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Research Article
Effects of Essential Oils on Growth, Feeding and Food Utilization of *Spodoptera littoralis* Larvae

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Abstract

Background and Objectives: The intensive use of synthetic insecticides has increased environmental pollution, development of resistance of many insects and harmful effects on non-target species. These undesirable effects encouraged the use of insecticide alternatives particularly plant products and essential oils. In the present study, the bioactivity of five essential oils isolated from Egyptian plants was examined against *Sopdoptera littoralis*. **Methodology:** The essential oils of *Artemisia judaica*, *Citrus lemon*, *Origanum vulgare*, *Rosmarinus officinalis* and *Schinus molle* were isolated by hydrodistillation and their chemical compositions were identified by gas chromatograph/mass spectrometer. The oils were evaluated for their anti-nutritional, antifeedant and growth inhibitory activities against *Sopdoptera littoralis* larvae. **Results:** The oil of *C. lemon* caused the highest reduction of relative growth rate (RGR) at all of the tested concentrations (125, 250, 500, 1000 and 2000 mg L^{-1}). In addition, the tested oils significantly reduced efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD). On the other hand, the tested oils showed moderate antifeedant activity against the larvae. The tested oils showed remarkable growth inhibition of larvae with *C. lemon* being the most effective oil as the growth inhibition index value was 73.85% at 2000 mg L^{-1}. Moreover, the tested essential oils induced reduction in larval chitin formation and caused undifferentiated ovarioles of emerged females. The essential oil of *A. judaica* revealed the highest insecticidal activity on 4th instar larvae with LC_{50} value of 492.3 mg L^{-1}. **Conclusion:** These results indicate that the tested essential oils may be useful as alternative in management of *S. littoralis*.

Key words: *Spodoptera littoralis*, essential oils, nutritional indices, antifeedant, growth inhibition, chitin inhibition, insecticidal activity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is a serious polyphagous caterpillar damaging more than 85 host plants belonging to 40 plant families of economic importance. *S. littoralis* is distributed throughout the Southern Europe, Africa and the Middle East[1] and the Mediterranean area[2], being the native of Africa[3]. In addition to the direct damage caused by reducing photosynthetic area, the occurrence of larvae, feeding damage and excrement reduces marketability of vegetables and ornamentals[4].

Plants and their secondary metabolites, such as alkaloids, essential oils, terpenoids, steroids, polyphenols, lignans and sugars that protect the plants from insect pests have been receiving global research attention. These compounds have been evaluated and formulated as botanical pesticides for plant protection since they do not leave residues and have less toxic effect on the environment and humans[5]. More than 2000 species of plants are known to possess insecticidal activity, by containing either antifeedant, repellent, or insecticidal compounds[6]. Some of these botanical insecticides have growth regulatory effects due to the disruption of the hormonal regulation of metamorphosis and moulting process. These effects are manifested by changes in haemolymph ecdysteroid and juvenile hormone titres due to a blockage and/or delay in their release from neurohaemal organs, therefore, the plant substances are known to cause reproductive sterility in insects. Some of these compounds inhibit ovarian growth, testes growth and development, while others appear to induce fundamental changes in the chemical structure of nucleic acids (DNA and RNA). The natural compounds might be useful since all chemosterilants were found to be extremely hazardous compounds[7]. The insecticidal activity of several plant essential oils and other extracts has been evaluated against many insect pests of cereals and legumes[8].

Essential oils are defined as any volatile oil(s) that have strong aromatic components and that give a distinctive odor, flavor or scent to a plant. Essential oils are by-products of plant metabolism and are commonly referred to as volatile plant secondary metabolites[9,10]. The essential oils were found to have contact and fumigant toxicities and repellent and antifeedant effects[11,12].

In continuous studies on the chemistry and biological activities of aromatic Egyptian plants, the chemical composition, antifeedant, growth inhibitory and insecticidal activities of the essential oils isolated from five plants, viz. *Artemisia judaica*, *Citrus lemon*, *Origanum vulgare*, *Rosmarinus officinalis* and *Schinus molle*, on the fourth instar larvae of *Spodoptera littoralis* were examined. In addition, the effect of essential oils on food utilization has been evaluated.

MATERIALS AND METHODS

Insect rearing: A susceptible strain of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae), was reared under the laboratory conditions of 25±3°C and 70±5% R.H. on castor oil leaves, *Ricinus communis* L., according to El-Zoghaby[13].

Extraction and GC-MS analysis of essential oils: The plant samples of *Citrus lemon* (fruit peel), *Origanum vulgare* (aerial parts), *Rosmarinus officinalis* (leaves) and *Schinus molle* (leaves) were collected from Alexandria (31°13′N, 29°58′E) in August, 2013. *A. judaica* (aerial parts) was collected from Matrouh (31°19′N, 27°09′E) in August, 2013. The plant materials were identified by Prof. FathAllah Zaitoon of the Plant Pathology Department, Faculty of Agriculture, Alexandria University. Voucher specimens were deposited in Department of Chemistry of Pesticides, Faculty of Agriculture, Alexandria University. The essential oils obtained by subjecting plant materials to hydrodistillation using a Clevenger apparatus for 3 h. Anhydrous sodium sulfate was used to remove water after the oil extraction. Quantitative and qualitative analyses of essential oils were performed on a gas chromatography (Hewlett Packard 5890)/mass spectrometry (Hewlett Packard 5989B) (GC-MS) apparatus. The essential oils were diluted in diethyl ether and 0.5 µL was injected. The GC column was a 30 m (0.25 mm i.d., film thickness 0.25 µm) HP-5MS (5% diphenyl) dimethylpolysiloxane capillary column. The GC conditions were as follows: injector temperature, 240°C; column temperature, isothermal at 70°C and held for 2 min, then programmed to 280°C at 6°C/min and held at this temperature for 2 min; ion source temperature, 200°C; detector temperature, 300°C. Helium was used as the carrier gas at the rate of 1 mL min^{-1}. The effluent of the GC column was introduced directly into the ion source of the MS. Spectra were obtained in the EI mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 40-400 amu for 5 sec. The oil components were identified by comparison of their retention indices and mass spectra with the NIST Mass Spectral Library.

