

## **The Sublethal Effects of Both Essential Oils (*Cymbopogon citratus* and *Mentha piperita*) on *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae)**

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### **ABSTRACT**

*Spodoptera frugiperda* (J. E. Smith) is an invasive deleterious pest that causes huge economic losses to various crops, especially maize worldwide. Therefore, the insecticidal and antifeedant activities of lemongrass, *Cymbopogon citratus*, and peppermint, *Mentha piperita* essential oils (EOs) against the third larval instar of *S. frugiperda* were tested under laboratory conditions to find safer alternative approaches to managing *S. frugiperda*. The impact of sublethal concentrations (LC<sub>10</sub> & LC<sub>30</sub>) of tested EOs on the biological parameters and the activities of detoxifying enzymes of *S. frugiperda* were also evaluated. Their chemical composition was identified using gas chromatography-mass spectrometry (GC-MS). The major compounds in *C. citratus* EO were d-limonene (45.06%),  $\beta$ -citral (10.30%), and  $\alpha$ -citral (9.90%); whereas, in *M. piperita* EO were menthol (32.03%), menthone (30.18%), and *p*-menthan-3-one (11.53%). Bioassay results revealed that *C. citratus* (LC<sub>50</sub>= 725.2 mg/L) exhibited more toxicity on *S. frugiperda* larvae than *M. piperita* (LC<sub>50</sub>= 1024.2 mg/L) after 48 h of exposure. Both EOs revealed remarkable antifeedant effects, with the feeding deterrence index ranging from 30.67-43.06% against *S. frugiperda*. Sublethal concentrations of the tested EOs resulted in prolonged larval and pupal durations, reduced pupal weight of females and males, and decreased pupation and adult emergence percentages, compared to the control. The activities of carboxylesterases and glutathione *S*-transferase enzymes in *S. frugiperda* were dramatically suppressed, compared to the control, with dose-dependent effects. These results suggest that *M. piperita* and *C. citratus* EOs may be used to manage *S. frugiperda*.

**Keywords:** fall armyworm; lemongrass; peppermint; antifeedant activity; biological parameters; detoxifying enzymes.

### **INTRODUCTION**

Maize (*Zea mays* L.) is the predominant crop in Africa and a staple food for around fifty percent of the continent's population (Day *et al.*, 2017). The fall

armyworm, *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae), is a very damaging invasive pest that impacts various crops, notably maize, along with wheat, sorghum, sugarcane, cotton, rice, and different vegetables (Boregas *et al.*, 2013). It is a polyphagous insect pest that harms the stalks and leaves of over 350 distinct plant species from 76 different plant families (Montezano *et al.*, 2018). The initial report of this pest epidemic in Africa occurred in 2016 (Goergen *et al.*, 2016). In Egypt, the first occurrence of *S. frugiperda* was observed in 2019 in maize fields in Kom Ombo city of Aswan Governorate, Upper Egypt (Gamil, 2020).

Several insecticides with various mechanisms of action have previously been used against *S. frugiperda* (Gutiérrez-Moreno *et al.*, 2019 and Sisay *et al.*, 2019). However, resistance to the recommended insecticides has emerged as a result of the extensive application of these synthetic insecticides to control *S. frugiperda* (Van den Berg and du Plessis, 2022). Accordingly, there is a pressing need to find effective and sustainable alternatives to reduce the broad use of these synthetic chemicals, therefore delaying the development of pest resistance and limiting environmental pollution (Eldesouky *et al.*, 2019 and Hussein *et al.*, 2023).

The natural origins of botanical insecticides, along with their biodegradability and lack of harmful residues or by-products that could damage the environment, have made them good alternatives to synthetic insecticides for pest management (Kesraoui *et al.*, 2022 and Awad *et al.*, 2024). Essential oils (EOs) are botanical extracts that show promise as novel pesticides because they are repellent, attractant, and fumigant, and have contact properties against a variety of insect pests (Campolo *et al.*, 2018; Ma *et al.*, 2020 and Jayaram *et al.*, 2022). However, the precise mechanism of action of these EOs is still unknown.

