Lethal and sub-lethal effects of bio-and chemical insecticides on the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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**Abstract**

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is a destructive pest affecting the tomato crop causing a loss of 60–100% of the tomato crop production. In the present study, the lethal and sub-lethal effects of *Bacillus thuringiensis* (Bt), spinosad, and emamectin benzoate as bio-insecticides comparing with 2 synthetic insecticides: chlorpyrifos and indoxacarb against *T. absoluta* under laboratory conditions were evaluated. Take in consideration, life tables and reproductive parameters of treated larvae with LC$_{30}$ for each pesticide (bio- and chemical insecticides) by using leaf dipping technique were calculated. The results showed that Lepinox formulation of Bt was the most effective one against the early 2nd instar larvae with 0.01 × 10$^{-2}$ mg/l (ppm), followed by spinosad with 0.07 × 10$^{-2}$ ppm comparing to indoxacarb and chlorpyrifos (0.01 and 11.44 μg/ml, respectively). Sub-lethal effect of the Bt formulations significantly increased pupal mortality by 29.77, 33.30, and 26.94% for Lepinox, Dipel 2x, and Protecto, respectively. In addition to their efficiency, Bt formulations and emamectin benzoate increased larval developmental period and reduced adult emergency percentage to 70.28, 66.70, 73.06, and 75.55% for Lepinox, Dipel 2x, Protecto, and emamectin benzoate, respectively. All bio-insecticides including Bt, spinosad, and emamectin benzoate caused abnormalities in different life stages. The results suggest these bio-insecticides are promising alternatives to synthetic insecticides for *T. absoluta* management.

**Keywords:** *Tuta absoluta*, *Bacillus thuringiensis*, Bio-insecticides, Chemical insecticides, Lethal effect

**Background**

The tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), is originated in Chile then spread to South America, Europe, and South East Asia. It has also moved south from the lowest Mediterranean shores into Africa on different solanaceous crops (Retta and Berhe 2015). Since its arrival to the Mediterranean basin, it becomes a key pest of tomato crop in open field and protected cultivation, making more than 80% losses in the tomato crop yield (CFIA 2010). In 2009, *T. absoluta* was recorded as invasive insect pest attacking tomato plants in Egypt (Mohammed 2010).

*T. absoluta* belongs to multivoltine species which has high reproductive potential in a short time. Meanwhile, it could adapt itself in a broad variation temperature, whilst under Mediterranean condition, *T. absoluta* observed 12 generations per year and does not diapause (Garcia and Vercher 2010). Its control by using chemical pesticides is extremely difficult and quite challenging. So, it could be controlled by a combination of all available control measures including physical, cultural, biological methods, and use of registered pesticides (Retta and Berhe 2015). Bio-pesticides that utilize beneficial microbial agents could reduce the reliance of
chemical pesticides in its control. Thus, microbial agents could solve pressing needs as part of integrated pest management systems (Sayed and Behle 2017). Bio-pesticides based on B. thuringiensis (Bt) exhibited a potential role as an alternative strategy to control insect pests (Palma et al. 2014). The bio-insecticides spinosad and emamectin benzoate products have a high potent to a broad spectrum of lepidopterous pests on vegetables around the world, especially against T. absoluta (Gacemi and Guenaoui 2012).

It is a common phenomenon that arthropods are exposed to sub-lethal concentrations of pesticides because of their degradation after initial application in crops. Such exposure may lead to sub-lethal effects on all biological parameters, e.g., developmental rate, longevity, and fecundity (Chen et al. 2016). Therefore, determination of the sub-lethal effects of insecticides on insects is very important for elucidation the impact of pesticides on agro-ecosystem. These sub-lethal effects may be accompanied by an increase of some biological parameters such as fecundity and survival time, which was observed in the aphid species, Myzus persicae (Sulz.), after treatment with azadirachtin and imidacloprid (Culter et al. 2009).