Feeding bioassay: Feeding assay with the no-choice test technique was used to evaluate the bioactivity of essential oils against the newly molted fourth instar larvae of *S. littoralis*

by preparing leaf discs (5 cm in diameter) from leaves of castor oil as described by Morimoto *et al.*[14]. The essential oils were dissolved in acetone. For each essential oil, five concentrations were prepared (125, 250, 500, 1000 and 2000 mg L^{-1}), while the control treatment was conducted with acetone only. The leaf discs were immersed in essential oil solutions and in acetone (control) for five seconds and then allowed to dry before placing in plastic dishes (9 cm in diameter). Four replicates were carried out in each concentration and control. The larvae were allowed to feed for 72 h on treated discs that have been changed every 24 h and then the larvae were allowed to feed on untreated discs. Moistened cotton pad was placed in each dish to sustain humidity. All larvae were then kept under the laboratory conditions of 25±3°C and 70±5% R.H. Nutritional indices, antifeedant activity and growth inhibition index have been measured as described below:

Nutritional indices: The effect of essential oils on food consumption and utilization by the newly molted fourth instar larvae of *S. littoralis* was investigated. Known weights of fresh castor oil discs treated with different concentrations of essential oils dissolved in acetone (40 larvae for each concentration) were offered to the larvae. All larvae feces and unconsumed food were weighed every 24 h over the 72 h of feeding period. The nutritional indices, such as the relative growth rate (RGR) were determined according to Miller and Miller[15] and the efficiency of conversion of digested food (ECD) according to Klein and Kogan[16]. The formulae of Farrar *et al.*[17] were applied as follow:

RGR = Relative growth rate = ΔB ÷ Feeding period

Where:

ΔB = Change in body weight = (final weight-initial weight) ÷ No. of larvae

ECI = Efficiency of conversion of ingested food = ΔB ÷ I×100

Where:

I = Weight of the food consumed = Consumed food ÷ No. of larvae

ECD = Efficiency of conversion of digested food = ΔB ÷ (I-F)×100

Where:

F = Weight of the feces produced during the feeding period ÷ No. of larvae

Antifeedant activity: The antifeedant activities of the essential oils were tested using fresh leaf discs of castor bean. The tested oils were evaluated at 125, 250, 500, 1000 and 2000 mg L^{-1}. The feeding-deterrence index suggested by Isman *et al.*[18] was used as follows:

Feeding-deterrence index (FDI) = [(C-T)÷C]×100

where, C is the consumption of control discs and T is the consumption of treated discs).

Growth inhibition index: The growth inhibition indices of essential oils on newly molted fourth instar larvae of *S. littoralis* after 72 h of treatment with different concentrations were calculated according to the following Eq.:

$$\text{Growth inhibition index} = \frac{\text{CL - TL}}{\text{CL}} \times 100$$

where, CL is the larval weight gained in the control and TL is the larval weight gained in the treatment.

Chitin body wall formation: This experiment was conducted on the newly molted 6th instar larvae of *S. littoralis*. The fourth instar larvae were fed for 72 h on both fresh discs (control) and treated discs with the five essential oils at 2000 mg L^{-1}, following the procedure of Hughes *et al.*[19]. The ruptured larvae were weighed in the same age with the control larvae, anaesthetized by chilling, decapitated and dissected along the ventral surface. The gut, fat body and other internal tissues were removed. After rinsing under water, the body wall of each larva was placed in 3 mL of 10% (w/v) potassium hydroxide (KOH) at 100°C for 4 h, then allowed to stand overnight at room temperature. The remaining chitin from each larva was washed thoroughly with cold water. The trachea and spiracles were removed and the chitin extracts were oven-dried overnight at 80°C. After equilibration to room temperature, the extracts were weighed individually. In this way, the ratio of chitin dry weight to the larval fresh weight could be determined for the individual larva, as follows:

$$\text{Ratio of chitin formation} = \frac{\text{Chitin dry weight}}{\text{Larval fresh weight}}$$

Toxicity of essential oils against *S. littoralis* larvae: The mortality of fourth instar larval mortality of *S. littoralis* was recorded after 72 h of treatment. The lethal concentration causing 50% mortality (LC_{50}) expressed as mg L^{-1} was calculated from log-concentration mortality regression lines[20].

Reproductive tracts dissection: Adults of *S. littoralis* (1 day old) emerged from larvae treated with essential oils at 2000 mg L^{-1} for 72 h and from control were used in this experiment. The reproductive tracts of females were dissected from insects under a binocular microscope (10× magnification) in Ringer's solution (0.42 g KCl, 0.2 g $NaHCO_3$, 9.0 g NaCl, 0.48 g $CaCl_2$ in 1000 mL distilled water) according to the method of Junqueira and Carneiro[21].

Statistical analysis: Relative growth rate (RGR), efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD), feeding deterrent index (FDI) and growth inhibition index (GII) were subjected to one-way analysis of variance followed by Student-Newman-Keuls test to determine significant differences among mean values at the probability level of 0.05. The average mortality percentages were subjected to probit analysis for calculating LC_{50} and other statistic parameters using the SPSS14.0 (Statistical Package of Social Sciences Inc., USA) software. The values of LC_{50} were considered significantly different if the 95% confidence limits did not overlap.

RESULTS

Essential oils composition: The analysis of essential oils by GC/MS (Table 1) revealed that the monoterpenes β-thujone, limonene, pulegone, 1,8-cineole and α-phellandrene were present at the highest concentrations in the oils of *Artemisia judaica*, *Citrus lemon*, *Origanum vulgare*, *Rosmarinus officinalis* and *Schinus molle*, respectively. In addition to these compounds, each essential oil contains several major constituents as shown in Table 1. The major constituents of the essential oils mainly belonged to four chemical groups: Oxygenated monoterpenes (i.e. α- and β-thujone, chrysanthenone, pulegone, α- and β-citral, camphor and linalool); monoterpene hydrocarbons (i.e., limonene, γ-terpinene, β-pinene, α- and β-phellandrene and α-pinene); oxygenated sesquiterpenes (i.e. elemol, τ-muurolol and γ-eudesmol) and sesquiterpene hydrocarbons (i.e., σ-cadinene).

