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Evaluation of tea tree oil formulations contact and stomach toxicity against the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval, 1883) (Lepidoptera: Noctuidae)

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Abstract: The insecticidal potential of tea tree oil formulations was tested for contact and stomach poison toxicities against various stages of the *Spodoptera littoralis* ((Boisduval, 1883) (Lepidoptera: Noctuidae) larvae under laboratory conditions. The study was carried out at Yozgat Bozok University Faculty of Agriculture between 2020 and 2021. In the contact toxicity test, the formulations were tested at different stages of larvae by topical application. Among the tested formulations, TTO (100%), F14 (91.72%), and F15 (89.20%) formulations caused the highest mortality in the *S. littoralis* 3rd stage larvae after 72 h. Dose-response bioassay with the most promising formulations (TTO, F14 and F15) revealed that LD_{50} values were 0.016, 0.046, and 0.076 µg/larvae for TTO, F14, and F15, respectively. The stomach poison effects of the formulations were tested by applying a 0.16 µg/cm^2 dose to lettuce leaf discs. The F17 and F18 formulations produced the highest mortality with mortality rates of 75% and 65% after 10 days of incubation, respectively. The calculated LC_{50} values for these formulations were 0.027 and 0.042 µg/cm^2 for F17 and F18 formulations after 10 days of incubation, respectively. These results revealed that tea tree oil and its main components containing formulations have the potential in controlling this destructive lepidopteran pest species.

Key words: Contact toxicity, dose-response, *Spodoptera littoralis*, stomach poison, *Melaleuca alternifolia*

1. Introduction

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval, 1883) (Noctuidae: Lepidoptera), is a major pest of cotton and corn worldwide as this pest has been reported from 112 plant species with economic damage (Gordon, 1961; Temerak, 2002; Reda et al., 2016; Hamadah et al., 2020; Taha-Salaime, et al., 2020). The damage caused by the larvae is described by typical signs of feeding on the leaves of host plants and creating sieve-shaped holes. The larva also feeds on the flowers and fruits of the plant and causes major damage to the cash crops. This pest is on the A2 quarantine list of the EPPO (European and Mediterranean Plant Protection Organization) (OEPP/EPPO, 2015).

Conventional insecticides from different groups have been extensively used in the control of *S. littoralis* for decades, which has led to the development of resistance of this pest to nearly all major classes of insecticide groups (Sammour et al., 2008; Korrat et al., 2012). It is reported that this pest has developed resistance to many active compounds, including acephate, indoxacarb, and tebufenozide (Soderlund and Knipple 2003; Kasai, 2004; Wheelock et al., 2005; Elhadek et al., 2020; Hilliou et al., 2021; Abd El-Kareem et al., 2022). This limits available tools to control this destructive pest and, at the same time, increases environmental effects due to increased doses or new mixed formulations. Additionally, the outbreak of secondary pests has been reported in many areas as a result of pesticide pressure on natural enemies (Benelli, 2015; Naqqash et al., 2016).

Spodoptera littoralis is one of the insect pests that are the major reason for the large-scale loos of agricultural production, e.g., corn, cotton, tomato, and potato. Synthetic insecticides are toxic nontarget organisms, they pollute the environment and underground water, and endanger human health. Therefore, to protect the environment and human health, it has become important to research to develop alternative botanical pesticides, which are nontoxic to other organisms and do not pollute the environment and underground water.

Alternative control methods have been suggested and tested against the Egyptian cotton leafworm with varying

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success rates. However, especially cotton, corn, and vegetable growers urgently need a reliable alternative to the current insecticides to stay in the business (Koul et al. 2008; Khater, 2012; Acheuk et al. 2022; Abdelkhalek et al. 2022). Although there is a concern about the standardization and reliability of active compounds, various plant secondary metabolites have been tested against many important pest species including plant pathogen, insect, and mite species (Ikbal and Pavela, 2019; Badalamenti et al., 2021; Ghoneim et al., 2021; Abdelgaleil et al.,2022).

Plant terpenoids and especially essential oils obtained from various aromatic plant species have been screened on many important insect pests, mites, and even weed species (Pavela, 2018; Ammar et al., 2020; Benelli et al., 2020; Feng et al., 2022; Santana et al., 2022). Plant essential oils are thought to be low-risk products because their toxicity to mammals is low and also, they are extensively used in the pharmaceutical industry (Ebadollahi, 2013; Chellappandian et al., 2018). An increasing number of studies are underway, especially in the developing countries to find alternative control tools against the local pest species using local resources.

