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Sublethal Effects of Some Essential Oils on the Developmental and Reproduction of the Spodoptera littoralis (Boisduval)

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ABSTRACT

The cotton leafworm *Spodoptera littoralis* (Boisd.); is one of the most destructive pests. It can infest more than 120 economically important crops all year round and causing a great loss. The management of *S. littoralis* has been typically carried out by chemical pesticides, however, there has been an increasing interest in natural products, particularly those of plant origin, to control this pest. Plant essential oils (EOs) were tested for *S. littoralis* larvae. Responses varied according to EOs species and all EOs were proved to be toxic to the larvae. However, the highest mortality was observed in the oils of orange, sesamum, and camphor. The slope values indicated that the insect population was relatively heterogeneous in their susceptibility to tested EOs. Our results showed LC_{50} value, the range of toxicity was in the decreasing order f**or** orange> sesamum> camphor> pepper> pumpkin against both *S. littoralis* 2nd and 4th instars. The influence of LC_{25} values of biological parameters manifested in decreased the adult emergence and prolongation of the larval and pupal period. The percentage of larvae that survived and succeeded to pupate decreased the result of treatment by LC_{25} values. Longevity, fecundity, fertility, and natality were monitored in the surviving adults. The most significant effect of EOs was determined with respect to natality. Compared to the control, all the tested EOs caused a significant reduction in natality. While 1210.2 viable larvae were obtained in the control, the number of viable larvae obtained from those treated was lower, ranging from 35.8 % (for orange oil) up to 81.9 % (for pumpkin oil). Based on the presented results, EOs, orange, sesamum, and camphor. Generally, sublethal effects of EOs exhibit significant effects on the population dynamics of *S. littolaris* where, reduction of adult natality by more than 75 % in this pest.

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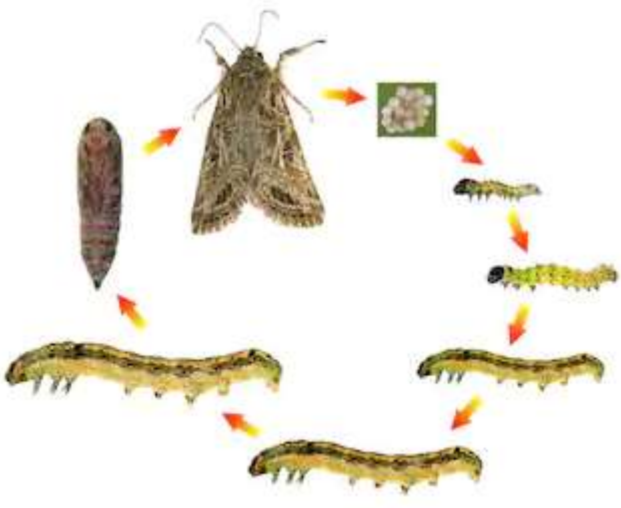
GRAPHICAL ABSTRACT



1. INTRODUCTION

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is a highly polyphagous destructive pest insect which seriously harms of many economically crucial crops in addition, it has high reproductive potential. Causing great losses in quantity and quality of the attacked crops and thereby significantly dropping the marketable yield [1]. Although the control of *S. littoralis*, is largely depending on the use of neurotoxic insecticides. But, difficult to control because of the insect's high capacity to develop resistance to the most of chemical pesticides. In addition, several environmental, health hazards and negative effects on domestic animals, and wildlife of chemical pesticides. Recently, the focus on many researchers targeted to find out new substances of plant origin, safe for the environment and health, which would become a suitable foundation of botanical pesticides thanks to their novel mechanism of action, and which will be able to resolve the present problems of plant protection, i.e. that such pesticides will be environmentally friendly, safe for the health, and will prevent any emergence of resistant pest populations. Essential oils (EOs) are generally considered as substances that may be suitable for the development of botanical insecticides thanks to their relative non-toxicity for homeothermic animals and high biological efficacy. The composition of EOs varies from every plant species and on the growth stage of the plant. EOs are formed by a mixture of several up to dozens of mono-, di-, sesqui-terpenes are the most representative molecules constituting 90% and allow a great variety of structures with diverse functions [2, 3]. At present, approximately 3,000 EOs are known, 300 of which are commercially important, However, various EOs and their constituents are recorded to show acute toxic effects against more than 200 species of insect-pests. The effect on octopamine receptors has been considered the principal mechanism of action of monoterpenes and phenols (as the major components of EOs), which block the octopaminergic nervous system as the site-of action in insects [4, 5, 6]. Different EOs had been used as antifeedant, repellent, fumigant, larvicide, ovicidal and adulticidal against different insect orders [7, 8, 9]. Also, lethal and sublethal doses of EOs significantly reduce both the percentage of surviving larvae as well as their fecundity and fertility [10, 11]. EOs may play an increasingly prominent role in pest management as an alternative to synthetic pesticides. Therefore, an excellent application is necessary in botanical insecticides based on EOs, to ensure the pests encounter direct contact with the fatal dose of the active substance. The importance of EOs is mainly recognized in the field of agriculture as they are safe, less hazardous, and cheap.

