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# Microbial biopesticides affected age-stage life table of the tomato leaf miner, *Tuta absoluta* (Lepidoptera – Gelechiidae)

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# **Abstract**

The tomato leaf miner, *Tuta absoluta* (Meyric), second instar larvae were exposed for 1 day to  $LC_{50}$  of *Bacillus thuringiensis* (Berliner) subsp. *kurstaki* or *Beauveria bassiana* (Balsamo) on treated tomato foliages. Treated larvae had the longest development period while non-treated larvae had the shortest development period. The highest survivorship ( $l_x$ ) of adults was obtained by the non-treated larvae while the lowest survivorship was obtained by *B. thuringiensis*-treated larvae. The lowest age-specific fecundity ( $m_x$ ) of females was obtained by individuals treated as second instar larvae with *B. bassiana*. The intrinsic rate of increase ( $r_m$ ) reached its maximum with non-treated individuals while this value decreased to the minimum values with biopesticide-treated individuals. Therefore, development, survival, and reproduction of treated individuals were lower than those of non-treated individuals. The reproduction period and adult longevity were the shortest considering biopesticide-treated individuals. The highest and lowest net reproductive rates ( $R_0$ ) were recorded for non-treated and treated individuals, respectively. The mean generation time was increased with biopesticide-treated individuals.

Keywords: Tuta absoluta, Microbial biopesticides, Survivorship, Population parameters, Life table

# **Background**

The tomato leaf miner, *Tuta absoluta* (Meyric) (Lepidoptera – Gelechiidae), is a pest of great economic importance in a number of countries. Its primary host is tomato although potato, eggplant, common weed, and various wild solanecous plants are also suitable hosts (Siqueira et al., 2000 and Lietti et al., 2005). The tomato leaf miner, *T. absoluta* larvae, can significantly reduce both yield and fruit quality by direct feeding and the secondary pathogens which may enter through the wounds caused (Silva et al., 2011). The infestation of tomato plants occurs throughout the entire crop cycle. Feeding damage is caused by all larval instars and throughout the whole plant. On leaves, the larvae feed on the mesophyll tissue, forming irregular leaf mines which may later become necrotic. Larvae also can form extensive galleries in the stems and attack fruits.

Since *T. absoluta* was detected in the Mediterranean Basin, the most common control practice has been based on the use of chemical insecticides (Bielza, 2010). Pesticides may have effects on the natural enemies and

In order to continue to evaluate potential of the entomopathogens, its effects on some development attributes and population parameters are needed. The population dynamics of any insect pest, the age-stage, and two-sex life table is the most important techniques which help in qualitative and quantitative female and male populations, the stage differentiation, and variable developmental rate among individuals (Chi and Liu, 1985 and Chi, 1988). Comparison of life table parameters of *T. absoluta* individuals treated with the microbial biopesticides with non-treated individuals may refer to important aspects that may be useful for future control programs.

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also may lead to insect resistance (Lietti et al., 2005). Microbial control include bacteria and fungi; the comparison of entomopathogens with convention chemical pesticides is usually solely from the perspective of their efficacy and cost (Lacey et al., 2001). As an alternative to chemicals, commercial formulations based on the entomopathogenic bacteria, *B. thuringiensis* subsp. *kurstaki* and entomopathogenic fungi, *B. bassiana* have been tested against *T. absoluta* (Giustolin et al., 2001; Marta et al., 2006 and Gonzalez-Cabrera et al., 2011).

The objective of this study is to elucidate novel aspects considering development, survival, and fecundity of the tomato leaf miner, *T. absoluta*, after treated the second instar larvae with the two microbial biopesticides, *Bacillus thuringiensis* (Berliner) subsp. *kurstaki* and *Beauveria bassiana*.