The present work was carried out to evaluate the biological activities of the bio-insecticides, Bt formulations, spinosad, and emamectin benzoate, compared to the chemical insecticides: chlorpyrifos and indoxacarb on T. absoluta. In addition, assessing the sub-lethal effects of each compound on the developmental rate and reproductive potentiality of the pest under laboratory conditions.

Materials and methods

Insect rearing

T. absoluta samples were collected from tomato fields at Giza and Kalubia Governorates, Egypt, and transferred to the laboratory, where they were divided into groups in plastic plates for rearing at 25 ± 2 °C, 60:70% RH, and 16:8 light photoperiod regime in an incubator. Fresh leaves were provided daily to maintain the culture up to the 3rd generation (Deleva and Harizanova 2014) (Table 1).

Tested bio-and chemical insecticides

Bioassays

Toxicity of the above insecticides on the 2nd instar larvae of T. absoluta was investigated, using the leaf dipping technique. Tomato leaves were collected from first to ninth node of the plants, where the leaves were greener and full of plant materials. Leaves were washed by water to ensure their cleanliness from any contaminants and cut in circular disks (3 cm diameter). Six concentrations of each bio-insecticide were used, while in untreated control, the disks were dipped in tap water. Each leaf disk was dipped for 10 s beginning with the lowest concentration and continued ascending. Tissue culture plates (6 wells, 33.9 mm, SPL Life Sciences Company) were used as vessels for study of toxicity. The bottom of each well of the plate was covered with thin layer of agar for moist. After drying at room temperature, five of 2nd instar larvae were transferred by thin brush to each well and kept in an incubator. The treatment for each concentration was replicated six times. After 24 h, the treated disks were replaced by untreated leaves disks for recording the daily mortality percent for 4 days. The insects were considered dead, when they were unable to move prodded with a fine brush (Galdino et al. 2011 and Deleva and Harizanova 2014).

Sub-lethal effects of tested bio-and chemical insecticides

The 2nd instar larvae of the 3rd generation of T. absoluta were used in experiments. The LC30 value of each insecticide was estimated as a sub-lethal concentration used in the study. The LC30 concentration was prepared by using the method described above. Three replicates, with 50 larvae each, were performed, and mortality rate was recorded and analyzed by probit analysis. In all treatments, the treated leaves after 24 h were replaced by untreated fresh leaves daily. Last instar larvae (4th instar larvae) were sexed following IRAC protocol (http://www.irac-online.org/methods/tuta-absoluta-larvae) and separated individually in glass tubes and then continued feeding till pupation. The pupae were weighted and transferred to clean

### Table 1: Tested bio-and chemical insecticides and their rates of application

| Insecticides (common name)          | Trade names | Formulation active ingredient (a.i. %) | Recommended rate (g or ml/fed.) |
|-------------------------------------|-------------|--------------------------------------|---------------------------------|
| Bacillus thuringiensis              | Lepinox    | 3.75% WP                             | 100 g                           |
|                                     | Dipel 2x    | 6.4% WP                              | 200 g                           |
|                                     | Protecto   | 9.4% WP                              | 300 g                           |
| Spinosad                            | Tracer      | 24% SC                               | 50 ml                           |
| Emamectin benzoate                  | Proclaim    | 5% SG                                | 60 g                            |
| Chlorpyrifos                        | Dursban     | 48% EC                               | 100–125 ml                      |
| Indoxacarb                          | Avaunt      | 15% EC                               | 25 ml                           |

All Bt formulations equivalent to 32,000 IU/mg of Bacillus thuringiensis sp. kurstaki strain
tubes to record pupation period. The sex ratio of adults was also recorded (Hafsi et al. 2012).

To assess the reproductive capacity, 4 groups of cross mating were used to elucidate the impact of sub-lethal effect. Cross mating experiment was conducted as follows: 1st group contained mating between untreated female + untreated male (control), 2nd group between treated female + untreated male, 3rd group between untreated female + treated male, and the 4th group between treated female + treated male. Number of deposited eggs was counted daily and transferred into glass jars till hatching to calculate hatchability rate, ovipositional periods, fecundity, and longevity of adults.

