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Microbial Control of the Tomato Leaf-miner, *Tuta absoluta* (Povolny) (Lepidoptera: Gelechiidae) by Biopesticides in Relation to some Biological Parameters

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ABSTRACT

The tomato leaf miner, *Tuta absoluta* (Povolny) (Lepidoptera: Gelechiidae) was treated as newly hatched and 3rd instar larvae by two biopesticide formulations; Protecto and Biover as well by the mixture of both to evaluate their toxicities to the pest. Biological and life table parameters of *T. absoluta* were reported when treated as newly hatched larvae by LC_{50}s of the biopesticides. Obtained results showed that Protecto was the much effective compound used, followed by Biover and then the mixture. The later mixture gave antagonistic effect against the pest. Newly hatched larvae were more susceptible than 3rd instar larvae. Larval and pupal durations, adult longevity, post ovipositional period and life cycle of *T. absoluta* were increased when treated by LC_{50}s, except for the mixture treatment. Percentages of larval mortality and sterility were decreased in all treatments. The biopesticides had decreasing effects on egg laying rate, egg hatchability, pupation, percentages of adult emergence ovipositional period and fecundity compared with the control. On the other hand, the life table parameters of *T. absoluta* under biocides treatments were affected. The progeny/female (Mx) and survival rate (Lx) of *T. absoluta* were decreased, especially in case of Protecto. Also, the biopesticides treatments decreased the net reproductive rate (Ro), increase rate (intrinsic rate of natural increase (r_m) and finite rate of increase (e^{rm})), compared with the control. Vice versa, Protecto, followed by Biover increased the time of generation (T) and doubling time (DT).

Key words: Microbial Control, *Tuta absoluta*, Biopesticides, Biological Parameters.

INTRODUCTION

Tuta absoluta (Povolny) (Lepidoptera: Gelechiidae), namely tomato leaf-miner, tomato borer, South American tomato moth, is a devastating tomato pest. It is originated from South America. Recently, *T. absoluta* has become a serious threat to tomato production in the Mediterranean region. The pest has crossed the boarders and devastating tomato production in both protected and open fields. Infestation with *T. absoluta* was also reported on potato, Aborigine and common beans. It is a very challenging pest to control depending on multiple applications of insecticides. Effectiveness of chemical control is limited due to insect's nature of damage, as well as its rapid capability for development of insecticidal resistant (Straten *et al.*, 2011). Efficacy of the bio-insecticides, *Bacillus thuringiensis* Berliner, when sprayed four times at weekly intervals, showed a pest reduction from 35 to 70% (Nannini *et al.*, 2011).

Biopesticides are effective in the agricultural pest control without causing serious harm to ecological chains or worsening the environmental pollution. Development of practical applications of biopesticides greatly mitigates environmental pollution caused by chemical pesticide residues and promotes sustainable development of agriculture; therefore biopesticides can gradually replace chemical pesticides. Many of them are ideal substitutes for the traditional chemical counterparts in pollution-free agricultural production, but some have displayed certain toxicity, which should be taken into consideration by the researchers in the fields (Leng *et al.*, 2011). On the other hand, the biopesticides have been reported as safe on the beneficial insects (Amer and El-Nemaky, 2012).

The genus *Bacillus* is a diverse group of spores forming bacteria that consists of more than 20 species. The species *thuringiensis* is common in terrestrial habitats, including soil, dead insects and granaries 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 (endotoxin). When eaten by the insect larvae, the endotoxin becomes activated and binds to the larval 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).

Beauveria bassiana (Balsamo) is a pathogenic fungus with a large host range, where it is used for insect biological control. *B. bassiana* infects and kills the pest when it comes in contact with the fungal spores. Once the fungal spores attach the cuticle of insect, they germinate sending out structures (hypha) that penetrate and proliferate in the body of insect. It may take 3-5 days for insects to die, but the 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).

The present study aimed to evaluate the toxicity of two biopesticides; Protecto and Biover to the tomato leaf-miner, *T. absoluta,* as well their effects on biological and life table parameters of the pest.

MATERIALS AND METHODS

Biopesticides

- **Protecto**: is a commercial formulation of *Bacillus thuringiensis kurstaki.* It is a product of a Research Unit for Producing Bioinsecticide, Plant Protection Research Institute, Agriculture Research Center, Giza, Egypt, with 32000 international units per mg. The active ingredient is 6.4% W.P. and the application rate is 300gm/feddan.
- **Biover:** is a commercial formulation of *Beauvaria bassiana* (Balsamo). It is a product of the same Unit, with 32000 viable spores per mg. The active ingredient is 10% W.P. The recommended application rate is 200gm/100 liter water/Feddan (Acre).

