

RESEARCH ARTICLE

TOXICITY OF SELECTED BOTANICALS TO THE COTTON LEAF WORM, SPODOPTERA LITTORALIS (BOISD.)

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Manuscript Info	Abstract		
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<i>Manuscript History</i> Received: 03 January 2017 Final Accepted: 04 February 2017 Published: March 2017	The sensitivity of <i>Spodoptera littoralis</i> 2 nd and 4 th instar larvae towards the essential oils (mint and lavender oils) and aqueous plant extracts (<i>Artemisia herba-alba</i> and <i>Catharanthus roseus</i>) were investigated under laboratory conditions and the effect of sublethal concentrations on the feeding deterrence were evaluated on the insect. Results revealed		
Key words:- Spodoptera littoralis, lavender oil, mint oil, Catharanthus extract, Artemisia extract, toxicity, feeding deterrency.	that 2^{nd} and 4^{th} instar larvae of <i>S. littoralis</i> were more susceptible to lavender oil than mintoil as it has higher LC ₅₀ values. In addition, the results showed that the mean feeding deterrence (FDI%) of essential oils and botanicals extracts was concentration-dependent. Therefore, these botanicals could be important as eco-friendly accessible pest control alternatives against <i>S. littoralis</i> and other closely related species.		
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Introduction:-

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is one of the most destructive pests of several crops such as cotton, corn, peanut, clover, vegetables and various fruits in Egypt as well as in Mediterranean and Middle East countries (El-Sinaryet al. 2008 and El-Zoghbyet al. 2011). The continuous and unwise use of insecticides to control agricultural pests usually lead to development of resistance, adverse effects on beneficial insects and residues in foods (Rizket al. 2010 and Ehab 2012). The essential oils and other plant extracts, as a new class of natural products for controlling insect pests environmentally friendly have begun to play an increasing prominent role as alternatives to synthetic insecticides (El-Sinaryet al. 2008; Tripathiet al. 2009 and Ragaei and Sabry 2011). The selected essential oils and the botanical extracts used in this study are among those compounds under investigation as potential biopesticides.

Materials and Methods:-

Insect rearing:-

The colony of cotton leafworm, *Spodoptera littoralis* (Boisd.), was obtained from the division of the cotton leafworm, Plant Protection Research Institute, Dokki, Egypt. Larval stages were reared on castor bean leaves at $27 \pm 2^{\circ}$ C and $65 \pm 5\%$ R.H. and photoperiod of 16:8 hr (L:D) as described by **El-Dafrawi***et al.* **1964**.

Commercial essential oils:-

Lavender oil and mint oil (containing carvone, menthol, menthone, sinod, kadenin and limonene as major constituents) were obtained from El Captain Company, Cairo, Egypt.

Preparations of aqueous botanical extracts:-

Healthy plants *Catharanthus roseus* (family: Apocynaceae) and *Artemesia herba-alba* (family: Asteraceae) were collected in the morning hours from the medicinal plant garden, Faculty of Pharmacy, Cairo university and after separating the leaves to test their insecticidal properties against *S. littoralis*, they were washed with distilled water and left to dry in the shade. Finally, they were transferred to an oven (70°C) for 24 hour and the dried leaves were blendered to make fine powder. Fifty gram of dried powder were stirred with 1 Liter distilled water for 1 hour and incubated for 48 hour at 4°C and then stirred for additional 1 h and filtered twice through whatman No.1 filter paper. The volume was made up to 500 ml and it was considered as stock solution of the extract. This stock extract was maintained in a refrigerator until being used and the diluted concentration of the extract were made up.

Insect Bioassay:-

Leaf-dip method as described by **Tabashniket al. 1991** was followed using castor leaves. Fresh castor leaves, of almost the same size, were dipped in different concentrations (0.1, 0.5, 0.75, 1, 1.5 and 2%) in case of treatment each of *S. littoralis* 2^{nd} and 4^{th} instars with essential oils but dipped in (1, 5, 10, 15, 20 and 25%) concentrations for botanical extracts treatments. The dipping lasted for ca. 5-10 seconds and left to dry in air from excess solution. The treated leaves were transferred singly in plastic cups where 10 individuals of 2^{nd} and 4^{th} instar larvae were allowed to feed on these treated leaves. Treated leaves were offered to larvae for 48 hrs. Three replicates of each concentration were performed. The untreated castor bean leaves (control) were dipped in distilled water for the same period of time as treated ones. Insect mortality were recorded daily starting after 24h from treatment. The experiment was conducted at laboratory temperature of $27 \pm 2 \circ C$, $70 \pm 5\%$ R.H. with photoperiod of 16:8 (L: D) hr.