To estimate abnormalities and deformations, each treatment and control was replicated 3 times (50 larvae of the 2nd larval instar each), and percentage of abnormalities was calculated according to El-Tantawy and Salem (1976).

**Data analysis**

Mortality data from leaf dip bioassay were corrected, using Abbott’s formula (Abbott 1925) to account natural mortality recorded in the control treatment. The statistical analysis program LDP line was used to determine the lethal and sub-lethal concentration values (LC₃₀, LC₅₀, and LC₉₀) for each bio-insecticide (with 95% confidence limits). All biological parameters were performed using one-way ANOVA, followed by Duncan’s multiple range test with the SPSS statistical analysis software.

**Results and discussion**

**Toxicity of different bio-and chemical insecticides on 2nd instar larvae of T. absoluta**

Data setup in Table 2 showed the LC₃₀, LC₅₀, and LC₉₀ values estimated for the tested insecticides against the 2nd instar larvae of *T. absoluta* under laboratory conditions using leaf dipping technique after 4 days of treatments. The results indicated that Lepinox “Bt” showed significantly the highest toxicity than spinosad with LC₅₀ values of 0.0007 mg/l and 0.002 mg/l, respectively, followed by emamectin benzoate 0.009 mg/l. LC₅₀ value was 0.11 mg/l for Dipel 2× “Bt” and 0.09 mg/l for indoxacarb. In addition, LC₅₀ was 0.10 mg/l for Protecto “Bt”, while chlorpyrifos showed the lowest toxicity effect with 21.30 mg/l. These results indicated that lepinox and Dipel 2× formulations of *Bt* and emamectin benzoate were the highly effective ones than indoxacarb and chlorpyrifos. These results are similar to those obtained by (Rausell et al. 2000 and Hashemitassuji et al. 2014) who found that 2nd and 3rd larval instars of *T. absoluta* that were susceptible to *Bt*. Hashemitassuji et al. (2014) mentioned that spinosad was more effective in the control of *T. absoluta* than *Bt*. Moreover, López et al. 2010 showed that emamectin benzoate was the most effective insecticide for control several lepidopteran pests and causing a reduction in the fecundity of females. While Hafsi et al. (2012) evaluated the toxicity of 13 insecticides against *T. absoluta*, and reported that the most 3 effective insecticides were *Bt*, spinotram, and Spinosad, meanwhile, they were statistically similar. Thus, *Bt* caused a significant mortality percent after treated the 2nd instar larvae of *T. absoluta* with different products of *Bt* such as Protecto, Dipel 2×, and Lepinox (Giustolin et al. 2001). Worthwhile, the inferior effectiveness of chlorpyrifos and indoxacarb may be due to their intensive used against *T. absoluta*, and consequently the insect became more resistant to these both insecticides. Moreover, insecticides are subjected to degradation after application. Consequently, the target pest was exposed to low concentrations of insecticides. Therefore, studies on the sub-lethal effects of insecticides on the target pests of great importance are to explore their indirect effectiveness, especially in the case of bio-insecticides or insect growth regulators.

**Table 2** Toxicity of the tested bio-and chemical insecticides against the early 2nd instar larvae of *Tuta absoluta* (treated by leaf dipping technique)

