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Antifeedant activity and some biochemical effects of garlic and lemon essential oils on *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae)

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Abstract
The present study was aimed to evaluate the larvicidal activity, antifeedant activity and some biochemical studies of garlic and lemon essential oils on *Spodoptera littorals* (Boisduval) larvae by leaf dipping method. The results showed that both garlic and lemon oils have larvicidal effects against *S. littoralis* larvae, with $LC_{90,}$ LC_{50} and LC_{30} of 39.18, 19.95 and 16.60% for garlic oil, respectively; and it was 47.04, 24.20% and 20.09 % for lemon oil, respectively. The antifeedant activity and the starvation percentage decreased significantly in larvae treated with garlic and lemon oils. Biochemically, the total midgut proteins and lipids contents decreased significantly while the carbohydrate levels increased in larvae treated with LC_{30} of both garlic and lemon oils. α-amylase and lipase decreased significantly in treated larvae. The present study showed that both garlic and lemon essential oils were toxic to *S. littoralis* larvae and these results could be useful for the development of new insecticide formulations to control this pest. The effects of essential oils on larvicidal and antifeedant activities are discussed.

Keywords: Botanicals, garlic and lemon essential oils, Egyptian cotton leafworm

1. Introduction
The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is a destructive, polyphagous, and multivoltine insect pest [1]. This pest is widely distributed all over the world; in Egypt and other Middle East countries, Southern Europe, and in temperate zones in Asia and Africa [2]. It has high reproductive potential and three generations during the cotton season in Egypt [1]. In addition to the cotton, the *S. littoralis* larvae infest more than 90 important plant species belonging to 40 families causing great losses in quantity and quality of the attacked crops [3, 4, 5]. Mechanical control and chemical synthetic insecticides have been used widely to control this pest. But the extensive uses of chemical insecticides lead to several environmental and health problems such as pesticide poisoning, residual toxicity in water and soil and harmful effects to beneficial insects [6]. So it is demanded to develop selective and environmentally safe methods that will result in better control of *S. littoralis.* Recently, great attempts have been done at screening plants in order to develop new botanical insecticides as an alternative to the existing insecticides [7, 8]. Botanicals are plant-derived materials and can be used as a major component in IPM for controlling insect pests. Botanical insecticides are fast biodegradable; have little or no harmful effect on the environment and non-target organisms, cheap, easily produced and may retard the development of resistance [9]. There are four major types of botanical insecticides used for insect control including pyrethrum, rotenone, neem, and essential oils [10]. Generally essential oils are secondary metabolites, volatile, with strong odor and are formed of mixture of several up to dozens of mono-,di-,sesqui-terpenes [11]. The composition of essential oils varies with every plant species and also on the growth stage of the plant [12]. Commonly the essential oils are used as flavoring agent food additives or in medicine however, various essential oils and their constituents are recorded to show acute toxic effects against different insects [13, 14]. Different essential oils have been used as repellent, fumigant, larvicidal, ovicidal and adulticidal against different insect orders [12, 15]. Many researchers have documented the larvicidal, antifeedant and physiological effects of several essential oils such as neem oil, jojoba oil, peppermint oil, and ginger oils against the *S. littoralis* larvae [16]. Moreover, numerous essential oils from different plants showed high fumigant activity against *S. littoralis* [11, 17]. Both garlic (*Allium Sativum*, Liliaceae) and lemon (*Citrus lemon*) essential oils were selected in this study based on their medicinal properties and their safety to human and environment [14, 18, 19].

The major bioactive components responsible for the benefits of garlic are assumed to be allylic sulfur compounds and limonene for the lemon oil [20, 21]. Several studies showed that garlic and lemon plants are not only beneficial as medicinal plants, but they can be used also as insecticidal, acaricides and as insect repellent to some plant pests [18, 21]. The present study aims to evaluate the larvicidal effects, antifeedant activity of both garlic and lemon essential oils against *S. littoralis* 4th instar larvae. Also the effects of the sub-lethal concentration (LC_{30}) of each essential oil on the total midgut proteins, carbohydrates, and lipids as well as the digestive enzymes activity (α-amylase and lipase) were evaluated.

