Journal of Plant Protection Research eISSN 1899-007X

ORIGINAL ARTICLE

Toxicological, biological, and biochemical impacts of the egyptian lavender (*Lavandula multifida* L.) essential oil on two lepidopteran pests

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Vol. 64, No. 2: 127–138, 2024

DOI: 10.24425/jppr.2024.150245

Received: December 27, 2023
Accepted: February 09, 2024
Online publication: May 20, 2024

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Responsible Editor:
Jolanta Kowalska

Abstract

The use of essential oils as an eco-friendly tool in pest management stems from their natural origin and the presence of bioactive compounds that exhibit pesticidal properties, offering a sustainable alternative to synthetic chemical pesticides. This study explores the toxicity of *Lavandula multifida* (lavender) essential oil (EO), as a botanical pesticide against two widespread and destructive Noctuidae pests, *Spodoptera littoralis* (Boisd.) and *Agrotis ipsilon* (Hufnagel). GC-MS was employed to characterize 23 compounds in the EO, with 1,3,3-trimethyl-2-oxabicyclo [2.2.2] octane (eucalyptol) (39.84%), being the primary component. The leaf dipping technique was utilized to assess the toxicity of the EO to both insects. At 96 hours post-treatment, the LC_{50} of lavender EO to *S. littoralis* and *A. ipsilon* larvae were 2.350 and 2.991 mg · ml^{-1}, respectively. Concerning its biological effect, both concentrations of the EO (LC_{15} and LC_{50}) significantly shortened the duration of the larval (to 15.24 and 14.23 days) and pupal (to 11.19 and 10.55 days) stages of *S. littoralis*. Biochemical assays revealed that the LC_{50} of lavender EO significantly inhibited α-esterase activity in *S. littoralis* at 72- and 96 hours post-treatment (0.031 and 0.063 mmol · min^{-1} · mg^{-1}), and *A. ipsilon* at 96 hours post-treatment (0.129 mmol · min^{-1} · mg^{-1} protein). Given its significant toxicological, biological, and biochemical effects on *S. littoralis*, it is suggested that lavender EO could be considered for use in integrated pest management strategies while ensuring its safe application to protect non-target organisms.

Keywords: *Agrotis ipsilon*, essential oil, Gas Chromatography-Mass Spectrometry (GC-MS lavender, *Spodoptera littoralis*

Introduction

Noctuidae is considered the 2nd largest moth family in the order Lepidoptera (Regier *et al.* 2017). It contains over 12,000 described species in around 1,150 genera (Keegan *et al* 2021), placed in 16 sub-families (Kirti and Dar 2013). It is distributed all over the world and is thought to include critical agricultural pests. This family has several important moth pests such as the cotton leafworm (*Spodoptera littoralis*) (Boisd) (Khalifa *et al.* 2023), black cutworm (*Agrotis ipsilon*), (Hufnagel), fall armyworm, *S. frugiperda* (J.E. Smith) (Palli *et al.* 2023), beet armyworm, *S. exigua* (Hübner) (Zhao *et al.* 2023), cotton bollworm, *Helicoverpa armigera* (Hübner), corn earworm, *H. zea* (Boddie), European corn borer, *Ostrinia nubilalis* (Hübner), and cabbage moth, *Mamestra brassicae* (Linnaeus, 1758). As generalist herbivores, these noctuid pest moths are

known to damage a variety of important agricultural crops including cotton, maize, soybean, vegetables, and others, leading to global losses to crop production estimated in the billions annually through yield reductions and management costs (Le Goff and Nauen 2021).