| Bio-and chemical insecticides formulations | LC₃₀ (mg/l), 95% CL | LC₅₀ (mg/l), 95% CL | LC₉₀ (mg/l), 95% CL | Slope ± SE |
|-------------------------------------------|---------------------|---------------------|---------------------|-----------|
| Lepinox (Bt)                              | 0.01 × 10⁻⁷ (0.06 × 10⁻⁷–0.08 × 10⁻⁷) | 0.07 × 10⁻² (0.09 × 10⁻²–0.13 × 10⁻²) | 31085 IU/mg | 0.02 (0.03–0.05) | 0.70 ± 0.06 |
| Dipel 2× (Bt)                             | 0.09 × 10⁻¹ (0.03 × 10⁻¹–0.20 × 10⁻¹) | 0.11 (0.06–0.50) = 203.66 IU/mg | 9.89 (8.66–16.14) | 0.48 ± 0.05 |
| Protecto (Bt)                             | 0.14 × 10⁻¹ (0.59 × 10⁻²–0.22 × 10⁻¹) | 0.10 (0.05–0.13) = 224.0 IU/mg | 11.79 (3.35–23.77) | 0.65 ± 0.06 |
| Spinosad                                  | 0.07 × 10⁻¹ (0.04 × 10⁻¹–0.001) | 0.002 (0.001–0.004) | 0.08 (0.05–0.21) | 0.85 ± 0.07 |
| Emamectin benzoate                        | 0.03 × 10⁻¹ (0.01 × 10⁻¹–0.05 × 10⁻¹) | 0.009 (0.06 × 10⁻¹–0.12 × 10⁻¹) | 0.10 (0.07–0.25) | 1.22 ± 0.18 |
| Chlorpyrifos                              | 11.44 (5.7–15.9) | 21.30 (13.21–32.78) | 97.24 (72.75–272.9) | 1.94 ± 0.16 |
| Indoxacarb                                | 0.01 (0.04 × 10⁻¹–0.20 × 10⁻¹) | 0.09 (0.05–0.17) | 24.87 (10.68–78.19) | 0.53 ± 0.04 |

*LC₃₀* concentration causing 30% mortality  
*LC₅₀* concentration causing 50% mortality  
*LC₉₀* concentration causing 90% mortality  
*CL*: confidence limits

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**References**

Hafsi et al. (2012), Rausell et al. 2000, Hashemitassuji et al. 2014, Giustolin et al. 2001, López et al. 2010, Kandil et al. Egyptian Journal of Biological Pest Control 2020.
Latent effects of bio-and chemical insecticides on the 2nd instar larvae

Data in Table 3 evaluated the latent effect of the tested bio-insecticides on the 2nd instar larvae of T. absoluta due to exposure to sub-lethal concentration of LC30 for 24 h. Results showed elongating developmental period of larvae by about 6.64–1.44 days at all treatments. Time to pupate was significantly longer for Dipel 2x by about 2.6 days, conversely indoxacarb larvae lasted (6.98 days) to pupate compared to the control (6.62 days). The pupation percent decreased significantly (48.67 ± 1.76%) in case of the emamectin benzoate; however, it was slightly differ than the control in case of the larvae treated with chlorpyrifos, as it was (93.61 ± 1.85%) and for untreated larvae as it was (98.00%). Concerning the pupal weight, spinosad showed a significant increase in female weight (0.31 ± 0.01 mg) and Lepinox (0.25 ± 0.00 mg) than the control (0.20 ± 0.01 mg). However, emamectin benzoate showed an increase in male pupal weight (0.35 ± 0.00 mg), at variance indoxacarb (0.24 ± 0.01 mg) compared to the control (0.22 ± 0.01).

Latent effect of bio-and chemical insecticides on adult stage

Results in Table 4 present the developmental parameters of adult stage. Emergence % was highly reduced post treatment with Dipel 2x; meanwhile, chlorpyrifos did not exhibit any significant difference than control. Regarding the sex ratio of emerged adults, as shown in the results, spinosad led to increase adult sex ratio to 2:1 female: male ratio, although emamectin benzoate caused a slight change in this ratio to be 2/3:1 2:1.2. For the adult longevity, the results showed fluctuated effect. All tested agents, except emamectin benzoate, decreased the adult longevity, especially in case of Protecto, which decreased adult longevity to 14.67 and 12.6 days for both females and males, respectively. In all bio-insecticides treatments, the pre-ovipositional period was increased, especially in case of Dipel 2x (with 8.0 days). However, synthetic insecticides (indoxacarb and chlorpyrifos) did not differ than control. Moreover, it is clear from the results that all insecticides treatments had negative affect on the ovipositional period.