The mortality % was corrected according to Abbott's formula (Abbott, 1925) as follows:

100 - control mortality %

Probit analysis was determined to calculate the median lethal concentration values (LC_{50}) and related parameters, according to **Finney** (1971).

Feeding deterrent activity (non-choice method):-

Feeding deterrent activity of the botanical solutions was assayed against *S. littoralis* 2^{nd} and 4^{th} instar larvae using a leaf-dip bioassay in no-choice situations. For this purpose the concentrations (LC₂₅ and LC₅₀) of essential oils and botanical extracts were prepared for each instar. The Leaf discs of (\emptyset = 8 cm) were impregnated for 5-10 seconds in each concentrations and the control leaf discs were impregnated in distilled water for the same time. In each plastic Petri dish (1.5 cm x 9 cm) wet filter paper was placed to avoid early drying of the leaf discs and ten larvae per replicate of either 2^{nd} or 4^{th} instar were introduced. Progressive consumption of leaf weight by the larvae after 24 hrs was recorded in control and treated discs. Amount of leaf eaten by the larva in essential oils and botanical extracts treatments was corrected from control. Three replicates were maintained for each treatment with 10 larvae per replicate (total, n= 30). Feeding deterrent activity was assessed by calculating the Feeding deterrence Index by the formula of **Saleh et al. 1986**:

Feeding Deterrence Index (FDI);

Statistical Analyses:-

Using the computed percentage of mortalities versus corresponding concentrations, Probit analysis was adopted according to **Finney (1971)** using a software computer program (**SAS**, 2002). This yields determination of the toxicity indices (LC₂₅ and LC₅₀) as well as the related parameters (95% confidence intervals, slope and Chi-square, χ^2) for established toxicity regression lines.

Obtained data were statistically analyzed using one-way analysis of variance (ANOVA) supported by Duncan's multiple range test (Duncan, 1955) running on Costat statistical software, 1990. Means were compared using L.S.D. (5% significance level).

Results and Discussion:-

Toxicity of tested botanicals to S. littoralis:-

Table (1 & 2) revealed that the LC₂₅ and LC₅₀ values were 0.179 and 0.379 %, respectively for lavender compared with 0.296 and 0.417 %, respectively for mint against 2nd instar larvae. The LC₂₅ and LC₅₀ values were 0.293 and 0.504 %, respectively for lavender compared with 0.39 and 0.839 %, respectively for mint against 4th instar larvae. The essential oils act at multiple levels in the insects, so the possibility of generating resistance is little probable (**Gutierrez** *et al.* **2009**). The main components of lavender oil were linalool acetate and linalool while the mint oil consisted of menthol in high percentage, menthone (*iso*), β -pinene, and menthyl acetate (**Karamaouna***et al.* **2013**).

In addition, the LC₂₅ and LC₅₀ values were 2.297 and 5.016 %, respectively for *Catharanthus* compared with 2.633 and 6.527 %, respectively for *Artemisia* extract against 2nd instar larvae. While the LC₂₅ and LC₅₀ values were 3.456 and 6.56 %, respectively for *Catharanthus* compared with 3.818 and 8.332 %, respectively for *Artemisia* against 4th instar larvae. The slope values indicated that the insect population was relatively heterogeneous in their susceptibility toward tested essential oils and botanical extracts by leaf-dip method. Our results showed LC₅₀ values, the range of toxicity was in the decreasing order of lavender> mint> *Catharanthus* > *Artemisia* against both *S*. *littoralis* 2nd and 4th instars.

The Artemisia sp. belonging to the important family Asteraceae (Compositae) has known to possess several important biological properties, such as insecticidal activity (Saleh 1984). Hifnawy et al. (2001) reported larvicidal activity of A. herba-alba against cotton leafworm, S. littoralis (Biosd.) larvae.

Among the plants found to contain insecticidal or growth regulatory effects of insects, plants from the genus *Ageratum* and *Artemisia* were reported to have insecticidal activity (**Anjoo and Ajay 2008**). *Artemisia herba-alba*, is rich in terpenoids like monoterpene hydrocarbons (**Behtariet al. 2012**), oxygenated monoterpenes (**Hudaib and Aburjai 2006**) and sesquiterpenes(**Laid et al. 2008 and Paoliniet al. 2010**). **Ramya et al. (2008) and Alaguchamy and Jayakumararaj (2015**) studied the effect of leaf aqueous extract of *C. roseus* and they recommended that it can potentially be used as ecofriendly bio-pesticide to control the devastating damage caused by larvae of *Helicoverpa armigera*.

