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The Efficiency, Mode of Action and Side Effects of Two Essential Oil Formulations on *Spodoptera littoralis* and Its Predator

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Abstract : The insecticidal effect of some essential oils which prepared as natural formulations namely (mento and basicide) against 4th instar larvae of *Spodoptera littoralis* and their side effects on the predator *Cocconella undociumpanctata* L. (Coleoptera: Coccinellidae) were studied. The results indicated that Growth, morphogenetic and developmental period were influenced by two formulations, since, mento formulation was more effective than basicide in all tested parameters after feeding treatment. Concerning the predation rate of *C. undecimpunctata* adult, mento formulation had no significant effect (238.75±74.63) compared to control (272.73±37.85), Also, the mode of action of these formulations including the enzymatic activity of the digestive enzymes revealed a significant inhibition in the treated larvae which was pronounced in mento formulation treatment than basicide. Also, transaminase enzymes activity (AST and ALT) reduced after treatment, the maximum inhibition in AST and ALT was induced by mento giving 51.13% and 37.29 respectively.
Key words : Natural compounds, *Spodoptera littoralis*, natural enemies, mode of action, enzymatic activity.

Introduction

In agriculture, insects affect directly on the growing part of the crop and causes severe damage, resulting in revenue loss. Crop loss due to insect pests is estimated between ten and thirty percent for major crops Ferry *et al.*[1] *Spodoptera littoralis* (Fab). (Lepidoptera: Noctuidae) is considered one of the major polyphagous pest attacking various important crops Kandil *et al.,*[2].

In recent years the environmental problems caused by overuse of conventional pesticides have been the matter of concern for both scientists and public. The move toward green chemistry processes and the Continuing need for developing new crop protection. Some researchers Study the activity of green pesticides which prepared as formulations based on plant products, essential oils and their constituents against important agricultural insect pests Sammour *et al.*,[3]; Issa *et al.*,[4] ; Ateyyat *et al.*,[5]; and Castresan *et al.*, [6] and Abdel-Aziz *et al.*,[7].

Also, biological control agent such as the predator *Coccinella septempunctata* L. ladybird beetle (Coleoptera: Coccinellidae) is benefit for the reduction of pest population in agriculture crops. It is mainly free-living predatory species that consumes a large number of preys during lifetime. Therefore, it is potential to control many insect pests on which it feeds voraciously the immature as wells as mature stages Liu and Stansly[8].

The biochemical studies of mode of action of tested formulations which deal with the carbohydrate metabolizing enzymes (amylase, invertase and trehalase), and transaminases (AST &ALT).

Impairment of these enzymes could be effective in impairing the feeding and reproductive competence at the insect population level Abd-elaziz[9].

The aim of this work is to study the insecticidal effect of some essential oils which prepared as natural formulations namely (mento and basicide) against *Spodoptera littoralis* and their side effects on the predator *Cocconella undociumpanctata* L. (Coleoptera: Coccinellidae). Also, the mode of action of these formulations will be included.

Materials and methods

Preparation of the essential oils

Dried aerial parts of *Mentha longifolia* (wild mint) and *Ocimum basilicum* (basil) were grinded and possessed by hydro -distillation for 3 h in a Clevenger-type apparatus to obtain the essential oils. The collected oils were separated and dried on anhydrous sodium sulphate and after filtration stored at 4°C until use.

Preparation of the tested formulated compounds:

The formulations were prepared as emulsifiable concentrate (EC) as follow:

The formulations namely Mento and Basicide were prepared by mixing oil of *M. longifolia* and *O. basilicum* respectively with proper amount of different emulsifiers and natural solvent (mineral and vegetable oils). Emulsion of each compound in water was sprayed at concentration of 5%.

Insects cultures.

Stock culture of *Spodoptera littoralis* was reared in the laboratory at 27 ±28C and 65 ±5% RH. The new moult of the fourth instar larvae (25–30 mg) were chosen from the stock culture for bioassay study.

The laboratory stock cultures of the predator *C. undecimpunctata* was reared according to the method described by Bahy El-Din[10]. Predaceous stages of the predator was bred on the durante aphid, *Ap*his *punicae* Pass ,only full grown nymphs were offered to experimental larvae as a standard prey material in excess of that required by the insect for normal development. While prey aphids were obtained directly from the field.

Physiological aspects

Topical treatment

Freshly-moulted 4th instar larvae of S. *littoralis* was treated topically with 1µl / larva of concentration 5% for each formulation. Daily observations were included changes in body weight and in the durations of the developmental stages of the test insect.