Target Pest

Infested samples from *T. absoluta* were collected from tomato fields at Wadi El-Natron, Al-Alamein road, Cairo-Alexandria desert road, Egypt in 2012. The pest was reared on tomato seedlings under laboratory conditions. Larvae were placed on tomato seedlings in glass jars, closed tightly with muslin and rubber bands. Fresh tomato seedlings were added when needed until pupation. Newly emerged (5 pairs of moths) were placed in glass cages (17 cm height and 7-12 cm in diameter), supplied with tomato seedlings to lay eggs and a peace of cotton saturated with sugar solution 10%, as source of food. The eggs were collected daily and kept in jars until hatching.

Bioinsecticidal Treatments

Four concentrations; 3.2 x10^6, 6.4 x10^6, 9.6 x10^6 &12.8 x10^6 IU/L of Protecto or Biover were prepared. In case of the mixture, the concentrations were 1:1 (Protecto: Biover). Three replicates/ each concentration were used. Tomato seedlings were dipped in each concentration; transferred onto white clean paper until water evaporated. Tomato seedlings, treated with the biopesticide formulations, were placed in clean glass jars with 20 newly hatched or 3rd instar larvae of *T. absoluta*/ replicate (60 larvae/ concentration). Tomato seedlings treated with water only was used as control. The jars were closed tightly with muslin and rubber bands and kept at 27±2°C. Data was recorded, 72 hours post treatments. Mortality percentages were corrected by Abbott's formula (1925). LC_{50} and LC_{90} values were obtained by the software computer probane. Efficiency of the bioinsecticides was measured by using Sun's equation (1950) as follows:

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

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

Biological Parameters

T. absoluta was treated, as newly hatched larvae, by LC_{50}'s of Protecto, Biover and their mixture. The following biological parameters were investigated;

1- Larval and pupal durations, and adult longevity (in days)

2- Pupation and moths' emergence percentages

3- Larval and adult mortality percentages (Corrected by Abbott's formula (1925)).

4- Pre-ovipositional, ovipositional and post-ovipositional periods. 2-5 pairs of newly emerged moths were placed in clean glass cages (17 cm height and 7-12 cm in diameter), containing tomato seedlings and peaces of cotton, saturated with 10% sugar solution. Ovipositional periods were estimated.

5- Egg laying and egg hatchability percentage. The hatchability percentage was calculated as follows:

$$\frac{\text{No. egg hatchability}_{(\text{check})} - \text{No. egg hatchability}_{(\text{treated})}}{\text{No. egg hatchability in check}} \times 100$$

6- Fecundity percentage: calculated according to Crystal and Lachance (1963) as follows:

$$\%\ \text{Fecundity} = \frac{\text{No. eggs/ treated female}}{\text{No. eggs/ untreated female}} \times 100$$

7- Sterility observed and corrected percentages: calculated according as follows:

$$\%\ \text{Sterility observed} = 100 - \text{Egg hatchability percentage}$$

$$\%\ \text{corrected sterility} = \frac{\%\ \text{Sterility observed - Check}}{100\ \text{- Check}} \times 100$$

8- Life cycle

Statistical analysis

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

Life table parameters

Data of life table study were analyzed, using life 48 basic computer program of (Abou-Setta *et al.*, 1986). Output data included; 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 dayx (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 of e (the base of natural logarithm to the power of (r_m)). The program printed out the precise life table sheet parameters of the data that study the sum of RML, the mean time between generations time (T) by [Σ ((X)(Lx)(Mx))/Ro], the net reproductive rate (Ro) by [Σ((Lx)(Mx))], the doubling time (DT) that resulted from the division of the normal logarithm on r_m and the intrinsic rate of natural increase (r_m) that was calculated by [ln (Ro)/T] and the finite rate of increase (e^{rm}) which is the natural antilogarithm of the intrinsic rate of increase and the number of times which the population multiplies in a unit time (doubling time, DT) were determined. Also, the sex ratio was calculated.