2. Materials and Methods

2.1 Insect rearing

A susceptible strain of the cotton leaf worm, *S. littoralis* was reared in the laboratory for more than 10 generations. The egg masses were obtained from Plant Protection Department, Faculty of Agriculture, Assiut University. The egg masses were kept in a plastic Petri dish (9 cm in diameter) in an incubator at 26± 2° C and 65±5 % R.H., with 8:16 L:D h photoperiod. All the larval instars were fed on fresh castor leaves until pupation. The feces were collected daily and fresh castor leaves were provided daily. The pupae were collected daily and kept in a large glass jar provided with a piece of cotton soaked in a 10% sugar solution for feeding the adult moths. Each jar was provided with branches of tafla plant, *Nerium oleander*, as an oviposition site and the egg masses were collected daily. All experiments were carried out in Entomology laboratory, Zoology and Entomology department, Science, Assiut, Egypt. The experiments were done on the 4th instar larvae during September, October and November in 2014 and in January and February in 2015.

2.2 Plant essential oils

The two essential oils: garlic and lemon were purchased from EL-Captain Company for natural oils & herbs extraction (CAP PHARM), Assiut city, Egypt.

2.3 Larvicidal bioassay

Determination of the lethal concentration values (LC_{30}; LC_{50}; LC_{90}) of garlic and lemon oils against 4th larval instar of *S. littoralis* was done by leaf dipping technique. Five aqueous concentrations (15, 20, 25, 30, and 35%) of the essential oils were prepared using one ml of Triton X-100 as emulsifier. Equal discs of fresh castor bean leaves (10 cm diameter) were dipped in each tested concentration of the oil for 20 s and left in the laboratory to dry. At the same time, fresh castor leaves were dipped in the control solution (dist. Water + one drop of Triton X-100). The dried leaves were put singly in plastic Petri dishes. Ten starved 4th instar larvae were transferred into each Petri dish and allowed to feed on the treated and untreated leaves. Both treated and untreated larvae were kept in the incubator under the same laboratory conditions mentioned above. Five replicates for each concentration were performed. Mortality counts were recorded 24 h post-treatment and corrected according to Abbott formula [22].

2.4 Antifeedant bioassay

This bioassay was done to check the antifeedant activity of the sub-lethal concentration (LC_{30}) of each essential oil against the 4th instar larvae. 180 4th instar larvae were starved for more than four hours, divided into 3 groups of 60 larvae each, two groups for the treatments and one group for the control. The leaf dipping technique was done as mentioned above in the larvicidal bioassay. Ten larvae were transferred individually into ten Petri dishes and allowed to feed individually on the treated and untreated leaves for 3 days. Fresh concentrations were prepared every day and six replicates for each treatment were done. The larvae were weighed individually and the leaves were daily weighed before and after feeding for 3 days. The amount of consumed food for each larva was recorded and the antifeedant index was calculated according to Ben Jannet *et al*. [23]

2.5 Starvation bioassay

This bioassay was set up to ensure the antiffedant activity of the two essential oils. 240 larvae of the 4th instar larvae of *S. littoralis* treated by the sub-lethal concentration (LC_{30}) of each essential oil were starved, divided into 4 groups of 60 larvae each, two groups for the treatments, one group for the control and one group as starved larvae. The insects were treated by the leaf dipping technique as mentioned above. Before treatments, all larvae were weighed. The castor leaves were placed individually in plastic Petri dishes and ten individual larvae were transferred into ten Petri dishes and allowed to feed on the treated and untreated leaves for 24h. The starved larvae were left without feeding for 24h. Six replicates for each treatment were carried out. The larvae were then reweighed, where the starvation percentages of tested larvae were calculated according to the equation of Abdel-Mageed *et al.* [24].

2.6 Biochemical studies

After feeding the 4th instar larvae on castor leaves treated with the LC_{30} of the two oils for 24 h, the surviving larvae were collected and the midgut was taken out by dissection and then homogenized in sodium phosphate buffer (pH 7.2). Homogenates were placed in 1.5 ml eppendorf tubes and centrifuged at 10000 rpm for 15 min at 4 °C in cooling centrifuge. The supernatant was kept at -20 °C till use for biochemical assays. The total midgut proteins, carbohydrates, lipids as well as the activity of lipase and α-amylase enzymes were evaluated**.** The results of treatment were compared to those of the controls (positive control using castor leaves only and negative control dipped in Triton X-100). All assays were performed in 5 replicates.

2.7 Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA). Mean values were compared by using the least significant difference (LSD) test at 5% level. Corrected mortality values were subjected to probit analysis to estimate LC_{30}, LC_{50} and LC_{90} values using SPSS statistical program software [25].