Within the family Noctuidae, there are two major pests that are widely distributed over Egypt's fields: the cotton leafworm, *S. littoralis* (Fouad *et al.* 2022; Moustafa *et al.* 2023a) and the black cutworm, *A. ipsilon* (Moustafa *et al.* 2022). Both pests cause extensive damage to cotton and many other key hosts including soybean and vegetable crops through defoliation by its larvae. The polyphagous nature of these two noctuid pests and the development of resistance due to frequent applications of insecticides necessitates effective integrated pest management (Ahmed *et al.* 2022). This includes using chemical insecticides (Othman *et al.* 2020), transgenic crops (Dutton *et al.* 2005), biopesticides (Moustafa *et al.* 2022), and integrated strategies, given the societal call for the creation of more eco-friendly molecules (Ngegba *et al.* 2022). Consequently, the need to reduce the use of synthetic pesticides or substitute them with natural alternatives has led to the exploration of environmentally friendly methods for pest control.

In recent years, essential oils (EOs) have gained popularity because they are readily available in various plants, and they exhibit low toxicity to mammals and high degradation patterns (Garrido-Miranda *et al.* 2022). These oils contain allelochemicals such as terpenic compounds, alcohols, aldehydes, esters, ethers, and ketones that plants produce to defend themselves against bacteria, viruses, fungi, insects, and other plants (Wagner *et al.* 2021).

Research on the use of EOs as pesticides has increased dramatically because sustainable agriculture has gained greater acceptance and the preference for organic or ecological crops has become increasingly popular throughout the world (Giunti *et al.* 2019; Tortorici *et al.* 2022; Dunan *et al.* 2023; Qi *et al.* 2024). On the other hand, the US Food and Drug Administration (FDA) has certified that EOs are safer than synthetic pesticides (Al-Harbi *et al.* 2021). EOs are aromatic and volatile substances with an oily consistency, extracted from different parts of the plant such as the leaf, stem, flower, bark and fruit (Ebadollahi *et al.* 2021). They exhibit insecticidal (Abdelaal *et al.* 2021), antifeedant (Valcárcel *et al.* 2021), growth regulator (Dyadiuchenko *et al.* 2020), and repellent (Lee 2018) effects. When judiciously incorporated as a component of a systems-oriented approach that underscores biological and cultural controls, essential oils present a diminished mammalian toxicity profile, minimal issues related to residues, and a reduced propensity for insect resistance in comparison to broad-spectrum insecticides (Raveau *et al.* 2020). Using essential oils as crop protectants in integrated pest management (IPM) has its pros and cons. They help delay resistance development with their multiple action mechanisms and selective toxicity, which reduces selection pressure (Isman and Machial 2006). In addition, incorporating essential oils into IPM programs could extend the effectiveness of chemical and genetic control methods by lessening the dependence on a single type of pesticide (Lengai *et al.* 2020). However, issues such as inconsistent oil composition, high production costs, and short residual activity have so far hindered their extensive commercial development (Koul *et al.* 2008). Continued teamwork across various disciplines is crucial to fine-tune utilizing essential oils in IPM systems.

The Lamiaceae plant family encompasses a diverse array of aromatic herbs, spanning genera like *Lavandula* L., Mentha L., *Origanum* L., *Perilla* L., *Rosmarinus* L., *Salvia* L., *Satureja* L., *Thymus* L., and more. These plants have been studied for the pesticidal properties of their EOs extracted from leaves, stems, and flowers (Ebadollahi *et al.* 2020). Whether utilized in natural insect repellents, diffusers, or household products, Lamiaceae EOs present a safer option for pest management, benefiting both humans and the environment. Additionally, their pleasant fragrances make them a preferred choice for environmentally conscious individuals seeking sustainable pest control methods.