Effect on relative growth rate (RGR): Table 2 shows the relative growth rate (RGR) values estimated for those larvae treated with essential oil at concentrations of 125, 250, 500, 1000 and 2000 mg L^{-1}. The results showed that all of the tested oils significantly lowered RGR, particularly at the higher concentrations of 500, 1000 and 2000 mg L^{-1}. The oil of *C. lemon* revealed the greatest reduction in RGR at the tested concentrations. RGR was recorded 14.89 mg day^{-1} in the control, while *C. lemon* oil recorded 8.53, 5.78, 4.96, 4.25 and 3.55 mg day^{-1} at 125, 250, 500, 1000 and 2000 mg L^{-1}, respectively. In general, the values of RGR reduced with increasing the tested oil concentrations.

Effect on food consumption: Table 3 illustrates the efficiency of conversion of ingested food (ECI) values, which have been measured the overall ability of the insect to convert ingested food into body matter. At the 125 mg L^{-1}, the ECI values were not differed significantly between the all tested essential oils and control. However, at higher concentrations (250, 500, 1000 and 2000 mg L^{-1}), significant reduction of ECI was induced by the tested essential oils. The essential oil of *R. officinalis* at 250 mg L^{-1} may stimulate the fourth instar larvae of *S. littoralis* to feed and convert ingested food into

Table 1: Major constituents of essential oils isolated form five Egyptian plants

Essential oil	Major components (%)
Artemisia judaica	β-Thujone (49.83), Chrysanthenone (10.88), α-Thujone (8.21), 1,8-Cineole (4.91), L-Camphor (3.0), Artemisia alcohol (2.20)
Citrus limon	Limonene (56.30), β-Pinene (8.81), γ-Terpinene (6.42), α-Citral (4.96), β-Citral (3.83), α-Terpineol (3.38)
Origanum vulgare	Pulegone (77.45), Menthone (4.86), *cis*-Isopulegone (2.22), Piperitenone (2.13), dl-Limonene (1.08), β-Myrcene (0.66)
Rosmarinus officinalis	1,8-Cineole (19.60), Camphor (17.01), α-Pinene (15.12), Verbenone (9.55), Endo-Borneol (8.17), L-Linalool (5.32)
Schinus molle	α-Phellandrene (29.87), β-Phellandrene (21.08), Elemol (13.00), τ-Muurolol (5.35), γ-Eudesmol (4.48), σ-Cadinene (3.99)

Table 2: Effect of essential oils on relative growth rate (RGR) of the fourth instar larvae of *Spodoptera littoralis* after 72 h of treatment with different concentrations

	Relative growth rate (mg day^{-1}) at concentrations (mg L^{-1})				
Oil	125	250	500	1000	2000
Control	14.89±0.29^{a}	14.89±0.29^{a}	14.89±0.29^{a}	14.89±0.29^{a}	14.89±0.29^{a}
*A. judaic*a	10.36±1.15^{bc}	9.45±1.48^{bc}	9.58±0.72^{b}	7.38±0.48^{bc}	5.83±0.48^{c}
C. lemon	8.53±0.59^{c}	5.78±0.70^{c}	4.96±0.59^{d}	4.25±0.48^{d}	3.55±0.45^{d}
O. vulgare	12.84±1.37^{ab}	8.25±1.25^{bc}	6.76±0.29^{c}	6.0±0.34^{cd}	5.80±0.42^{c}
R. officinalis	14.22±0.78^{a}	11.65±2.0^{ab}	9.41±0.26^{b}	7.80±1.19^{bc}	8.83±0.72^{b}
S. molle	10.75±2.59^{bc}	9.27±0.64^{bc}	8.93±0.26^{b}	8.5±0.06^{b}	7.88±0.58^{b}

Mean values within a column sharing the same letter are not significantly different at the 0.05 probability level

Table 3: Effect of essential oils on food consumption by fourth instar larvae of *Spodoptera littoralis* after 72 h of treatment with different concentrations

	Efficiency of conversion of ingested food (ECI) (%±SE) (mg L^{-1})				
Oil	125	250	500	1000	2000
Control	20.87±0.27^{a}	20.87±0.27^{a}	20.87±0.27^{a}	20.87±0.27^{a}	20.87±0.27^{a}
*A. judaic*a	18.96±0.76^{a}	12.53±1.40^{b}	14.66±0.35^{b}	12.43±0.69^{c}	9.44±0.38^{c}
C. lemon	16.67±0.94^{a}	13.87±1.72^{b}	12.37±0.37^{c}	10.08±1.07^{c}	6.39±1.09^{c}
O. vulgare	16.89±1.84^{a}	14.07±0.58^{b}	10.37±0.38^{c}	9.72±0.65^{c}	9.98±0.92^{c}
R. officinalis	20.46±1.28^{a}	23.69±1.60^{a}	16.57±0.15^{b}	15.05±0.95^{b}	14.22±1.79^{b}
S. molle	15.99±1.12^{a}	13.04±0.27^{b}	11.81±1.46^{c}	11.16±0.19^{c}	10.54±1.06^{c}

Mean values within a column sharing the same letter are not significantly different at the 0.05 probability level

Table 4: Effect of essential oils on food digestion by fourth instar larvae of *Spodoptera littoralis* after 72 h of treatment with different concentrations

	Efficiency of conversion of digested food(ECD) (%±SE) (mg L^{-1})				
Oil	125	250	500	1000	2000
Control	23.27±0.16^{a}	23.27±0.16^{a}	23.27±0.16^{a}	23.27±0.16^{a}	23.27±0.16^{a}
*A. judaic*a	20.83±1.79^{a}	17.56±0.61^{c}	17.11±1.07^{b}	12.80±0.56^{c}	10.44±0.90^{b}
C. lemon	16.13±0.31^{b}	15.34±0.07^{d}	11.18±1.42^{c}	10.05±1.00^{d}	7.79±1.16^{b}
O. vulgare	19.54±1.0^{ab}	13.59±0.07e^{d}	11.52±0.53^{c}	11.24±0.28^{cd}	10.63±1.04^{b}
R. officinalis	23.19±1.42^{a}	20.53±1.26^{b}	16.87±0.64^{b}	15.69±0.90^{b}	14.67±1.67^{b}
S. molle	20.48±2.33^{a}	17.86±1.84^{c}	16.32±2.88^{b}	14.47±1.09^{b}	14.07±0.37^{b}

Mean values within a column sharing the same letter are not significantly different at the 0.05 probability level

