

Research Article

## Anti-nutritional, antifeedant, growth-disrupting and insecticidal effects of four plant essential oils on *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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**Abstract:** Essential oils of four aromatic plants, *Artemisia monosperma* Del., *Callistemon viminalis* (Sol.ex Gaertn.) G. Don, *Citrus aurantifolia* (Christm.) Swingle and *Cupressus macrocarpa* Hartw. ex Gordon, were evaluated for their anti-nutritional, antifeedant, growth inhibitory and insecticidal activities against *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). The essential oils of *A. monosperma* and *C. aurantifolia* caused the highest reduction in relative growth rate (RGR) at the tested concentrations (125, 250, 500, 1000 and 2000mg/l). The RGR values ranged between 8.63 and 3.05 mg/day for *A. monosperma*, and between 10.74 and 2.89 mg/day for *C. aurantifolia* compared with 14.89 mg/day for control after 72 h of treatment. In general, the results showed that the values of relative growth rate (RGR) decreased with increasing the concentration of the tested oils. In addition, the tested oils significantly reduced efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) values, particularly at the higher concentrations of 500, 1000 and 2000mg/l. On the other hand, the tested oils showed antifeedant activity against the larvae of *S. littoralis* with *A. monosperma* and *C. aurantifolia* oils being more active than *C. viminalis* and *C. macrocarpa* oils. The tested oils showed remarkable growth inhibition effect as the growth inhibition index values were increased from 37.63 to 79.80% for *A. monosperma*, from 21.69 to 52.12% for *C. viminalis*, from 16.55 to 28.59% for *C. aurantifolia* and from 37.64 to 52.32% for *C. macrocarpa* when the concentration increased from 125 to 2000mg/l. Based on chitin formation ratio values, the tested essential oils induced reduction in chitin formation. *A. monosperma* and *C. macrocarpa* essential oils revealed the highest insecticidal activity on 4<sup>th</sup> instar larvae of *S. littoralis*. Examination of reproductive tracts of adult females emerged from treated larvae indicated that the tested oils caused undifferentiated ovarioles.

**Keywords:** Essential oils, *Spodoptera littoralis*, nutritional indices, feeding deterrence, chitin formation, growth inhibition

### Introduction

The cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is a

serious polyphagous caterpillar damaging more than 85 host plants belonging to 40 plant families of economic importance. *Spodoptera littoralis* is distributed throughout the world in Southern Europe, Africa, the Middle East (Abo-El-Ghar, *et al.*, 1986; Sadek 2003; El-Sabrou, 2013), and the Mediterranean area (Pineda *et al.*, 2007). This noctuid insect is native in Africa (Tanani *et al.*, 2016, Shonouda and

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Osman, 2000). The insect attacks several economic crops and causes a great yield loss. It causes direct damage by reducing plant photosynthesis and reduces market value of vegetables and ornamentals due to the presence of larvae, their excrements and feeding damage in these products (El-Sheikh and Amir, 2011). Therefore, this pest remains one of the most destructive agricultural lepidopterous pests within its subtropical and tropical range.

Plants have been receiving global attention and also their secondary metabolites, such as alkaloids, essential oils, terpenoids, steroids, polyphenols, lignans and sugars that protect the plants from insect pests. 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 (Duke, 1985; El-Sabrou, 2009). Botanical insecticides offer a more natural, “environmentally friendly” approach to pest control than synthetic insecticides do, because the continuous use of the chemical pesticides leads to development of resistance. Over 2000 species of plants are known to possess insecticidal activity, by containing either antifeedant, repellent, or insecticidal compounds (Ladhari *et al.*, 2013). Some of these botanical insecticides have revealed insect growth regulatory effects. They disrupt the hormonal regulation of metamorphosis and moulting process. These effects are manifested by changes in hemolymph ecdysteroid and juvenile hormone levels due to retardation and/or delay in their release from neurohaemal organs. Therefore, the plants metabolites are known to cause damages in insect reproductive organs. Some of these metabolites inhibit growth and development of ovaries and testes, and others appear to induce major changes in the chemical structure of nucleic acids. The natural compounds might be useful since all chemosterilants were found to be extremely hazardous compounds (1992, El-Sabrou, 2009). The insecticidal potential of plant essential oils and extracts has been assessed against agricultural and public health insects. The plant families, Meliaceae, Rutaceae, Labiateae, Asteraceae, Convolvulaceae and Pedaliaceae are

among the most promising sources of plant-based insecticides (Isman, 2000).

Essential oils are defined as mixture of volatile compounds that give a distinctive odor, flavor or scent to a plant. They are plant secondary metabolites containing mainly monoterpenes, sesquiterpenes, and their oxygenated derivatives (Koul *et al.*, 2008; Saeidi and Hassanpour, 2014; Choudhary *et al.*, 2017). The essential oils were found to have contact and fumigant toxicities, and repellent and antifeedant effects (Mohamed and Abdelgaleil, 2008; Jalali Sendi and Ebadollahi, 2014; Yazdani *et al.*, 2014).

The purpose of the present study was to evaluate the antifeedant, growth inhibitory and insecticidal activities of the essential oils isolated from four plants grown in Egypt, viz. *Artemisia monosperma* Del., *Callistemon viminalis* (Sol. ex Gaertn.) G. Don, *Citrus aurantifolia* (Christm.) Swingle and *Cupressus macrocarpa* Hartw. ex Gordon, on the fourth instar larvae of *S. littoralis*. In addition, the effect of essential oils on food utilization was examined.