Deformations shapes in different developmental stages

Data in Table 5 demonstrate the means of deformation of different developmental stages. The results indicated that Lepinox showed the highest impact, and the incidence of deformation of larval and pupal percent was 28.33 ± 0.33% and 10.83 ± 2.49%, respectively. However, emamectin benzoate caused a slight deformed larval percent (2.85 ± 1.43%) than the control, and the highest deformed adult’s percent with 38.42 ± 3.24% for adult females and 26.85 ± 3.34% for adult males. In addition, spinosad caused the lowest deformed pupae percent with 2.63 ± 2.59% and also in females with 2.56 ± 2.56%. As well, the lowest deformed males were found in case of Protecto with 11.43 ± 5.95%. Thus, all Bt formulations had obtained deformations in all life stages expect egg stage, contrary of chlorpyrifos, and indoxacarb, which did not cause any deformations.

Latent effect on fecundity and fertility

A broad range of variations in fecundity of T. absoluta was detected in all treatments (Fig. 1a). The results of mating between treated females × treated males showed that the females’ fecundity was 267.33 ± 97.27 egg/female after treated the larvae with chlorpyrifos (Fig. 1a); otherwise, Dipel 2x gave the lowest number of eggs per female (43.27 ± 24.22 eggs) compared to control (314.87 ± 19.48 eggs). Besides, the total numbers of eggs of mating between treated females + untreated males were 73.63 ± 48.86 eggs, when treated the 2nd instar larvae with Dipel 2x (Fig. 1c), while emamectin benzoate

| Insecticides formulations | Duration (mean ± SE) | Pupal mortality % | Pupation % | Pupal weight (mg) |
|---------------------------|----------------------|------------------|------------|------------------|
|                           | *Larval              | **Pupal          | Female     | Male             |
| Control                   | 6.08 ± 0.91          | 6.62 ± 0.96      | 6.08 ± 0.68| 98.00 ± 0.00     | 0.20 ± 0.01 | 0.22 ± 0.01 |
| Lepinox (Bi)              | 9.12 ± 0.20          | 7.41 ± 0.16      | 29.77 ± 0.55| 67.33 ± 3.71     | 0.25 ± 0.00 | 0.27 ± 0.01 |
| Dipel 2x (Bi)             | 8.37 ± 0.11          | 9.22 ± 0.33      | 33.30 ± 1.43| 70.00 ± 1.15     | 0.27 ± 0.00 | 0.26 ± 0.01 |
| Protecto (Bi)             | 12.72 ± 0.71         | 8.02 ± 0.21      | 26.94 ± 6.02| 66.67 ± 6.67     | 0.30 ± 0.01 | 0.30 ± 0.00 |
| Spinosad                  | 7.52 ± 0.18          | 7.13 ± 0.28      | 21.39 ± 2.9 | 52.00 ± 4.0      | 0.31 ± 0.01 | 0.33 ± 0.01 |
| Emamectin benzoate        | 10.44 ± 0.26         | 7.84 ± 0.26      | 24.45 ± 3.17| 48.67 ± 1.76     | 0.27 ± 0.01 | 0.35 ± 0.00 |
| Chlorpyrifos              | 8.3 ± 0.18           | 7.45 ± 0.16      | 6.39 ± 1.85 | 93.61 ± 1.85     | 0.28 ± 0.01 | 0.28 ± 0.01 |
| Indoxacarb                | 8.05 ± 0.15          | 6.98 ± 0.2       | 13.93 ± 0.97| 60.00 ± 1.15     | 0.26 ± 0.01 | 0.24 ± 0.00 |

Values marked with the same letters are not significantly different (P > 0.05: Duncan’s multiple range tests).