Kumar and Yadav (2013) showed that screened phytochemical constituents of *Catharanthus roseus*(family: Apocynaceae) possesses carbohydrates, anthraquinone glycosides, flavanoids, saponins, and alkaloids. Also, the work on the isolation of a possible insect growth regulator (IGR) from *C. roseus* is in progress (Summarwar and Pandey 2015).

(-1)				
Phytochemicals	LC ₂₅ (Conc. %) 95% confidence	LC ₅₀ (Conc. %) 95%	Slope \pm SE	χ^2
	interval	confidence interval		
Mint	0.296 (0.12 - 0.52)	0.417 (0.34 - 0.67)	2.44 ± 0.31	4.17
Lavender	0.179 (0.12 - 0.37)	0.379 (0.26 - 0.77)	2.41 ± 0.35	2.13
Artemisia	2.633 (1.96 - 3.27)	6.527 (4.81 - 7.96)	1.71 ± 0.24	4.83
Catharanthus	2.297 (1.11 - 3.27)	5.016 (4.81 - 7.96)	1.98 ± 0.26	3.42
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Table (1): Toxicity indices (LC₂₅ and LC₅₀) of the essential oils (Mint and Lavender) and the botanical extracts (*Artemisia herba-alba* and *Catharanthus roseus*) against *Spodoptera littoralis*2nd instar larvae.

* LC₂₅ and LC₅₀ values are significant (p < 0.05) whenever confidence intervals do not overlap.

Table (2): Toxicity indices (LC₂₅ and LC₅₀) of the essential oils (Mint and Lavender) and the botanical extracts (*Artemisia herba-alba* and *Catharanthus roseus*) against *Spodoptera littoralis* 4^{th} instar larvae.

Phytochemicals	LC ₂₅ (Conc. %) 95% confidence	LC ₅₀ (Conc. %) 95%	Slope ± SE	χ^2
	interval	confidence interval		
Mint	0.390 (0.13 - 0.49)	0.839 (0.53 - 0.99)	2.96 ± 0.30	3.42
Lavender	0.293 (0.19 - 0.58)	0.504 (0.33 - 0.78)	3.02 ± 0.35	3.67
Artemisia	3.818 (1.75 - 6.29)	8.332 (6.00 - 11.17)	1.12 ± 0.24	1.52
Catharanthus	3.456 (1.97 - 5.78)	6.56 (4.40 - 7.71)	1.15 ± 0.23	3.41

* LC_{25} and LC_{50} values are significant (p < 0.05) whenever confidence intervals do not overlap.

Feeding deterrence activity:-

Data presented in table (3) showed that the mean feeding deterrence activity (based on feeding deterrence index values) was significantly different (P < 0.05) between lavender and mint oil treatments on 2^{nd} instar larvae at both

 LC_{25} and LC_{50} where mean feeding deterrent values at LC_{25} were higher in case of mint oil (68.369 %) than that in case of lavender oil (65.833 %) for four days after treatment. while for the same instar at LC_{50} levels, the mean feeding deterrent values were higher in case of lavender oil (79.151 %) compared to that in case of mint oil (75.272%). In connection with the 4th instar, also the mean feeding deterrent values were significantly different between lavender and mint oil treatments either at LC_{25} or LC_{50} where mean feeding deterrent values at LC_{25} were higher in case of mint oil (63.561%) than that in case of lavender oil (60.408 %) for four days after treatment. Also, at LC_{50} levels, the mean feeding deterrent values were still higher in case of mint oil (73.413 %) compared to lavender oil (70.837%) (table 3).

Depending on the data, the mint oil exhibited relatively more feeding deterrent effect than lavender oil treatments. The higher feeding deterrence index normally indicates decreased rate of feeding. Also, the *Mentha pulegium* oil significantly inhibits the feeding of fall armyworm, *Spodoptera frugiperda* (Zalkowet al. 1979). Any substance that reduces food consumption by an insect can be considered as antifeedant or feeding deterrent (Isman 2002).

Abd El-Galeil and Nakatani (2003) indicated that the antifeedant activity was dose-dependent in some of the isolated compounds. **Elumalaiet al. (2010)** reported that all tested essential oils are showed moderate antifeedant activity against 4th instar larvae of *S. litura*; however, the highest antifeedant activity was observed in the essential oils of *Cuminum cyminu, Mentha pipertia, Rosmarinus officinalis, Thymus vulgaris. Coriandrum sativum* exhibited (100%) complete antifeedant activity at 6 mg/cm².