The aim of our study was to assess the toxicity of the tested EOs, against the second and fourth instars larvae of *S. littoralis*. Also, effect of sublethal concentration of EOs on the development of surviving larvae and on the subsequent fertility, fecundity, and natality of *S. littoralis* adults.

2. EXPERIMENTAL

2.1. Experimental insects

The larvae of the cotton leafworm (*S. littoralis*) was obtained from a laboratory population. The culture was reared under laboratory conditions on fresh castor beans leaves (*Ricinus communis* L.) and under controlled conditions at 25 ± 1 °C, ≈70% RH, with a 16: 8 L: D photoperiod. This experiment was performed with pre-weighed newly molted *S. littoralis* L2-larvae & L4-larvae.

2.2. Essential oils (EOs)

The EOs from the study were purchased from the Department of Pharmacology, Faculty of Pharmacy, University of Alexandria, Egypt (Table 1). The EOs were selected based on their safety for human and environment, in addition to the criterion of their biological efficacy, their commercial availability, and price.

Table 1. Investigated EOs

Scientific name	***Family***	***Common name***
Citrus sinensis	Rutaceae	Orange
Cucurbita pepo	Cucurbitaceae	Pumpkin
Capsicum annuum	Cucurbitaceae	Pepper
Sesamum indicum	Pedailiaceae	Sesamum
Eucalyptus camaldulensis	Myrtaceae	Camphor

2.3. Bioassays

2.3.1. Toxicity

The toxicity of EOs, measured as mortality after 48 hours of exposure, was determined by dipping technique to early 2nd & 4th larval instars of *S. littoralis*. The stock solutions to EOs (Table 1) were formulated as an emulsion in water containing 0.5% triton X-100. A series of five concentrations of each EOs was tested immediately after preparation. The fresh castor bean leaves were dipped in each concentration of the EOs for 20 seconds and left to dry before being offered to *S. littoralis* larvae. Fifty larvae distributed among five replicating (10 larvae/replicate), were used for each concentration. Larvae were fed on leaves immersed in only water containing 0.5% triton X-100 as a control. The glass jars were placed for 48 hours of a growth chamber (L16:D9, 25°C). Death was recorded when the larvae did not respond to prodding with forceps.

2.3.2. Sublethal effects

The LC_{25} estimated in Table 2 was chosen to determine the effect of sublethal of the EOs on some biological aspects *S. littoralis*. The method was processed by the same means as those stated in paragraph “Toxicity”. Fifty 4th instar larvae were always selected for every variant and control. The larvae were left for 24 hrs in separated cages with treated leaves. The surviving larvae were maintained in clean glass jars, covered with a perforated cap that ensured sufficient air circulation during the whole period of larval development. Then checked daily to recorded larval and pupal duration, pupal weight, adult emergence%, adult longevity, and sex ratio. Newly-adults, which separated one female and one meal of emerged adults were placed together to maximize successful mating in wood box were used as breeding containers and were fed a 10 % sugar solution. Their oviposition was allowed on a filter paper placed inside the wood box. The number of eggs laid per single female of *S. littoralis* was supervised each day. In order to determine the fertility of the adults, 100 freshly-laid eggs were taken on the first 3 successive days of oviposition. The eggs were then placed in plastic dishes (diameter of 12 cm and height of 6 cm) and the number of emerging larvae was evaluated. Adult fertility was expressed as a percentage of larvae born from the eggs. Natality, being an important indicator of the reproductive capacity of the population, was calculated using the formula [12]:

$$NAT = \frac{(ExF)}{L}$$

Where: E is the sum of all eggs oviposited by the adults during their lifetime; F is fertility percentage; and L is the number of larvae entering the experiment. Natality inhibition was expressed as the percentage of reduced natality of one generation of the larvae treated with LC_{25} values of EOs, compared to the control.