3. Results

3.1 Larvicidal effects

The data of larvicidal activity of the garlic and lemon essential oils against the 4th instar larvae of *S. littoralis* are shown in Table 1. The results revealed that the mortality percentage increased with the increase in the concentration and the values (LC_{30}, LC_{50}, and LC_{90}) indicate that the garlic oil is more toxic than the lemon one. Moreover the higher slope value of the log dose-probit line of garlic relative to that of lemon was concomitant with the lethal concentration values (LC_{30}, LC_{50}, LC_{90}) for garlic essential oil relative to the lemon essential oil, confirming the superiority of garlic essential oil over lemon essential oil as larvicide (Table 1).

Table 1: Toxicity of garlic and lemon essential oils to the 4th instar larvae of *S. littoralis*.

Plant essential oil	Lethal concentrations %		95% Confidence limits		Slope	χ2 (d.f)
			Lower	Upper		
Garlic	LC_{30}	16.60	10.04	21.06	5.75	$\chi2_{(3)}$=1.05
	LC_{50}	19.95	16.75	23.77		
	LC_{90}	39.18	28.75	113.23		
Lemon	LC_{30}	20.09	18.9	21.09	5.03	$\chi2_{(2)}$=0.17
	LC_{50}	24.20	19.60	29.35		
	LC_{90}	47.07	31.83	52.29		

Units LC_{30}, LC_{50} and LC_{90} = % / w, applied for 24h, a95% lower and upper fiducial limits are shown.

3.2 Antifeedant activity

Table 2 shows the antifeedant activities of the sub-lethal concentration (LC_{30}) of the tested essential oils against the larvae after 24, 48 and 72 hours of feeding on treated castor leaves. Data showed that both garlic and lemon essential oils exhibit antifeedant activity against the 4th larval instar and the antifeedant index increases with the increase of time exposure. The antifeedant effect of garlic essential oil was significantly ($P<0.05$) higher than that of lemon essential oils at all the interval times except for the third day, where the difference was not significant (Table 2).

Table 2: Antifeedant activity of garlic and lemon essential oils against the 4th instar larvae of *S. littoralis* fed on castor leaves treated with the LC_{30}

Plant essential oil	Antifeedant index (%)			Mean*
	Days post-treatment			
	1st	2nd	3rd	
Garlic	62.0±3.9[a]	69.9±2.9[a]	74.4±5.5[a]	68.77
Lemon	47.8±3.3[b]	47.3±3.9[b]	57.2±4.6[b]	50.77

Data are expressed as mean ± SE, * total mean of each treatment at different time intervals, values were analyzed by one-way ANOVA, where means within each column followed by different letters are significantly different ($P< 0.05$ by LSD).

3.3 Starvation percentage

Data presented in Table 3 show the starvation percentage of the 4th instar larvae of *S. littoralis* treated by the sub-lethal concentration (LC_{30}) of both garlic and lemon oils. It was noticed that starvation percentage of larvae treated by garlic essential oil was about 3.39 times more than that of larvae treated with lemon essential oil (Table 3). Therefore, garlic essential oil proved to be more effective antifeedant than lemon essential oil. The average weight of larvae 24h post-treatment was about 67.45% and 90.74% of the control for larvae treated with garlic and lemon essential oils, respectively; emphasizing the superiority of garlic essential oil over the lemon essential oil as antifeedant.

Table 3: Starvation percentage (%) of the 4th instar larvae of *S. littoralis* treated with LC_{30} of garlic and lemon essential oils.

Treatments	Mean weight at zero time (mg/larva)	Mean weight after 24h (mg/larva)	Difference* (mg/larva)	Starvation %
Garlic	33.14	49	+15.86	55.71
Lemon	33.83	65.94	+32.11	16.42
Control larvae	33.75	72.65	+38.9	------
Starved larvae	33.15	30.7	-2.45	------

*Average weight after 24h – Average weight at zero time.

3.4 Biochemical effects

3.4.1 Total protein, carbohydrate and lipid contents of the midgut

The data in Table 4 show the changes in the total protein, carbohydrate and lipid content of the midgut tissues of the larvae treated with the LC_{30} of garlic and lemon essential oils. From the data recorded, it was shown that there was a highly significant decrease ($P<0.05$), in the level of total protein and lipid contents of treated larvae compared to control. Also within the treated larvae there was a significant reduction in total midgut proteins of the larvae treated with garlic essential oil compared to the larvae treated with lemon essential oil and there was no significant difference in the total lipid contents in both treated larvae (Table 4). On the other hand the total midgut carbohydrate contents increased significantly in the larvae treated with garlic essential oil and there was no significant difference in larvae treated with lemon oils compared to control.

