</i> + <i>B. bassiana</i>	13 ^c (10-15)	56 ^c (52-60)	8 ^c (7-9)	44 ^b (40-48)	23 ^a (22-24)	42 ^b (38-46)	2 ^a (2-2)	10 ^b (7-13)	11 ^a (10-12)	2 ^b (2-2)
Control	14 ^{bc} (13-15)	10 ^d (8-12)	10 ^c (7-13)	90 ^a (88-92)	21 ^b (19-23)	88 ^a (86-90)	2 ^a (2-2)	12 ^a (11-13)	7 ^b (6-8)	2 ^b (1-3)
LSD _{0.05}	1.708	31.500	5.944	27.240	1.000	28.770	0.000	1.000	2.000	2.500
SS	70.670	8930.300	242.250	6760.300	45	7529	0	39	44	68.250
df	8	8	8	8	8	8	0	8	8	8
F	1.967	360.800	15.280	222.680	0.670	248.300	0	0.800	12	12.500
P	0.1976 NSD	0.000 SD	0.0011 SD	0.000 SD	0.5957 NSD	0.000 SD	0	0.5279 NSD	0.0025 SD	0.0022 SD
Error mean square	5.080	8.250	4.500	10	4.500	10	0	3.750	1	1.500

NSD = No Significant Difference. SD = Significant Difference.

bassiana decreased about one day than control, the values were 17, 15, 13 and 14 days for *B. thuringiensis* var. *kurstaki*, *B. bassiana*, *B. thuringiensis* var. *kurstaki* + *B. bassiana* and control as in Table (2).

The same clear tendency in pupal duration of *T. absoluta* that increased especially in *B. thuringiensis* var. *kurstaki* treatment which had the highest increase (19 days), followed by *B. bassiana* (12 days). Opposite, *B. thuringiensis* var. *kurstaki* + *B. bassiana* decreased to 8 days compared to the control value that was 10 days, as illustrated in Table (2). All the treatments used gave the same duration (23 days) for *T. absoluta* adult moth compared to control value (21 days) as shown in the same table.

Pupation and adult emergence percentages

Table 2 shows that the pupation percentages of *T. absoluta* were affected when treated with biopesticides. Pupation percentages were decreased to (27% and 42%) with *B. thuringiensis* var. *kurstaki* and *B. bassiana* (42%) respectively and to (44%) with the combination of *B. thuringiensis* var. *kurstaki* + *B. bassiana* compared with control pupation (90%). The same trend was found in adult eclosion percentage parameter of *T. absoluta*. All the compounds decreased the eclosion percentages to 20, 40 and 42% for *B. thuringiensis* var. *kurstaki*, *B. bassiana* and *B. thuringiensis* var. *kurstaki* + *B. bassiana*, respectively compared to normal adult emergence percentage (88%).

Larval and adult mortality percentages

The results in the table 2 show that the larval mortality percentage of *T. absoluta* treated as newly hatched larvae with *B. thuringiensis* var. *kurstaki* reached 83%, followed by *B. bassiana* 68% and then 56% in *B. thuringiensis* var. *kurstaki* + *B. bassiana* treatment, while the value was 10% in the control.

On the other hand, percentage of adult mortality of *T. absoluta* increased to 7% in *B. thuringiensis* var. *kurstaki* only. Meanwhile, the other treatments of *B. bassiana* and *B.*

thuringiensis var. *kurstaki* + *B. bassiana* had the same adult mortality percentage than the control (2%) as described in the aforementioned Table.

Pre-oviposition, oviposition and post-oviposition periods

Table (2) shows that there are not differences among the biopesticides used in the effect on pre-oviposition period of *T. absoluta*, noted every two days after treatment. Also the same time in control value (2 days). The previous table demonstrates that normal oviposition period of *T. absoluta* was 12 day; this value decreased to about 10 days in the females obtained from newly hatched larvae treated with *B. thuringiensis* var. *kurstaki*, *B. bassiana* and *B. thuringiensis* var. *kurstaki* + *B. bassiana*. The biopesticide, *B. thuringiensis* var. *kurstaki* when used alone or mixed with *B. bassiana* gave the same result of post-oviposition period when the *T. absoluta* treated as newly hatched larvae with the LC₅₀s of the previous biopesticides. The values were 11 days which increased in over 4 days the control (7 days) as described in table (2).