# Materials and methods

# The insect colony

The tomato leaf miner, Tuta absoluta, colony was established with larvae collected from tomato fields at EL-Natron Valley area (30° 30′ 26″ N, 13° 30′ 03″ E). The moth adults emerged from larvae reared on leaves of different tomato cultivars were collected to be used in the development of the colony. In order to obtain the same age eggs, 15 pairs of both sexes of the moth were kept inside a plastic oviposition container (30 cm diameter, 20 cm height) sealed at the top with a fine mesh net. After 24 h, the laid eggs were collected from the container. Each egg was transferred into a 10-cm Petri dish. Fresh tomatoes' leaves were provided for larval feeding in dishes and were replaced every other day. The fourth instar larvae were transferred into small plastic tubes (3 cm diameter, 6 cm depth) till pupation. Experiments began following the rearing of three generations of T. absoluta under laboratory conditions. The insect colony and the experiments were conducted in a  $(3 \times$ 4 m) greenhouse equipped with a drip irrigation system located at Biological Control Laboratory, Agricultural Research Center, Giza. The average temperature during the experiments was  $25 \pm 3$  °C with relative humidity ranging from 70 to 85% and a photoperiod of 14:10 h (L:D).

# The microbial biopesticides

The first microbial biopesticide, *Bacillus thuringiensis subsp. kurstaki*, is a member of the genus *Bacillus*, adverse group of spore-forming bacteria Dipel-2x. The commercial formulation (wettable powder) of *B. thuringiensis kurstaki* (32,000 IU/mg) was used in this bioassay. The second biopesticide used in this study was Bio-Bower, the commercial formulation of entomopathogenic fungus, *Beauveria bassiana* provided by T. Stanes and Company Limited, India.

# Bioassay activity of the microbial biopesticides.

This study was designed to determine the efficacy of *B. thuringiensis* and *B. bassiana* when *T. absoluta* larvae fed on treated tomatoes leaves. Five concentrations of 0.05, 0.1, 0.2, 0.4, and 0.5 mg *B. thuringiensis* powder/ml  $\rm H_2O$  and also five concentrations of  $1\times10^7$ ,  $0.5\times10^7$ ,  $0.25\times10^7$ ,  $0.125\times10^7$ , and  $0.063\times10^7$  *B. bassiana* Bio-Bower/ml  $\rm H_2O$  were used. Five replicates (each replicate involved 10 larvae) per concentration were tested. Tomato seedlings' pots of approximately 45 days old were

embedded in each concentration then transferred to a clean filter paper for allowing water to evaporate. The second instar larvae (2 days old) were used in this bioassay. The larvae were starved for 24 h before the begging of feeding. After 24 h of treatment, treated larvae were transferred to new clean Petri dishes (10 cm  $\times$  3 cm) with new clean fresh tomato seedling pots. The numbers of larval mortality were recorded daily and also the pots of tomatoes were changed daily until pupation. The average cumulative mortality percentage of larvae was calculated for each concentration. Mortality was corrected with Abbott's formula (Abbott, 1925), and all *B. thuringiensis* or *B. bassiana* data were subjected to probit analysis (Finney, 1971) also the LC<sub>50</sub>, LC<sub>90</sub>, and 95% confidence limits.

# Host plant source

Young foliage (45 days old) of tomato plants was used in this study. All foliage tested in this laboratory experiments was collected from field grown plants free of pesticide and chemical fertilizers.

## Development and survival of the immature stages

Ten female and ten male moths (newly emerged) were collected from larvae reared on normal host plants. The moths were provided with 10% sucrose solution and allowed to mate for 1-2 days in containers ( $25 \times 15 \times$ 10 cm). The mated moths were transferred to new cages  $(15 \times 10 \times 5 \text{ cm})$ , one female/cage. The top of the cage was cut-off and replaced with a covering of fine mesh gauze. Host plant leaves were replaced with a fresh one on a daily basis. Thirty 2-day-old larvae (second instar) were treated with LC<sub>50</sub> of *B. thuringiensis kurstaki* while other 30 larvae of the same age were treated with the LC<sub>50</sub> of B. bassiana and left for 24 h. After treatment, the larvae were transferred to new clean Petri dishes provided by normal tomatoes' foliage. Non-treated 30 larvae of the same age used as control. Development of larvae and pupae were observed in the growth chamber under similar conditions. Survival rate and developmental time were recorded daily for all immature stages; also, the sex of emerged adults was determined.