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Among the 400–500 commercially produced EOs are those belonging to the lemongrass family (*Cymbopogon* spp.). Lemongrass EO has insecticidal properties attributed to its diverse secondary metabolites, including bioactive cyclic and acyclic terpenes (Eden *et al.*, 2020). These compounds cause disruptions in insect neurotransmitters (Zibae, 2015). Moreover, it has been found that lemongrass extracts contain additional secondary metabolites, including carotenoids, flavonoids, and alkaloids (Avoseh *et al.*, 2015), suggesting lemongrass's potential as a bio-insecticide. Furthermore, tannin compounds have the potential to function as enzyme activity inhibitors during insect digestion (Rahayu and Mairawita, 2018). Citral (a combination of geranium and neral) is assumed to be responsible for the insecticidal effect of lemongrass EO (Solomon *et al.*, 2012), coming from its interaction with oxidative stress and intracellular oxygen radicals (Sanches *et al.*, 2017).

*Mentha piperita* L., or peppermint, is a perennial aromatic plant that is significant for medicine and belongs to the Lamiaceae family. It is widely cultivated in temperate regions around the world, including Asia, North Africa, Europe, and North America (Pang *et al.*, 2020). It has also been shown that EO from the mint genus has insecticidal and repellent properties against a variety of insect pests (Kumar *et al.*, 2011). According to Pavela *et al.* (2014), it is commonly utilized in the food sector as a natural flavoring and food ingredient.

Significant detoxification enzymes involved in the metabolism of xenobiotics in living organisms are glutathione *S*-transferase and carboxylesterases. Their actions have been regarded as indicators of chemical stress and environmental pollution (Hilliou *et al.*, 2021).

The current study aimed to assess the toxicity and sublethal effects of *C. citratus* and *M. piperita* EOs on the antifeedant, biological and biochemical activities of *S. frugiperda* under laboratory conditions. The goal was to ascertain the potential of these EOs as a safe replacement for chemical insecticides in integrated pest management programs.

## MATERIALS AND METHODS

### Insect rearing

The larvae of *S. frugiperda* were originally collected from the infested field of maize, *Zea mays* L. at the Experimental Farm in El-Nubaria Agricultural Research Station, EL-Beheira, Egypt, in June 2022. The insect population was maintained for several generations in an incubator at  $26 \pm 1$  °C,  $65 \pm 5\%$  relative humidity, and 14L: 10D h photoperiod, fed on fresh castor bean leaves (*Ricinus communis* L.). *S. frugiperda* larvae were identified using morphological characteristics and taxonomic keys at the Department of Applied

Entomology and Zoology, Faculty of Agriculture, Alexandria University, Egypt.

### Extraction of essential oils

The leaves of *M. piperita* and *C. citratus* were gathered in different regions of Alexandria, Egypt. Fresh leaves of the tested plants were washed, allowed to dry in the shade, and then clipped into little pieces. In a flask (1-L), 100 g of each plant was added to 500 ml of distilled water. Using the hydrodistillation method, EOs were separated and dried over anhydrous sodium sulfate in a glass Clevenger-style apparatus after three hours. The extracted oils were stored at 4 °C before usage in closed glass flasks (Salem *et al.*, 2020).

### GC-MS analysis

The chemical composition of *C. citratus* and *M. piperita* EOs was analyzed utilizing a Trace GC-TSQ Evo 9000 mass spectrometer (Thermo Scientific, Austin, TX, USA) at the Atomic and Molecular Physics Unit, Atomic Energy Authority, Inshas, Cairo, Egypt, employing a direct capillary column TG-5MS (30 m × 0.25 mm × 0.25 µm film thickness). Helium served as the carrier gas at a flow rate of 1 ml/min, with the oven temperature programmed to increase from 45 to 165 °C at a rate of 4 °C/min, followed by an increase from 165 to 280 °C at a rate of 15 °C/min, concluding with a post-run phase at 280 °C. Samples (1 µl) were injected at 250 °C using a split/splitless injector with a 50:1 split ratio in splitless mode at a flow rate of 10 ml/min. The solvent delay was 2 min, and 1 µl diluted samples were automatically injected utilizing the Auto-sampler AS3000 in split mode with the gas chromatograph. In full scan mode, electron ionization (EI) mass spectra were collected at 70 eV ionization voltages across the *m/z* 40–550 range. The temperatures of the transfer line and ion source were adjusted to 200 and 250 °C, respectively. The constituents were distinguished using a comparative analysis of their retention times and mass spectra against the mass spectral databases of Wiley 09, mainlib, replib, and NIST 11 (Adams, 2005).