## Materials and Methods

### Insect rearing

A susceptible strain of the cotton leafworm, *S. littoralis* was reared on castor leaves, *Ricinus communis* L. at  $25 \pm 3$  °C and  $70 \pm 5\%$  R.H, according to El-Zoghaby (1980). Egg-masses were confined in sterilized jars and tapped with muslin covers. Upon hatching, fresh and clean castor oil leaves were provided as food. Jars were daily cleaned out where fresh leaves were substituted for the used ones. Upon pupation, pupae were sexed prior to moth emergence. Adult moths were supplied by 10% sugar solution in which a cotton wick was immersed for feeding. In addition, two leaves of *Nerium oleander* L. were provided as oviposition sites. Deposited egg-masses were daily collected and the hatched larvae were reared again for another generation.

### Extraction and GC–MS analysis of essential oils

The plant sample of *A. monosperma* (leaves) was collected from Matrouh ( $31^{\circ}19'N$ ,

27°09'E) and samples of *C. viminalis* (leaves), *C. aurantifolia* (fruit peel) and *C. macrocarpa* (leaves) were collected from Alexandria (31°13'N, 29°58'E) in August, 2013. The essential oils were obtained by subjecting plant materials to hydrodistillation using a Clevenger apparatus for 3h. Anhydrous sodium sulfate was used to remove water after the oil extraction. The chemical composition (Table 1) of essential oils was determined by GC-MS as described by Abdelgaleil *et al.* (2016).

**Table 1** Major constituents of essential oils isolated from four Egyptian plants.

Essential oil	Major components (%)
<i>A. monosperma</i>	Capillene (36.86), capillin (14.68), $\gamma$ -Terpinene (12.46), $\beta$ -Pinene (7.85), <i>cis</i> -Ocimene (3.26), 4-Terpineol (2.59)
<i>C. viminalis</i>	1,8-Cineole (71.77), $\alpha$ -Pinene (11.47), Terpinen-4-ol (3.18), Octadecanoic acid (3.08), 1-Phellandrene (1.30), Terpinen-4-ol (1.22)
<i>C. aurantifolia</i>	Limonene (40.19), $\beta$ -Pinene (19.65), $\alpha$ -Citral (8.14), $\gamma$ -Terpinene (6.34), $\alpha$ -Terpineol (3.71), Terpinen-4-ol (2.62)
<i>C. macrocarpa</i>	Terpinen-4-ol (20.29), Sabinene (18.67), $\beta$ -Citronellol (13.01), $\gamma$ -Terpinene (7.59), Campher (6.66), $\alpha$ -Terpinene (4.50)

### Feeding bioassay

Feeding assay with the no-choice test technique was used to evaluate the bioactivity of essential oils against the newly moulted fourth instar larvae of *S. littoralis* by preparing leaf discs of 2 cm in diameter from leaves of castor oil as described by Morimoto *et al.* (2006). The essential oils were dissolved in acetone. For each essential oils, five concentrations were used (125, 250, 500, 1000 and 2000mg/l), while the control treatment was conducted with acetone only. The discs were immersed in acetone solutions of essential oils for 2 seconds and left for 2 minutes for solvent evaporation before introducing to petri dishes. Four replicates were carried out for each concentration. In each replicate, 10 larvae were released in a plastic Petri dish (9 cm in diameter) containing 5 treated discs. The larvae were allowed to feed for 72 h on treated discs that were 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 at  $25 \pm 3$  °C,  $70\% \pm 5\%$  R.H., and a photoperiod regime of 14: 10h (light/dark).

### Nutritional indices

The essential oils were investigated on food consumption and utilization by the newly molted fourth instar larvae of *S. littoralis*. 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 fourth instar larvae. All larvae feces and unconsumed food were weighed every 24h, all over 72h feeding period. The nutritional indices, including the relative growth rate (RGR) were determined according to Miller and Miller (1988) and the efficiency of conversion of digested food (ECD) according to Klein and Kogan (1974). The formulae of Farrar *et al.* (1989) were applied as follow:

Relative growth rate (RGR) =  $\Delta B$  / feeding period.

Change in body weight ( $\Delta B$ ) = (final weight – initial weight) / No. of larvae.

Efficiency of conversion of ingested food (ECI) =  $\Delta B$  / I  $\times$  100.

where I = weight of the food consumed = consumed food / No. of larvae.

Efficiency of conversion of digested food (ECD) =  $\Delta B$  / (I – F)  $\times$  100.

where F = weight of the feces produced during the feeding period / No. of larvae.

### Antifeedant activity

The antifeedant activity of the essential oils was tested using fresh leaf discs of castor bean. The tested oils were evaluated at 125, 250, 500, 1000 and 2000mg/l. The feeding-deterrence index suggested by Isman *et al.* (1990) was used as follows:

The feeding-deterrence index (FDI) =  $[(C - T) / C] \times 100$ . 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 fourth instar larvae of *S. littoralis* after 72h of treatment with different concentrations were calculated according to the following formula:

$$\text{Growth inhibition index} = [(CL - TL) / CL] \times 100.$$

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 6<sup>th</sup> instar larvae of *S. littoralis*. The larvae were fed for 72h on both fresh discs (control) and discs treated with the tested four essential oils (2000mg/l). The procedures followed as those of Hughes *et al.* (1989). The ruptured larvae were weighed in the same age as 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 4h, 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:

Ratio of chitin formation = Chitin dry weight/Larval fresh weight.