# Reproduction and population growth parameters Adult longevity and reproduction

Moths emerged from larvae treated as second instar by the LC<sub>50</sub> of *B. thuringiensis* or *B. bassiana* and also those emerged from non-treated larvae were allowed to mate for 1-2 days. Mated moths were transferred to a new container ( $25 \times 15 \times 10$  cm, one female per container) and supplied with 10% sucrose solution. The females were provided with fresh foliage every day for oviposition. The foliage was collected every day to determine the number of eggs deposited until the death of

each adult female. The pre-oviposition period, oviposition period, post-oviposition period, adult longevity, and age-specific fecundity were determined.

# The intrinsic rate and life table parameters

Data on the developmental times of immature individuals and adult reproduction were combined to create the effects of the two biopesticides on the life tables of *T. absoluta*. For each experiment, the following parameters, as defined by Carey (1993) and Gotelli (1998), were calculated depending on the basic concept of life table theory (Lotka-Euler formula).

$$\sum_{x=0}^{\infty} e^{-r_m x} l_x m_x = 1$$

where x is the age (days),  $l_x$  is the age-specific survival, and  $m_x$  is the average number of female offspring of a female at age x. The  $r_m$  is the intrinsic rate of increase for the population. In addition to  $r_m$ , the other life table parameters, including net reproductive rate  $(R_0 = \Sigma \ l_x \ m_x)$ , generation time  $(T = \Sigma \ x l_x m_x / R_0)$ , finite rate of increase  $(\lambda = e^{rm})$ , and population doubling time (DT =  $\ln 2/r_m$ ) were calculated.

# Statistical analysis

Firstly, variables were tested for normality before analysis. Data were analyzed by one-way analysis of variance (ANOVA) followed by comparison of the means with Tukey test at  $\alpha = 0.05$  using software GraphPad Instat (2009).

# Results and discussion

## Toxicity of biopesticides

Toxicity of *B. thuringiensis* subsp. *kurstaki* and *B. bassiana* against second instar larvae of *T. absoluta* is shown in Table 1. Obtained data showed that the  $LC_{90}$  of *B. thuringiensis* was 17.3-folds more than the  $LC_{50}$  of the same pathogen with a slope of  $1.33 \pm 0.34$ . The obtained *B. thuringiensis*- $LC_{90}$  was 4.16 mg/ml while the obtained  $LC_{50}$  was 0.24 mg/ml. On the other hand, the  $LC_{90}$  of *B. bassiana*  $(48.9 \times 10^7 \text{ spore/ml})$  was 27.2-folds than the obtained  $LC_{50}$   $(1.8 \times 10^7 \text{ spore/ml})$  with a slope of 0.48  $\pm$  0.24.

# Development of treated second instar T. absoluta larvae

Table 2 and Fig. 1 show the developmental period and survival of *T. absoluta* treated as second instar larvae by

the LC<sub>50</sub> values of *B. thuringiensis* or *B. bassiana*. All eggs hatched successfully; the duration of egg hatching ranged from  $5.93 \pm 0.13$  to  $6.3 \pm 0.12$  days. Total periods of larval stage were  $14.75 \pm 0.03$ ,  $15 \pm 0.04$ , and  $10.96 \pm$ 0.15 days considering B. thuringiensis-treated, B. bassiana-treated, or non-treated larvae, respectively. Statistically, significant difference was found when comparing B. thuringiensis- or B. bassiana-treated larvae with nontreated larvae ( $F_{(2.58)} = 98.87$ ; P < 0.0001). The longest period of larval duration was determined with B. bassiana-treated larvae while the shortest developmental period was determined with the non-treated larvae. The pupal period was influenced in the treated and nontreated larvae. The pupal duration varied from 10.94 ± 0.26 days in case of B. thuringiensis-treated larvae to  $8.29 \pm 0.14$  days for non-treated larvae. There was a significant difference between treated (B. thuringiensis or B. bassiana) and non-treated larvae ( $F_{(2.58)} = 43.47$ ; P <0.0001). The longest pupal period was determined in those larvae treated with B. thuringiensis while the shortest pupal period was determined in non-treated larvae. For the total development time, the shortest one was estimated for the non-treated larvae (25.21 ± 0.38 days). On the other hand, the total developmental period increased to  $31.57 \pm 0.42$  and  $32.07 \pm 0.51$  days, considering B. thuringiensis- and B. bassiana-treated larvae, respectively. The microbial biopesticides (B. thuringiensis and B. bassiana) significantly affected the total developmental period in comparison with non-treated larvae ( $F_{(2,58)} = 175$ ; P < 0.0001).