Feeding treatment

Castor bean leaf discs of 2.5 cm diameter were treated with 5% concentrations of the two tested formulations for 2 minutes and dried at room temperature. Treated discs were placed separately in Petri dishes (diameter 9 cm) and offered to 4th instar larvae to test its effect on parameters as mention above.

The side effects of the tested formulations on the *predator C. undecimpunctata*.

The same concentration of two tested formulations were restricted only to the (3rd) instar larvae and adults of *C. undecimpunctata*. The durations of the developmental stages and predation rate (PR) of the predator were recorded.

Biochemical studies

Fourth instar larvae of *S. littoralis* were treated with the tested formulations. After 72 h of treatment the larvae were kept under freezing conditions at -20°C.

Tissue preparation

The treated larvae were homogenized in distilled water (1 g insect body/5 ml water) using a mortar for 3 min. Homogenates were centrifuged at 3000 rpm for 15 min under a cooling centrifuge and the supernatant was used (as enzyme solution) directly or stored at –20°C until use for biochemical determinations. A control experiment was set up using the supernatant of untreated larvae.

Enzyme assay

(i) Determination of the digestive enzyme. Amylase ,invertase and trehalase activities were assayed colorimetrically according to the methods described by Ishayaa and Swirski[11]

(ii) Determination of transaminase enzymes (AST and ALT).

AST (aspartate aminotransaminase) and ALT (alanine aminotransaminase) were determined colorimetrically according to the method of Reitman and Frankle [12].

Statistical analysis

Data obtained from experimental and control insects were compared by paired Student`s *t*- test. All collected data were subjected to analysis of variance (ANOVA) using Co-Stat test; the means were calculated and statistically analysed using Duncan's multiple range tests Duncan[13].

Results and discussion

Growth, morphogenetic and developmental period as influenced by two formulations when applied to the 4th larval instar of *S. littoralis* were studied.

Data obtained in (Table1) illustrate that both mento and basicide formulations at 5% concentration had remarkable effects on larval, pupal, development and growth. Fourth instar larvae topically treated with both tested formulations showed a significant prolongation in their life stages. The larval development of the treated ones lasted (13.9± 1.6) and (11.2±0.21) days from the 4th instar till prepupal stage for the two formulations, respectively, while the control larvae lasted (11.7±0.8) days on the average. In the same table data showed that 4th instar larvae treated with mento formulation led to reduction in the body weight but not significant (308.3±47.7mg), while in the basicide formulation the larval weight was reduced in a highly significant (253.07±37.47mg) compared with untreated control (317.7±41.3mg).

The resulting pupae had reduction in body weight in both formulations but not significant when compared with control. While the pupal duration was significantly decreased in case of treatment with basicide (10±1.04) days with regard to the control (12.5±3 days) see Table1.

On the other wise pupae resulted from treatment with mento were significant increase in pupal duration (16.2 ±1.3 days) compared with control.

Table(1): Effect of the two tested formulations when applied topically on the 4th instar larvae of cotton leafworm *Spodoptera littoralis*.

Treatment	No. Test larvae	Larval weight Mg (means±sd)	Larval duration Days (means± sd) (range)	Pupal weight Mg (means±sd)	Pupal duration Days (means±sd) (range)	Mortality prior to within pupation		Enclosing adults %
Control	15	317.7±41.3	11.7±0.8 (10-12)	336.1±24.7	12.5±3 (10-15)	0	0	100
Mento	15	308.3±47.7^{ns}	13.9±1.6* (12-17)	312.8±37.9^{ns}	16.2±1.3* (14-19)	2	8	33.3
Basicide	15	253.07±37.47*	11.2±0.21^{ns} (11-12)	306.66±48.68^{ns}	10±1.04* (9-11)	1	5	60

* Significant of differences from "un-treated" control (taking into account the Student`s t-test): p=0.05. ns: denotes not significant

Results in Table (2) showed variety degrees of antifeedant activity of the two formulations at concentration 5% against *S. littoralis* larvae after feeding treatment. Formulation of mento was the most effective as antifeedant activity 83.3% for larval mortality till pupation since, only 16.7% of the larvae success to reach the adult stage. In case of basicide only30% died as larvae and 70% of the larvae reached the adult stage. Concerning the developmental parameters, the mean of larval body weight treated with mento was significantly reduced to (142.43±10.42 mg) when compared with control (199.06±17.68 mg). To the contrary, the basicide had low antifeedant activity since the mean of larval weight was (187.1±2.73mg).