Melaleuca alternifolia (Maiden & Betche) Cheel, 1924, (Myrtaceae) is known for its antiseptic, antimicrobial, and antiinflammatory properties (Australian Aboriginals used TTO many years ago). Tea tree oil has been extensively studied in pest management as well as in the pharmaceutical and cosmetic industry, and products based on components of tea tree oil are on the market (Belaiche, 1985; Altman, 1989). Tea tree oil contains various terpenoids including terpinen-4-ol (40%), γ-terpinene (23%), α-terpinene (20.4%), 1.8%-cineole (5.1%) (Carson et al., 2006; Borotová et al., 2022; Yuan et al., 2022). Tea tree oil's various toxic and behavioral effects including antiovipositon have been reported in previous studies (Benelli et al., 2013; Liao et al., 2017; Ático Braga et al., 2020; Zimmermann et al., 2021; Zhang et al., 2021). While tea tree oils significantly inhibited 3 enzymes in the rice weevil, *Sitophilus zeamais* (Motschulsky, 1855) (Coleoptera: Curculionidae), terpinene-4-ol was the most effective compound among them (Liao et al., 2016). Tree tea oils have also both behavioral and toxic effects on medically important species, e.g., *Culex* spp. (Diptera: Culucidae). It was also reported that tea tree oil has a repellent effect on *Culex pipiens* (Linnaeus,1758) (Diptera: Culucidae) females and also has a larvicide effect on *Culex quinquefasciatus* (Say, 1823) (Diptera: Culucidae) (Kang et al., 2009; Pavela, 2009). Tea tree oil is also shown to be an effective repellent against livestock infesting insects. When applied to wool, 3% TTO formulation repelled the female of *Lucilia cuprina* (Wiedemann, 1830) (Calliphoridae: Diptera) which infest sheep and cause death, application repelled the female fly of *L. cuprina* and prevented oviposition for 6 weeks (Callander and James, 2012) also formulations containing 1% TTO caused 100% mortality of *L. cuprina* eggs and first instar stages (Callander and James, 2012).

Although tea tree oils have been tested against various insect pest species, the whole or crude tea tree oil was used. In the current study, formulations that contain various ratios of different components of tea tree oils were tested on various development stages of the Egyptian cotton leafworm as contact and stomach poison. Additionally, the dose-response bioassay was also performed to compare the most promising formulations to fully explore the potential of tea tree oil components as bioinsecticide especially for organic growers.

2. Materials and methods

2.1. Tea tree oil formulations

Tea tree oil and five tea tree oil formulations, named F14, F15, F16, F17, and F18, were obtained from BioAust Pty Ltd. (Stafford Heights, Queensland, Australia). The formulations contain different components of tea tree oils with varying ratios. The main components of these formulations are terpinen-4-ol in F14, monoterpinens form tea tree oil in F15, γ-terpinene in F16, linalool in F17, and eugenol in F18. Alongside these formulations, the pure tea tree oil was also tested (Table 1).

2.2. *Spodoptera littoralis* rearing under laboratory conditions

Spodoptera littoralis larvae were collected from the soybean leaves in Adana (Turkey) in August 2020. The larvae were transferred in 5-L plastic containers with fresh food sources to the Entomology Laboratory of the Department of Plant Protection at Yozgat Bozok University. The larvae were reared on artificial media prepared according to Saljoqi et al. (2015) at 25 ± 1 °C, 16 L: 8 D photoperiod, and 60 ± 10% RH until they became pupae.

The pupae were sexed according to Aydın (2002). Around 20 pupae were transferred into 3-L plastic containers and incubated at the above conditions until adults emerge. Male and female adults were incubated in the plastic containers and folded about five 10-cm wide and 20-cm length folded wax paper was added into each container for egg-laying. Twenty milliliters of 10% sugar solution was transferred into a 30-mL plastic cup and a dental wick was placed through a perforated lid. Three sugar solutions were supplied as adults' food for each container until all adults died. Eggs were harvested and placed into Petri dishes and placed into the incubator under the abovementioned conditions. Neonate larvae were collected and transferred into 90-mm Petri dishes with artificial food sources. F1 generations were used in the toxicity studies. The larvae were incubated until they reached the desired stage under the above conditions.