In this study, the mean larval periods varied from 10.96 to 15 days considering the non-treated and *B. bassiana*-treated larvae. Pereyra and Sanchez (2006) found out that at 25 °C the larval periods of the tomato leaf miner were 12.14 and 14 days on tomato and potato plants, respectively. Erdogan and Babaroglu (2014) reported that total larval period of *T. absoluta* was 10.97 days at 25 °C when fed on non-treated tomato cultivars; the same result was obtained also by Nouri-Ganbalani et al. (2016). The pupal period of the tomato leaf miner varied from 8.29 to 10.94 days taken into consideration the non-treated and *B. thuringiensis*-treated larvae, respectively. Torres et al. (2001) found the pupal period of the tomato leaf miner ranged from 7 to 9 days when the larvae reared on normal (non-treated) tomato cultivar.

In our study, the total developmental time of *T. absoluta* varied from 25.21 days on non-treated cultivar to 31.57 and 32.07 days on *B. thuringiensis*- or *B. bassiana*-

**Table 1** Lethal concentrations of *B. thuringiensis* and *B. bassiana* against second instar larvae of *T. absoluta* 

Biopesticides	LC <sub>50</sub>	95% confidence limits	LC <sub>90</sub>	95% confidence limits	Slope
B. thuringiensis (mg/ml)	0.24	0.16-0.48	4.16	1.35-6.48	1.33 ± 0.34
B. bassiana (spore/ml)	$1.8 \times 10^{7}$	$1.2 \times 10^7 - 9.5 \times 10^7$	$48.9 \times 10^7$	$10.9 \times 10^7 - 100.4 \times 10^7$	$0.48 \pm 0.24$

Biopesticides	Developmental perio	Developmental period (mean ± SE) days					
	Egg	Larva	Pupa	Total developmental time			
B. thuringiensis	5.93 ± 0.13a	14.75 ± 0.03a	10.94 ± 0.26a	31.57 ± 0.42a			
B. bassiana	$6.3 \pm 0.12a$	$15 \pm 0.04a$	$10.77 \pm 0.24a$	32.07 ± 0.51a			
Control	$5.96 \pm 0.14a$	$10.96 \pm 0.15b$	$8.29 \pm 0.14b$	$25.21 \pm 0.38b$			
F(df)	2.95 (2,87)	98.87 (2,58)	43.47 (2,58)	175 (2,58)			
P value	0.057	< 0.0001	< 0.0001	< 0.0001			

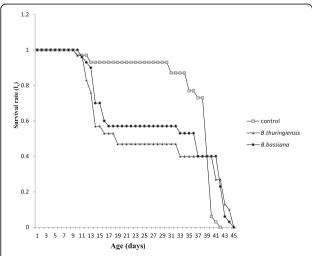
**Table 2** Development of T. absoluta treated as second instar larvae by  $LC_{50}$  values of B. thuringiensis or B. bassiana

Means followed by the same letter, in the same column, are not significantly different (P > 0.05)

treated cultivar, respectively. According to Cuthbertson (2011), the mean developmental time of *T. absoluta* was 35 days under 25 °C while Erdogan and Babaroglu (2014) showed that the mean total of developmental time of *T. absoluta* was 31.18 days on non-treated tomato leaves at 25 °C. The treated *T. absoluta* in our study developed slowly compared to the non-treated individuals. The relatively longer developmental time of *T. absoluta* on microbial biopesticide-treated cultivar may be attributed to the mode of action of the microbial biopesticides. The developmental time of the herbivore insects are strongly affected by the nutritional qualities of the host plant, which in turn influences its population growth (Du et al., 2004).