The *Lavandula* genus, includes some promising plants that have antimicrobial, insecticidal, parasiticidal, and herbicidal effects (Zuzarte *et al.* 2012). *Lavandula* essential oil, or lavender, has demonstrated insecticidal properties against various insects (Eesa *et al.* 2017; Sayada *et al.* 2022), due to the main terpenoid compounds, such as 1,3,3-trimethyl-2-oxabicyclo [2.2.2] octane (eucalyptol), linalool, linalyl acetate, and camphor. However, few studies have evaluated the bioactivity of EO from *Lavandula* sp. in agricultural pests. Additionally, it is important to study the sublethal effects of EOs on insect pests, because EOs contain volatile or non-volatile substances that can have a positive or negative effect on insects (Costa *et al.* 2023). While volatile substances in essential oils can be attractive or repellent to insects, affecting host plant location, egg laying and feeding of herbivorous insects, non-volatile substances can exert deterrent or arresting effects on insects, thus affecting the biological performance of insect pests (Piesik *et al.* 2016). Therefore, evaluating the physiological impact of any toxic agent on insects can assist in formulating effective pest control strategies and understanding the mechanism of action.

In this work, we examined *L. multifida* EO chemical composition on two lepidopteran pests, *S. littoralis* and *A. ipsilon* which previously had not been reported.

Therefore, in this work, we examined the EO chemical composition and its influence on the development of surviving larvae, pupae, and adults' sex ratio of both insects at lethal and sublethal concentrations. Further, we investigated the impact of lavender EO on the detoxification enzymes to gain insights into the latent effects of this EO on both insects.

Materials and Methods

Insects

Susceptible strains of cotton leafworm *Spodoptera littoralis* (Boisd.) and black cutworm *Agrotis ipsilon* (Hufnagel.) were reared under laboratory conditions (26 ± 1°C, 65 ± 5% relative humidity, 16:8 L/D photoperiod) at the Economic Entomology and Pesticides Department, Faculty of Agriculture, Cairo University, Giza, Egypt (Moustafa *et al.* 2023b). Over 12 generations, the larvae of both insects were kept insecticide-free and fed daily fresh castor oil leaves. To avoid *A. ipsilon* larvae cannibalism, the third instar larvae were individually reared (Awad *et al.* 2022). Adults of both insects were provisioned with a cotton wool segment, saturated with a 10% sucrose solution. For the oviposition of *S. littoralis* adults, filter paper was employed as a surface and was regularly replaced (Othman *et al.* 2020). In contrast, for the oviposition of *A. ipsilon* adults, dark net strips were utilized as sites for egg deposition by mated female moths (Moustafa *et al.* 2021). The eggs from both insect species were gathered daily, relocated to fresh jars, and allowed to hatch. Bioassays were conducted using the second-instar larvae of both insects.

Chemicals

The following chemicals were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA): α-naphthyl acetate, fast blue B salt, 1-chloro-2,4-dinitrobenzene (CDNB), L-glutathione reduced (GSH), P-nitroanisole (PNOD), and β-nicotinamide adenine dinucleotide phosphate (reduced β NADPH).

Extraction of lavender essential oil

Fresh leaves of *Lavandula multifida* were obtained from the farms of the Medicinal and Aromatic Plants Research Department, El-Kanater El- Khairiya, Qalubeya Governorate, Egypt. The EO was extracted from leaves using the method described by Moustafa *et al.* (2021). Briefly, the leaves were thoroughly rinsed with tap water and finely chopped using an excelsior. The essential oil was extracted from 100g of fresh sample via hydro-distillation using a Clevenger-type apparatus with water heated to 70°C. This process continued until no further increase in the EO was observed (2.5 to 3 hours). Following distillation, hexane was added to the mixture, yielding two separate phases: an aqueous phase known as aromatic water, and an organic phase, which, being less dense than water, contained the essential oil. The essential oil was subsequently isolated using a conical separating funnel, dried with anhydrous sodium sulfate, and stored in dark brown sealed vials at 4°C in the refrigerator until required for chemical analysis. The yield of EO was 1.04%.