RESULTS AND DISCUSSION

Efficacy of Protecto and Biover against *T. absoluta*

As shown in table (1), Protecto was the most potent compound against the newly hatched larvae of *T. absoluta* (LC_{50}: 1.53x10^6 IU/L), followed by Biover (LC_{50}: 3.35 x10^6 IU/L) then the mixture (LC_{50}: 5.19 x10^6 IU/L). The same effect of the two biopesticides was found against the 3rd instar larvae, presented in the same table, where Protecto was the most toxic (LC_{50}: 3.71 x10^6 IU/L), followed by Biover (LC_{50}: 4.19 x10^6 IU/L) and then the mixture (LC_{50}: 7.46 x10^6 IU/L).

Protecto was the most efficacious against newly hatched and 3rd instars larvae (Toxicity index = 100) at the two levels of LC_{50} and LC_{90}. While, Biover had lower efficacy than Protecto (Toxicity index = 45.67) according to LC_{50} for newly hatched and 88.5 according to LC_{50} for 3rd instars larvae. On the other hand, the mixture had an antagonistic effect against the pest at the two instars. At LC_{90} levels, the mixture gave higher efficacy than treatments of Biover only (Table 1). On the contrary, Amer and El-Nemaky (2008) reported that the mixture gave synergistic effect against the newly hatched larvae of *Pectinophera gossypiella* than Protecto and Biover when used singly.

Biological Parameters of the *T. absoluta* Treated with Biopestcides

1-Larval, Pupal and Adult Durations

The larval duration of *T. absoluta* increased as compared to the control with those treated as newly hatched larvae by LC_{50}'s of Protecto, followed by Biover. Mixture treatment decreased the larval duration about one day than control. The values were 17, 15, 13 and 14 days for Protecto, Biover, mixture treatments and control, respectively (Table 2). The same trend was observed in pupal duration of *T. absoluta* that increased, especially in Protecto treatment hading highest increase (19 days), followed by Biover (12 days). Meanwhile, the mixture decreased the period to 8 days as compared to the control (10 days) (Table 2). All the treatments gave the same duration (23 days) for *T. absoluta* moths as compared to the control (21 days) (Table 2).

Pupation percentages of *T. absoluta* were affected by the treatments with the two biopesticides. The pupation percentage decreased in Protecto treatment (27%), followed by Biover (42%) and then the mixture (44%) as compared to the control (90%) (Table 2). The same effect was found in adult emergence percentage parameter of *T. absoluta*. All the formulations decreased emergence percentages to 20, 40 and 42% for Protecto, Biover and the mixture, respectively, compared to the control (88%).

2- Pupation and Adult Emergence

Pupation percentages of *T. absoluta* were affected by the treatments with the two biopesticides. The pupation percentage decreased in Protecto treatment (27%), followed by Biover (42%) and then the mixture (44%) as compared to the control (90%) (Table 2). The same effect was found in adult emergence percentage parameter of *T. absoluta*. All the formulations decreased emergence percentages to 20, 40 and 42% for Protecto, Biover and the mixture, respectively, compared to the control (88%).

3- Larval and Adult Mortality

Table (2) shows that larval mortality percentage of *T. absoluta* treated as newly hatched larvae by Protecto reached 83%, followed by Biover (68%) and then the mixture (56%), while it was (10%) in the control. On the other hand, percent of adult mortality of *T. absoluta* was 7% in Protecto, while it was (2%) in the other treatments of Biover, the mixture, and the control (Table 2).

4- Ovipositional Periods

Pre-ovipositional period of *T. absoluta* showed insignificant difference among different treatments, as well the control as it was (2 days). Ovipositional period *T. absoluta* attained 12 days, decreased to about 10 days in the females initiated from newly hatched larvae treatments by Protecto, Biover and the mixture. Protecto alone or mixed with Biover gave the same result for the post-ovipositional period when *T. absoluta* was treated as newly hatched larvae with the LC_{50}'s of the biopesticides. The values were 11 days, about 4- days longer than the control (7- days) (Table 2).

Table (1): Efficacy of the biocide compounds against *T. absoluta*.