In the same table data showed a significant prolongation in their larval stage (10.86±0.40 days) treated with basicide while the control larvae (9.30±0.39 days). The resulting pupae had decreased in body weight (225.69±9.34 mg), (231.87±13.49 mg) for mento and basicide respectively compared with control(275.00±35.36 mg). Concerning the pupal duration mento caused significant reduction (8.19±0.55 days) with regard to control.

Table (2): Developmental aspects of 4th instar larvae of *Spodoptera littoralis* after feeding of castor- bean leaves with two formulations.

Treatment	No. Test larvae	Larval weight mg (means±sd)	Larval duration Days (means± sd) (range)	Pupal weight mg (means±sd)	Pupal duration Days (means±sd) (range)	Mortality prior to within pupation		Enclosing adults %
Control	10	199.06±17.68	9.30±0.39 (9-12)	275.00±35.36	9.4±0.34 (7-10)	0	0	100
Mento	12	142.43±10.42*	9.2±1.47^{ns} (5-11)	225.69±9.34*	11.50±0.71* (11-12)	9	1	16.7
Basicide	10	187.1±2.73^{ns}	10.86±0.40* (9-11)	231.87±13.49*	8.19±0.55* (7-10)	3	0	70

* Significant of differences from "un-treated" control (taking into account the Student`s t-test): p=0.05. ns: denotes not significant

A majority of reports have shown an increase in larval duration by using plant products Senthil Nathan[14]. Here the results of our study clearly manifested that the increase in larval duration and reduced the weight of larvae of *S. littoralis* when treated with the two tested formulations. This is supported by Wondafrash, *et al.*,[15] who reported a significant reduction in weight of larvae for *Helicoverpa armigera* after treatment with *Azadirachtin indica*. Many natural products inhibited the larval growth, increased the larval-pupal duration and multiplied the abnormalities of emerged adults of *H. armigera* and *S. littoralis* Baskar, *et al.,*[16].

Our results showed that, the selected medicinal plants oils tested against fourth instar larvae of *S. littoralis* had larvicidal activity which mainly depends on the presence of toxic materials present in plants oils.

These results are also coinciding with the findings of Jayasankar, *et al.,*[17] Elumalai, *et al.*,[18] and Elanchezhiyan, *et al.,*[19] they reported that high larvicidal effects in the essential oils of *Mentha pulegium* and *Ocimum basilicum*. Duraipandiyan, *et al.*,[20] found that rhein isolated from *Cassia fistula* flower have larvicidal activity against some lepidopteron pests *S. littoralis* and *H. armigera*. Malformation of larvae, pupae and pharat adults in both two formulations were observed. (Fig1. A,b,C).

(a) (b) (c)

Fig. 1. Showing malformation resulting from treatment of 4th instar *S. littoralis* larva with two plant extracts

(a) Normal pupa. (b)pupal-adult intermediate (Mento). (c) pupal-adult intermediate (Basicide).

The side effect of the tested formulations on the natural enemies

The results of the tested formulations on_the predation rate of *C. undcimpunctata* larvae table (3) showed a significant increasing in case of mento (323.25±110.1) while basicide showed a significant reduction in predation rate (127.44±113.5) on aphids relative to that of the control (243.08±25.52). The two tested formulations used were found to variably effect on the developmental period of the insect. Mento did not had significant effect in the larval and pupal period 7.8±2.22 6.8±1.54 respectively, but basicide showed a shortage to half when compared to mento and control in larval and pupal periods.

Table (4) compares the data obtained on predation rate of *C. undecimpunctata* adult as affected by the two formulations when applied as direct topically, mento had no significant in the predation rate (238.75±74.63) on aphid relative to that of the control (272.73±37.85), but the highest reduction in the predation rate (123.86±27.56) was obtained by basicide treatment. Mento and basicide proved to have no effect on the developmental rate of adults. Adults treated with mento were less affected since only 25% mortality compared to 18.1% for control, basicide was much dramatic it showed 60% mortality.

Table (3): Predation efficiency on *Aphis punicae* nymph of *C. undecimpunctata* larvae as influenced by direct topically treatment at 3rd instar with two tested formulations.

Treatments	No. of Tested larvae	Predation Rate (mean ± SD)	Larval duration In days (means ± SD)	Pupal duration in days (means ± SD)	Mortality prior to within pupation		Enclosing adult %
Control	12	243.08±25.52	7.42±0.49	6.42±1.1	0	0	100
Mento	12	323.25±110.1***	7.8±2.22ns	6.8±1.54ns	0	2	83.3
Basicide	12	127.44±113.5***	4.54±0.49***	3.50±1.12***	8	4	0

(Predation rate): Average number of prey larvae devoured by an individual predator during larval stage.