Table 5: Antifeedant activity of essential oils on fourth instar larvae of *Spodoptera littoralis* after 72 h of treatment with different concentrations

	Feeding deterrent index (FDI)(%±SE) (mg L^{-1})				
Oil	125	250	500	1000	2000
Control	0.00±0.0^{c}	0.00±0.0^{d}	0.00±0.0^{e}	0.00±0.0^{d}	0.00±0.0^{c}
*A. judaic*a	2.18±0.58^{bc}	6.03±0.36^{c}	10.44±1.22^{c}	13.04±0.79^{b}	16.82±2.34^{b}
C. lemon	12.02±1.05^{a}	18.78±0.85^{a}	31.19±1.1.42^{a}	32.28±1.30^{a}	31.73±1.98^{a}
O. vulgare	4.25±0.42^{b}	5.44±0.25^{c}	6.67±0.66^{d}	9.66±1.10^{c}	14.19±2.36^{b}
R. officinalis	13.52±1.00^{a}	9.59±0.55^{b}	13.58±0.80^{b}	12.57±1.00^{b}	13.15±1.16^{b}
S. molle	13.63±0.94^{a}	17.85±0.85^{a}	7.18±0.80^{d}	1.28±0.00^{d}	1.59±0.16^{c}

Mean values within a column sharing the same letter are not significantly different at the 0.05 probability level

body matter more than the same larval age in control, where the ECI value was 23.69% as compared with that value of control (20.87%). It was clearly noticed that the ECI values were gradually decreased as the concentration of the tested essential oil increased. Again the essential oil of *C. lemon* was the most effective oil showing the least ECI (6.39%) at the concentration of 2000 mg L^{-1}.

Effect on food digestion: The efficiency of conversion of digested food (ECD), indicating the percentage of assimilated food converted into body matter, was reduced at all tested concentrations (Table 4). Except for 125 mg L^{-1}, the essential oils significantly decreased the ECD values at all of the tested concentrations. The reduction of ECD was concentration dependent. Among the tested plants, *C. lemon* and *O. vulgare* caused the greatest reduction of ECD.

Antifeedant activity: The values of feeding deterrent index (FDI) of the five tested essential oils on the fourth instar larvae of *S. littoralis* after 72 h of treatment are given Table 5. The data showed that the tested oils possessed different levels of antifeedant activity. *Citrus lemon* oil revealed the highest antifeednat activity at all of the tested concentrations. FDI values of this oil were 31.19, 32.28 and 31.73% at 500, 1000 and 2000 mg L^{-1}, respectively. In contrast, the oil of *S. molle* displayed the weakest antifeedant activity at concentrations of 1000, 2000 mg L^{-1}.

Effect of essential oils on *S. littoralis* larval growth: The obtained data in Table 6 show the effect of the five tested essential oils on growth of the fourth instar larvae of *S. littoralis* after 72 h of treatment at different concentrations of 125, 250, 500, 1000, 2000 mg L^{-1}. The tested oils revealed pronounced larval growth inhibition even at the lowest concentration of 125 mg L^{-1}. The growth inhibition index (GII) values increased gradually as the tested concentrations of essential oils increased. The oil of *C. lemon* caused the greatest reduction in growth inhibition, followed by *R. officinalis* and *A. judaica*, while the oil of *R. officinalis* was the less effective one. At 2000 mg L^{-1}, the values of GII

Table 6: Effect of essential oils on growth of fourth instar larvae of *Spodoptera littoralis* after 72 h of treatment with different concentrations

	Growth inhibition index (%±SE) (GII) (mg L^{-1})				
Oil	125	250	500	1000	2000
Control	0.00±0.0[c]	0.00±0.0[d]	0.00±0.0[d]	0.00±0.0e	0.00±0.0[e]
*A. judaic*a	16.90±0.84[b]	22.01±2.25[c]	36.19±2.16[c]	53.03±1.16[bc]	60.30±2.55[b]
C. lemon	34.49±2.14[a]	52.84±1.39[a]	60.73±1.33[a]	64.50±0.90[a]	73.85±3.97[a]
O. vulgare	23.87±3.33[b]	33.77±3.28[b]	54.35±0.58[a]	58.12±2.37[b]	58.48±2.06[b]
R. officinalis	17.91±0.77[b]	28.53±1.52	34.45±1.78[c]	36.30±2.45[d]	36.78±1.34[d]
S. molle	17.32±1.66[b]	34.04±1.72[b]	42.81±4.00[b]	48.98±3.11[c]	49.29±2.20[c]

Mean values within a column sharing the same letter are not significantly different at the 0.05 probability level

Table 7: Comparative toxicity of essential oils against fourth instar larvae of *Spodoptera littoralis*

		95% confidence limits (mg L^{-1})				
Oil	LC_{50} (mg L^{-1})[a]	Lower	Upper	Slope±SE[b]	Intercept±SE[c]	(x^2)[d]
A. judaica	492.3	423.4	571.5	1.92±0.16	-5.17±0.44	2.59
C. lemon	546.2	267.1	1183.0	1.62±0.15	-4.43±0.42	13.2
O. vulgare	>2000.0	-	-	-	-	-
R. officinalis	590.6	475.3	788.8	1.43±0.20	-3.97±0.53	0.53
S. molle	788.7	612.2	1155.3	1.39±0.21	-4.02±0.52	0.69

[a]Concentration causing 50% larval mortality, [b]Slope of the concentration-mortality regression line±standard error, [c]Intercept of the regression line±standard error, [d]Chi square value

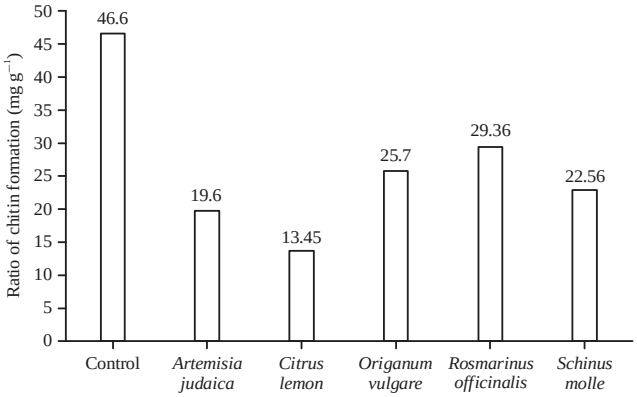

Fig. 1: Effect of the tested essential oils at a concentration of 2000 mg L^{-1} on chitin formation of *Spodoptera littoralis* larvae. x-axis: Essential oils

were 60.30, 73.85, 58.48, 36.78 and 49.29% for *A. judaica*, *C. lemon*, O. vulgare, *R. officinalis* and *S. molle*, respectively.