Biocides	LC_{50} (IU/L) 95%Confidence limit	LC_{90} (IU/L) 95%Confidence limit	Toxicity index	
			LC_{50}	LC_{90}
	Newly hatched larvae			
Protecto	1.53x10^{6} (0.91 x10^{6}±2.18 x10^{6})	10.06 x10^{6} (5.63 x10^{6} ±43.3 x10^{6})	100	100
Biover	3.35 x10^{6} (2.08 x10^{6}±8.368 x10^{6})	45.5 x10^{6} (13.8 x10^{6}±59.8 x10^{6}	45.67	22.1
Protecto + Biover	5.19 x10^{6} (3.97 x10^{6}±6.70 x10^{6})	22.1 x10^{6} (14.6 x10^{6}±48.8 x10^{6})	29.5	45.52
	3rd instars larvae			
Protecto	3.71 x10^{6} (1.61 x10^{6}±5.08 x10^{6})	10.2 x10^{6} (7.49 x10^{6}±22.3 x10^{6})	100	100
Biover	4.19 x10^{6} (0.736 x10^{6} ±6.22 x10^{6})	20.6 x10^{6} (11.8 x10^{6}±63.5 x10^{6})	88.5	49.5
Protecto + Biover	7.46 x10^{6} (5.84 x10^{6}±9.18 x10^{6})	16.6 x10^{6} (12.5 x10^{6}±32.6 x10^{6})	49.7	61.4

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

Biocides	Larval duration (days)	Larval Mortality %	Pupal duration (days)	% Pupation	Adult duration (days)	% Adult emergency	Female adult longevity (days)			% Adult mortality
							Pre-oviposition period	oviposition period	Post-oviposition period	
Protecto	17^{a}	83^{a}	19^{a}	27^{c}	23^{a}	20^{c}	2^{a}	10^{b}	11^{a}	7^{a}
Biover	15^{b}	68^{b}	12^{b}	42^{b}	23^{a}	40^{b}	2^{a}	10^{b}	11^{a}	2^{b}
Protecto+Biover	13^{c}	56^{c}	8^{c}	44^{b}	23^{a}	42^{b}	2^{a}	10^{b}	11^{a}	2^{b}
Control	14^{bc}	10^{d}	10^{c}	90^{a}	21^{b}	88^{a}	2^{a}	12^{a}	7^{b}	2^{b}
$LSD_{0.05}$	1.708	31.50	5.944	27.24	1.00	28.77	0.0	1.00	2.00	2.5

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

Biocides	Egg laying rate (No. of egg/ female)	Egg hatchability %	Control of hatchability %	Fecundity %	Sterility observed %	Corrected sterility %	Life cycle (days)
Protecto	175^{c}	74^{c}	17.78^{a}	41.67^{c}	26^{a}	17.78^{a}	40^{a}
Biover	185^{b}	80^{b}	11.11^{b}	47.22^{bc}	20^{b}	11.11^{b}	31^{b}
Protecto+Biover	189^{b}	85^{b}	5.58^{c}	49.44^{b}	15^{c}	5.56^{c}	25^{c}
Control	260^{a}	90^{a}	-	100^{a}	10^{d}	-	28^{c}
$LSD_{0.05}$	48.85	6.849	7.608	27.14	6.849	7.611	7.632

5- Egg Laying Rate and Hatchability Percentage

The egg laying rate of *T. absoluta* females in the control reached 260 eggs/ female. It decreased to 175 eggs/ female when treated as newly hatched larvae by Protecto, followed by Biover (185 eggs/ female) and the mixture (189 eggs/female).On the other hand, the deposited eggs by the females emerged from the newly hatched larvae, treated with Protecto had the lowest hatchability percentage (74%), followed by Biover treatment (80%) and then the mixture (85%) compared to the control (90%) (Table 3).

6- Hatchability Percentage

Deposited eggs by the females of *T. absoluta,* when treated as newly hatched larvae by Protecto and Biover mixture, reached 5.58% hatchability. The value increased to 11.11 and 17.78% in case of Protecto and Biover, respectively (Table 3).

7- Fecundity Percentage

Protecto and Biover mixture gave highest fecundity percentage (49.44%), when the moths were initiated from *T. absoluta*, treated as newly hatched larvae, followed by Biover (47.22%) and Protecto (41.67%) (Table 3).

8- Sterility Percentages

Sterility observed in the control was 10%. The value increased to 15% in the adult females initiated from newly hatched larvae treatment by the mixture, followed by Biover treatment (20%) and reached 26% in Protecto treatment. The mixture treatment gave the lowest corrected sterility (5.56%), when the adult females of *T. absoluta* emerged from treated newly hatched larvae. The value increased to 11.11% in Biover treatment and 17.78% in the Protecto treatment (Table 3).