### Bioassays

The toxicity of *C. citratus* and *M. piperita* EOs against the third larval instar of *S. frugiperda* was determined using the leaf-dipping method. Six concentrations of each EO (100, 200, 500, 1000, 2000, and 4000 mg/L) were prepared in distilled water with a small amount of Tween-20 (10 mg/L) added as an emulsifier. Fresh castor bean leaves were dipped in each concentration and air-dried for half an hour. The control leaves were only immersed in water containing Tween-20. The treated leaves were placed in Petri plates (12 cm diameter) containing filter papers. Twenty larvae of *S. frugiperda* were transferred to each plate. Each treatment was replicated five times. The mortality percent was recorded after 48 hours of exposure.

### Antifeedant activity

The feeding deterrence effect of the sublethal concentrations (LC<sub>10</sub> & LC<sub>30</sub>) of the tested EOs against the third larval instar of *S. frugiperda* was assessed. Each concentration was applied to fresh castor bean leaves and left to air dry. The control treatment was done with water mixed with Tween-20 only. Five replicates of each concentration were used, with twenty larvae per replication. Following 48 hours of exposure, the feeding deterrence index (FDI) was determined using the following formula (Rahman *et al.*, 2022):

FDI = [(C - T) / (C + T)] × 100, where C and T represent the weights of treated and control leaves that *S. frugiperda* consumed, respectively.

### Biological parameters

To assess the impact of the tested EOs on *S. frugiperda* development, sublethal concentrations at LC<sub>10</sub> & LC<sub>30</sub> values were employed. Castor bean leaves were dipped in each concentration, as described in the bioassays section. Each treatment included one hundred *S. frugiperda* larvae. After 48 hours, the remaining individuals were placed on untreated castor bean leaves. Fresh leaves were added each day. Larval duration (days), pupation (%), pupal duration (days), pupal weight (g), and adult emergence (%) were all noted during the trial.

### Biochemical assays

One of the tested EO concentrations, LC<sub>10</sub> or LC<sub>30</sub>, was applied to the third larval instar of *S. frugiperda*. After 48 h of exposure, the fresh body weight of the remaining larvae was homogenized in a cold 0.1 M phosphate buffer. The pH of the buffer was adjusted to 6.5 for glutathione *S*-Transferase (GST) and 7.0 for carboxylesterase (CarE). The homogenates were centrifuged using a Cryofuge 20-3 Heraeus Christ centrifuge at 12,000 rpm for 30 min at 4°C. To assess the protein content and the activity of detoxifying enzymes, the clear supernatants were immediately frozen at -20°C. There were five replicates utilised for each treatment. The Coomassie brilliant blue assay was used to measure the protein content (Bradford, 1976).

### Carboxylesterase (CarE) activity assay

Van Asperen (1962) and Cao *et al.* (2008) determined the activity of CarE, including α- and β-esterase, with slight modification. A 30 μL portion of the homogenate was incubated with 100 μL of 30 mM α- or β-naphthyl acetate for 15 min at 25°C. The reaction was stopped by adding 50 μL of a stop solution consisting of fast blue b (2%) and sodium dodecyl sulfate (5%). The Jenway-7205UV/Vis Spectrophotometer was used to measure the hydrolysis of α- and β-naphthyl acetate at 600 nm and 550 nm,

respectively. CarE activity was determined based on α- and β-naphthyl acetate standard curves.

### Glutathione *S*-transferase (GST) activity assay

According to Habig *et al.* (1974), the activity of GST was determined. The reaction solution included 10 μL of enzyme stock solution, 25 μL of 1-chloro-2, 4-dinitrobenzene (30 mM), and 25 μL of glutathione (50 mM). Measurement was carried out at 340 nm using Jenway-7205UV/Vis spectrophotometer for a period of 3 min at 25°C.