Egg laying rate and hatchability percentage

The egg laying rate of *T. absoluta* normal females in control was of 260 eggs/female as shown in table (3), this value decreased to 175 eggs/female in females treated as newly hatched larvae with *B. thuringiensis* var. *kurstaki*, followed by *B. bassiana* (185 eggs/female) and *B. thuringiensis* var. *kurstaki* + *B. bassiana* (189 eggs/female).

On the other hand, eggs laid by the females initiated from newly hatched larvae of *T. absoluta* treated with *B. thuringiensis* var. *kurstaki* compound had the lowest hatchability percentage (74%), followed by *B. bassiana* treatment that had 80% and then *B. thuringiensis* var. *kurstaki* + *B. bassiana* treatment (85%) compared to control (90%) as in the same table.

Control of hatchability percentage

Table (3) describes that eggs laid by the females of *T. absoluta* treated as newly hatched

Table 3: Effect of tested biopesticides on the egg, fecundity, sterility and life cycle of *T. absoluta* treated as newly hatched larvae

Biopesticides	Egg laying rate (N° of egg/ female)	% Egg hatchability	% Control of hatchability	% Fecundity	% Sterility observed	% Corrected sterility	Life cycle (days)
<i>B. thuringiensis</i> var. <i>kurstaki</i>	175 ^c (170-180)	74 ^c (70-78)	17.78 ^a (12.7- 22.8)	41.67 ^c (38.6-44.7)	26 ^a (23-29)	17.78 ^a (15.7- 19.8)	40 ^a (36-44)
<i>B. bassiana</i>	185 ^b (178-192)	80 ^b (75-85)	11.11 ^b (8.1-14.1)	47.22 ^{bc} (42.2-52.2)	20 ^b (18-22)	11.11 ^b (10.1- 12.1)	31 ^b (28-34)
<i>B. thuringiensis</i> var. <i>kurstaki</i> + <i>B.</i> <i>bassiana</i>	189 ^b (182-196)	85 ^b (83-88)	5.58 ^c (3.5-7.8)	49.44 ^b (44.4-54.4)	15 ^c (11-19)	5.56 ^c (3.5-7.6)	25 ^c (22-28)
Control	260 ^a (250-270)	90 ^a (84-96)	-	100 ^a (100-100)	10 ^d (8-12)	-	28 ^c (26-30)
LSD _{0.05}	48.850	6.8490	7.608	27.140	6.849	7.611	7.632
SS	14098.250	594.670	761.490	6748.400	488.250	539.320	454
df	8	8	8	8	8	8	8
F	81.630	6.848	11.567	149.840	17.060	77.230	13.260
P	0.000 SD	0.0134 SD	0.0028 SD	0.000 SD	0.0008 SD	0.0000 SD	0.0018 SD
Error mean square	55.750	20.830	17.830	14.750	8.250	2.250	9.500

SD = Significant Difference.

larvae with the *B. thuringiensis* var. *kurstaki* and *B. bassiana* mixture were 5.58% control of hatchability, this value increased to 11.11% and 17.78% when the *B. thuringiensis* var. *kurstaki* and *B. bassiana* were used alone, respectively.

Fecundity percentage

The biopesticides of *B. thuringiensis* var. *kurstaki* and *B. bassiana* mixture gave the highest fecundity percentage (49.44%) on adult moths initiated from *T. absoluta* newly hatched larvae treated with it as shown in table (3), followed by *B. bassiana* (47.22%) and *B. thuringiensis* var. *kurstaki* (41.67%) as analysis data show in table (3).