GC-MS analysis of the lavender EO

The analysis of lavender EO constituents was conducted using GC Ultra-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA), which is equipped with a direct capillary column TG–5MS (30 m × 0.25 mm × 0.25 µm film thickness). The essential oil was first diluted in n-hexane solvent (1 : 1000 ratio) before being introduced to the GC–MS. Helium served as the carrier gas at a flow rate of 1 ml · min^{-1}. A solvent delay of 3 min was implemented, and a 2 µl diluted sample was automatically injected in splitless mode using an Autosampler AS1310 coupled with the GC. The operating conditions were programed from 50 to 250°C at a rate of 5°C · min^{-1}, held for 2 min, then the final temperature was increased to 310°C at a rate of 25°C · min^{-1} and held for another 2 min. The injector and MS transfer line temperatures were maintained at 270 and 260°C, respectively. EI mass spectra were collected at the m/z range of 50–650 with ionization voltages set at 70 eV. The ion source temperature was set at 250°C. The components were identified using their match factor and their mass spectrum by comparison of their mass spectra with those of WILEY 09 and NIST14 mass spectral database.

Bioassays

The toxicity of lavender EO on the second instar larvae of *S. littoralis* and *A. ipsilon* was evaluated using the leaf dipping method (Moustafa *et al.* 2023b). Preliminary tests led to the selection of six concentrations ranging from 8 to 0.5 mg · ml^{-1}, with 2X serial dilutions, to determine the LC-values. These concentrations were diluted using water and the surfactant polysorbate Tween-20 (0.5%). Castor plant leaves were dipped in each prepared concentration for 20 s before drying at room temperature (29 ± 2°C) for 1 h. For each concentration, 50 larvae (ten larvae × five replicates) were allowed to feed on treated castor oil leaves (4 cm diameter) in a 0.25 l glass jar, covered with a clean muslin cloth, for 24 hours. The surviving larvae

were then moved to clean jars with untreated leaves and mortality was recorded daily. The bioassays were repeated twice.

Insect development studies

The latent effects of lavender EO on the larvae, pupae, and adults of *S. littoralis* and *A. ipsilon* were examined using LC_{15} and LC_{50}. A bioassay method was employed, involving 15 second-instar larvae in triplicates for each insect species at each concentration (Moustafa *et al.* 2023a). Surviving larvae were individually relocated into clean cups containing sufficient sawdust 96 hours post-treatment and were daily provided with fresh castor oil leaves until pupation. Upon pupation, each pupa was sexed and weighed. The following parameters were recorded for both insects: larval and pupal durations (days), pupation percentages, pupal weight (g), adult emergence percentage, and sex ratio. The parameters of the treated insects were compared with those of a control group, which was exposed to castor oil leaves dipped in a solution of water and Triton-20.

Enzyme assays

Sample preparation

The second-instar larvae of *S. littoralis* and *A. ipsilon* were exposed to lavender EO at two different concentrations (LC_{15} and LC_{50}). After 24-, 48-, 72-, and 96-hours of this treatment, the surviving larvae were collected, weighed, and rinsed with distilled water. To evaluate the activity of detoxifying enzymes, namely α-esterase, glutathione S-transferase, and cytochromes P450, three separate samples were prepared, each of which contained 50 milligrams of fresh larval weight. These samples were specifically prepared for each enzyme determination and were promptly stored at –20°C for subsequent analysis.

α-esterase assay

The larvae were homogenized in a cold 0.1 M phosphate buffer with a pH of 7. Subsequently, the homogenates were centrifuged at 12,000 rpm for 10 minutes. The α-esterase activity was determined according to the method described by Asperen (1962) and Moustafa *et al.* (2021). In brief, the larval homogenate was incubated with 30 mM of α-naphthyl acetate at 25°C for 15 minutes. The reaction was stopped by adding a mixture of 2% Fast Blue B and 5% sodium dodecyl sulphate. The enzyme activity was measured at an endpoint of 600 nm using a Jenway-7205UV/Vis Spectrophotometer and the absorbance levels were compared with a standard curve for known concentrations of α-naphthol. The specific activities of α-esterase were reported as μmoles per minute per milligram of protein.