### Statistical analysis

Probit analysis was used to estimate the sublethal (LC<sub>10</sub> & LC<sub>30</sub>) and lethal (LC<sub>50</sub>) concentrations of the tested EOs against *S. frugiperda* (Finney, 1971). One-way analysis of variance (ANOVA), followed by the Tukey's HSD test (Cohort Software Inc., 1985), was performed to determine the differences among treatments ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

### The chemical composition of *C. citratus* and *M. piperita* EOs

GC-MS analysis of EOs isolated from *C. citratus* and *M. piperita* leaves revealed a total of 34 components (Table 1). The major chemical constituents in *C. citratus* EO were d-limonene (45.06%), β-citral (10.30%), α-citral (9.90%), sulcatone (3.55%), and limonene oxide (3.32%). However, in *M. piperita* EO were menthol (32.03%), menthone (30.18%), *p*-menthan-3-one (11.53%), cis-carane (8.09%), d-limonene (6.13%), pulegone (2.55%), and piperitone (2.55%). Prior research aligned with our findings, but with differences in the oil's relative composition and minor constituents. The major compounds in *C. citratus* EO were previously determined to be β-citral, geranial (α-citral or citral A), and β-myrcene, with percentages of 43.63, 41.51, and 12.37%, respectively (Mansour *et al.*, 2020). Similarly, Moustafa *et al.* (2021) found that α-citral (35.91%) and β-citral (35%) were the two main constituents of *C. citratus* EO. According to Rosato *et al.* (2018) reported on the chemical analysis of EO from *M. piperita* leaves and found that its main components were menthol (68.0%), menthone (9.5%), isomenthone (8.4%), and menthyl acetate (2.4%). Furthermore, Jayaram *et al.* (2022) found that the main component present in *M. piperita* EO was neo-isomenthol (38.64%), which was followed by menthone (29.54%), neo-menthyl acetate (7.55%), menthofuran (6.49%), and 1, 8-cineole (6.31%). As reported by Sayed *et al.* (2022), carvone (61.16%), α-cubebene (10.99%) and d-limonene (4.08%) were the main components of *M. piperita* EO. Various production conditions, including harvest time, location, seasonal variations, and storage duration, might result in variations in the components of

**Table 1. Chemical composition of *C. citratus* and *M. piperita* EOs analysed by GC-MS**

No.	Retention Time (min)	Compound	Plant species	
			<i>C. citratus</i>	<i>M. piperita</i>
1	4.22	D-Limonene	45.06	6.13
2	5.47	Eucalyptol	1.36	-
3	8.26	6-methylheptan-3-ol	-	1.33
4	8.95	6-Methyl-5-hepten-2-one	1.31	-
5	11.34	cis-Limonene oxide	1.74	-
6	11.67	Limonene oxide	3.32	-
7	12.49	Citronellal	1.92	-
8	13.27	Menthone	-	30.18
9	13.70	cis-Carane	-	8.09
10	14.16	<i>p</i> -Menthan-3-one	-	11.53
11	14.28	<i>p</i> -Menth-1-en-9-ol	0.81	-
12	14.82	Menthol	-	32.03
13	15.11	2,8-p-Menthadien-1-ol	0.73	-
14	15.33	Levomenthol	-	0.53
15	16.04	$\alpha$ -Terpineol	0.52	1.00
16	16.66	cis-Isopulegone	-	0.34
17	17.00	Pulegone	-	2.55
18	18.57	$\beta$ -Citral or Neral	10.30	-
19	19.00	D-Carvone	1.33	0.61
20	19.27	Carvone	0.51	-
21	19.73	Piperitone	-	2.55
22	19.75	$\alpha$ -Citral or Geranial	9.90	-
23	22.68	Isophorone	0.38	-
24	25.28	Caryophyllene oxide	-	0.84
25	27.32	2-Isopropylimidazole	0.19	-
26	28.16	3-Methyl-2-furoic acid	0.24	-
27	28.64	Allethrolon	1.00	-
28	28.82	Sulcatone	3.55	-
29	31.40	$\beta$ -Citronellol	0.45	-
30	36.64	Benzofuran	0.59	-
31	39.50	Myrtanal	1.05	-
32	41.14	Bioallethrin	1.86	-
33	41.30	Nerolic acid	0.32	-
34	42.02	Carbamothioic acid	0.62	-
Total area (%)			89.06	97.71

The underline means not detected

EO within the same plant species (Aungtikun and Soonwera, 2021). Therefore, more research on essential oil standardization and plant cultivation is required.