# Age-specific survival

The survival rate  $(l_x)$  of T. absoluta treated with B. thuringiensis or B. bassiana is shown in Fig. 2 and Table 3. The highest survival rates of egg, larval, and pupal stages was obtained by larvae fed on non-treated foliages. The survival of the egg stage was 100% while survival of larval and pupal stages was 93.3 and 90%, respectively. Survival of T. absoluta treated with  $LC_{50}$ -B. thuringiensis or B. bassiana showed a similar pattern including high

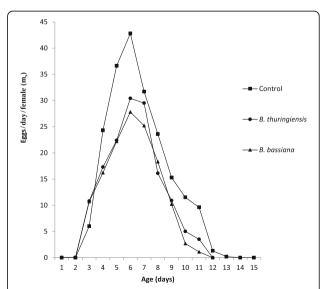


**Fig. 1** Age-specific survivorship  $(I_x)$  of *T. absoluta* treated as second instar larvae by  $LC_{50}$  values of *B. thuringiensis* or *B. bassiana* 

mortality occurring during larval growth which gradually decreased during the pupal stage. The survival rates obtained when larvae were fed on *B. thuringiensis*- or *B. bassiana*-treated leaves were 46.6% or 56.6% considering the larval stage, respectively. Also, it was found that the survival rates of pupa were 40% and 46.6% considering larvae treated with *B. thuringiensis* and *B. bassiana*, respectively.

# **Adult longevity**

Data in Table 4 shows the life span of adult T. absoluta treated as second instar larvae by the LC<sub>50</sub>-B. thuringiensis or B. bassiana. Female and male moths from larvae reared on non-treated foliages lived longer than moths from larvae reared on treated foliages. Female life span from non-treated larvae was  $13.2 \pm 0.26$  days in comparison to  $10.75 \pm 0.41$  days and  $10.8 \pm 0.33$  days considering B. thuringiensis- or B. bassiana-treated larvae, respectively. Statistically, a significant difference was obtained when comparing life span of treated and non-treated females ( $F_{(2,30)} = 33.51$ ; P < 0.0001). Adult male longevity showed the same trend (Table 4). Adult males



**Fig. 2** Fecundity  $(m_x)$  of *T. absoluta* treated as second instar larvae by LC<sub>50</sub> values of *B. thurinaiensis* or *B. bassiana* 

**Table 3** Age-specific mortality of *T. absoluta* treated with the two tested microbial pesticides

Age stages (x)	Microbial biopesticides	Number alive at begging of $x(l_x)$	Number dying during (x)	% mortality (100qx)	% cumulative surviving (100rx)	
Egg	Control <i>B. thuringiensis</i>	30 30	0	0	100 100	
	B. bassiana	30	0	0	100	
Larva	Control B. thuringiensis B. bassiana	30 30 30	2 16 13	6.6 53.3 43.3	93.3 46.6 56.6	
Pupa	Control B. thuringiensis B. bassiana	28 14 17	1 2 3	3.3 6.6 10	90 40 46.6	
Adult	Control B. thuringiensis B. bassiana	27 12 14			90 40 46.6	

x developmental stage,  $I_x$  number entering stage, 100qx percent apparent mortality,  $100r_x$  percent cumulative surviving

from non-treated larvae lived  $11.17 \pm 0.32$  days, which was longer than males from larvae treated with *B. thuringiensis* which lived  $9.48 \pm 0.44$  days and males from larvae treated with *B. bassiana* which lived  $9.63 \pm 0.48$  days. Statistically, a significant difference was obtained in comparison to adult male longevity of treated and non-treated larvae ( $F_{(2.25)} = 8.88$ ; P = 0.0012).

Obtained data indicated that the microbial biopesticides can also affect survival, growth, and reproduction of herbivore insects. Insect individuals receiving lethal concentration ( $LC_{50}$ ) of the microbial biopesticides may survive and complete their development but with differences in growth and development as a result of their varying nutritional requirements (Lacey et al., 2001). Differences in life span and reproduction period of T. absoluta in this study indicated that the microbial biopesticides had deleterious effects on the treated individuals. The longest female life span was obtained by the non-treated individuals, whereas the shortest life span was obtained by the treated individuals. The same result was obtained considering male life span.