Effect of essential oils on chitin formation of *S. littoralis* larvae: The measurements of body wall chitin of *S. littoralis* larvae are presented in Fig. 1. The ratio of chitin formation expressed as mg chitin/g body wall. The obtained results indicated that *C. lemon* was the most effective essential oil causing the interruption of chitin formation. The values of chitin formation ratio were 46.60 mg g^{-1} in the control. When larvae treated with essential oils at 2000 mg L^{-1}, these values were 19.60, 13.45, 25.7, 29.36 and 22.56 mg g^{-1} for *A. judaica*, *C. lemon*, *O. vulgare*, *R. officinalis* and *S. molle*, respectively.

Toxicity of essential oils against *S. littoralis* larvae: Based on LC_{50} values, the essential oils showed moderate to weak insecticidal activity. The LC_{50} values were 492.3, 546.2, >2000, 590.6 and 788.7 mg L^{-1} for *A. judaica*, *C. lemon*, *O. vulgare*, *R. officinalis* and *S. molle*, respectively, after 72 h of treatment (Table 7). Therefore, the essential oil of *A. judaica* revealed the highest insecticidal activity, while the oil of *O. vulgare* showed the least insecticidal activity against the 4th instar larvae of *S. littoralis*.

Malformation effects of essential oils on *S. littoralis*: The daily observations for the development post the larval treatment with the essentials oils of *A. judaica*, *C. lemon*, *O. vulgare*, *R. officinalis* and *S. molle* proved their effects yielding abnormal stages. The cuticle colour of the treated larvae turned to a uniform dark grey, losing the spotted pattern characteristic of the species (*S. littoralis*). At higher concentrations (1000 and 2000 mg L^{-1}) of the tested essential oils, some larvae either become dwarf and swollen in all their body or at least in thoracic segments and eventually the cuticle become ruptured after 72 h post-treatment, along the interface between the intersegmental membrane and more sclerotized portions of the abdominal segments. Normal Larvae (Fig. 2c) were reduced in size to be dwarfed (Fig. 2a) and other become deformed larvae (Fig. 2b). The formation of larval-pupal intermediates was also observed. On the other hand, the female reproductive tracts were tested to check the ovarioles, accessory glands and bursa copulatrices. The present results illustrated the undifferentiated ovarioles

Fig. 2(a-c): (a) Dwarfed larvae after 2 days from treatment, (b) Deformed larvae with defect in front legs tarsi, stretch prologs and mouth parts resulted after 4 days from essentials oils treatment and (c) Normal larva

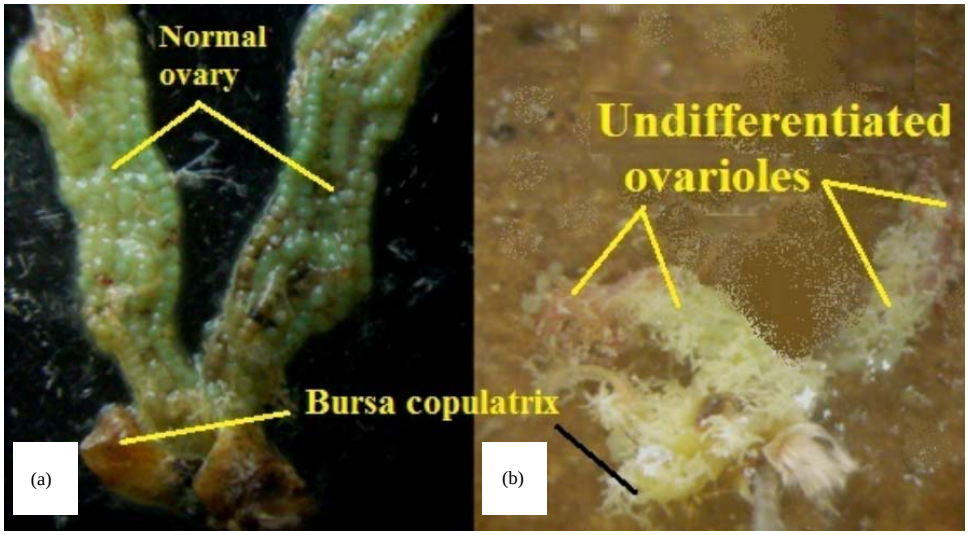

Fig. 3: (a) Normal ovary and (b) Undifferentiated ovarioles of adult resulted from larvae treated with tested essentials oils

(Fig. 3a and b) of an adult resulted from larvae fed on fresh discs of castor bean leaves (control) and another fed on treated discs with the tested essentials oils.

DISCUSSION

The chemical compositions of the extracted essential oils from *C. limon*, *S. molle* and *R. officinals* as shown by GC-MS analysis were similar to those previously reported[22-24]. On the other hand, the major constituents of the essential oils extracted from *A. judaica* and *O. vulgare* differed from those previously reported on the chemistry of these oils[25,26]. The differences in essential oil compositions may be due to several factors, such as geographical location, season, environmental conditions and the nutritional status of the plants[27].

In this study, the essential oils isolated from *A. judaica*, *C. lemon*, *O. vulgare*, *R. officinalis* and *S. molle* showed anti-nutritional effects on *S. littoralis*. All of the tested oils significantly decreased RGR, ECI and ECD. The effects of the tested essential oils on nutritional indices of *S. littoralis* were not previously reported. However, other essential oils and plant extracts have been reported to alter nutritional indices of *S. littoralis*. For example, Pavela and Chermenskaya[28] studied the effect of 18 plant extracts on nutritional indices of the 3rd instar larvae of the *S. littoralis* and found that the extracts caused clear reduction in RGR, ECI and ECD. Also, reductions of nutritional indices of the 5th instar larvae of *S. littoralis* induced by *Reynoutria* sp. extract were reported by Pavela *et al.*[29]. Azadirachtin (the most effective compound of the neem tree, *Azadirachta indica*) has been

described to cause reduction in the RGR of the 3rd instar larvae *S. littoralis*[30]. In addition, the jojoba and sesame oils have been shown to alter nutritional indices of 4th instar larvae of *S. littoralis* [31].