2.4. Statistical analysis

The data were corrected using Abbott’s formula [13] for the mortalities. Then, using the computed percentage of mortality versus corresponding concentrations, Probit analysis was adopted according to [14] using the SPSS 20.0 Software Package (SPSS Inc., Chicago, IL, USA). This yields determination of the toxicity indices (LC_{25} and LC_{50}) as well as the related parameters (95% confidence limits, and slope) for established toxicity regression lines. Obtained data were statistically analyzed using One-way ANOVA followed by the Tukey’s HSD test in JMP 11.1.1. (SAS Institute Inc. 2013, Cary, NC, USA).

3. RESULTS AND DISCUSSION

3.1. Toxicity

Toxicity of EOs on 2nd & 4th larvae instars of *S. littoralis* is presented in Table 2. Based on the estimate LC_{50} values, EOs of orange, sesamum and camphor exhibited the highest efficiency followed by pepper and pumpkin oils. Where, orange oil proved to be the most toxic to both 2nd & 4th instars larvae; the corresponding LC_{50} values was 9.933 & 14.492 ppm, respectively followed by sesamum and camphor. By comparison, the other two oils showed low toxicity LC_{50} values of 26.114 & 38.758 and 30.014 & 40.130 for pepper and pumpkin oils, respectively. Concerning the relative potency levels based on the LC_{50} values, the larvacidal activity values of orange, sesamum, camphor, and pepper oils were 3.02 & 2.77, 2.20 & 1.82, 1.48 & 1.30 and 1.15 & 1.04 times as toxic as a larvacidal action of pumpkin oil against the 2nd & 4th larvae instars of *S. littoralis*, respectively. All tested EOs, proved to be toxic to the cotton leafworm, although they differed with their efficacy. In addition, results showed that the toxicity of EOs decreased with the advancement of larval instar where second instar larvae were more sensitive than the fourth instar larvae. The toxic and repellent effects of the main constituents of these oils, have been demonstrated by other researchers, [15] showed that orange oil was exhibited toxicity against the larvae (L_3) of *S. littoralis* because the toxic effect of orange oil upon insect pest may be due to the high percentage of limonene components (92 – 96 %) in its constituents. Limonene component was reported as a toxic agent against some insect pest. Many other EOs was shown to be active on *S. littoralis* larvae as *Artemisia absintium* [16], *Slavia officinalis* [17] also many studies had been conducted showing the sensitivity of other pest with various EOs, [18, 19, 20].

Table 2. Insecticidal activity of EOs against 2nd & 4th larval instars of *Spodoptera littoralis*

	2nd instar						
EOs	***Toxicity parameters***						
	LC_{25} (ppm)	LC_{50} (ppm)	Confidence limits 95%		Slope ± SE	Toxicity Index	Relative potency levels
			Lower	Upper			
Orange	1.42	9.931	6.570	12.44	0.97±0.234	100	3.02
Sesamum	2.80	13.626	7.622	20.42	0.95±0.281	72.88	2.20
Camphor	4.25	20.214	7.029	10.276	1.70±0.193	49.13	1.48
Pepper	5.17	26.114	13.093	36.267	1.77±0.204	38.03	1.15
Pumpkin	7.39	30.014	17.569	44.308	1.52±0.681	33.09	1.0
	4th instar						
EOs	***Toxicity parameters***						
	LC_{25} (ppm)	LC_{50} (ppm)	Confidence limits 95%		Slope ± SE	Toxicity Index	Relative potency levels
			Lower	Upper			
Orange	8.24	14.492	10.411	15.203	1.41±0.599	100	2.77
Sesamum	9.74	21.996	18.810	25.418	1.84±0.201	68.88	1.82
Camphor	13.02	30.986	22.883	33.113	1.28±0.139	46.77	1.30
Pepper	16.58	38.758	33.456	42.182	1.98±0.455	37.93	1.04
Pumpkin	24.83	40.130	38.597	61. 539	1.68±0.936	36.11	1.0