Table 1. Compounds in formulations and their ratios.

Formulations	Compounds	Rate (%)
TTO	Pure tea tree oil	100
F14	Terpinen- 4-ol	25.2
F15	Monoterpinens form tea tree oil	29.2
F16	γ-terpinene	28.9
F17	Linalool	25.9
F18	Eugenol	25.9

2.3. Contact toxicity studies

Prior to the single-dose contact toxicity test, preliminary studies were conducted to decide a dose for each stage. The larvae were collected from the stock culture and segregated according to pronotum width, and 10 larvae in the same stage were placed into a Petri dish.

The stock solutions of tea tree oil and its formulations were prepared using 50% acetone containing 0.8% Tween 80 (v/v) as surfactant. Application of the concentration to the dorsal side of the individual larva using a microapplicator (HAMİLTON, Pb-600-1 Repeating Dispenser, 50 µL Gastight & Microliter Syringe) gave the dose of 0.025, 0.1, 0.2, 0.3, and 0.4 µg/larva for the 2nd, 3rd, 4th, 5th, and 6th stage larvae, respectively. The larvae were left to dry for 10 min at room temperature. About 2 g of the newly prepared artificial food was provided to each Petri dish. In the corresponding control group, the larvae were treated with 1 µL of 50% acetone containing 0.8% Tween 80. The larvae were incubated under the abovementioned conditions for 72 h. Mortality was recorded every 24 h, and the dead larvae were removed from the Petri dishes to prevent disease development. The experiment was set up in a randomized block design. Each block contains all treatments and control groups. The experiment was repeated on three different dates. A total of 630 larvae were used for each stage.

The dose-response bioassay was performed on 3rd larval stage with TTO, F14, and F15 formulation based on the single-dose contact dose study. Six different concentrations (0.125%, 0.25%, 0.35%, 0.5%, 0.75%, and 1%) were prepared from the stock concentrations using 50% acetone containing 0.8% Tween 80. The larvae were obtained from the stock culture and 10 larvae that were in the 3rd larval stage were placed into a Petri dish. One microliter of suspension was applied to the dorsal side of each larva and the larvae were left to dry for 10 min. The larvae were placed into a new Petri dish containing about 2 g of artificial food source. In the control groups, the larvae were treated with 1 µL of 50% acetone containing 0.8% Tween 80. The larvae were incubated under the above conditions for 72 h. The mortality was recorded every 24 h for 72 h and each time the dead larvae were removed from the Petri dishes. A randomized block design was used for performing the experiment. Each block contains all doses and the control group. The experiment was repeated on three different dates. For each treatment, a total of 630 3rd instar larvae were used.

2.4. Stomach poison tests

The stomach poison test was performed on 3rd stage instar of the Egyptian cotton leafworm larvae. The larvae were collected from the stock culture and segregated according to stages as described in Section 2.3. In the single-dose screening test, the concentrations were prepared as outlined above using 50% acetone containing 0.8% Tween 80. Leaf discs of 1 cm^2 in diameter were cut from fresh lettuce leaves that were grown in a greenhouse without any fertilizer and pesticide application at Yozgat Bozok University. One milliliter of a suspension of treatment, giving 0.16 µg/cm^2 dose, was applied with a micropipette (Rainin Pipet-Lite XLS) and spread on the disc surface using a glass rod. The leaf discs were left to dry for 15 min at room temperature. Each disc was transferred into a Petri dish and then a 3rd stage larva was placed into the dish. Prior to transferring into Petri dishes, the larvae were starved for 3 h. The larvae were incubated under the abovementioned conditions. Each day, newly treated leaf discs were provided to larvae for 10 days. The mortality was recorded every 24 h for 10 days. The experiment was set up in a randomized block design and each block contained all treatment and control groups. The experiment was repeated on three different dates and a total of 210 larvae were used.

The dose-response bioassay was carried out with F17 and F18 formulations based on the single-dose bioassay. The suspension was prepared to give 0.0083, 0.01, 0.04, 0.08, 0.12, 0.16 µg/cm^2 doses. Third instar stage larvae were obtained from the stock culture and they were placed individually in 90-mm Petri dishes. The larvae were starved for 3 hours prior to transferring the leaf discs. The leaf discs were treated as described above and incubated at room temperature for 15 min. A disc was transferred into a Petri dish and the larva was incubated under the above conditions for 10 days. Each day, a freshly treated leaf disc was provided to each larva. The experiment was set up using a randomized block design and each block contained all tested doses and control. The experiment was repeated on five different dates. A total of 350 larvae were used for each formulation.