Table (3): Percentage feeding deterrent indices (mean \pm SE) of *S. littoralis* 2nd and 4th instars larvae treated with LC₂₅ and LC₅₀ of essential oils (Mint and Lavender).

Treatment	LC ₂₅		LC ₅₀	
	2 nd instar	4 th instar	2 nd instar	4 th instar
Mint	68.369± 4.195 ^a	63.561± 3.895 ^a	75.272± 4.613 ^b	73.413± 4.504 ^a
Lavender	65.833± 4.116 ^b	60.408± 3.706 ^b	79.151± 4.850 ^a	70.837± 4.342 ^b
L.S.D.5%	2.173	2.199	2.217	2.249

*Within the same column, means followed by the same letter are not significantly different (P > 0.05).

While table (4) showed that the mean feeding deterrent activity (based on antifeedant index values) was significantly different (P < 0.05) between *Artemisia* and *Catharanthus* treatments on 2nd instar larvae at both LC₂₅ and LC₅₀ where mean antifeedant values at LC₂₅ were higher in case of *Catharanthus* (63.503 %) than that in case of *Artemisia* (56.46 %) for four days after treatment. Also, for the same instar at LC₅₀ levels, the mean antifeedant values were still higher in case of *Catharanthus* (70.903 %) compared to *Artemisia* (63.617 %).

In connection with the 4th instar, also the mean feeding deterrence values were significant different between *Catharanthus* and *Artemisia* treatments either at LC₂₅ or LC₅₀ where mean antifeedant values at LC₂₅ were higher in case of *Catharanthus* (57.65%) than that in case of *Artemisia* (54.342%) for four days after treatment. Also, at LC₅₀ levels, the mean feeding deterrent values were still higher in case of *Catharanthus* (67.75%) compared to *Artemisia* (63.871%) (table 4).

It is obvious from data that the *Catharanthus* extract exhibited more feeding deterrent effect than *Artemisia* extract. In addition, the data indicates that feeding deterrency of both botanical extracts has increasing trend till the 4th day after treatments. Also, it is interesting to notice that the feeding deterrent activity of both botanical extracts was higher in 2^{nd} instar than 4^{th} instar larvae.

The extracts of *Artemisia monosperma*, *Calotropia procera* and *Tagetes patula* were the powerful antifeeding effect against *S. littoralis* larvae (**Ahmed 1985**). In general, the antifeeding effect of plant extracts depend mainly on insect species, however, the plant structure-activity relationship associated with its components on insect feeding is complex and no clear trends emerge (**Bruno** *et al.* **2002**).

Erturk (2006) reported that the extracts derived from different plants Artemisia absinthum, Aesculus hippocastanum, Viscum album, Sambucus nigra, Buxus sempervirens, Diospyros kaki, Alnus glutiosagoertn, Origanum vulgare, Hypericum androsaemum and Ocimum basilicum had antifeeding effect against the $3^{rd} - 4^{th}$ instar larvae of the Thaumetopoae solitaria (Lepidoptera).

While **Summarwar and Pandey (2015)** observed that at 5% of leaf extract of *C. roseus* the percent feeding of *S. litura* 4th instar larvae was reduced to 47.77 compared to 82.47 % in control. Also, the antifeedant activity caused a reduction in food consumption and chronic toxicity leading to delayed growth, development and increased mortality (**Vattikonda***et al.* **2015**).

Table (4): Percentage feeding deterrent indices (mean \pm SE) of *S. littoralis* 2nd and 4th instars larvae treated with LC₂₅ and LC₅₀ of botanical extracts (*Artemisia herba-alba* and *Catharanthus roseus*).

Treatment	LC ₂₅		LC_{50}	
	2 nd instar	4 th instar	2 nd instar	4 th instar
Artemisia	56.460± 3.462 ^b	54.342± 3.332 ^b	63.617± 3.902 ^b	63.871± 3.920 ^b
Catharanthus	63.503± 3.896 ^a	57.650± 3.535 ^a	70.903± 4.349 ^a	67.750± 4.157 ^a
L.S.D.5%	2.203	2.162	2.203	2.361

*Within the same column, means followed by the same letter are not significantly different (P > 0.05).

Conclusion:-

Our results confirmed that the tested botanicals either oils or extracts resulted in increased mortality, reduced food consumption via their feeding deterrent effect and exert a adverse impact on *S. littoralis* growth and development. These effects were dose-dependent. The findings may be helpful and effective for studying the efficacy of such botanicals as a part of the Integrated Pest Management (IPM) against this pest and closely related ones.

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