#### **Toxicity of *C. citratus* and *M. piperita* EOs to *S. frugiperda* larvae**

The lethal and sublethal toxicity of *C. citratus* and *M. piperita* EOs to the third larval instar of *S. frugiperda* indicated that *C. citratus* was more lethal ( $LC_{50} = 725.2$  mg/L) than *M. piperita* ( $LC_{50} = 1024.2$  mg/L), after 48 h of exposure (Table 2). The sublethal effects of both tested EOs on the antifeedant, biological, and biochemical activities of *S. frugiperda* were

estimated using the  $LC_{10}$  and  $LC_{30}$  concentrations. Previously, Park *et al.* (2017) determined that the  $LC_{50}$  of *C. aurantium* EOs was 92.58 and 113.26 mg/L against *Pochazia shantungensis* nymphs and adults, respectively. Furthermore, Moustafa *et al.* (2021) showed that *C. citratus* had  $LC_{15}$  and  $LC_{50}$  values of 427.67 and 2623.06 mg/L on the 2<sup>nd</sup> instar larvae of *Agrotis ipsilon*. Several plant species from the *Mentha* genus have shown remarkable efficiency against different insect pests (Saeidi & Mirfakhraie, 2017; Benelli *et al.*, 2018; Kavallieratos *et al.*, 2022 and Sayed *et al.*, 2022).

**Table 2. Toxicity of *C. citratus* and *M. piperita* EOs to the third larval instars of *S. frugiperda* after 48 h of exposure**

Essential oil	LC <sub>10</sub> (mg/L) (95% CL)	LC <sub>30</sub> (mg/L) (95% CL)	LC <sub>50</sub> (mg/L) (95% CL)	Slope ± SE	χ <sup>2</sup>
<i>C. citratus</i>	69.5 (41.6 - 101.5)	277.8 (209.4 - 349.6)	725.2 (592.9 - 889.4)	1.26 ± 0.108	0.95
<i>M. piperita</i>	108.3 (69.2 - 150.9)	408.2 (319.7 - 502.1)	1024.2 (843.0 - 1263.8)	1.31 ± 0.111	0.77

CL = Confidence limit; SE = Standard error; χ<sup>2</sup> = Chi-square value.

Our results align with those of Rajkumar *et al.* (2019), who observed that *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst.) adults were susceptible to insecticidal effects from *M. piperita* EO. Furthermore, *M. piperita* EO showed contact toxicity to *T. castaneum*, *Lasioderma serricornis*, and *Liposcelis bostrychophila*, according to Pang *et al.* (2020).

#### Antifeedant effect of *C. citratus* and *M. piperita* EOs against *S. frugiperda*

Both tested EOs, showed remarkable antifeedant effects against the third larval instars of *S. frugiperda* after 48 hours of exposure to LC<sub>10</sub> and LC<sub>30</sub> concentrations as shown in Table (3). *C. citratus* EO (FDI = 35.17% and 43.06%) showed substantially greater feeding deterrent activity than *M. piperita* EO (FDI = 30.67% and 39.01%) at LC<sub>10</sub> and LC<sub>30</sub> values, respectively. Essential oils can prevent feeding in addition to being toxic to various species. According to Kumar *et al.* (2011), this property is related to terpene compounds that are often present in essential oils, such as linalool, thujone, limonene, and geraniol. Also, sugars and amino acids may interfere with the perception of feeding stimulant receptors, while others may generate unpredictable bursts of electrical impulses in the neurological system, leading to feeding deterrents (Khamis *et al.*, 2016). Skuhrovec *et al.* (2020) found that the EOs have a significant impact on the feeding

behavior of the potato beetle *Leptinotarsa decemlineata*. *M. piperita* EO showed moderate antifeedant effects against *Spodoptera littoralis* (Valcárcel *et al.*, 2021).