Glutathione – S- transferase (GST) assay

The larvae were homogenized in a cold 0.1 M phosphate buffer with a pH of 6.5. The homogenates were then centrifuged at 10,000 rpm for a duration of 10 minutes. The GST activity was determined using 30 mM of 1-chloro-2,4-dinitrobenzene (CDNB) and 50 mM GSH, as outlined by Habig *et al.* (1974). The optical density was monitored at 340 nm for 3 minutes, with readings taken at 1-minute intervals using a Jenway-7205 UV/Vis Spectrophotometer. The specific activities of GST were reported as mmoles per minute per milligram of protein.

Cytochromes P450 assay

The larvae were homogenized in a cold 0.1 M phosphate buffer with a pH of 7.4. The activity of P450 was determined using p-nitroanisole (PNOD) and NADPH, as described by Hansen and Hodgson (1971). Briefly, the larval homogenate was incubated with 2mM p-nitro anisole at 27°C for 2 minutes. The reaction was then initiated by adding 9.6 mM NADPH. The optical density was measured at 405 nm using a Clindiag-MR-96 microplate reader (ISO09001:2008, Steenberg, Belgium). The specific activities of P450S were reported as nmol per minute per milligram of protein.

Protein determination

Bradford's method (Bradford 1976) was used to determine the total protein content using Coomassie brilliant blue dye and bovine serum albumin as a standard.

Data analysis

Mortality data were corrected when needed using Abbott's formula (Abbott 1925). Data were analyzed using SPSS (V.22). The data were tested to ensure that they met the assumptions of parametric tests. Continuous variables were subjected to the Shapiro-Wilk and Kolmogorov-Smirnov tests for normality. Probability and percentile data were standardized for normality using the Arcsine Square Root method. The LC values of lavender EO were calculated using Probit analysis (EPA Probit analysis software, version 1.5). Concerning the recorded developmental parameters and enzyme activities, one-way ANOVA were performed for the experimental groups (Control, LC_{15} and LC_{50}) for both insects. Data were presented as the mean and standard deviation. Post-hoc analysis was conducted using Tukey's pairwise comparison; *p*-values were considered significant at <0.05. The Chi-square test was utilized to compare the observed and expected

frequencies of both sexes of the investigated insects using MiniTab (V 14). When necessary, data visualization was carried out using R studio (V 2022.02.4.)

Results

Chemical composition of the essential oil of lavender (*Lavandula multifida)*

GC-MS analysis of the essential oil (EO) of lavender (*Lavandula multifida)* led to the identification of 23 compounds. Percentages of these compounds ranged from 0.35 to 39.84%. The analysis highlighted a significant presence of terpenoids, with 1,3,3-trimethyl-2-oxabicyclo [2.2.2] octane (Eucalyptol) being the predominant monoterpenoid (39.84%). Other notable constituents included (1*S*,4*S*)-1,7,7--trimethylbicyclo [2.2.1] heptan-2-one (Camphor) (18.86%), 2,6,6-trimethylbicyclo [3.1.1] hept-2-ene (alpha-pinene) (8.86%), and 66,6-dimethyl-2-methylidenebicyclo [3.1.1] heptane (beta-pinene) (7.46%) (Table 1 and Fig. 1).

Toxicity of lavender EO to *Spodoptera littoralis* and *Agrotis ipsilon* larvae

Table 2 presents the stomach toxicity of *L. multifida* EO to the 2nd instar larvae of *S. littoralis* and *A. ipsilon* 96 hours post-treatment, and the relative potency of the EO to both species. The EO exhibited significant toxicity against *S. littoralis* larvae, with LC_{15} and LC_{50} of 0.401 and 2.350 mg/ml, respectively. It also demonstrated high toxicity to *A. ipsilon*, with corresponding values of 0.829 and 2.991 mg/ml. The relative potency indicated that the EO was 1.27 times more toxic to *S. littoralis* larvae than *A. ipsilon* larvae.