### Toxicity of essential oils against *S. littoralis* larvae

Larval mortality of *S. littoralis* was recorded after feeding on castor leaves treated with essential oils at concentrations of 125, 250, 500, 1000 and 2000 mg/l for three days. There were 10 larvae in each replicate and four replicates in each concentration. Mortality data were subjected to probit analysis to estimate the lethal concentration values (LC<sub>50</sub>) of essential oils (Finney, 1971).

### Reproductive tracts dissection

Reproductive tracts of both treated and control female adults were dissected 24 h after emergence. The female reproductive tracts were dissected from insects under a binocular microscope (10 × magnification) in Ringer's solution (0.42 g KCl, 0.2 g NaHCO<sub>3</sub>, 9.0 g NaCl, 0.48 g CaCl<sub>2</sub> in 1000 ml distilled water) according to the method of Junqueira and Carneiro (1980).

### Statistical analysis

Data on 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 (Cohort Software Inc. 1985) to determine significant differences among mean values at the probability level of 0.05. The mortality data were subjected to probit analysis for calculating LC<sub>50</sub> and other statistic parameters (Finney, 1971) using the SPSS14.0 (Statistical Package of Social Sciences Inc., USA) software. The values of LC<sub>50</sub> were considered significantly different if the 95% confidence limits did not overlap.

### Results

#### Chemical composition of essential oils

The analysis of essential oils by GC/MS (Table 1) revealed that the main components of the essential oils were capillene (36.86%), capillin (14.68%), and  $\gamma$ -terpinene (12.46%) in *Artemisia monosperma*; 1,8-cineole (71.77%),  $\alpha$ -pinene (11.47%) and terpinen-4-ol (3.18%) in *Callistemon viminalis*; limonene (40.19%),  $\beta$ -pinene (19.65%) and  $\alpha$ -citral (8.14%) in *Citrus aurantifolia*; terpinen-4-ol (20.29%), sabinene (18.67%) and  $\beta$ -citronellol (13.01%) in *Cupressus macrocarpa*. The major constituents of the essential oils mainly belonged to three chemical groups: oxygenated monoterpenes (i.e. 1,8-cineole,  $\alpha$ -citral,  $\beta$ -citronellol and terpinen-4-ol); monoterpene hydrocarbons (i.e. limonene,  $\alpha$ - and  $\beta$ -pinene,  $\gamma$ -terpinene and sabinene) and polyacetylenes (i.e. capillene and capillin).

**Effects of essential oils on the nutritional indices of *S. littoralis* larvae**

The nutritional indices of *S. littoralis* larvae allowed to feed on fresh discs treated with four

essential oils extracted from *A. monosperma*, *C. viminalis*, *C. aurantifolia* and *C. macrocarpa* were calculated and are summarized in Tables 2, 3, 4 and 5.

**Table 2** Effect of essential oils on relative growth rate (RGR) (mg/day  $\pm$ SE) of fourth instar larvae of *Spodoptera littoralis* after 72h of treatment with different essential oil concentrations.

Oil	Concentration (mg/l)				
	125	250	500	1000	2000
Control	14.89 $\pm$ 0.29a	14.89 $\pm$ 0.29a	14.89 $\pm$ 0.29a	14.89 $\pm$ 0.29a	14.89 $\pm$ 0.29a
<i>A. monosperma</i>	8.63 $\pm$ 0.18c	5.22 $\pm$ 0.62b	4.73 $\pm$ 0.57c	4.07 $\pm$ 0.37d	3.05 $\pm$ 0.28d
<i>C. viminalis</i>	14.55 $\pm$ 0.95a	13.78 $\pm$ 0.49a	13.78 $\pm$ 0.11a	12.11 $\pm$ 1.62b	10.78 $\pm$ 0.90b
<i>C. aurantifolia</i>	10.74 $\pm$ 1.65bc	6.87 $\pm$ 1.55b	5.67 $\pm$ 0.33c	5.22 $\pm$ 0.06d	2.89 $\pm$ 0.11d
<i>C. macrocarpa</i>	13.35 $\pm$ 0.68ab	11.32 $\pm$ 1.45a	7.91 $\pm$ 0.95b	8.28 $\pm$ 0.61c	6.85 $\pm$ 0.36c

Data are means of four replications of 10 insects each.

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 (ECI) (%  $\pm$ SE) by fourth instar larvae of *Spodoptera littoralis* after 72h of treatment with different essential oil concentrations.