*number of days from 2nd instar larvae till pupation, **number of days from pupae till the eclosion
Table 4 Effects of tested bio-and chemical insecticides on the adult stage of Tuta absoluta results from treated the 2nd instar larvae with the sub-lethal concentration of LC30 value

| Bio-and chemical insecticides formulations | Mean ± SE | Emergence % | Longevity (days) | Pre-oviposition (days) | Oviposition period (days) | Post-oviposition (days) |
|-------------------------------------------|-----------|-------------|------------------|-----------------------|--------------------------|-------------------------|
|                                           |           | Females     | Males            | Females               | Males                    | Females                 |
| Control                                   | 99.32 ± 0.86 | 52.05 ± 0.6 | 47.95 ± 0.69     | 18.00 ± 0.58          | 1.00 ± 0.00              | 13.67 ± 0.67            |
| Lepinox (Bt)                              | 70.28 ± 0.6 | 60.19 ± 0.95| 39.81 ± 0.59     | 16.75 ± 0.33          | 4.33 ± 0.33              | 6.67 ± 0.33             |
| Dipel 2x (Bt)                             | 66.70 ± 1.43| 61.47 ± 2.01| 38.52 ± 2.01     | 16.75 ± 0.33          | 8.00 ± 0.00              | 9.67 ± 0.33             |
| Protecto (Bt)                             | 73.06 ± 0.6 | 61.39 ± 3.58| 38.61 ± 3.85     | 14.67 ± 0.33          | 2.00 ± 0.00              | 11.33 ± 0.33            |
| Spinosad                                  | 78.61 ± 2.9 | 67.06 ± 1.03| 32.94 ± 1.03     | 16.75 ± 0.33          | 2.00 ± 0.00              | 12.67 ± 0.33            |
| Emamectin benzoate                        | 75.55 ± 3.17| 45.92 ± 0.23| 54.08 ± 0.23     | 17.33 ± 0.33          | 2.00 ± 0.00              | 13.67 ± 0.33            |
| Chlorpyrifos                              | 91.63 ± 1.85| 54.44 ± 1.00| 45.56 ± 1.00     | 16.75 ± 0.33          | 1.00 ± 0.00              | 14.00 ± 0.00            |
| Indoxacarb                                | 86.00 ± 1.0 | 54.44 ± 1.00| 36.89 ± 5.35     | 16.75 ± 0.33          | 1.00 ± 0.00              | 14.00 ± 0.00            |

Values marked with the same letters are not significantly different (P > 0.05: Duncan’s multiple range test)

Table 5 Malformations % ± SE of Tuta absoluta after treated the 2nd instar larvae with LC30 value of the tested bio-and chemical insecticides

| Bio-and chemical insecticides formulations | Larval deformation % | Pupal deformation % | Normal pupae % | Deformation adults | Normal adults |
|-------------------------------------------|----------------------|---------------------|----------------|--------------------|---------------|
|                                           |                      |                     |                | Female % | Male % | Female % | Male % |
| Control                                   | 0.00 ± 0.00          | 0.00 ± 0.00         | 100.00 ± 0.00  | 0.00 ± 0.00 | 1.52 ± 1.52 | 100.00 ± 0.00 | 98.48 ± 1.52 |
| Lepinox (Bt)                              | 28.33 ± 0.33         | 10.83 ± 2.49        | 71.67 ± 0.33   | 7.90 ± 1.06 | 11.33 ± 1.9 | 92.10 ± 1.06 | 88.27 ± 1.9 |
| Dipel 2x (Bt)                             | 10.48 ± 1.91         | 4.29 ± 2.51         | 89.52 ± 1.91   | 9.20 ± 2.06 | 18.70 ± 4.06 | 90.87 ± 2.06 | 81.30 ± 4.06 |
| Protecto (Bt)                             | 18.05 ± 6.09         | 4.51 ± 2.77         | 84.96 ± 6.12   | 13.49 ± 3.71 | 11.43 ± 5.95 | 85.61 ± 3.71 | 88.57 ± 5.95 |
| Spinosad                                  | 0.00 ± 0.00          | 2.63 ± 2.59         | 100.00 ± 0.00  | 2.56 ± 2.56 | 0.00 ± 0.00 | 73.44 ± 2.56 | 100.00 ± 0.00 |
| Emamectin benzoate                        | 2.85 ± 1.43          | 6.84 ± 2.59         | 97.15 ± 1.43   | 38.42 ± 3.24 | 26.85 ± 3.34 | 61.58 ± 3.24 | 73.15 ± 3.34 |
| Chlorpyrifos                              | 0.00 ± 0.00          | 0.00 ± 0.00         | 100.00 ± 0.00  | 0.00 ± 0.00 | 0.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 |
| Indoxacarb                                | 0.00 ± 0.00          | 0.00 ± 0.00         | 100.00 ± 0.00  | 0.00 ± 0.00 | 0.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 |