#### Sublethal effect of *C. citratus* and *M. piperita* EOs on developmental aspects of *S. frugiperda*

As shown in Table (4), the tested EOs significantly affected the development of *S. frugiperda*. Both EOs, when applied to third larval instars at the LC<sub>10</sub> and LC<sub>30</sub> concentrations, led to an extended length of both larval and pupal stages compared to the control. A more prolonged larval duration was noted with the LC<sub>30</sub> in comparison to the LC<sub>10</sub>. The pupation and adult emergence percentages were significantly decreased after treatment with the LC<sub>10</sub> and LC<sub>30</sub> of the tested EOs compared to the control. Furthermore, the two concentrations significantly lowered male and female pupal weights (Table 4). Our findings revealed that the tested EOs at sublethal concentrations not only caused insect mortality but also interfered with the development of the insects, hence preventing the production of new generations. Several studies have also found that EOs include a range of secondary metabolites with insecticidal activity (Lambert *et al.*, 2020), such as larval mortality, delayed larval duration, pupation decrease, and inhibition of adult emergence (Moustafa *et al.*, 2021; 2023).

**Table 3. Antifeedant activity of LC<sub>10</sub> and LC<sub>30</sub> concentrations of *C. citratus* and *M. piperita* EOs on the third larval instar of *S. frugiperda* after 48 h of exposure**

Treatment	Conc. (mg/L)	Mean weight of leaf consumed (g)	Feeding deterrence index (FDI)*
Control	-	0.98 ± 0.04 <sup>a</sup>	-
<i>C. citratus</i>	69.5	0.47 ± 0.05 <sup>c</sup>	35.17 ± 2.4 <sup>c</sup>
	277.8	0.39 ± 0.03 <sup>e</sup>	43.06 ± 1.8 <sup>a</sup>
	108.3	0.52 ± 0.02 <sup>b</sup>	30.67 ± 2.1 <sup>d</sup>
<i>M. piperita</i>	408.2	0.43 ± 0.02 <sup>d</sup>	39.01 ± 1.6 <sup>b</sup>

\*FDI = [(C - T) / (C + T)] × 100; where C and T are the weights of control and treated leaves consumed by *S. frugiperda*, respectively (Rahman *et al.* 2022). Means ± standard error followed by the same letter do not differ significantly by the Tukey's HSD test (P < 0.05).



**Table 4. Sublethal effects of LC<sub>10</sub> and LC<sub>30</sub> concentrations of *C. citratus* and *M. piperita* EOs on the development of *S. frugiperda* after treating the third larval instars**