Oil	Concentration (mg/l)				
	125	250	500	1000	2000
Control	20.87 $\pm$ 0.27a	20.87 $\pm$ 0.27a	20.87 $\pm$ 0.27a	20.87 $\pm$ 0.27a	20.87 $\pm$ 0.27a
<i>A. monosperma</i>	9.0 $\pm$ 1.29b	7.44 $\pm$ 0.60c	8.09 $\pm$ 0.91c	6.83 $\pm$ 0.68c	6.08 $\pm$ 0.47d
<i>C. viminalis</i>	25.15 $\pm$ 1.76a	23.69 $\pm$ 1.60a	23.02 $\pm$ 2.01a	18.80 $\pm$ 2.25a	17.23 $\pm$ 2.0a
<i>C. aurantifolia</i>	16.09 $\pm$ 1.32c	11.95 $\pm$ 0.91b	11.09 $\pm$ 0.61bc	10.10 $\pm$ 0.34c	5.27 $\pm$ 0.39d
<i>C. macrocarpa</i>	15.94 $\pm$ 1.46c	14.13 $\pm$ 1.95b	13.40 $\pm$ 0.23b	14.20 $\pm$ 0.87b	10.93 $\pm$ 0.85b

Data are means of four replications of 10 insects each.

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 (ECD) (%  $\pm$ SE) by fourth instar larvae of *Spodoptera littoralis* after 72h of treatment with different essential oil concentrations.

Oil	Concentration (mg/l)				
	125	250	500	1000	2000
Control	23.27 $\pm$ 0.16b	23.27 $\pm$ 0.16b	23.27 $\pm$ 0.16b	23.27 $\pm$ 0.16a	23.27 $\pm$ 0.16a
<i>A. monosperma</i>	9.81 $\pm$ 1.00d	7.77 $\pm$ 1.25d	7.88 $\pm$ 0.12d	7.21 $\pm$ 0.73d	6.39 $\pm$ 0.47c
<i>C. viminalis</i>	29.93 $\pm$ 0.64a	27.87 $\pm$ 1.33a	28.69 $\pm$ 0.93a	22.46 $\pm$ 1.51a	21.40 $\pm$ 1.30a
<i>C. aurantifolia</i>	21.32 $\pm$ 1.74b	15.55 $\pm$ 0.29c	12.04 $\pm$ 0.51a	11.08 $\pm$ 0.14c	5.49 $\pm$ 0.51c
<i>C. macrocarpa</i>	15.94 $\pm$ 1.46c	14.13 $\pm$ 1.95c	13.12 $\pm$ 0.37c	14.20 $\pm$ 0.87b	10.93 $\pm$ 0.85b

Data are means of four replications of 10 insects each.

Values within a column sharing the same letter are not significantly different at the 0.05 probability level.

**Table 5** Antifeedant activity (FDI) (%  $\pm$ SE) of essential oils on fourth instar larvae of *Spodoptera littoralis* after 72h of treatment with different essential oil concentrations.

Oil	Concentration (mg/l)				
	125	250	500	1000	2000
Control	0.0 $\pm$ 0.0c	0.0 $\pm$ 0.0d	0.0 $\pm$ 0.0c	0.0 $\pm$ 0.0c	0.0 $\pm$ 0.0d
<i>A. monosperma</i>	11.32 $\pm$ 1.08ab	16.86 $\pm$ 1.14a	17.65 $\pm$ 0.90b	19.38 $\pm$ 1.27b	30.13 $\pm$ 0.75a
<i>C. viminalis</i>	9.57 $\pm$ 0.16b	11.14 $\pm$ 0.58b	21.88 $\pm$ 1.66a	20.63 $\pm$ 1.18b	21.67 $\pm$ 1.50b
<i>C. aurantifolia</i>	13.86 $\pm$ 0.65a	17.59 $\pm$ 1.54a	27.0 $\pm$ 1.15a	28.43 $\pm$ 2.08a	28.59 $\pm$ 1.92a
<i>C. macrocarpa</i>	8.22 $\pm$ 1.33b	7.11 $\pm$ 1.38c	22.98 $\pm$ 2.03a	19.79 $\pm$ 1.77b	14.99 $\pm$ 2.12c

Data are means of four replications of 10 insects each.

Values within a column sharing the same letter are not significantly different at the 0.05 probability level.

**Effect on relative growth rate (RGR)**

Table 2 shows the relative growth rate (RGR) values estimated for those larvae treated with different tested concentrations of 125, 250, 500, 1000 and 2000 mg/l. The results showed that all of the tested oils significantly decreased RGR, particularly at the higher concentrations of 500, 1000 and 2000mg/l. The oil of *A. monosperma* and *C. aurantifolia* revealed the greatest reduction in RGR at the tested concentrations. RGR was recorded 14.89 mg/day in the control, while *A. monosperma* oil recorded 8.63, 5.22, 4.73, 4.07 and 3.05 mg/day, *C. aurantifolia* recorded 10.74, 6.87, 5.67, 5.22 and 2.89 mg/day at 125, 250, 500, 1000 and 2000mg/l, respectively. In general, the values of RGR reduced with increasing the tested oil concentrations (Table 2).

**Effect on food consumption**

Table 3 illustrates the efficiency of conversion of ingested food (ECI) values, the overall ability of the insect to convert ingested food into body matter. At all of the tested concentrations, significant reduction of ECI was induced by the tested essential oils except for *C. viminalis*. The essential oil of *C. viminalis* at 125, 250 and 500mg/l may stimulate the fourth instar larvae of *S. littoralis* to feed and convert ingested food into body matter more than the same larval age in control, where the ECI values were 25.15, 23.69 and 23.02%, respectively, as compared with that of control (20.87%). It was clearly noticed that the ECI values were gradually decreased as the concentration of the tested essential oils increased. Again the essential oil of *C. aurantifolia* was the most effective oil showing the least ECI (5.27%) at the concentration of 2000mg/l (Table 3).