* Significant of differences from "un-treated" controls.

Table (4): Predation efficiency on *Aphis punicae* nymph of *C. undecimpunctata* adult as influenced by topical treatment with two tested formulations

Treatments	No. of Tested adults	Predation Rate (mean ± SD)	Adult duration In days (means ± SD) (Range)	Mortality %
Control	11	272.73±37.85	6.5±0.9 (4-7)	18.1
Mento	12	238.75±74.63^{ns}	6±1.7^{ns} (3-7)	25
Basicide	10	123.86±27.56***	6.14±1.86^{ns} (1-7)	60

For further details, see footnote table(3).

Study the mode of action of the tested formulations

The enzymatic activity of the digestive enzymes and transaminase enzymes of *S. litturalis* larvae treated with the tested formulations are presented in table (5).

Digestive enzymes (amylase ,invertase and trehalase)

Digestive enzymes have a major role in the body of insects since they convert

complex food materials into smaller molecules that are necessary to provide energy and metabolites Senthil Nathan *et al*.,[21].

The results of the three enzymatic activity revealed a significant decrease in the larvae treated with mento and basicide respectively, since it was (40.08 ±0.82 and 56.62± 6.16µm/min/g tissue) for amylase activity, compared to control (84.81 ±5.57 µm/min/g).

As for invertase and trehalase activity ,the results showed that treatment with mento and basicide had significantly inhibited with (41.09%, 16.17%) and (66.00% , 44.51%) respectively.

So, mento formulation was more pronounced than basicide in inhibiting the activity of the three tested enzymes. This confirms the obtained results in which mento was more effective than basicide in inhibited feeding and subsequent larval growth of *S. littoralis.*

In agreement, Ishaaya and Ascher[22] recorded that disturbance in amylase and invertase activities could interfere with feeding ability. Also, Jbilou and Sayah,[23] reported that the reduction of these enzymes activity could be due to a cytotoxic effect of different extracts on epithelial cells of midgut that synthesize a-amylase.

Impairment of the substrate availability and inhibition of peristaltic movement of the gut might have inhibited the digestive enzymes activity in the treated insects Senthil Nathan et al., [21].

Transaminases enzymes (AST and ALT)

Data in Table (5) illustrated the effect of the two tested formulations on the enzymatic activity of transaminases in the tissue of *S. littoralis* larvae. It could be noticed from the table that the maximum inhibition in AST and ALT was induced by Mento giving 51.13% and 37.29 respectively. Basicide caused moderate effect on both enzymes and was more pronounced in AST than ALT, it was 37.5% and 17% reduction respectively. So, these formulations greatly affected the enzymatic activity of AST and ALT. These enzymes are important anaplerotic enzymes providing oxaloacetate and pyruvate respectively as precursors of Kreb's cycle intermediates for the tricarboxylic (TCA) cycle through anaplerosis were recorded as target enzymes which could affect the aerobic metabolism of the larvae Icen *et al*.,[24].

Impairment of Kreb's cycle as the major aerobic pathway for getting energy (ATP) could be easily affect the normal reproductive and growth rate of the treated insects Azmi *et al*.,[25].

In conclusion, our data showed that, the formulated compound mento was relatively safe to the *C. septempunctata* adults and larvae and was effective in the controlling *S. littoralis* when compared with basicide.

Table (5): Enzymatic activity of fourth instar larvae of *S. littoralis* treated with the two formulations.

Enzyme samples	Control		Mento		Basicide	
	*Activity	% inhibition	*Activity	% inhibition	*Activity	% inhibition
Digestive enzymes (µ mol /min/g tissue)						
Amylase	84.81 ±5.57 a **	----	40.08 ±0.82 c	52.74	56.62± 6.16 b	33.23
Invertase	85.77 ±7.12 a **	----	50.52 ±4.35 b	41.09	71.9± 2.42 a	16.17
Trehalase	81.01 ± 1.00 a**	----	28.56 ± 1.00 c	66.00	46.61± .50 b	44.51
Transaminase enzymes (U/g tissue)						
AST	8. 8±0.76 a **	----	4.3±0.57 b	51.13	5.5±0.86 b	37.5
ALT	3.11±0.29 a**	----	1.95±0.08 b	37.29	2.58±0.28 ab	17

*Enzyme activity is presented as the mean of four replicates ± S.D.

**Means followed by the same letters in the same row are not significantly differ at $p<0.01$ and $p<0.05$ level of probability according to Duncan's multiple range test.

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