# Oviposition periods and age-specific fecundity

Table 5 shows the oviposition periods of treated and non-treated second instar *T. absoluta* larvae. The pre-

**Table 4** Comparison of T. absoluta life span treated as second instar larvae by  $LC_{50}$  values of B. thuringiensis or B. bassiana

Biopesticides	Life span			
	Male Mean ± SE (range)	Female Mean ± SE (range)		
B. thuringiensis	9.48 ± 0.44a (7–11)	10.75 ± 0.41a (9–12)		
B. bassiana	9.63 ± 0.48a (7–12	10.8 ± 0.33a (9–12)		
Control	11.17 ± 0.32b (9–12)	13.2 ± 0.26b (12-15)		
F(df)	8.88 (2,25)	33.51 (2,30)		
P value	0.023	0.012		

Means followed by the same letter, in the same column, are not significantly different (P > 0.05)

oviposition period of non-treated T. absoluta was 2.4 ± 0.13 days. On the other hand,  $2.13 \pm 0.13$  and  $2 \pm 0.1$  days were obtained considering pre-oviposition period of B. thuringiensis- and B. bassiana-treated females, respectively. Statistically, no significant difference was obtained in comparing the pre-oviposition periods of treated and non-treated *T. absoluta* ( $F_{(2.32)} = 2.84$ ; P = 0.294). The oviposition periods of females from non-treated larvae were statistically significant against those of females from B. thuringiensis- or B. bassiana-treated larvae  $(F_{(2..32)} = 9.34; P = 0.045)$ . The oviposition periods obtained were  $8.93 \pm 0.23$ ,  $7.85 \pm 0.24$ , and  $7.5 \pm 0.31$  days considering non-treated, B. thuringiensis-treated, and B. bassiana-treated individuals, respectively. Also, a significant difference between the post-oviposition periods of treated and non-treated *T. absoluta* larvae ( $F_{(2,32)} = 21.9$ ; P < 0.0001) was produced. There were no differences among females from larvae treated with B. thuringiensis and those from larvae treated with B. bassiana considering pre-oviposition, oviposition, and post-oviposition periods as shown in Table 5.

The fecundity was affected significantly by the microbial biopesticide treatments as shown in Table 5. The fecundity of female from non-treated larvae was  $202.4 \pm 3.95$  eggs with daily oviposition rate of 22.78 ± 0.55 eggs. Fecundity of those females from B. thuringiensis- or B. bassiana-treated larvae were 146.87 ± 4.4 or  $134.4 \pm 7.3$  eggs with daily oviposition rate of  $18.46 \pm 1.1$  and  $18.07 \pm 1.03$  eggs, respectively. Statistically, a significant difference of fecundity was found between the non-treated larvae and treated larvae  $(F_{(2.32)} = 53.18; P = 0.018)$ . Similarly, a significant difference was obtained considering the daily ovipositional rate of females from non-treated and treated larvae ( $F_{(2,32)} = 11.16$ ; P = 0.033). No significant differences were obtained in comparison with the fecundity and daily ovipositional rate of females from B. thuringiensis- or B. bassiana-treated larvae (Table 5).

**Table 5** Oviposition periods and fecundity (mean  $\pm$  SE) of *T. absoluta* treated as second instar larvae by LC<sub>50</sub> values of *B. thuringiensis* or *B. bassiana* 

Parameters	(Mean ± SE)			F(df)	P value
	Control	B .thuringiensis	B. bassiana		
Pre-oviposition period (day)	2.4 ± 0.13 a	2.13 ± 0.13 a	2 ± 0.1 a	2.84(2,32)	0.294
Oviposition period (day)	8.93 ± 0.23 a	$7.85 \pm 0.24 \text{ b}$	$7.5 \pm 0.31 \text{ b}$	9.34(2,32)	0.045
Post-oviposition period (day)	1.67 ± 0.21 a	$0.5 \pm 0.02 \text{ b}$	$0.3 \pm 0.02 \text{ b}$	21.9(2,32)	< 0.0001
Fecundity (no.)	202.4 ± 3.95 a	146.87 ± 4.4 b	134.4 ± 7.34 b	53.18(2,32)	0.018
Daily ovipositional rate	22.78 ± 0.55 a	18.46 ± 1.1 b	18.07 ± 1.03 b	11.16 (2,32)	0.033

Means followed by the same letter, in the same row, are not significantly different (P > 0.05)

The age-specific fecundity  $(m_x)$  of *T. absoluta* females from non-treated larvae and those from B. thuringiensisor B. bassiana-treated larvae is shown in Fig. 2. The first oviposition occurred at age of 3 days of female lifetime considering non-treated and treated females. The highest daily fecundity  $(m_x)$  of T. absoluta females were 42.8, 30.4, and 27.8 female offsprings per female per day considering females from non-treated, B. thuringiensistreated, and B. bassiana-treated larvae, respectively. The highest daily fecundity was obtained at the age of 6 days. Treatment of *T. absoluta* larvae by the tested microbial biopesticide affected the age-specific fecundity of females. The obtained oviposition period of females from B. thuringiensis-treated larvae was  $7.85 \pm 0.24$  days while this period for treated larvae with *B. bassiana* was 7.5 ± 0.31 in comparison to oviposition period of 8.93 ± 0.23 days for females from non-treated larvae.