Values marked with the same letters are not significantly different (P > 0.05: Duncan’s multiple range test)

gave the highest number of eggs per female (274.03 ± 24.69 eggs). The females laid 363.67 ± 2.09 and 215.53 ± 79 eggs/female, respectively, when untreated females mated with treated males with chlorpyrifos or indoxacarb (Fig. 1b).

Considering the number of eggs that actually hatch, fertility %, (Fig. 1b), in case of mating treated female + treated male results, indicated that emamectin benzoate and chlorpyrifos had a slight effect on fertility percentage than untreated females and males. In addition, chlorpyrifos and indoxacarb showed the same results in results in mating (Fig. 1b, c).

The abovementioned data showed that the larvae survived after exposure to sub-lethal concentration (LC30) of the tested bio-insecticides that had a significant reduction in developmental rate including larval, pupal duration, pupal weight, and pupation% of 2nd instar larvae till emergence comparing to chlorpyrifos and indoxacarb. This finding is expected because chlorpyrifos is an inhibitor for cholinesterase activity. Also, indoxacarb acts by blocking sodium channel and caused hyperpolarization. This means that both compounds have acute neurotoxic effects. However, Bt formulations had long acting effects, and this action gave chance to enhance their latent effects. In contrast, in some other studies, it was found that sub-lethal concentrations of indoxacarb (Wang et al. 2011) on P. xylostella inhibited insect development. In the present study, the highest reduction percentage in the development of T. absoluta including larval and pupal durations, pupal mortality, pupation%, and emergence% occurred in case of using Lepinox or Dipel 2x formulation of Bt. Both bio-insecticides, Bt or spinosad (tracer) insecticides, showed a high efficacy in controlling larvae of T. absoluta (Hamdy et al. 2013; Khidr et al. 2013 and Hashemittassi et al. 2014). Obtained results were in agreement with (Moustafa et al. 2016) who demonstrated the effect of emamectin benzoate and spinosad on cabbage moth, Mamestra brassicae larvae, and found a significant increase in larval and pupal durations than control with a significant decrease in adult emergency percentage than control.
Conclusions
This study elucidated the efficiency and sub-lethal effects of different bio-insecticide formulations against the tomato leaf miner, *T. absoluta*. The significance of the results that the bio-insecticides, *Bt* formulations and emamectin benzoate, had a good potential against *T. absoluta* and is recommended to be effectively used in the pest management. Further evaluation under field conditions is still needed.

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Authors’ contributions
The authors carried out all the experiments including the bioassay tests, analytical part, analysis of data, and write the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials
All data of the study have been presented in the manuscript and the materials which are used in this study are highly quality and grade.

Ethics approval and consent to participate
Not applicable.

Consent for publication
This study does not contain any individual person’s data.

Competing interests
The authors declare that they have no competing interests.

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