9- Life Cycle

As shown in table (3), when the newly hatched larvae of *T. absoluta* were treated by the mixture, the value was 23 days, which was lower with about 2-days than the control (25 days), but in the other two treatments of Protecto and Biover, when used alone, the life cycle lasted 31 and 40 days, respectively.

Obtained results agree with those of Amer (2007) who mentioned that Dipel-2x increased pupal and adult longevity, life cycle and the percentages of larval and pupal mortality and sterility. On the other hand, it decreased egg laying and egg hatchability. On the other hand, the results of this study were not in line with Amer and El-Nemaky (2008) who reported that Protecto+Biover was much better than Protecto or Biover alone in toxicity and biological and prediction parameters of *P. gossypiella.*

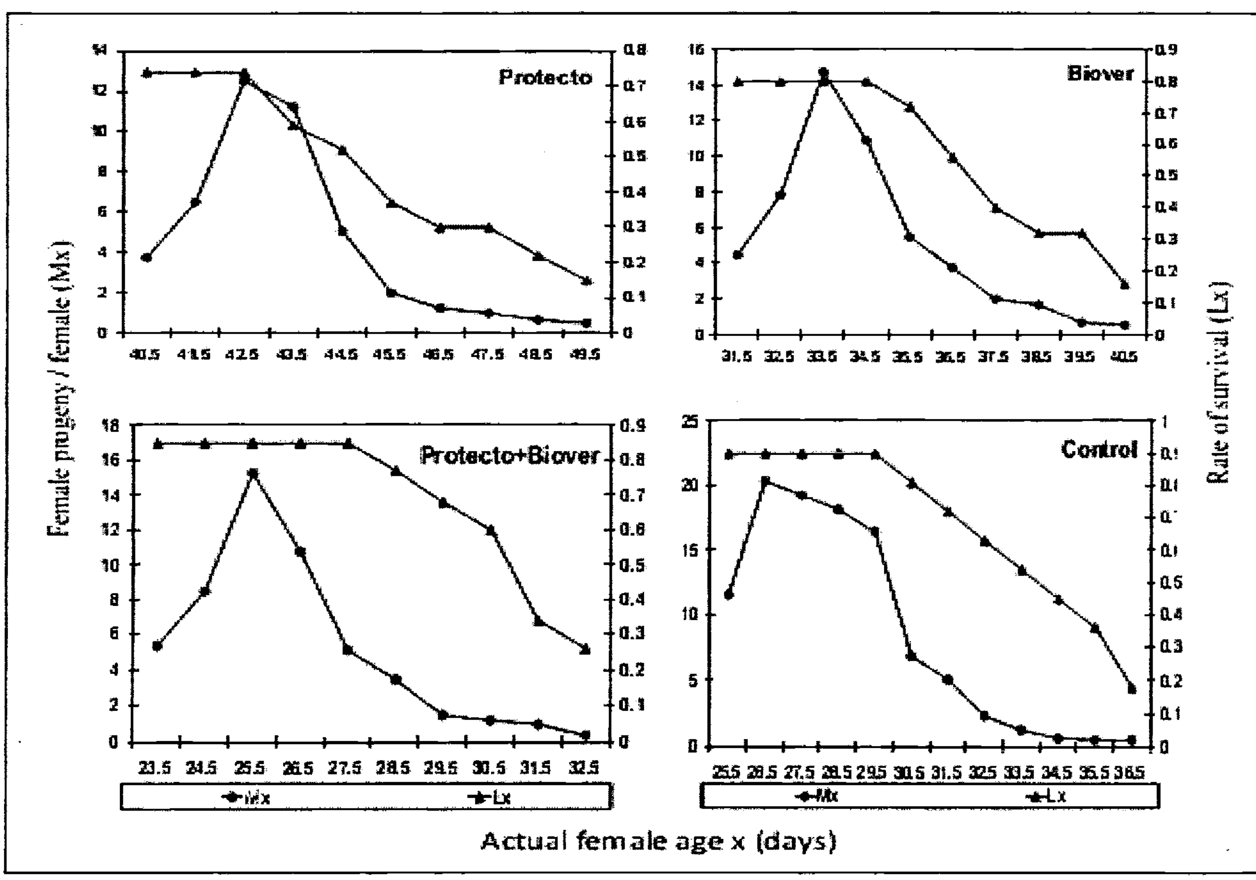

Fig. (1): Effect of tested biopesticides on the female progeny/ female (Mx) and survival rate (Lx) of *T. absoluta*.