Table 4: Total midgut proteins, carbohydrates and lipids in the the 4th instar larvae of *S. littoralis* fed on castor leaves treated with the LC_{30} of garlic and lemon essential oil.

Treatment	Total protein (µg/mg)±SE	Total carbohydrate (µg/mg)±SE	Total lipid (µg/mg)±SE
Garlic	22.16±0.3[b]	69.2±5.0[a]	4.47±0.22[b]
Lemon	13.1±0.6[c]	37.85±1.9[b]	3.67±0.17[b]
Positive control	33.91±1.0[a]	34.55±1.9[b]	10.1±0.7[a]
Negative control	32.2±1.0[a]	33.16±2.4[b]	9.21±0.3[a]

Data are expressed as mean ± SE (n=5), values were analyzed by one-way ANOVA, where means within each column followed by different letters are significantly different ($P< 0.05$ by LSD).

3.4.2 Enzymes activity

Both garlic and lemon essential oils significantly decreased ($P<0.05$) the activity of *α*-amylase and lipase in the midgut of 4th instar larvae (Table 5). Among the treatments, *α*-amylase decreased significantly in larvae treated with garlic oil compared to lemon, and the lipase activity did not differ significantly between garlic and lemon oils.

Table 5: α-Amylase and lipase activities of the midgut 4th instar larvae of *S. littoralis* treated with the LC_{30} of garlic and lemon essential oils.

Treatment	α-Amylase (µmol/min/mg)±SE	lipase (µmol/min/mg)±SE
Garlic	1.71±0.06^{c}	0.70±0.03^{b}
Lemon	2.07±0.03^{b}	0.60±0.06^{b}
Positive control	2.86±0.1^{a}	1.24±0.07^{a}
Negative control	2.69±0.1^{a}	1.28±0.04^{a}

Data are expressed as mean ± SE (n=5), values were analyzed by one-way ANOVA, where means within each column followed by different letters are significantly different (P< 0.05 by LSD).

4. Discussion

Natural pesticides, especially those derived from plants, are promising elements for pest control and are considered an alternative to synthetic pesticides as it reduces the negative impacts on the human health and the environment [9]. Essential oils are very important botanicals that can act as a fumigants, insecticides, repellents, and antifeedants [26].

4.1 The larvicidal activity: In the present study, *A. sativum* essential oil showed larvicidal activity against *S. littoralis*. Similarly, garlic extracts have shown a considerable toxicity to a number of species of different insect orders and to different developmental stages [27, 28, 29]. For example, garlic oil showed toxic effects against *Callosobruchus maculatus* [30], *Tribolium confusum* and *Ephestia kuehniella* [31]. Also, it was found that the natural garlic essential oil may be used in IPM against grasshopper, *Heteracris littoralis* [32]. Moreover, garlic essential oils were used as a fumigant against different insect pests and this could be considered as a component of IPM without risk for consumers and the environment [33]. Likewise**,** *Citrus limon* essential oil was potential in controlling *S. littoralis* larvae. These results are in agreement with those of other researchers working on the dengue fever mosquito, *Aedes albopictus* [34], and the soybean weevils, *Sternechus pinguis* and *Rhyssomatus subtilis* treated with *C. limon* oil [35]. Similar to the garlic essential oil, lemon essential oil was used also as fumigant for *Alphitobius diaperinus* [29]. Other essential oils such as thyme, bitter and neem showed larvicidal and growth inhibitory activities against 2nd and 4th instar larva of *S. littoralis* [36]. There are several factors affecting the insecticidal properties of the essential oils, including, for example, the mode of entry and the bioactive chemicals [37]. It is known that each of the constituents of the essential oils has its own mode of action [38]. Most monoterpenes of the essential oils showed cytotoxic effects to plant and animal tissues causing a reduction in the number of insect mitochondria and Golgi bodies, blocking respiration and decreasing cell membrane permeability [39, 40, 13]. Similarly, we found in the present study various histological changes in the midguts cells including necrosis, vacuolation, and destruction of midgut cells, muscles and peritrophic membrane (unpublished data).