**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 500mg/l (*C. viminalis*) and 1000mg/l (*C. macrocarpa*), 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, *A. monosperma* and *C. aurantifolia* (2000mg/l) caused the greatest reduction of ECD.

**Antifeedant activity**

The values of feeding deterrent index (FDI) of the four tested essential oils on the fourth instar larvae of *S. littoralis* after 72h of treatment are given Table 5. The data showed that the tested oils possessed different levels of antifeedant activity. The oil *C. aurantifolia* revealed the highest antifeedant activity at the concentrations of 125, 250, 500 and 1000mg/l. Moreover, this oil and *A. monosperma* oil displayed the highest antifeedant activity at concentration 2000mg/l.

**Effect of essential oils on *S. littoralis* larval growth**

The obtained data in Table 6 show the effect of the four tested essential oils on growth of the fourth instar larvae of *S. littoralis* after 72h of treatment at different concentrations of 125, 250, 500, 1000, 2000mg/l. The tested oils revealed pronounced larval growth inhibition even at the lowest concentration of 125mg/l. The growth inhibition index (GII) values increased gradually as the tested concentrations of essential oils increased. The oil of *A. monosperma* caused the greatest growth inhibition, followed by *C. macrocarpa* and *C. viminalis*, while the oil of *C. aurantifolia* was the least effective one. At 2000mg/l, the values of GII were 79.80, 52.12, 28.59 and 52.32% for *A. monosperma*, *C. viminalis*, *C. aurantifolia* and *C. macrocarpa*, 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 Figure 1. The ratio of chitin formation is expressed as mg chitin/g body wall. The obtained results indicated that the essential oils of *A. monosperma* and *C. aurantifolia* were the most effective in causing the interruption of chitin formation. The values of chitin formation ratio were 46.60mg/g in the control. When larvae

were treated with essential oils at 2000mg/l, these values were: 17.86, 22.8, 18.14 and 21.81 mg/g for *A. monosperma*, *C. viminals*, *C. aurantifolia* and *C. macrocarpa*, respectively.

### Toxicity of essential oils against *S. littoralis* larvae

The results of feeding toxicity assay revealed that the tested oils had pronounced insecticidal activity against the fourth instar larvae of *S.*

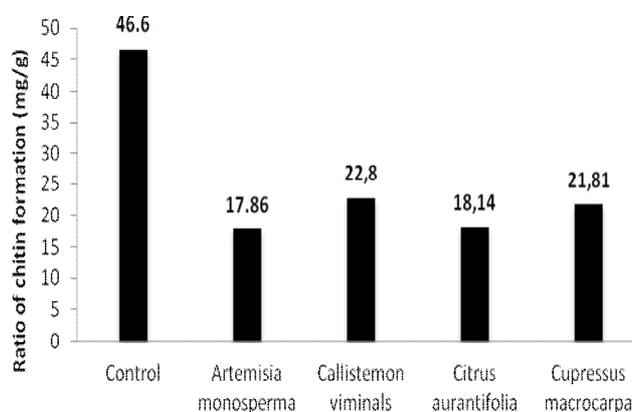
*littoralis* except the oil of *C. viminals*. The LC<sub>50</sub> values were 408.4, >2000, 605.8 and 408.4mg/l for *A. monosperma*, *C. viminals*, *C. aurantifolia* and *C. macrocarpa*, respectively, after 72h of treatment (Table 7). Therefore, the essential oils of *A. monosperma* and *C. macrocarpa* were significantly more toxic than the oil *C. aurantifolia* to the larvae and the oil of *C. viminals* was the least insecticidal.

**Table 6** Effect of essential oils on growth inhibition index (GII) (%±SE) of fourth instar larvae of *Spodoptera littoralis* after 72h of treatment with different essential oil concentrations.

Oil	Concentration (mg/l)				
	125	250	500	1000	2000
Control	0.0 ± 0.0c	0.0 ± 0.0e	0.0 ± 0.0e	0.0 ± 0.0d	0.0 ± 0.0d
<i>A. monosperma</i>	37.63 ± 1.27a	59.52 ± 0.78a	69.65 ± 1.48a	72.59 ± 2.27a	79.80 ± 1.66a
<i>C. viminals</i>	21.69 ± 2.15b	23.54 ± 2.00c	33.53 ± 3.03c	33.50 ± 2.84c	52.12 ± 1.73b
<i>C. aurantifolia</i>	16.55 ± 2.00b	15.91 ± 1.37d	27.0 ± 1.15d	28.43 ± 2.08c	28.59 ± 1.92c
<i>C. macrocarpa</i>	37.64 ± 1.83a	38.82 ± 4.21b	48.21 ± 1.20b	50.25 ± 3.75b	52.32 ± 2.45b

Data are means of four replications of 10 insects each.

Values within a column sharing the same letter are not significantly different at the 0.05 probability level.