2.5. Statistical analysis

The single-dose contact and stomach toxicity data were calculated as a percentage and then normalized using arcsin transformation. The data were then subjected to variance analysis (ANOVA) ($p \leq 0.05$) and then the Tukey test ($p \leq 0.05$) for differentiating treatments using

SPSS® 20 statistical software program. Lethal dose and lethal concentration values were calculated with a 95% confidence invertal by probit analysis. The statistical analysis was performed with SPSS® 20 program (Zhang et al., 2021).

3. Results

3.1. Contact toxicity of tea tree oil formulations against *Spodoptera littoralis* larvae

All tested tea tree oil formulations and TTO caused some mortality in the Egyptian leafworm larvae. The mortality rates of various developmental stages of *S. littoralis* larvae are presented in Table 2. The mortality rates varied between stages as high mortality rates were observed in early larval stages, e.g., 2nd and 3rd instar larvae, the rates drop to around 40% for the most toxic formulations. The formulations F14, F15, and the tea tree oils appeared to be more toxic to tested stages compared with other formulations. The mortality rates of the larvae increased in parallel to the extension of the incubation time (data not presented). The mortality rates of larvae recorded for 72 h are presented in Table 2. This time period was presented because the mortality rates did not increase after 72 h.

Tea tree oil and all tested formulations caused some mortality ranging from 33% to 100% in the 2nd stage of the Egyptian larvae and there were significant differences between the toxicity of the formulations after 72 h ($F = 74.39$; df: 6, 56; $p < 0.05$). TTO, F14, F15, and F17 formulations produced the greatest contact toxicity in the 2nd instar stage larvae with 100%, 99%, 90%, and 96% mortality rates, respectively.

A similar trend was also observed in the 3rd instar larvae but the toxicity of the formulations increased for F16 and F18 formulations in this stage. TTO produced the highest (100%) contact toxicity and killed all tested larvae after 72 h. It was followed by F14 with 92% mortality. F15, F17, F16, and F18 also showed considerable contact toxicity with mortality rates over 70%, but they were significantly less toxic than TTO in this stage ($F = 51.47$; df: 6, 35; $p < 0.05$).

Although TTO killed all the tested larvae after 72 h in the 4th stage larvae, the other formulations' efficacy decreased as compared to the previous stages. Among the formulations, F14 caused the greatest mortality but it was 58% and significantly lower than TTO toxicity ($F = 79.86$; df: 6, 56; $p < 0.05$). F15 toxicity was nearly halved and the mortality decreased to 39%. The most dramatic decrease in toxicity of the formulations was observed in F16 and F18 formulations as their mortality rates dropped from 76% and 69% to 8% and 5%, respectively.

TTO and the tested formulations caused some mortality on the 6th stage larvae but their toxicity to the insect decreased in this stage. TTO was the most toxic among treatments with 46% mortality, but its efficacy was nearly halved in this stage. A similar decrease in mortality rates was also observed for all formulations. F14 was the most toxic formulation with 35% mortality and it was followed by F15 with 23% mortality. F16 and F18 produced rather low mortality. There were significant differences between the toxicity of formulation to this stage larvae ($F = 5.89$; df: 6, 35; $p < 0.05$) (Table 2).

The dose-response bioassay with TTO, F14, and F15 formulations confirmed the results of the single-dose toxicity test. TTO was the most toxic formulation among the tested treatments with the lowest LD_{10}, LD_{50}, and LD_{90} values with 0.006 µg/larva, 0.016 µg/larva, and 0.042 µg/larva, respectively. It had also the steepest slope with 3.13. Although the F14 LD_{10} value (0.017 µg/larva) was not significantly different from TTO LD_{10}, LD_{50}, and LD_{90} were significantly higher than TTO LD_{50} and LD_{90} ($p \leq 0.05$).

Table 2. Contact toxicity of tea tree oil formulations on various stages of *Spodoptera littoralis* larvae after 72 h of incubation.