On the other hand, the effects of essential oils on nutritional indices of other insects have been studied. The essential oils were found to cause significant reduction in RGR, ECI and ECD of *Plodia interpunctella* larvae[32] and *Rhyzopertha dominica* adults[33]. Moreover, the essential oils altered nutritional indices of the adults of *Sitophilus oryzae*[34], the 4th instar larvae and adults of *Leptinotarsa decemlineata*[35] and 4th instar larvae of *Glyphodes pyloalis* [36].

Nutritional indices measure the efficiencies of digestion or utilization of diets by insects. Lower nutritional indices, such as RGR, ECI and ECD perhaps lead to insect growth retardation and formation of smaller insect life stages, which results in reduced fertility, fecundity and longevity of the adult insect and makes them susceptible to diseases and natural enemies[37]. The tested essential oils significantly decreased nutritional indices of *S. littoralis* larvae. Therefore, these oils may be useful for reducing the population or controlling *S. littoralis*.

The results of antifeedant experiment indicated that the tested essential oils have variable levels of antifeedant activities against *S. littoralis* larvae with *C. lemon* oil being the most potent one. These findings are supported by previous studies explaining the antifeedant activity of essential oils and their major components, monoterpenes on *S. littoralis*[38-40]. In general, the antifeedants can inhibit insect feeding through sensory perception, such as having an unpalatable taste to insects[41] or through postingestive effects[42]. Essential oils possess aromatic properties and make insects disgusted by food, reducing or stopped feeding[43]. Some essential oils and monoterpenes were reported to possess inhibitory effect on α-amylase and other digestive enzymes[44-46]. In addition, as shown in this study, the essential oils may inhibit insect feeding through changes in nutritional indices.

Moreover, the essentials oils of *A. judaica*, *C. lemon*, *O. vulgare*, *R. officinalis* and *S. molle* were found to have a deleterious action on the insect growth even after the feeding period has ceased, likely as a result in the reduction of food intake and to the ability of converting food into biomass and this is consistent with research on testing certain essential oil against several other lepidopteran insects. Similarly, *Artemisia campestris* extracts caused pronounced larval growth inhibition of *S. littoralis*[47]. Although, the tested oils showed pronounced larval growth inhibition, the oils had moderate antifeedant activity. Similar findings were reported by Barnby and Klocke[48] on the bioactivity of azadirachtin against *S. littoralis*.

Also, the obtained data emphasized that *C. lemon* and *A. judaica* inhibited the larval growth of *S. littoralis*, which indicates that the tested essential oils could be considered as inhibitors of chitin synthesis. These interpretations are in accordance with the findings of many authors such as Hughes et al.[19] who explained the inhibition of growth and development of the tobacco hornworm, *Manduca sexta*.

In the present study the essential oils of *A. judaica*, *C. lemon*, *O. vulgare*, *R. officinalis* and *S. molle* showed moderate to weak insecticidal activity against the fourth instar larvae of *S. littoralis* after 72 h of treatment. The reasons for this weak insecticidal activity of tested oils may be attributed to the use of residual film assay and the antifeedant activity of essential oils. In our earlier studies, the essential oils and monoterpenes showed potent toxicity against *S. littoralis* in fumigant assay while they showed a weak activity in both topical application and residual film assays[49,50]. This indicates that the bioassay method is crucial when evaluating the toxicity of essential oils. Similar conclusion has been stated on the insecticidal activity of essential oils against stored product insects by Kim *et al.*[51]. Also, antifeedant potential of tested oils decreased food intake and exposure of insects to oils which resulted in weak insecticidal activity particularly after short periods of treatment.

The plants substances are known to cause reproductive sterility in insects. Some of these compounds inhibit ovarian growth, testes growth and development[7]. The present results illustrated the undifferentiated ovarioles of *S. littoralis* adults developed from larvae fed for three days on castor bean leaves treated with essential oils. Similar observations have been noticed by Root and Dauterman[52]. Also, the results of Martinez and Van Emden[30] on the effects of azadirachtin on *S. littoralis* confirmed our findings. The occurrence of some of permanent copulations in moths may be due to malformation of the general reproductive system and premature sclerotisation[53].

CONCLUSION

In conclusion, the essential oils from *A. judaica*, *C. lemon*, *O. vulgare*, *R. officinalis* and *S. molle* decreased nutritional indices (RGR, ECI and ECD) of *S. littoralis*. Also, the tested oils revealed pronounced antifeedant, growth inhibitory and insecticidal activities against the insect. In addition, the tested oils reduced chitin formation and disrupted female reproductive system. The results also indicated that the essential oils caused their effects on insects through several modes of action as they act as toxicants, antifeedants, growth inhibitors and sterilants. These multi-modes of action of

essential oils delayed the development of resistance. Therefore, the results of current study suggest that the essential oils should be developed as potential natural insecticides for integrated pest management of *S. littoralis*.

SIGNIFICANCE STATEMENT

This is the first study describing the anti-nutritional, antifeedant and growth inhibitory activities of the tested essential oils against *Sopdoptera littoralis* larvae. Based on the results of this study, it is possible to use essential oils for reducing the development and population of *S. littoralis*. The findings of this study will encourage the development of new eco-friendly essential oil-based products for control *S. littoralis*.