Sterility observed and corrected percentages

The sterility observed of *T. absoluta* control was 10% as mentioned in table (3), this value increased to 15% in adult females initiated from newly hatched larvae treated with *T. absoluta* by *B. thuringiensis* var. *kurstaki* + *B. bassiana* compound, followed by *B. bassiana* treatment (20%) and reached to 26% in *B. thuringiensis* var. *kurstaki* treatment. The corrected sterility percentage was lower with *B. thuringiensis* var. *kurstaki* + *B. bassiana* treatment (5.56%) than both *B. bassiana* (11.11%) and *B. thuringiensis* var. *kurstaki* (17.78%) as illustrated in table (3).

Life cycle

Results in table 2 showed that *T. absoluta* treated with *B. thuringiensis* var. *kurstaki* + *B. bassiana*, had a life cycle of 23 days which was 25 days in the control, but in the other two treatments with *B. thuringiensis* var. *kurstaki* and *B. bassiana* alone they were increased to 31 and 40 days, respectively. The results obtained agreed with AMER (2007) who mentioned that Dipel-2x increased pupal duration, adult longevity, life cycle, percentages of larval & pupal mortality and sterility. On the other hand, it decreased egg laying and egg hatching. Times additionally, the result of

this work agree too with the results of Amer and EL-NEMAKY (2008) who reported that *B. thuringiensis* var. *kurstaki* + *B. bassiana* is considered the best compound over *B. thuringiensis* var. *kurstaki* or *B. bassiana* alone in toxicity, biological and prediction parameters of *P. gossypiella*.

The eradication (Life table) parameters of *T. absoluta* treated by the biopesticides

Female progeny/female (Mx) and rate of survival (Lx)

Figure 1, illustrates that female progeny/female (Mx) of normal *T. absoluta* ranged be-