	% Mortality ± SE*				
Treatment	2nd instar	3nd instar	4th instar	5th instar	6th instar
Control	0.00 ± 0.0 e[a]	0.00 ± 0.00 c	0.00 ± 0.00 e	0.00 ± 0.00 b	0.00 ± 0.00 c
TTO	100.00 ± 0.00 a	100 ± 0.00 a	100 ± 0.00 a	53.42 ± 0.46 a	46.13 ± 1.00 a
F14	98.66 ± 0.59 ab	91.72 ± 0.39 ab	58.06 ± 0.20 b	50.00 ± 0.96 ab	34.54 ± 2.43 ab
F15	89.90 ± 0.49 b	89.20 ± 0.62 b	38.91 ± 0.49 bc	49.74 ± 0.51 ab	22.87 ± 1.10 ab
F16	66.50 ± 0.31 c	76.43 ± 0.63 b	8.20 ± 0.59 d	36.34 ± 0.20 ab	5.08 ± 1.14 bc
F17	95.81 ± 0.66 ab	87.69 ± 0.67 b	19.67 ± 0.44 cd	56.58 ± 0.71 a	13.60 ± 0.79 abc
F18	33.33 ± 0.85 d	68.67 ± 1.07 b	5.60 ± 0.63 de	23.28 ± 2.90 b	9.00 ± 1.03 abc

[a] Different lowercase letters following the averages in the same column indicate that the means are statistically significantly different (ANOVA $p < 0.05$, Tukey test)

* Standard error

There was no significant difference between LD_{10}, LD_{50}, and LD_{90} values of F14 and F15 formulations while F14 had a steeper slope than F15 (Table 3).

3.2. Stomach poison toxicity of tea tree oil formulations against *Spodoptera littoralis* larvae

The mortalities caused by ingestion of TTO and its formulations are presented in Table 3. After 1 and 2 days of the treatment, the tested formulations did not cause any mortality (Data is not presented). Formulation F17 and F18 produced some mortality (7% and 2%, respectively) but there was no significant difference between treatments after 3 days ($F = 3.73$; df: 6, 203; $p < 0.05$). A similar trend was also observed after 4 days; thus, the mortality rates regarding the initial 4 days are not presented in Table 4.

On day 5, the treatments F17, F18, and F16 caused 35%, 21%, and 21% mortalities in the 3rd stage larvae and they were significantly different from that of the control group ($F = 5.33$; df: 6, 203 $p < 0.05$). Interestingly, TTO treatment did not cause any mortality at this time interval. The efficacy of the tested formulations increased in parallel to the incubation period. The F17 formulation killed half of the tested larvae while the F18 formulation caused around 40% mortality. These two formulations also caused the highest mortality on day 7. This trend continued until the end of the experiment. On day 10, the greatest stomach poison effect was observed in the F17 formulation with a mortality rate of 75% and it was followed by the F18 formulation with a mortality rate of 66%. These treatments were significantly different ($F = 6.12$; df: 6, 203 $p < 0.05$) (Table 4) from TTO and the control group on day 10.

Stomach poison dose response bioassays were performed with the F17 and F18 formulations that showed the greatest stomach poison effect during 10 days of the incubation period. The calculated LC_{10}, $LC_{50,}$ $LC_{90,}$ and slope values of the F17 and F18 formulations are presented in Table 5. While there was no significant difference between calculated LC_{10} and LC_{50} values of the formulations ($p \geq 0.05$), LC_{90} values were significantly different ($p \leq 0.05$). The F17 formulation appeared to be more toxic to the larvae than the F18 formulation as it has 0.123 μg/cm^2 LC_{90} value and a steeper slope compared to the F18 formulation.

4. Discussion

The study showed that different formulations of tea tree oil have both contact and stomach poison effects against *S. littoralis* larvae. The biological activity of tea tree oil against fungi, bacteria, and insects was reported in previous studies (Braga et al. 2020; Chidi et al. 2020; Lee & Oh, 2020; Tavares et al., 2020; Iseppi et al., 2020; Roana et al., 2021; Zimmermann et al., 2021). Tea tree oil contains high amounts of terpinene4-ol, γ-terpinene, 1.8-cineol, 1.8-cineol, and α-terpinene components, and these are known to have biological activities against various pest species (Hammer et al., 2003; Liao et al., 2017; Brun et al., 2019; Sevik et al., 2021). Liao et al. (2017) reported the acetylcholinesterase (AChE) and glutathione S-transferase (GST) inhibitory effect of tea tree oil. The current results are in parallel to previous ones (Abbassy et al., 2009; Pavela, 2014) and the presence of these components is thought to be related to the toxicity of tea tree oil formulations against *S. littoralis* larvae.