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REFERENCES

1. Sadek, M.M., 2003. Antifeedant and toxic activity of *Adhatoda vasica* leaf extract against *Spodoptera littoralis* (Lep., Noctuidae). J. Applied Entomol., 127: 396-404.
2. Pineda, S., M.I. Schneider, G. Smagghe, A.M. Martinez and P. Del Estal *et al*., 2007. Lethal and sublethal effects of methoxyfenozide and spinosad on *Spodoptera littoralis* (Lepidoptera: Noctuidae). J. Econ. Entomol., 100: 773-780.
3. Shonouda, M.L. and S.I. Osman, 2000. New botanical derivatives, used in medicinal preparations, showing bioactive action on insect pests. I- Toxicological effect on the development of *Spodoptera littoralis* Boisd. J. Egypt. Ger. Soc. Zool., 31: 227-234.
4. El-Sheikh, E.S.A. and M.M. Aamir, 2011. Comparative effectiveness and field persistence of insect growth regulators on a field strain of the cotton leafworm, *Spodoptera littoralis*, Boisd (Lepidoptera: Noctuidae). Crop Prot., 30: 645-650.
5. Duke, J.A., 1985. CRC Handbook of Medicinal Herbs. 1st Edn., CRC Press, Inc., Florida, USA.
6. Ladhari, A., A. Laarif, F. Omezzine and R. Haouala, 2013. Effect of the extracts of the spiderflower, *Cleome Arabica*, on feeding and survival of larvae of the cotton leafworm, *Spodoptera littoralis*. J. Insect Sci., Vol. 13. 10.1673/031.013.6101.
7. Tunaz, H. and N. Uygun, 2004. Insect growth regulators for insect pest control. Turk. J. Agric. For., 28: 377-387.
8. Isman, M.B., 2000. Plant essential oils for pest and disease management. Crop Protect., 19: 603-608.
9. Koul, O., S. Walia and G.S. Dhaliwal, 2008. Essential oils as green pesticides: Potential and constraints. Biopestic. Int., 4: 63-84.
10. Saeidi, K. and B. Hassanpour, 2014. Efficiency of *Mentha piperita* L. and *Mentha pulegium* L. essential oils on nutritional indices of *Plodia interpunctella* Hubner (Lepidoptera: Pyralidae). J. Entomol. Acarol. Res., 46: 13-17.
11. Sendi J.J. and A. Ebadollahi, 2014. Biological Activities of Essential Oils on Insects. In: Recent Progress in Medicinal Plants (RPMP): Essential Oils-II. Govil, J.N. and S. Bhattacharya (Eds.)., Vol. 37, Studium Press LLC., India, pp: 129-150.
12. Yazdani, E., J.J. Sendi and J. Hajizadeh, 2014. Effect *of Thymus vulgaris* L. and *Origanum vulgare* L. essential oils on toxicity, food consumption and biochemical properties of lesser mulberry pyralid *Glyphodes pyloalis* walker (Lepidoptera: Pyralidae). J. Plant Prot. Res., 54: 53-61.
13. El-Zoghaby, F., 1980. Studies on the effects of some materials from plant origin on insects. Ph.D. Thesis, Faculty of Agriculture, Alexandria University, Egypt.
14. Morimoto, M., H. Fukumoto, M. Hiratani, W. Chavasiri and K. Komai, 2006. Insect antifeedants, pterocarpans and pterocarpol, in heartwood of *Pterocarpus macrocarpus* Kruz. Biosci. Biotechnol. Biochem., 70: 1864-1868.
15. Miller, J.R. and T.A. Miller, 1988. Insect-Plant Interactions. Springer-Verlag, New York.
16. Klein, I. and M. Kogan, 1974. Analysis of food intake, utilization and growth in phytophagous insects-A computer program. Ann. Entomol. Soc. Am., 67: 295-297.
17. Farrar, R.R., J.D. Bardour and G.G. Kennedy, 1989. Quantifying food consumption and growth in insects. Entomol. Soc. Am., 82: 593-598.
18. Isman, M.B., O. Koul, A. Luczynski and J. Kaminski, 1990. Insecticidal and antifeedant bioactivities of neem oils and their relationship to azadirachtin content. J. Agric. Food Chem., 38: 1406-1411.
19. Hughes, P.B., W.C. Dauterman and N. Motoyama, 1989. Inhibition of growth and development of tobacco hornworm (Lepidoptera: Sphingidae) larvae by cyromazine. J. Econ. Entomol., 82: 45-51.
20. Finney, D.J., 1971. Probit Analysis. 3rd Edn., Cambridge University Press, Cambridge, UK., Pages: 318.
21. Junqueira, L.C. and J. Carneiro, 1980. Basic Histology. 3rd Edn., Lange Medical Publications, Maruzen Asia Limited, Tokyo, pp: 8-18.
22. Chalchat, J.C., R.P. Garry, A. Michet, B. Benjilali and J.L. Chabart, 1993. Essential oils of rosemary (*Rosmarinus officinalis* L.). The chemical composition of oils of various origins (Morocco, Spain, France). J. Essent. Oil Res., 5: 613-618.
23. Viuda-Martos, M., Y. Ruiz-Navajas, J. Fernandez-Lopez and J.A. Perez-alvarez, 2009. Chemical composition of mandarin (*C. reticulate* L.), grapefruit (*C. paradise* L.), lemon (*C. limon* L.) and orange (*C. sinensis* L.) essential oils. J. Essent. Oil Bear. Plants, 12: 236-243.