3.2. Sublethal effects

The sublethal effects of EOs on main biological aspects are shown in Table 3 & 4. Data in Table (3) clarified that the larvae that survived LC_{25} application and finished their larval development transformed to pupae without any observable abnormalities. All EOs caused mortality (larvae and pupae) significantly higher compared to the control (Table 3). The highest larval and pupal mortality were found for orange oil (67.7 % and 22.7 %, respectively) and the lowest for pumpkin oil (26.0 % and 7.6 % respectively). However, although good transformation to the pupal stage occurred, subsequent mortality of the pupae reached 18 - 22 % in the treated variants, which is a value significantly higher compared to the control (4.1 %). All EOs showed significant prolongation in larval and pupal duration. In addition, all EOs significantly reduced the weight of the pupae compared to control. There was a remarkable decrease in pupal weight as it was 167.2 mg of orange oil comparing to 288.0 mg of control. The number of emergence adults was expressed as the percentage of viable adults out of the total number of larvae entering the experiment treated with LC_{25} of all EOs caused a significant reduction in the number of adults compared to the control, where 98 % of adult emergence of the pupae. However, the lowest number of emergence adults after treatments of EOs, orange (67.9 %), sesamum (76.6 %), and camphor, (84.5 %). The highest number of emergence adults after treatments of the two oils pumpkin (92 %) and pepper (90.8 %). It is obvious that the larval stage was the most sensitive one than the other stages pupae and adults), where it became too long as a latent effect of EOs. The sex ratio significantly decreases at all treatments.

The emergence, adults mated without any apparent abnormal behavior. No significant difference in fecundity was found between individual treatments, i.e., in the number of eggs per female (Table 4). Every female hatched 2136 eggs, on average, in the control. After treatment with EOs, orange, sesamum, and camphor, females did oviposit approximately 400 - 700 fewer eggs; however, this inhibition was not statistically significant ($P<0.05$). The effect of EOs on fertility is shown in the same Table. Only the two EOs orange, and sesamum, caused significantly ($P<0.001$) low fertility (74.6 and 79.3 %, respectively) compared to the control (97 %). It is possible that EOs influences the physical condition of females, thus reducing reproductive capacity. These data agree with those of [10, 11]; who suggested the lethal and sublethal doses of EOs significantly reduce both the percentage of surviving larvae as well as their fecundity and fertility.

The obtained results (Table 4) showed that in the control, longevity of the adults (males and females) 4.6 and 5.4 days, respectively. EOs orange, sesamum, and camphor reduced longevity considerably, by 2 – 3.5 days for males. Also reduced longevity of the females range 3 – 3.9 days; but this reduction cannot be considered significant. Natality of the generation entering the experiment is shown in Table 4. Compared to the control, all the tested EOs caused a significant reduction in natality ($P<0.001$). While 1210.2 viable larvae were obtained in the control, the number of viable larvae obtained from those treated was lower, where inhibition of larvae ranging from 35.8 % (for pumpkin oil) up to 81.9% (for orange pumpkin oil). The data show that the effect of sublethal concentration on reduced natality of the next generation of the important polyphagous pest S. littoralis. It could be concluded that there is a positive correlation between the toxicity of the EOs at LC25 value and influence behavior of S. littoralis. The aforementioned results suggested that sublethal concentration of each EOs may be interfered with physiological balances and hormonal changes of the insect thereby affecting the S. littoralis from through the development time lengthened, indicating that EOs could be accumulated in the larval body; thus, a cumulative toxicity against S. littoralis was exhibited. There are many searches interested to study the role of EOs in reducing the damage to cotton leafworm and confirm with the obtained results of [7, 21, 22, 23] whom reported negative impacts on different EOs on insect biological parameters which suppressed the population of the target pest.