In the single-dose toxicity studies, there were significant differences between the efficacies of different formulations of tea tree oil against *S. littoralis* larvae. TTO, F14, and F15 formulations showed the highest contact toxicity against different larval stages of *S. littoralis*. The main component of these formulations was terpinen- 4-ol for F14 and monoterpinens from tea tree oil for F15. The toxicity of these components to other insect species, e.g., *S. zeamais* (Liao et al., 2016; Yang et al., 2020), *Helicoverpa armigera* (Hübner, 1808) (Lepidoptera: Noctuidae) (Liao et al., 2017) were reported. In the stomach poison study, F17 and F18 formulations showed the greatest toxicity. The main component of F17 and F18 were linalool and eugenol, respectively. Eljazi et al. (2017) stated that linalool had

Table 3. Dose-response results of TTO, F14, and F15 formulations in 3rd stage larvae of *Spodoptera littoralis* after 24 h.

Treatment	LD_{10} (μg/larvae) (%95 CI*)	LD_{50} (μg/larvae) (%95 CI)	LD_{90} (μg/larvae) (%95 CI)	Slope ± SE	X^2
TTO	0.006 (0.005–0.007)	0.016 (0.014–0.018)	0.042 (0.036–0.050)	3.13 ± 0.229	7.66
F14	0.017 (0.013–0.021)	0.046 (0.041–0.052)	0.122 (0.101–0.161)	4.02 ± 0.410	8.85
F15	0.026 (0.019–0.031)	0.076 (0.056–0.091)	0.223 (0.164–0.365)	2.65 ± 0.345	2.79

* CI = Confidence interval

Table 4. Stomach poison toxicity of TTO and its formulations against 3rd instar larvae of *Spodoptera littoralis* over time.

% Mortality ± SE*						
Treatment	**5th day**	**6th day**	**7th day**	**8th day**	**9th day**	**10th day**
Control	0.00 ± 0.00 b[a]	0.00 ± 0.00 c	0.00 ± 0.00 c	0.27 ± 1.17 c	0.27 ± 1.51 c	0.27 ± 1.77 c
TTO	0.00 ± 0.00 b	1.09 ± 0.52 bc	6.69 ± 31.77 bc	6.69 ± 2.11 bc	12.84 ± 2.07 bc	20.61 ± 2.02 bc
F14	4.32 ± 0.97 ab	12.84 ± 1.51 abc	12.84 ± 0.27 bc	12.84 ± 0.27 bc	20.61 ± 0.27 bc	34.54 ± 0.27 ab
F15	6.69 ± 1.17 ab	16.54 ± 1.65 abc	29.66 ± 1.51 abc	29.66 ± 1.51 abc	29.66 ± 1.77abc	34.54 ± 2.02 ab
F16	20.61 ± 1.77 a	29.66 ± 1.96 ab	34.54 ± 1.96 ab	34.54 ± 1.96 ab	39.60 ± 1.96 ab	44.77 ± 2.02 ab
F17	34.54 ± 2.02 a	50.00 ± 2.11 a	65.45 ± 2.02 a	70.33 ± 2.02 a	75.00 ± 2.07 a	75.00 ± 2.10 a
F18	20.61 ±1.77 a	39.60 ± 2.07 a	44.77 ± 2.02 ab	50.00 ± 1.96 ab	60.39 ± 1.87 ab	65.45 ± 1.87 ab

[a]Different lowercase letters following the averages in the same column indicate that the means are statistically significantly different (ANOVA $p < 0.05$, Tukey test)
* Standard error

Table 5. Stomach poison dose-response bioassay results of F17 and F18 formulations in 3rd stage larvae of *Spodoptera littoralis* (Boisduval) after 10 days.