24. Bendaoud, H., M. Romdhane, J.P. Souchard, S. Cazaux and J. Bouajila, 2010. Chemical composition and anticancer and antioxidant activities of *Schinus molle* L. and *Schinus terebinthifolius* Raddi berries essential oils. J. Food Sci., 75: C466-C472.
25. Sahin, F., M. Gulluce, D. Daferera, A. Sokmen and M. Sokmen *et al*., 2004. Biological activities of the essential oils and methanol extract of *Origanum vulgare* ssp. vulgare in the Eastern Anatolia region of Turkey. Food Control, 15: 549-557.
26. Mohamed, M.I.E. and S.A.M. Abdelgaleil, 2008. Chemical composition and insecticidal potential of essential oils from Egyptian plants against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Applied Entomol. Zool., 43: 599-607.
27. Perry, N.B., R.E. Anderson, N.J. Brennan, M.H. Douglas, A.J. Heaney, J.A. McGimpsey and B.M. Smallfield, 1999. Essential oils from Dalmatian sage (*Salvia officinalis* L.): Variations among individuals, plant parts, seasons and sites. J. Agric. Food Chem., 47: 2048-2054.
28. Pavela, R. and T. Chermenskaya, 2004. Potential insecticidal activity of extracts from 18 species of medicinal plants on larvae of *Spodoptera littoralis*. Plant Prot. Sci., 40: 145-150.
29. Pavela, R., N. Vrchotova and B. Sera, 2008. Growth inhibitory effect of extracts from *Reynoutria* sp. plants against *Spodoptera littoralis* larvae. Agrociencia, 42: 573-584.
30. Martinez, S.S. and H.F. van Emden, 2001. Growth disruption, abnormalities and mortality of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) caused by Azadirachtin. Neotropical Entomol., 30: 113-125.
31. Marei, S.S., E.M. Amr and N.Y. Salem, 2009. Effect of some plant oils on biological, physiological and biochemical aspects of *Spodoptera littoralis* (Boisd). Res. J. Agric. Biol. Sci., 5: 103-107.
32. Shahab-Ghayoor, H. and K. Saeidi, 2015. Antifeedant activities of essential oils of *Satureja hortensis* and *Fumaria parviflora* against Indian meal moth *Plodia interpunctella* Hubner (Lepidoptera: Pyralidae). Entomol. Ornithol. Herpetol., Vol. 4. 10.4172/2161-0983.1000154.
33. Bahrami, R., F. Kocheili and M. Ziaee, 2016. Effects of asafoetida, geranium and walnut leaves essential oils on nutritional indices and progeny reduction on *Rhyzopertha dominica* adults (Coleoptera: Bostrychidae). J. Crop Prot., 5: 369-375.
34. Benzi, V., N. Stefanazzi and A.A. Ferrero, 2009. Biological activity of essential oils from leaves and fruits of pepper tree (*Schinus molle* L.) to control rice weevil (*Sitophilus oryzae* L.). Chilean J. Agric. Res., 69: 154-159.
35. Saroukolai, A.T., G. Nouri-Ganbalani, H. Rafiee-Dastjerdi and J. Hadian, 2014. Antifeedant activity and toxicity of some plant essential oils to Colorado potato beetle. Plant Prot. Sci., 50: 207-216.
36. Yazdani, E., J.J. Sendi and A. Aliakbar, 2013. Chemical composition, toxicity and physiological effects of essential oil of *Rosemarinus officinalis* on lesser mulberry pyralid, *Glyphodes pyloalis* Walker (Lepidoptera: Pyralidae). J. Crop Prot., 2: 461-476.
37. Khosravi, R., J. Sendi and M. Ghadamyari, 2010. Effect of *Artemisia annua* L. on deterrence and nutritional efficiency of lesser mulberry pyralid (*Glyphodes pylolais* Walker) (Lepidoptera: Pyralidae). J. Plant Prot. Res., 50: 423-428.
38. Sabbour, S. and S.A.E. Aziz, 2002. Efficacy of some botanical oils formulated with microbial agents against the cotton leafworm and greasy cutworm attaching cotton plants. Bull. Entomol. Soc. Egypt, 28: 135-151.
39. Abdelgaleil, S.A.M., M.A. Abbassy, A.S.H. Belal and M.A.A. Abdel Rasoul, 2008. Bioactivity of two major constituents isolated from the essential oil of *Artemisia judaica* L. Bioresour. Technol., 99: 5947-5950.
40. Ali, A.M., D.S. Mohamed, E.H. Shaurub and A.M. Elsayed, 2017. Antifeedant activity and some biochemical effects of garlic and lemon essential oils on *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). J. Entomol. Zool. Stud., 5: 1476-1482.
41. Chapman, R.F., 1995. Chemosensory Regulation of Feeding. In: Regulatory Mechanisms in Insect Feeding, Chapman, R.F. and G. de Boer (Eds.)., Chapman and Hall, New York, pp: 101-136.
42. Glendinning, J.I., 1996. Is chemosensory input essential for the rapid rejection of toxic foods? J. Exp. Biol., 199: 1523-1534.
43. Arasu, M.V., N.A. Al-Dhabi, V. Saritha, V. Duraipandiyan, C. Muthukumar and S.J. Kim, 2013. Antifeedant, larvicidal and growth inhibitory bioactivities of novel polyketide metabolite isolated from *Streptomyces* sp. AP-123 against *Helicoverpa armigera* and *Spodoptera litura*. BMC Microbiol., Vol. 13. 10.1186/1471-2180-13-105
44. Basak, S.S. and F. Candan, 2010. Chemical composition and *in vitro* antioxidant and antidiabetic activities of *Eucalyptus camaldulensis*. Essential oil. J. Iran. Chem. Soc., 7: 216-226.
45. Sudha, P., S.S. Zinjarde, S.Y. Bhargava and A.R. Kumar, 2011. Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. BMC Complement. Altern. Med., Vol. 11. 10.1186/1472-6882-11-5.
46. Kohl, K.D., E. Pitman, B.C. Robb, J.W. Connelly, M.D. Dearing and J.S. Forbey, 2015. Monoterpenes as inhibitors of digestive enzymes and counter-adaptations in a specialist avian herbivore. J. Comparat. Physiol. B, 185: 425-434.
47. Pavela, R., 2011. Screening of Eurasian plants for insecticidal and growth inhibition activity against *Spodoptera littoralis* larvae. Afr. J. Agric. Res., 6: 2895-2907.

48. Barnby, M.A. and J.A. Klocke, 1987. Effects of azadirachtin on the nutrition and development of the tobacco budworm, *Heliothis virescens* (Fabr.) (Noctuidae). J. Insect. Physiol., 33: 69-75.
49. El-Aswad, A.F. and S.A.M. Abdelgaleil, 2008. Insecticidal, antifeedant and molluscicidal potential of essential oils extracted from Egyptian plants. J. Egy. Soc. Toxicol., 38: 81-91.
50. Abdelgaleil, S.A.M., 2010. Molluscicidal and insecticidal potential of monoterpenes on the white garden snail, *Theba pisana* (Muller) and the cotton leafworm, *Spodoptera littoralis* (Boisduval). Applied Entomol. Zool., 45: 425-433.
51. Kim, S.I., J.Y. Roh, D.H. Kim, H.S. Lee and Y.J. Ahn, 2003. Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis*. J. Stored Prod. Res., 39: 293-303.
52. Root, D.S. and W.C. Dauterman, 1996. Cyromazine toxicity in different laboratory strains of the tobacco hornworm (Lepidoptera: Sphingidae). J. Econ. Entomol., 89: 1074-1079.
53. Navon, A. and H.Z. Levinson, 1976. Oral application of D-glucoascorbic acid to adult *Spodoptera littoralis*(Boisduval)(Lepidoptera, Noctuidae) inducing sterility by spermatophore malformation. Bull. Entomol. Res., 66: 437-442.