Life Table Parameters of *T. absoluta* treated with biopesticides

1-Female Pprogeny/female (Mx) and Rate of Survival (Lx)

Fig. (1) illustrates that female progeny/ female (Mx) of untreated *T. absoluta* ranged between 0.55 to 20.35. The last value decreased in females when treated as newly hatched larvae with Protecto as it ranged 0.50 to 12.50 female progeny/ female. Moreover, it ranged 0.56 to 14.73 females' progeny/female in Biover treatment. The same trend was found in the mixture treatment as the Mx values ranged 0.38 to 15.28 female progeny/female that initiated from *T. absoluta* newly hatched larvae treated with the mixture. The (Lx) parameter (rate of survival) ranged 0.18 to 0.90 in untreated females. The females, treated as newly hatched larvae with Protecto, had survival rate ranged 0.15 to 0.74. In Biover treatment, it ranged 0.16 to 0.80. Survival rate of females developed from the treated newly hatched *T. absoluta* larvae with the mixture had survival rate ranged 0.26 to 0.85, which was the highest when compared with each biopesticide when used alone (Figure 1).

2-Generation Time (T)

T. absoluta treated as newly hatched larvae lasted a generation time of 42.71 days at Protecto treatment, followed by Biover (33.83 days). While, the mixture of Protecto + Biover reduced the period to 27.78 days, compared with the Protecto and Biover when used alone and when compared with the control (29.85 days) (Table 4).

3- Net Reproductive Rate (Ro)

The tested biopesticides caused high reduction in females' capacity to increase the population in each generation, when *T. absoluta* treated as newly hatched larvae. Biover treatment was 38.08 females/ female in one generation, the last value decreased to 27.75 females/ female in Protecto treatment. The mixture (Protecto+Biover) had the least destructive reduction from net reproductive rate (43.12 females/female) compared to the untreated *T. absoluta* (89.09 females/female) (Table 4).

4- Increase Rate

4.1- Intrinsic Rate of Natural Increase (r_m)

Intrinsic rate of natural increase (r_m) where the ability of inheriting increase of *T. absoluta* untreated female was 0.161 times/female/day was calculated. While, the females of *T. absoluta*, treated as newly hatched larvae with Protecto and Biover reduced intrinsic rate compared with the control to become 0.078 and 0.108 times/ female/ day, respectively. On the other hand, the mixture had the least reduction in intrinsic rate to reach 0.146 times/ female/day (Table 4).

4.2- Finite Rate of Increase (e^{rm})

Daily population of the untreated *T. absoluta* increased to 1.175 times/ female/ day. Also, the females developed from the newly hatched larvae treated by the mixture had the capacity of 1.157 times/ female/ day, close to the control, followed by Biover (1.114 times/ female/ day) and then Protecto, which had the lowest population capacity (1.081 times/ female/ day) Table 4).

5- Doubling Time (DT)

Doubling time (DT) depends on the intrinsic rate of natural increase (r_m), which could be affected by many factors, as survival rate, generation time, female in progeny and fecundity. *T. absoluta* had a population that multiplies every 4.31 days in the control (Table 4). These days increased to 6.42 and 8.89 days, when *T. absoluta* treated as newly hatched larvae with Biover and Protecto, respectively. The treatment of the mixture was close to the control (4.75 days).

6- Sex Ratio

Sex ratio was calculated as females/ total. In the control, it was 0.55. This ratio, in case of the treatment as newly hatched larvae, became close to the control. The values were 0.5, 0.56 and 0.57 for Protecto, Biover and the mixture, respectively.

The aforementioned results agree with those of Amer (2006) who reported that Dipel-2x (*B. thuringiensis kurstaki*) decreased the rate of survival (Lx) and r_m. while, it increased generation time of the pink bollworm. On the contrary, Amer and El-Nemaky (2008) reported that Protecto + Biover had a potentate effect on most of the biological parameters of the pink bollworm than each biocide alone.

In conclusion, Protecto was the best compound used in this study, followed by Biover which showed less toxicity and less effect on the life table parameters of *T. absoluta*. Meanwhile, the mixture compound of Protecto + Biover had an antagonistic effect and gave the lowest destructive impact on most of the survival parameters of *T. absoluta*.

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