Treatment	**Number of tested insects**	**LC_{10} (µg/cm²) (%95 CI*)**	**LC_{50} (µg/cm²) (%95 CI)**	**LC_{90} (µg/cm²) (%95 CI)**	**Slope ± SE**	***X*²**
F17	350	0.006 (0.002–0.010)	0,027 (0.19–0.035)	0.123 (0.084–0.241)	3.06 ± 0.487	0.99
F18	350	0.007 (0.002–0.013)	0.042 (0.030–0.057)	0.237 (0.139–0.79)	2.34 ± 0.462	0.48

* CI = Confidence intervals

high insecticidal activities against various insect species. Pure eugenol toxicity was reported to be more toxic than it was used as a component of essential oil (Prates et al., 2019).

The difference between the formulations could cause variation both in contact and stomach poison toxicity against the Egyptian leafworm larvae. Similar variations were observed in the toxicity studies of other essential oils components against different insect species (Kim & Park 2008; López et al., 2008; Cardiet et al., 2012; Kim & Lee 2014). Yıldırım et al. (2013) tested terpinen4-ol, α-terpinene, and 1,8-cineol against *S. zeamais* and reported varying insecticidal activities. Similarly, Saad et al. (2018) reported different efficacy of essential oil components against *Sitophilus oryzae* (Linnaeus, 1763) (Coleoptera: Curculionidae).

The tested formulation showed similar toxicity in various stages, e.g., in the 2nd, 3rd, and 4th stage larvae. TTO, F14, and F15 formulations in contact toxicity showed similar effects in different larval stages. Generally, in single-dose screening tests, the dose is kept constant in different larval stages (Alouani et al., 2009; Chegini & Abbasipou 2017). In the current study, the dose was increased depending on the larval stage. It is thought to produce a more reliable comparison of different formulations on various stages of the targeted insect pests since there were many reports showing that the efficacy of the tested compound decreased as the larval stages increased (Karakoç & Gökçe 2012; Alkan et al., 2017; Karakoç et al., 2020). In the current study, it was observed that mortality in the later larval stages, e.g., 6th stage larvae, was lower compared to early stages. This result could be related to the preparation of the larvae for the pupal stage as the physiology of insects is dramatically altered in the final larval stages (Davidowitz & Nijhout 2004). This could explain the lower efficacy of the tested formulations in the 6th stage larva.

There was a difference between the contact and stomach poison toxicity of the same formulations. The most dramatic difference was observed in the TTO formulation. While it produces the greatest toxicity in the contact toxicity test, it was the least toxic formulation in the stomach poison toxicity test. That is also true for the F14 and F15

formulations. This could be the result of morphological and physiological differences between the application sites. The digestive system has similar layers to the insect exoskeleton except for the midgut where possibly the active component of the formulation was absorbed in the stomach poison toxicity test. That could explain the differences in the same formulations' efficacy in different tests (Catae et al., 2014; Aljedani et al., 2017).

In contact toxicity dose-response studies, TTO has the greatest toxicity against *S. littoralis* larvae than other tested formulations including F14 and F15. This formulation contains multiple components of the tea tree oil, which lead to a synergistic effect of different components or cumulative effects of the components. Similar results were reported in the literature showing the toxic effects of tea tree oils' superiority over its components (Machial et al., 2010; Pazinato et al., 2014; Birol, 2015; Liao et al., 2017).

The possibility of using tea tree oil formulations against *S. littoralis* as contact and stomach poison insecticides was tested under laboratory conditions. Although the biological activity of tea tree oil against fungi, bacteria, and insects was reported in previous studies, the present study showed the contact and stomach toxicities of different components as formulations against *S. littoralis*. This is the first research reporting the biological activities of tea tree oil components against the Egyptian cotton leafworm. Additionally, the study shows that TTO and the formulations produced toxic effects on *S. littoralis* larvae in different ways. In particular, pure tea tree oil and F14 and F15 formulations caused contact toxicity on larvae, and F17 and F18 formulations produced stomach poison effect. The dose levels required to ensure effective control of the targeted pest were also calculated for the first time with the most promising formulations. These data could provide the first concrete step in the use of tea tree oil and its formulations against the major pest of *S. littoralis* both in conventional and organic agriculture. When the current status of the Egyptian cotton leafworm is taken into consideration, this study could provide some solutions in line with the integrated pest management program. Moreover, the result could contribute to the management of insecticide resistance of this major pest species.

The TTO and the formulations could be further developed and registered as a plant-based control tool. However, the current study was performed under laboratory conditions and produced promising results. The study showed that TTO and the formulations are toxic to the *S. littoralis* and have the potential to control this destructive pest.

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