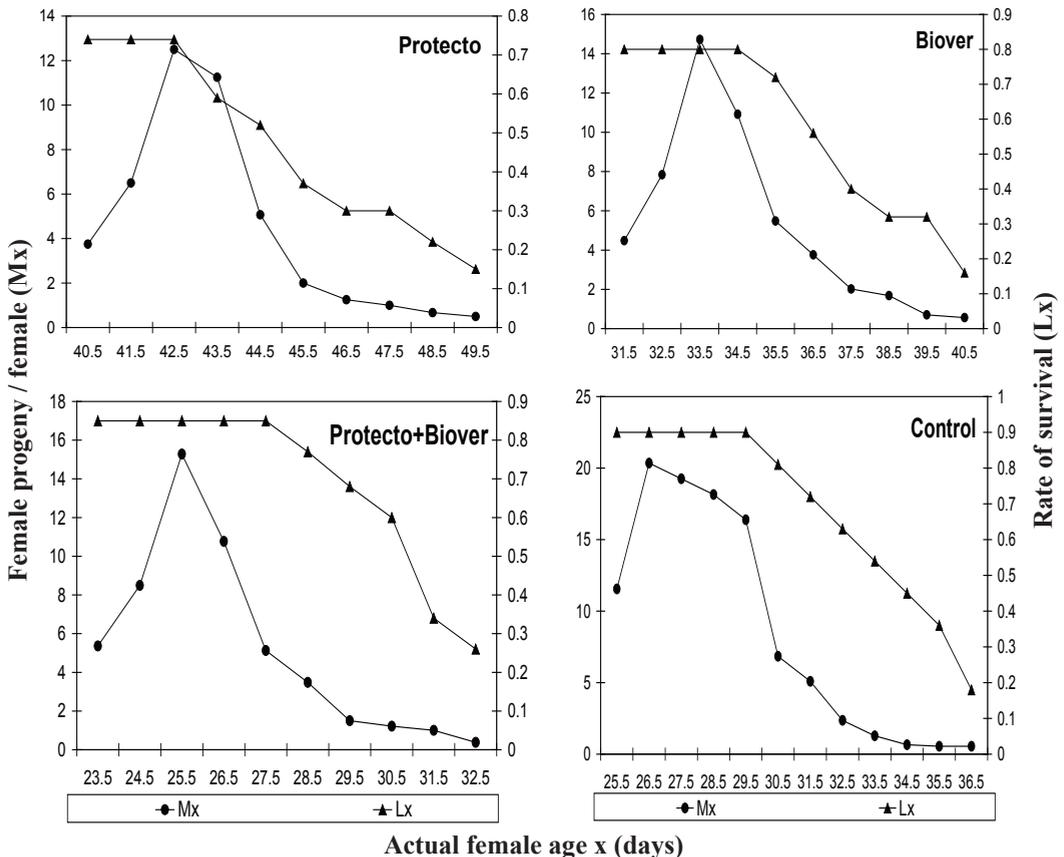


Figure 1: Effect of tested biopesticides on the female progeny/female (Mx) and survival rate (Lx) of *T. absoluta*

tween 0.55 to 20.350, the last values decreased in females initiated from newly hatched larvae treated with *B. thuringiensis* var. *kurstaki*, it ranged between 0.500 to 12.500 females progeny/female. Moreover, it ranged between 0.560 to 14.730 females progeny/female in *B. bassiana* treatment. The same trend was found in *B. thuringiensis* var. *kurstaki* + *B. bassiana* treatment, the Mx values, it ranged between 0.380 to 15.280 females progeny/female that initiated from *T. absoluta* newly hatched larvae treated with biopesticide mixture (*B. thuringiensis* var. *kurstaki* + *B. bassiana*). The (Lx) parameter (rate of survival) ranged between 0.180 to 0.900 times in *T. absoluta* normal females. The females treated as newly hatched larvae with *B. thuringiensis* var. *kurstaki* had a survival rate ranged between 0.150 to 0.740 times, while in *B. bassiana* treatment ranged between 0.160 to 0.800 times. Also, the females initiated from *T. absoluta* newly hatched larvae treated with *B. thuringiensis* var. *kurstaki* + *B. bassiana* had a survival rate ranged between 0.260 to 0.850 times which was the greatest increase when compared with the biopesticides used alone.

Generation time (T)

The tomato leaf miner, *T. absoluta* treated as newly hatched larvae spent 42.71 days un-

der *B. thuringiensis* var. *kurstaki* treatment as in Table (4), followed by *B. bassiana* (33.83 days) which had greater values than the control. Also, the mixture of *B. thuringiensis* var. *kurstaki* + *B. bassiana* caused reduction to 27.78 days) compared with *B. thuringiensis* var. *kurstaki* and *B. bassiana* when used alone and compared with the control (29.85 days).

Net reproductive rate (Ro)

The tested biopesticides caused high reduction of net reproductive rate (Ro) when *T. absoluta* was treated as newly hatched larvae as shown in table (4). The *B. bassiana* treatment result was 38.08 females/female in one generation, the last value decreased to 27.75 females/female under *B. thuringiensis* var. *kurstaki* treatment. The biopesticide mixture (*B. thuringiensis* var. *kurstaki* + *B. bassiana*) had the least destructive reduction effect from net reproductive rate (43.12 females/female) compared with the untreated *T. absoluta* (89.09 females/female).

Increase rate

- Intrinsic rate of natural increase (r_m)

Table (4) shows that intrinsic rate of natural increase (r_m) which means the ability to inherit increase for the *T. absoluta* normal female was 0.161 times/female/day. Also females of *T. ab-*

Table 4: Life table parameters of *T. absoluta* treated as newly hatched larvae with LC₅₀'s of tested biopesticides