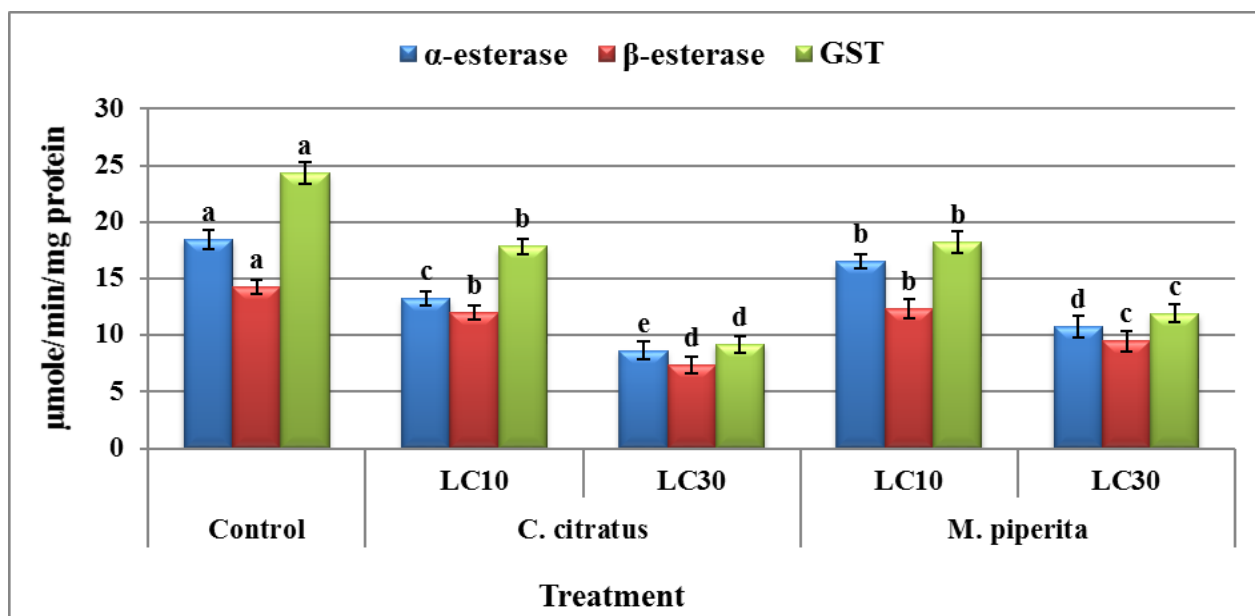
Treatment	Conc. (mg/L)	Larval duration (days)	Pupation (%)	Pupal duration (days)	Pupal weight (g)		Emergence (%)
					Female	Male	
Control	-	16.24 ± 1.18 <sup>e</sup>	96.42 ± 2.85 <sup>a</sup>	9.78 ± 1.14 <sup>d</sup>	0.27 ± 0.08 <sup>d</sup>	0.25 ± 0.02 <sup>d</sup>	94.52 ± 4.18 <sup>a</sup>
<i>C. citratus</i>	69.5	18.06 ± 1.35 <sup>c</sup>	87.83 ± 3.65 <sup>c</sup>	11.25 ± 1.39 <sup>b</sup>	0.35 ± 0.02 <sup>b</sup>	0.33 ± 0.03 <sup>b</sup>	86.34 ± 2.38 <sup>c</sup>
	277.8	19.38 ± 1.26 <sup>a</sup>	82.34 ± 3.52 <sup>d</sup>	12.52 ± 1.28 <sup>a</sup>	0.39 ± 0.05 <sup>a</sup>	0.36 ± 0.06 <sup>a</sup>	80.74 ± 3.62 <sup>e</sup>
<i>M. piperita</i>	108.3	17.52 ± 0.98 <sup>d</sup>	93.22 ± 2.15 <sup>b</sup>	10.43 ± 1.19 <sup>c</sup>	0.31 ± 0.07 <sup>c</sup>	0.29 ± 0.04 <sup>c</sup>	88.45 ± 2.56 <sup>b</sup>
	408.2	18.76 ± 1.19 <sup>b</sup>	86.74 ± 2.60 <sup>c</sup>	12.08 ± 1.23 <sup>a</sup>	0.36 ± 0.04 <sup>b</sup>	0.32 ± 0.03 <sup>b</sup>	83.21 ± 3.42 <sup>d</sup>

Means ± standard error followed by the same letter do not differ significantly by the Tukey's HSD test ( $P < 0.05$ ).

### Detoxification enzymes activity

The CarE and GST enzyme activities of *S. frugiperda* were determined after 48 hours of exposure to LC<sub>10</sub> and LC<sub>30</sub> concentrations of *C. citratus* and *M. piperita* EOs, and the results are presented in Figure (1). The  $\alpha$ -esterase activity in *S. frugiperda* larvae after treatment with LC<sub>10</sub> value was 13.24 and 16.52  $\mu\text{mole}/\text{min}/\text{mg}$  protein, and with LC<sub>30</sub> value was 8.65 and 10.74  $\mu\text{mole}/\text{min}/\text{mg}$  protein, as compared to 18.45  $\mu\text{mole}/\text{min}/\text{mg}$  protein in the control for *C. citratus* and *M. piperita*, respectively. Likewise, the activity of  $\beta$ -esterase in *S. frugiperda* larvae after treatment with LC<sub>10</sub> value was 11.96 and 12.33  $\mu\text{mole}/\text{min}/\text{mg}$  protein, and with LC<sub>30</sub> value was 7.38 and 9.46  $\mu\text{mole}/\text{min}/\text{mg}$  protein, as compared to 14.26  $\mu\text{mole}/\text{min}/\text{mg}$  protein in the control for *C. citratus* and *M. piperita*, respectively. In addition, GST activity significantly inhibited after