**Figure 1** Effect of essential oils on chitin formation of *Spodoptera littoralis* larvae treated with 2000mg/l.

**Table 7** Comparative toxicity of essential oils against larvae of *Spodoptera littoralis*.

Oil	LC <sub>50</sub> (mg/l) <sup>1</sup>	95% Confidence limits (mg/l)		Slope ± SE <sup>2</sup>	Intercept ± SE <sup>3</sup>	(χ <sup>2</sup> ) <sup>4</sup>
		Lower	Upper			
<i>A. monosperma</i>	408.4	326.5	502.1	1.30 ± 0.15	-3.38 ± 0.39	1.93
<i>C. viminals</i>	> 2000	-	-	-	-	-
<i>C. aurantifolia</i>	605.8	524.6	714.2	2.43 ± 0.33	-6.77 ± 0.90	2.03
<i>C. macrocarpa</i>	408.4	305.3	509.8	1.52 ± 0.31	-3.96 ± 0.83	1.37

1. The concentration causing 50% mortality.

2. Slope of the concentration-mortality regression line ± standard error.

3. Intercept of the regression line ± standard error.

4. Chi square value.

Data are means of four replications of 10 insects each.

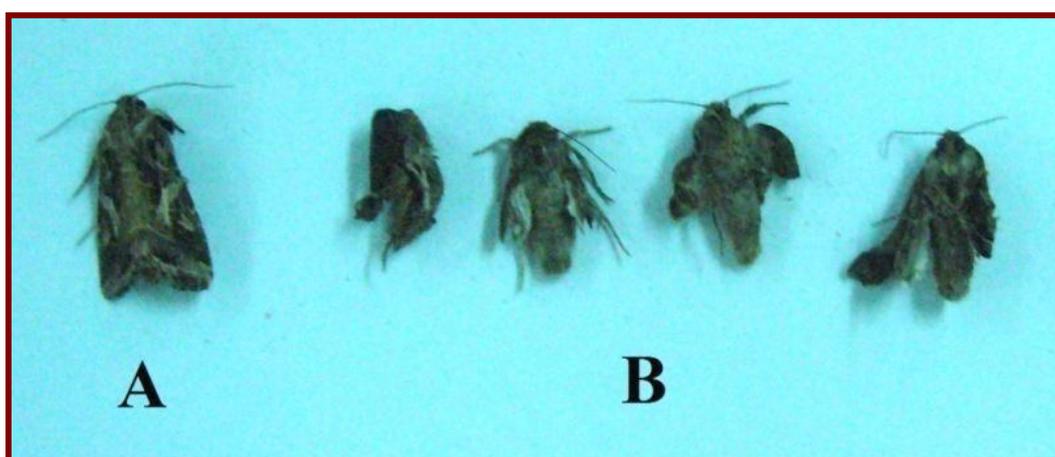
**Hormonal effects of essential oils on *S. littoralis***

The daily observations of the development post the larval treatment with the essential oils of *A. monosperma*, *C. viminalis*, *C. aurantifolia* and *C. macrocarpa* proved their effects on 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 2000mg/l) of the tested essential oils, some larvae either became dwarf and swollen in all their body or at least in thoracic segments and eventually the cuticle ruptured after 72h post-treatment, along the

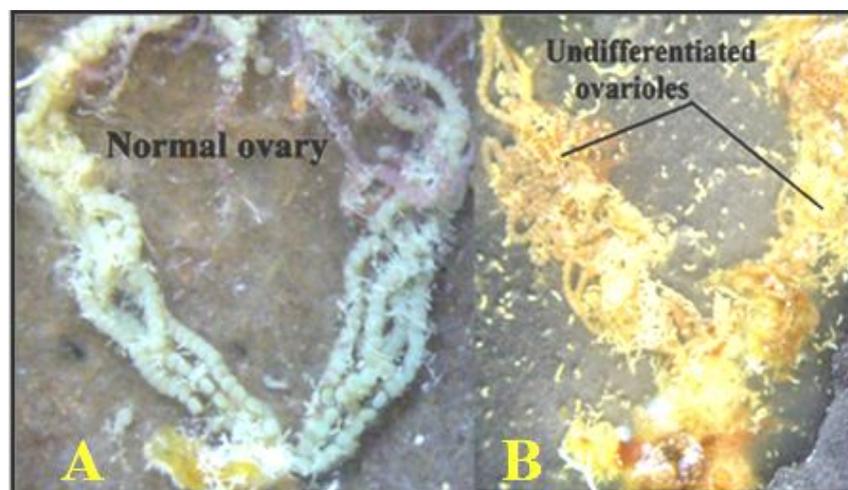
interface between the inter segmental membrane and the more sclerotized portions of the abdominal segments. Larvae were reduced in size to be dwarfed (Figure 2A) and/or were deformed (Figure 2B). Figure 3B shows the formation of larval-pupal intermediates as compared with control (normal pupa) (Figure 3A). Also the female reproductive tracts were tested to check the ovarioles, accessory glands and bursa copulatrix. The results as illustrated in Figure 4 indicate that the ovarioles of adults developed from larvae that fed on leafdiscs treated with the tested essential oils were undifferentiated (Figure 4).



**Figure 2** Dwarfed larvae that resulted after treated with *A. monosperma* essential oil at 2000mg/l.



**Figure 3** (A): Normal adult; (B): Some abnormal adults emerged from larvae fed on fresh discs of castor bean leaves treated with *A. monosperma* essential oil at 2000mg/L.