Differences in life span and reproduction period of T. absoluta in this study indicated that the microbial biopesticides had deleterious effects on the treated individuals. The longest female life span was obtained by the non-treated individuals, whereas the shortest life span was obtained by the treated individuals. The same result was obtained considering male life span. The total female fecundity in this study ranged from 202.4 to 134.4 eggs considering non-treated and B. bassiana-treated individuals, respectively. The low number of eggs laid by B. thuringiensis- or B. bassiana-treated individuals could have been affected by the more indirect route of biopesticide treatment. It was observed that young tomato leaf miner individuals treated with the biopesticides fed less and developed more slowly than the non-treated individuals. Different values have been reported for preoviposition period, female fecundity, oviposition period, and longevity of T. absoluta.

# Population growth parameters

The population growth parameters of microbial biopesticides—treated and non-treated T. absoluta—are given in Table 6. The highest value of  $R_0$  was observed with T. absoluta adults from larvae fed on non-treated leaves ( $R_0 = 101.33 \pm 18.92$ ). The slowest value of  $R_0$  was

obtained by T. absoluta adults from B. thuringiensistreated larvae ( $R_0 = 38.73 \pm 11.98$ ) while  $R_0$  value of 44.57 ± 11.92 was observed with adults from B. bassiana-treated larvae. There were significant differences between the net reproductive rates  $(R_0)$  of *T. absoluta* fed on non-treated and B. thuringiensis- or B. bassianatreated larvae ( $F_{(2.87)} = 5.57$ ; P = 0.013). However, no significant difference was observed between  $R_0$  values of adults from B. thuringiensis- or B. bassiana-treated larvae. The intrinsic rates of increase  $(r_m)$  were also found to be significant when comparing non-treated and treated cohorts ( $F_{(2.87)} = 11.88$ ; P = 0.041) while no significant difference was obtained in comparing T. absoluta generation from larvae fed on B. thuringiensis or B. bassiana. The  $r_m$  values were 0.142 ± 0.006, 0.097 ± 0.01, and  $0.099 \pm 0.01 \text{ day}^{-1}$  considering *T. absoluta* fed as larvae on non-treated, B. thuringiensis-treated, or B. bassiana-treated foliages, respectively. The highest value of finite rate of increase  $(\lambda)$  was obtained by the nontreated individuals  $(1.15 \pm 0.01 \text{ day}^{-1})$  which was significantly different from B. thuringiensis- or B. bassianatreated individuals ( $F_{(2,87)} = 13.34$ ; P = 0.029) while no significant difference was obtained considering the finite rate of increase ( $\lambda$ ) from B. thuringiensis- and B. bassiana-treated individuals with values of  $1.1 \pm 0.009$  day<sup>-1</sup> and  $1.11 \pm 0.01 \text{ day}^{-1}$ , respectively. The mean generation time  $(T_0)$  was significantly different  $(F_{(2,87)} = 26.98; P <$ 0.0001), considering non-treated individuals (32.69 ± 0.4 days) compared to B. thuringiensis- or B. bassianatreated individuals  $(38.09 \pm 0.83 \text{ and } 38.57 \pm 0.58 \text{ days},$ respectively). The longest generation time was obtained by B. bassiana-treated individuals while the shortest one by the non-treated individuals. The doubling time (DT) was found to be significantly different in comparing non-treated and treated individuals ( $F_{(2.87)} = 13.62$ ; P <0.0001). The lowest doubling time was obtained by nontreated individuals  $(4.9 \pm 0.3 \text{ days})$  while the longest DT was by *B. thuringiensis*-treated individuals (7.1  $\pm$  0.4 days) with no significant difference with DT of B. bassianatreated individuals (7.0  $\pm$  0.3 days).