4.2 The antifeedant activity: Antifeedant is a chemical that inhibits the feeding without killing the insect pests directly, while it remains near the treated crops and dies through starvation [41]. Higher antifeedant index normally indicate decreased rate of feeding. Antifeedant effects of both garlic and lemon oils were assessed based on leaf area consumed by *S. littoralis* 4th instar larvae (antifeedant index). In the present finding, garlic and lemon essential oils showed antifeeding activity against *S. littoralis*. Russel and Lane [42] showed that plant metabolites produce toxic substances if ingested leading to rejection of the host plant. In garlic there are many secondary metabolites such as saponins, tannins, alkaloid steroids and glycosides that may affect the antifeedant [43]. Similarly, garlic extracts were found to act as antifeedants against different insect orders, for example, Coleoptera [44], Lepidoptera and Hemiptera [45, 46]. Likewise, lemon essential oil acts as antifeedant to *Anopheles* sp. [47], *C. pipiens* [48]. According to Isman [10] antifeedants have some physiological or toxic actions on insects, depending on the treatment concentrations. In addition to this, the antifeedant properties of botanicals also play an important role for the reduction of nutrients in the insects (unpublished data).

4.3 Essential oils and total midgut proteins, carbohydrates and lipids

In the present study, garlic and lemon essential oils significantly reduced the total midgut proteins and lipids while the carbohydrates contents increased significantly in *S. littoralis* larvae. Similarly, El-Naggar and Abdel- Fattah [49] found a reduction in the total proteins in the midgut content of *S. littoralis* larvae when treated with *Eucalyptus* oil and its combination with gamma radiation. Rawi *et al.* [50] recorded a decrease in the total protein and lipid in *S. littoralis* larvae fed on methylene chloride extract of *A. indica* and *Citrullus colocynthis*. Also, Marei *et al.* [51] found that sesame oil declined the total lipid of *S. littoralis*. The reduction in protein level could be associated to one or combination of many factors. It has been mentioned that the reduction in the protein contents is may be due to the insecticidal stress [52, 53]. And the same authors indicated that the reduction of the protein contents is a result of breakdown of these proteins into amino acids which are used in the compensatory mechanism to supply energy for the insect to recover from insecticidal stress [52, 53]. Moreover, Ali *et al*. [54] showed that the decrease in protein synthesis could also be related to reduce levels of nucleic acids.

The reduction of lipid levels in *S. littoralis* larvae treated with garlic and lemon oils may be due to the effect of these oils on the lipid metabolism, and due to the utilization of lipid reserves for energy generation as a result of induced stress [55]. Moreover, Anitha *et al.* [56] showed that the histopathological changes as we found in the present study (unpublished data) can be one of the indicators of the fat changes. Regarding the increase of carbohydrate contents in our study, several researchers found contrary results. Rawi *et al.* [50] observed a reduction in the glucose content in *S. littoralis* larvae treated with *A. indica* and *Citrullus colocynthis* methylene chloride extract. The increase in carbohydrate content in the present study may be due to hyperchitenase activity hence the peritrophic membrane of the midgut is formed mainly of chitin. Shakoori *et al.* [57] suggested that the decline in the carbohydrate metabolism may be result from the inhibition of chitenase synthesis. Abdel-Aziz *et al.* [36] found an increase in the chitenase activity of *S. littoralis* larvae treated with thyme, bitter and neem oils.

4.4 Essential oils and enzymatic activity: The decrease in the activity of α-amylase in the present study is in agreement with the results of several authors. For example, Krishnaveni *et al.* [58] found a decrease in the activity of the midgut α-amylase in *S. litura* larvae subjected to the neem and pongam oils. Yazdani *et al.* [59] recorded a decrease in the α-amylase activity of *Glyphodes pyloalis* treated with the essential oils *Thymus vulgaris* and *Origanum vulgare.* Yacoub [60] and Abdel-Rahman and Al-Mozini [61] reported a decrease in the activity of *S. littoralis* α-amylase due to treatment with

Calotropis procera and *Rhazya stricta* extracts. The reduction in the activity of this enzyme could be due to a cytotoxic effect of different plant materials on epithelial cells of the midgut which synthesize α-amylase [62]. This explanation extends to the results obtained in the current study where treatment of *S. littoralis* larvae with the essential oils of garlic and lemon revealed cytopathological symptoms. Lipases play a major role in storage and lipid mobilization. The decrease in the lipase activity of *S. littoralis* midgut in the present investigation agrees with the finding of Krishnaveri *et al.* [58] who found that pongam and neem oils declined lipase activity in *S. litura* larvae. Also, the same author found a decrease in the activity of the midgut lipase of *Glyphodes pyloalis* larvae treated with neem. In general the reduction of lipase and α-amylase of the present study is may be the binding of the antifeedant compounds of the oils to the enzyme proteins (zymogen) or to the digestive enzymes [63].

5. Conclusion

In conclusion both garlic and lemon oils showed negative impacts on 4th instar larvae (larvicidal), antifeedant activity, and protein and lipid contents. This shows the potency of these oils to be used as a natural insecticide.

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