**Figure 4** (A): Normal ovary; (B): Undifferentiated ovarioles of adult resulted when the larvae fed on fresh discs of castor bean leaves treated with *A. monosperma* essential oil at 2000mg/L.

## Discussion

The chemical compositions of the extracted essential oils from *C. viminalis*, *C. aurantifolia* and *C. macrocarpa* were in agreement with those previously reported on the chemistry of these oils isolated from other countries (Mubarak et al., 2014; Gamarra et al., 2006; Malizia et al., 2000). However, comparing the chemical analysis of the essential oil of *A. monosperma* with those described in previous studies revealed that there were dissimilarities in major constituents (Hifnawy et al., 1990; Khan et al., 2012). Similarly, Sobahi and Abdel-Mogib (2001) stated that the chemical constituents of *Artemisia* spp. are sensitive to the locality variations.

In this study, the essential oils isolated from *A. monosperma*, *C. viminalis*, *C. aurantifolia* and *C. macrocarpa* showed anti-nutritional effects on *S. littoralis*. All of the tested oils significantly decreased RGR, ECI and ECD. The essential oils and plant extracts have been reported to alter nutritional indices of *S. littoralis*. For example, Pavela and Chermenskaya (2004) studied the effect of 18 plant extracts on nutritional indices of the 3<sup>rd</sup> instar larvae of *S. littoralis* and found that the concentration 0.5% of *Artemisia vulgaris* L. extract affects the RGR (9.16 mg/day) and the

other 17 extracts caused clear reduction in RGR, ECI and ECD. Also, *Reynoutria japonica* extract was reported to reduce RGR (2.6 mg/mg d), ECI (10.95%) and ECD (18.8%) of the 5<sup>th</sup> instar larvae of *S. littoralis* fed on a diet treated with 7.5 mg/g of extract (Pavela et al., 2008). Treatment with azadirachtin (the most effective compound of the neem tree, *Azadirachta indica*) at 0.1 mg/kg has been described to cause reduction in the MRGR of the 3<sup>rd</sup> instar larvae *S. littoralis* to be 0.224 compared to 0.247 in control (Martinez and Van Emden, 2001). In addition, the jojoba and sesame oils have been shown to alter nutritional indices of 4<sup>th</sup> instar larvae of *S. littoralis* (Marei et al., 2009). The ethanolic extracts of different plant parts of *A. monosperma* were reported to influence the nutritional indices of sixth instar larvae of *S. littoralis* (El-Sabrou, 2009).

On the other hand, the effects of essential oils on nutritional indices of other insects have been studied. The essential oils isolated from *Zataria multiflora* Boiss., *Thymus daenensis* Celak., *Satureja hortensis* L. and *Fumaria parviflora* Lam. were found to induce significant reduction in RGR, ECI and ECD of *Plodia interpunctella* (Hübner) when larvae were fed on the treated flour discs (Saeidi and Yousefi, 2013; Shahab-Ghayoor and Saeidi, 2015). The oils of *Eucalyptus kruseana*

F. Muell., *Ferula assa-foetida* L., *Pelargonium hortorum* L.H. Bailey and *Juglans regia* L. have been shown to decrease RGR, RCR and ECI of *Rhyzopertha dominica* F. adults in concentration-dependent manner (Aref and Valizadegan, 2015; Bahrami *et al.*, 2016). Similarly, oils from leaves and fruits of *Schinus molle* L. at concentrations of 0.04 and 0.4% w/w modified the nutritional physiology of *Sitophilus oryzae* L., altering RGR, RCR, and ECI (Benzi *et al.*, 2009). Moreover, the essential oils of *Satureja khuzistanica* (Jamzad), *Ocimum basilicum* L., *Myrtus communis* L., *Thymus daenensis* Celak., *Mentha spicata* L., and *Eugenia caryophyllus* (Sprengel) Bullock & Harrison altered nutritional indices of the 4<sup>th</sup> instar larvae and adults of *Leptinotarsa decemlineata* (Say) (Saroukolai *et al.*, 2014). The essential oil of *Rosemarinus officinalis* L. was found to affect the nutritional indices of fourth instar larvae of *Glyphodes pyloalis* Walker (Yazdani *et al.*, 2013). This oil at 0.777% reduced ECI, ECD, RCR and RGR to be 4.13%, 4.22%, 1.36 mg/mg/day and 0.043 mg/mg/day, respectively.

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 (Khosravi *et al.*, 2010). 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 *A. monosperma* oil (30.13% at 2000mg/l) 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* (Sabbour and Abd El-Aziz, 2002; Abdelgaleil *et al.*, 2008; Santana

*et al.*, 2014; Ali *et al.*, 2017). Similarly, *A. monosperma* extracts have been shown to possess antifeedant activity against sixth instar larvae of *S. littoralis* (El-Sabrou, 2009).

In general, the antifeedant can inhibit insect feeding through sensory perception, such as having an unpalatable taste to insects (Chapman, 1995) or through postingestive effects (Glendinning, 1996). Essential oils possess aromatic properties that make insects disgusted by food, reducing or stopping feeding (Arasu *et al.*, 2013). Some essential oils and monoterpenes were reported to possess inhibitory effect on  $\alpha$ -amylase and other digestive enzymes and other enzymes that participate in metamorphosis and physiological functions (Basak and Candan, 2010; Sudha *et al.*, 2011; Kohl *et al.*, 2015). In addition, as shown in this study, the essential oils may inhibit insect feeding through changes in nutritional indices.