Biopesticides	T (days)	(Ro)	Increase rate		DT (days)	Sex ratio
			r_m	e^{rm}		
<i>B. thuringiensis</i> var. <i>kurstaki</i>	42.710	27.750	0.078	1.081	8.890	0.500
<i>B. bassiana</i>	33.830	38.080	0.108	1.114	6.420	0.560
<i>B. thuringiensis</i> var. <i>kurstaki</i> + <i>B. bassiana</i>	27.780	43.120	0.146	1.157	4.750	0.570
Control	29.850	89.090	0.161	1.175	4.310	0.550

(T)= The generation time. (Ro)= The net reproductive rate. (r_m)= The intrinsic rate of natural increase (e^{rm})= The finite rate of increase. (DT)= The doubling time

soluta treated as newly hatched larvae with *B. thuringiensis* var. *kurstaki* and *B. bassiana* had a reduced intrinsic rate compared to the control, down to 0.078 and 0.108 times/female/day, respectively. On the other hand, *B. thuringiensis* var. *kurstaki* + *B. bassiana* treatment showed the least reduction from intrinsic rate, reaching 0.146 times/female/day.

- Finite rate of increase (e^m)

The daily population of the normal *T. absoluta* increased to 1.175 times/female/day as represented in table (4). Also, the females initiated from *T. absoluta* newly hatched larvae treated with *B. thuringiensis* var. *kurstaki* + *B. bassiana* had a capacity (1.157 times/female/day) close to the control's, followed by *B. bassiana* treatment (1.114 times/female/day) and then *B. thuringiensis* var. *kurstaki* treatment which had the lowest population capacity (1.081 times/female/day).

Doubling time (DT)

The time for population become twice its number, (doubling time (DT)) depends on the intrinsic rate of natural increase (r_m) which is affected by many factors as the rate of survival, generation time, female in progeny and fecundity. The tomato leaf miner, *T. absoluta* in control doubled its population every 4.31 days as in table (4). These days increased to 6.42 and 8.89 days when *T. absoluta* was treated as newly hatched larvae with *B. bassiana* and *B. thuringiensis* var. *kurstaki*, respectively.

While, the treatment with *B. thuringiensis* var. *kurstaki* + *B. bassiana* gave values near from control time (4.75 days) for doubling.

Sex ratio

Sex ratio was calculated as females/total. In control of *T. absoluta*, the sex ratio was 0.55. Also, the sex ratio values were 0.5, 0.56 and 0.57 for *B. thuringiensis* var. *kurstaki*, *B. bassiana* and then *B. thuringiensis* var. *kurstaki* + *B. bassiana*, respectively.

The aforementioned results agree with AMER (2006) who reported that Dipel2x (*B. thuringiensis* var. *kurstaki*) decreased rate of survival (L_x) and r_m . On the other hand, it increased from generation time of the pink bollworm. Opposite, Amer and El-Nemaky (2008) reported that *B. thuringiensis* var. *kurstaki* + *B. bassiana* had potentiated effect against pink bollworm than usage of each biopesticide alone.

Generally, the biopesticide, *B. thuringiensis* var. *kurstaki* was the best compound used in this study, followed by *B. bassiana* that had lower effectivity than *B. thuringiensis* var. *kurstaki* in toxicity, biological and eradication parameters of *T. absoluta*. Meanwhile, the mixture biopesticide compounds of *B. thuringiensis* var. *kurstaki* + *B. bassiana* had antagonistic effects and yielded the lowest destructive effect on most of the biological and eradication parameters of the tomato leaf miner than when the same two biopesticides were used alone.

RESUMEN

REDA, A. M. A., A. E. HATEM. 2012. Efecto de dos productos biológicos sobre distintos parámetros biológicos de *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Bol San. Veg. Plagas*, **38**: 321-333