Table 3. Biological aspects of *S. littoralis* resulted from treated 4th instar larvae with EOs

EOs	Mortality (%)		Duration (days)		Pupae weight (mg)	Emergence of adults (%)	Six ratio (%)
	Larvae	Pupae	Larvae	Pupae			♂ : ♀
Orange	67.7^{e} ± 1.732	22.7^{c} ± 1.64	19.5^{a} ± 1.443	18.5^{a}± 1.155	167.2^{e} ± 3.2	67.9^{d} ± 1.8	0.94 : 1
Sesamum	64.6^{e} ± 1.547	20.1^{c} ± 1.04	18.0^{a} ± 1.732	18.0^{a}± 1.368	210.5^{d} ± 1.9	76.6^{c} ± 1.5	1.0 : 1
Camphor	51.3^{d}± 1.155	18.9^{b} ± 1.34	16.5^{a} ± 0.866	17.0^{a}± 1.663	229.7^{c} ± 3.1	84.5^{b} ± 2.4	1.2 : 1
Pepper	30.1^{c} ± 1.259	9.4^{a} ± 1.21	15.0^{a} ± 0.86	13.5^{a} ± 1.299	269.5^{b} ± 2.4	90.8^{a} ± 0.348	1.5 : 1
Pumpkin	26.0^{b} ± 1.21	7.6^{a} ± 1.10	14.5^{a} ± 1.52	13.0^{a}± 1.212	275.8^{b} ± 2.7	92.0^{a} ± 0.454	1.8 : 1
Control	2.4^{a} ± 0.289	4. 1^{a} ± 0.87	14.2^{a} ± 0.577	12.5^{a} ± 0.57	288.0^{a} ± 3.4	98^{a} ± 0.47	2.1 : 1

The data in the table are the means ± SE.
ANOVA parameters. Means followed in the same column by the same letter are not significantly different ($P \leq 0.05$; Tukey's HSD test)

CONCLUSION

In conclusion, the EOs are the promising materials which can be used as an important alternative for pest control because of their environmental and mammal's safety properties, reduce as possible the harmful and risks associated synthetic insecticides. From the results of the present study, could be concluded that the sublethal doses of tested EOs (orange, sesamum, pepper and camphor) possess toxic effect on all stages of *S. littoralis*. From this point of view could be recommending usage of the mentioned EOs for controlling the cotton leafworm as a biological control method throughout the integrated pest control program.

Table 4. Effect of EOs on longevity of adults, fecundity, fertility, and natality of *Spodoptera littoralis*.

EOs	Longevity of adults (days)		Fecundity indicators		Natality	
	♂	♀	Fecundity (No. eggs female)	Fertility (% egg hatchability)	Number of larvae/ larvae	Inhibition of natality (%)
Orange	2.0^{c}± 0.23	3.1^{c}± 0.25	1410.1^{f} ± 22.5	74.6^{c} ± 2.6	322.6^{d} ± 12.4	81.9^{d} ± 4.2
Sesamum	2.7^{bc}± 0.29	3.4^{c} ± 0.57	1576.5^{e} ± 23.09	79.3^{c} ± 3.5	387.7^{d} ± 21.9	75.7^{c} ± 2.9
Camphor	3.5^{ab}± 0.65	3.9^{bc}±0.12	1697.5^{d} ± 21.9	82.4^{b} ± 2.9	409.8^{c} ± 14.7	69.5^{b} ± 2.6
Pepper	4.0^{a}± 0.58	4.1^{b}± 0.49	2004.6^{c} ± 11.54	90.5^{a} ± 4.2	752.4^{b} ± 23.2	39.6^{a} ± 1.8
Pumpkin	4.2^{a}± 0.37	4.4^{b}± 0.32	2077.3^{b} ± 14.7	93.7^{a} ± 3.5	795.0^{b} ± 26.7	35.8^{a} ± 1.5
Control	4.6^{a}± 0.33	5.4^{a}± 0.21	2136.0^{a}± 25.98	97.0^{a} ± 2.1	1210.2^{a} ± 22.5	0.0

The data in the table are the means ± SE.
ANOVA parameters. Means followed in the same column by the same letter are not significantly different (P ≤ 0.05; Tukey's HSD test)

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