treating the 3<sup>rd</sup> larval instar of *S. frugiperda* with the LC<sub>10</sub> (17.84 and 18.26  $\mu\text{mole}/\text{min}/\text{mg}$  protein) and LC<sub>30</sub> (9.21 and 11.93  $\mu\text{mole}/\text{min}/\text{mg}$  protein) of the EOs of *C. citratus* and *M. piperita*, respectively, compared to the control (24.32  $\mu\text{mole}/\text{min}/\text{mg}$  protein). The mechanism of action of EOs is not fully known. Several studies have shown that EOs inhibit the detoxifying enzyme activity in insects (Czerniewicz *et al.*, 2018 and Huang *et al.*, 2020). This study observed inhibition of the detoxification enzymes in response to *C. citratus* and *M. piperita*. However, increased levels of both CarE and GST enzymes were observed in arthropod lines, demonstrating insecticide resistance. Confirming our findings, the LC<sub>15</sub> and LC<sub>50</sub> of *C. citratus* considerably suppressed the activity of detoxifying enzymes in *A. ipsilon* (Moustafa *et al.*, 2021), and in *S. littoralis* (Moustafa *et al.*, 2023).



**Figure 1. Detoxification enzyme activities of *S. frugiperda* after 48 h exposure to the LC<sub>10</sub> and LC<sub>30</sub> concentrations of *C. citratus* and *M. piperita* EOs**

## CONCLUSION

Based on the overall findings, *C. citratus* and *M. piperita* EOs demonstrate a potential approach as eco-friendly agents for the management of *S. frugiperda*. Tested EOs caused a remarkable effect on larval mortality, besides disruption in feeding behavior and development of *S. frugiperda*. In addition, these EOs significantly inhibited the activity of detoxifying enzymes. However, further research is necessary to evaluate these EOs under field conditions.

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## الملخص العربي

### التأثيرات غير القاتلة لكل من الزيوت العطرية (عشبة الليمون والنعناع الفلفلي) على دودة الحشد الخريفية

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العطري أكثر سمية على يرقات *S. frugiperda* من زيت النعناع الفلفلي بعد ٤٨ ساعة من التعرض. وكلا الزيتين العطرين لهما تأثيرات ملحوظة مانعة للتغذية، حيث يتراوح نسبة منع التغذية من ٣٠,٦٧ - ٤٣,٠٦%. كما أدت التركيزات دون القاتلة للزيوت المختبرة إلى إطالة مدة اليرقات والعذارى، وانخفاض وزن العذارى للإناث والذكور، وانخفاض نسبة التعذر ونسب ظهور الأطوار الكاملة، مقارنة بالكنترول. وأيضاً تم تثبيط نشاط إنزيمات الكربوكسيل استيريز وجلوتاثيون إس ترانسفيراز في *S. frugiperda* بشكل ملحوظ، مقارنة بالكنترول. وتشير هذه النتائج إلى الاستخدام المحتمل للزيوت العطرية المختبرة للتحكم في الـ *S. frugiperda*.

**الكلمات المفتاحية:** دودة الحشد الخريفية، عشبة الليمون، النعناع الفلفلي، التأثير المانع للتغذية، القياسات البيولوجية، أنزيمات إزالة السمية.

دودة الحشد الخريفية هي آفة غازية ضارة تسبب خسائر اقتصادية ضخمة لمختلف المحاصيل، وخاصة الذرة في جميع أنحاء العالم. فمن الضروري البحث عن طرق بديلة أكثر أماناً لإدارتها بشكل فعال. في هذه الدراسة، تم اختبار الأنشطة الإبادية ومضادات التغذية لزيت الليمون العطري (*Cymbopogon citratus*) والنعناع الفلفلي (*Mentha piperita*) ضد الطور اليرقي الثالث لـ *S. frugiperda* تحت الظروف المعملية. بالإضافة إلى تقييم تأثير التركيزات دون القاتلة ( $LC_{10}$  &  $LC_{30}$ ) للزيوت العطرية المختبرة على المعايير البيولوجية وأنشطة إنزيمات إزالة السمية لـ *S. frugiperda*. تم تحديد التركيب الكيميائي للزيوت المختبرة باستخدام كروماتوغرافيا الغاز - مطياف الكتلة (GC-MS). كانت المركبات الرئيسية في زيت الليمون العطري هي d-limonene (45.06%),  $\beta$ -citral (10.30%), and  $\alpha$ -citral (9.90%)؛ بينما في زيت النعناع الفلفلي كانت menthol (32.03%), menthone (30.18%), and p-menthan-3-one (11.53%). أظهرت نتائج اختبارات السمية أن زيت الليمون