Larvas neonatas y de tercer estadio de *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) fueron tratadas con los biopesticidas *B. thuringiensis* var. *kurstaki*, *B. bassiana* y una mezcla de ambos para evaluar su toxicidad contra la plaga. Así mismo, se calcularon las concentraciones letales medias (LC50) de cada uno de los insecticidas para larvas neonatas. Los resultados obtenidos mostraron que *B. thuringiensis* var. *kurstaki* fue mucho más tóxico que *B. bassiana*. Por su parte, la combinación de los dos productos mostró un efecto antagonístico con respecto al uso de los dos biopesticidas por separado. Las larvas neonatas fueron más susceptibles que las larvas de tercer estadio para los tres productos ensayados. Además, hubo una mayor duración de los periodos de desarrollo

larvario, de pupa, post oviposición y ciclo completo en individuos tratados con *B. thuringiensis* var. *kurstaki* y *B. bassiana*. Sin embargo, la combinación de los dos productos causó el efecto contrario, con parámetros por debajo de los obtenidos en el control. El biocida *B. thuringiensis* var. *kurstaki* fue el más activo contra larvas neonatas y de tercer estadio seguido de *B. bassiana* y la combinación de los dos productos. En todos los casos, los tratamientos aumentaron los porcentajes de mortalidad y esterilidad observada. Además, la fecundidad/hembra y el porcentaje de supervivencia fueron menores en los individuos tratados, sobre todo con *B. thuringiensis* var. *kurstaki*. Otros parámetros como la tasa de reproducción, tasa de crecimiento natural y finita fueron menores para los tratamientos que para el control.

Palabras clave: duración larval, duración pupal, tabla de vida, longevidad de los adultos.

REFERENCES

- ABBOTT, W.S. 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, **18**: 265-267.
- ABOU-SETTA, M.M., SORREL, R.W., CHILDERS, C.C. 1986. Life 48: A basic computer program to calculate life table parameters for an insect or mite species. *Florida Entomology*, **69** (4): 690-697.
- AMER, R.A. 2006. Effect of *Bacillus thuringiensis* (Kurs.) combined with gamma irradiation and the mixture of two bioinsecticides on the life table parameters of the pink bollworm. *Journal of Agriculture Science Mansoura University*, **31** (7): 4705-4714.
- AMER, R.A. 2007. Effect of certain bioinsecticides and gamma irradiation on some biological aspects of the pink bollworm. *Egypt Journal of Agriculture Research*, **85** (4):1285-1301.
- AMER, R.A., EL-NEMAKY, I.H. 2008. Effect of some bioinsecticides on the biological and prediction parameters of the pink bollworm, *Pectinophora gossypiella* (Saund.) (Lepidoptera: Gelechiidae). *2nd Arab Conference of Applied Biological Control in 7-10 April*.
- AMERICAN ACADEMY OF MICROBIOLOGY (2002): 100years of *Bacillus thuringiensis*: A critical assessment. <http://www.asm.org>.
- BARRIENTOS, Z.R., APABLAZA, H.J., NORERO, S.A., ESTAY, P.P. 1998. Temperatura base y constante térmica de desarrollo de la polilla del tomate, *Tuta absoluta* (Lepidoptera: Gelechiidae). *Ciencia e Investigación Agraria*, **25**:133-137
- CRYSTAL, M.M., LACHANCE, L.E. 1963. The modification of reproduction in insects treated with alkylating agents. Inhibition of ovarian growth and egg reproduction and hatchability. *Biology Bulletin*, **25**: 270-279.
- DESNEUX, N., WAINBERG, E., WYCKHUYS, K.A.G., BURGIO, G., ARPAIA, S., NARVÁEZ-VÁSQUEZ, C.A., GONZÁLEZ-CABRERA, J., RUESCAS, D.C., TABONE, E., FRANDON, J., PIZZOL, J., PONCET, C., CABELLO, T., URBANEJA, A. 2010. Biological invasion of European tomato crops by *Tuta absoluta*: ecology, history of invasion and prospects for biological control. *Journal of Pesticides Science*, **83**:197-215.
- DUNCAN, D.B. 1955. Multiple ranges and multiple F test. *Biometrics*, **11**:1-42.
- IRAC. 2009c. IRAC Mode of Action Classification. IRAC (Insecticide Resistance Action Committee). Accessed January 7, 2010. at: http://www.iraconline.org/documents/MoA%20classification_v6.3.3_28july09.pdf.
- ESTAY, P. 2000. Polilla del Tomate *Tuta absoluta* (Meyrick). Impresos CGS Ltda. Available online at: <http://www.inia.cl/medios/Descargas/CRI/Platina/Informativos/Informativo9.pdf>. Accessed 21 August 2007
- EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION (EPPO). 2006. Data sheets on quarantine pests. *Tuta absoluta*. Available online at: http://www.eppo.org/QUARANTINE/insects/Tuta_absoluta/DS_Tuta_absoluta.pdf. Accessed 11 August 2010.
- FINNEY, D.J. 1971. Probit Analysis, third ed. Cambridge University Press, Cambridge, United Kingdom.
- GONZÁLEZ-CABRERA, J., MOLLÁ, O., MONTÓN, H., URBANEJA, A. 2011. Efficacy of *Bacillus thuringiensis* (Berliner) in controlling the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *BioControl*, **56** (1): 71-80.
- LENG, P., ZHANG, Z., PAN, G., ZHAO, M. 2011. Applications and development trends in biopesticides. *African Journal of Biotechnology*, **10** (86): 19864-19873.
- LIETTI, M.M.M., BOTTO, E., ALZOGARAY, R.A. 2005. Insecticide Resistance in Argentine Populations of

- Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). *Neotropical Entomology*, **34** (1):113-119.
- LONG, D.W., DRUMMOND, G.A., GRODEN, E. 2000. Horizontal transmission of *Beauveria bassiana*. *Agricultural and Forest Entomology*, **2**:11-17.
- NANNINI, M., F. ATZORI, F., CHESSA, F., FODDI, F., MURGIA, G., PISCI, R., SANNA, F. 2011. Field experiments on management of the tomato borer *Tuta absoluta* (Meyrick) in Sardinian tomato greenhouses. *63rd International Symposium on Crop Protection* 24th May Ghent Belgium.
- SALVO, A., VALLADARES, G.R. 2007. Leafminer parasitoids and pest management. *Ciencia e Investigacion Agraria*, **34** (3):125-142.
- SIXSMITH, R. 2009. Call for integrated pest management as Mediterranean tomato pests spread to UK. *Horticulture Week* (October 9, 2009). Accessed November 5, 2009. <http://www.hortweek.com/news/search/943628/Call-integrated-pest-management-Mediterranean-tomato-pests-spread-UK>.
- STRATEN, M.J., POTTING, R.P., LINDEN, A.V. 2011. Introduction of the tomato leafminer *Tuta absoluta* into Europe. *PROC. NETH. ENTOMOL. SOC. MEET.* V(22).
- SUN, Y.P. 1950. Toxicity index on improved method of comparing the relative toxicity of insecticides. *Journal of Economic Entomology*, **43**: 45-53.
- TABASHNIK, B.E., CARRIERE, Y., DENNEHY, T.J., MORIN, S., SISTERTON, M.S., ROUSH, R.T., SHELTON, A.M., ZHAO, J.Z. 2003. Insect resistance to transgenic Bt crops. Lesson from laboratory and field. *Journal of Economic Entomology*, **96**: 1031-1038.
- TORRES GREGORIO, J., ARGENTE, J., ANGEL DÍAZ, M., YUSTE, A. 2009. Application of *Beauveria bassiana* in the biological control of *Tuta absoluta*. Aplicación de *Beauveria bassiana* en la lucha biológica contra *Tuta absoluta*. *Agrícola Vergel: Fruticultura, Horticultura, Floricultura, Citricultura, Vid, Arroz*, **28** (326):129-132.
- ZIDAN, H., ABDEL-MEGEED, M. I. 1987. New Trends in pesticides and pest control - Part II Al-Dar Al-Arabia for publishing and distribution, Cairo, Egypt.

(Recepción: 11 julio 2012)

(Aceptación: 27 diciembre 2012)