The population growth parameters of *T. absoluta* significantly varied in comparison with microbial

**Table 6** Life table parameters (mean  $\pm$  SE) of *T. absoluta* treated as second instar larvae by LC<sub>50</sub> values of *B. thuringiensis* or *B. bassiana* 

Population parameter	(Mean ± SE)			F(df)	P value
	Control	B. thuringiensis	B. bassiana		
The net reproductive rate $(R_0)$	101.3 ± 18.9 a	38.73 ± 11.98 b	44.57 ± 11.92 b	5.57(2,87)	0.013
The intrinsic rate of increase $(r_m)$	$0.142 \pm 0.01a$	$0.097 \pm 0.01 \text{ b}$	$0.099 \pm 0.01 \text{ b}$	11.88(2,87)	0.041
The finite rate of increase ( $\lambda$ )	1.15 ± 0.01 a	$1.10 \pm 0.01 \text{ b}$	$1.11 \pm 0.01b$	13.34(2,87)	0.029
The mean generation time ( $T_0$ )	$32.69 \pm 0.4$ a	$38.09 \pm 0.83 \text{ b}$	$38.57 \pm 0.58 \text{ b}$	26.98 (2,87)	< 0.0001
Doubling time (DT)	$4.9 \pm 0.3$ a	$7.1 \pm 0.4 \text{ b}$	$7.0 \pm 0.3 \text{ b}$	13.62(2,87)	< 0.0001

Means followed by the same letter, in the same row, are not significantly different (P > 0.05)

biopesticide-treated and non-treated tomato cultivars in this study. This indicated a deleterious effect of the biopesticides on the treated individuals. The net reproduction rate  $(R_0)$  is a key statistic in population dynamics (Richard, 1961) that summarizes the physiological traits of an insect related to its reproduction capacity. The net reproduction value of *T. absoluta* treated as second instar larvae with *B. thuringiensis* was the lowest. Moreover,  $R_0$  values observed from B. thuringiensis- or B. bassiana-treated individuals were significantly lower than  $R_0$  of non-treated individuals. The intrinsic rate of increase  $(r_m)$  reached its maximum for non-treated individuals while decrease to the minimum values for biopesticide-treated individuals. Intrinsic rate of increase is important because it reflects the overall effect of the biopesticides on development, reproduction, and survival of the insect species (Southwood and Henderson, 2000). Therefore, the reproduction, development, and survival of T. absoluta treated with B. thuringiensis or B. bassiana were low. The mean generation times  $(T_0)$  of biopesticide-treated individuals were longer than those of non-treated individuals. The daily finite rate of increase  $(\lambda)$  was significantly lower for biopesticide-treated individuals in comparison with that for non-treated individuals. Also, higher doubling times (DT) of T. absoluta were obtained with the treated individuals. In conclusion, the microbial biopesticides (B. thuringiensis subsp. kurstaki and B. bassiana) affected the development, survival, reproduction, longevity, and population parameters of T. absoluta. In this study, individuals of T. absoluta receiving lethal concentrations (LC<sub>50</sub>) of B. thuringiensis or B. bassiana and directly not dead, have long developmental time and lower  $m_x$  as well as the lowest  $r_m$  value of immature and low survival rate  $(l_x)$ . Such biopesticide effects could cause reductions in survival fitness of the pest insect. Also, prolonged developmental time could increase the exposure of the insect to its natural enemies.

#### Conclusion

*T. absoluta* is an important pest of tomato and many other plants of Solanaceae family. Because the primary management tactic for *T. absoluta* control is often the chemical methods although it is not a sustainable option

in the long run due to insecticide resistance. An attractive alternative tool to control this pest is the use of microbial biopesticides due to their eco-friendly and target selective characteristic. The microbial biopesticides (*B. thuringiensis and B. bassiana*) were affected the population parameters of *T. absoluta* in addition to their acute effects. Our study provides useful data for developing management tactics depending on the changes in the population density of the pest.

#### Authors' contributions

AAY and NZMZ conceived and designed the research. HAA and RF conducted the experiments. AAY and RF analyzed the data and wrote the manuscript. AAY, HAA and NZMZ made critical reviewed and approved the final version. All authors reviewed and approved the final manuscript.

## Competing interests

The authors declare that they have no competing interests.

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