Moreover, the essential oils of *A. monosperma*, *C. viminalis*, *C. aurantifolia* and *C. macrocarpa* 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. Pavela (2011) tested *Artemisia campestris* L. as larval growth inhibitor against *S. littoralis* and noticed that the larval mortality and growth inhibition were higher than 75% after application of 15 mg/g food. Although, the tested oils showed pronounced larval growth inhibition they had moderate antifeedant activity. Similar findings were reported by Barnby and Klocke (1987) on the bioactivity of azadirachtin against *S. littoralis*. They concluded, although azadirachtin treatment decreased food intake by *S. littoralis* larvae, this reduction alone would not explain the pronounced inability of the larvae to gain weight in the instar immediately after treatment.

Also, the obtained data emphasized that *A. monosperma* and *C. aurantifolia* 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.* (1989) who explained the inhibition of growth and development of the tobacco hornworm, *Manduca sexta*.

In the present study the essential oils of *A. monosperma*, *C. viminalis*, *C. aurantifolia* and *C. macrocarpa* 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 (El-Aswad and Abdelgaleil, 2008, Abdelgaleil, 2010). 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.* (2003). 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.

Effects of some botanical insecticides as hormonal regulators of metamorphosis and moulting process are manifested by changes in haemolymph ecdysteroid and juvenile hormone titres due to a blockage and /or delay in the release of those hormones from their 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 (Tunaz and Uygun, 2004; El-Zoghaby, 1992; El-Sabrouh, 2013). The present results illustrated that 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

(1996). Also, the results of Martinez and Van Emden (2001) 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 (Navon and Levinson, 1976).

In conclusion, the essential oils from *A. monosperma*, *C. viminalis*, *C. aurantifolia* and *C. macrocarpa* 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, i.e. as toxicants, antifeedants, growth inhibitors and sterilants. These multi-modes of action of essential oils delays the development of resistance. Therefore, we suggest that these essential oils be developed as potential natural insecticides for integrated pest management of *S. littoralis*.

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## اثرات ضد تغذیه‌ای، اختلال رشدی و حشره‌کشی چهار گونه اسانس گیاهی روی کرم برگ‌خوار مصری چغندر قند (*Spodoptera littoralis* (Lepidoptera: Noctuidae))

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**چکیده:** اثرات ضد تغذیه‌ای، بازدارنده رشد و حشره‌کشی اسانس چهار گونه گیاه معطر شامل *Artemisia monosperma* Del., *Callistemon viminalis* (Sol. ex Gaertn.) G. Don, *Citrus aurantifolia* (Christm.) Swingle و *Cupressus macrocarpa* Hartw. ex Gordon، روی کرم برگ‌خوار مصری چغندر قند (*Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae)) مورد بررسی قرار گرفت. اسانس گیاهان *A. monosperma* و *C. aurantifolia* موجب بیش‌ترین کاهش نرخ رشد نسبی در حشره شدند. نرخ رشد نسبی توسط *A. monosperma* برابر ۸/۶۳ تا ۳/۰۵ و برای اسانس *C. aurantifolia* معادل ۱۰/۷۴ تا ۲/۸۹ و برای شاهد ۱۴/۸۹ میلی‌گرم در هر میلی‌گرم در روز بود. به‌طور کلی نتایج نشان داد که با افزایش غلظت نرخ رشد نسبی کاهش می‌یابد. به‌علاوه، بازدهی تبدیل غذای خورده شده و بازدهی تبدیل غذای هضم شده در غلظت‌های بالای ۵۰۰، ۱۰۰۰، و ۲۰۰۰ میلی‌گرم در لیتر کاهش یافت. به‌عبارت دیگر، خواص ضد تغذیه‌ای اسانس *A. monosperma* و *C. aurantifolia* مؤثرتر از اسانس *C. viminalis* و *C. macrocarpa* روی لاروهای آفت بود. در صورتی که غلظت اسانس‌ها از ۱۲۵ تا ۲۰۰۰ میلی‌گرم در لیتر افزایش یابد شاخص ممانعت از رشد برای اسانس *A. monosperma* از ۳۷/۶۳ تا ۷۹/۸۰ درصد و برای *C. viminalis* از ۲۱/۶۹ تا ۵۲/۱۲ درصد و برای *C. viminalis* از ۱۶/۵۵ تا ۲۸/۵۹ درصد و برای *C. aurantifolia* این شاخص از ۳۷/۶۴ تا ۵۲/۳۲ درصد تغییر می‌نماید. هم‌چنین اسانس گیاهان *A. monosperma* و *C. macrocarpa* بیش‌ترین کاهش در سنتز کیتین و بیش‌ترین خاصیت حشره‌کشی در لاروهای سن چهارم آفت را داشتند. بررسی‌های به‌عمل آمده روی تخمدان حشرات ماده نشان می‌دهد که اسانس‌های آزمایش شده منجر به اختلال‌هایی در تمایزهای تخمدان می‌شوند.

**واژگان کلیدی:** اسانس‌های گیاهی، کرم برگ‌خوار مصری چغندر قند، شاخص‌های غذایی، بازدارنده تغذیه، تشکیل کیتین، ممانعت‌کننده رشد