Biological impact of lavender EO on *Spodoptera littoralis* and *Agrotis ipsilon*

Table 3 and Figure 2 show the impact of LC_{15} and LC_{50} of lavender EO on the biological characteristics of *S. littoralis* and *A. ipsilon*. The findings revealed that LC_{15} and LC_{50} of lavender EO caused a significant decrease in the duration of larval (to 15.24 and 14.23 days) and pupal (to 11.19 and 10.55 days) stages of *S. littoralis*,

Table 1. Chemical compounds identified in the EO of lavender (*Lavandula multifida*)

RT	Area [%]	IUPAC name (common name)	Match factor [MF]
2.02	0.87	2-ethyloxetane (2-ethyl-oxetane)	868
3.66	8.86	2,6,6-trimethylbicyclo [3.1.1]hept-2-ene (alpha-pinene)	931
3.93	2.51	2,2-dimethyl-3-methylidenebicyclo[2.2.1]heptane (Camphene)	949
4.30	2.12	4-methylidene-1-propan-2-ylbicyclo[3.1.0]hexane (Sabinen)	947
4.40	7.46	6,6-dimethyl-2-methylidenebicyclo [3.1.1] heptane (beta-pinene)	942
4.57	1.75	7-methyl-3-methylideneocta-1,6-diene (myrcene)	934
5.31	0.78	1-methyl-2-propan-2-ylbenzene (O-cymene)	938
5.47	39.84	1,3,3-trimethyl-2-oxabicyclo [2.2.2] octane (eucalyptol)	929
6.34	0.52	1-methyl-4-prop-1-en-2-ylcyclohexan-1-ol (beta-Terpineol)	906
7.05	0.71	3,7-dimethylocta-1,6-dien-3-ol (Linalool)	862
8.14	18.86	(1*S*,4S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (Camphor)	947
8.84	1.83	(1*R*,2*R*,4*R*)-1,7,7-trimethylbicyclo [2.2.1] heptan-2-ol (Isoborneol)	878
9.06	0.72	4-methyl-1-propan-2-ylcyclohex-3-en-1-ol (Terpinen-4-ol)	883
9.46	2.34	2-[(1*S*)-4-methylcyclohex-3-en-1-yl]propan-2-ol (alpha-Terpineol)	912
10.65	0.65	4-propan-2-ylbenzaldehyde (Cuminaldehyde)	784
11.28	0.70	(2*E*)-3,7-dimethylocta-2,6-dienal (Citral)	902
14.76	0.39	1,1,4,7-tetramethyl-1*a*,2,3,4,4*a*,5,6,7*b*-octahydrocyclopropa[e]azulene (alpha-Gurjunene)	936
15.07	0.86	(1*R*,4*E*,9*S*)-4,11,11-trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene (Caryophyllene)	931
17.28	4.85	(1*R*,5*R*)-1,8-dimethyl-4-propan-2-ylidenespiro[4.5]dec-8-ene	927
17.50	0.50	(1*S*,4*S*)-1,6-dimethyl-4-propan-2-yl-1,2,3,4-tetrahydronaphthalene (Calamenene)	900
18.92	1.64	(1*R*,4*R*,6*S*,10*R*)-4,12,12-trimethyl-9-methylidene-5-oxatricyclo[8.2.0.0^{4,6}]dodecane (4,11,11-Trimethyl-8--methylene-5-oxatricyclo(8.2.0.0(4,6))dodecane, (1R,4R,6R,10S)-	934
20.34	0.91	(1*S*,4*S*,4a*R*,8a*R*)-1,6-dimethyl-4-propan-2-yl-3,4,4*a*,7,8,8*a* hexahydro-2*H*-naphthalen-1-ol (Tau-cadinol)	911
21.48	0.35	3-ethenyl-3-methyl-6-propan-2-yl-2-prop-1-en-2-ylcyclohexan 1-ol (6-epi-shyobunol)	871

IUPAC – The International Union of Pure and Applied Chemistry

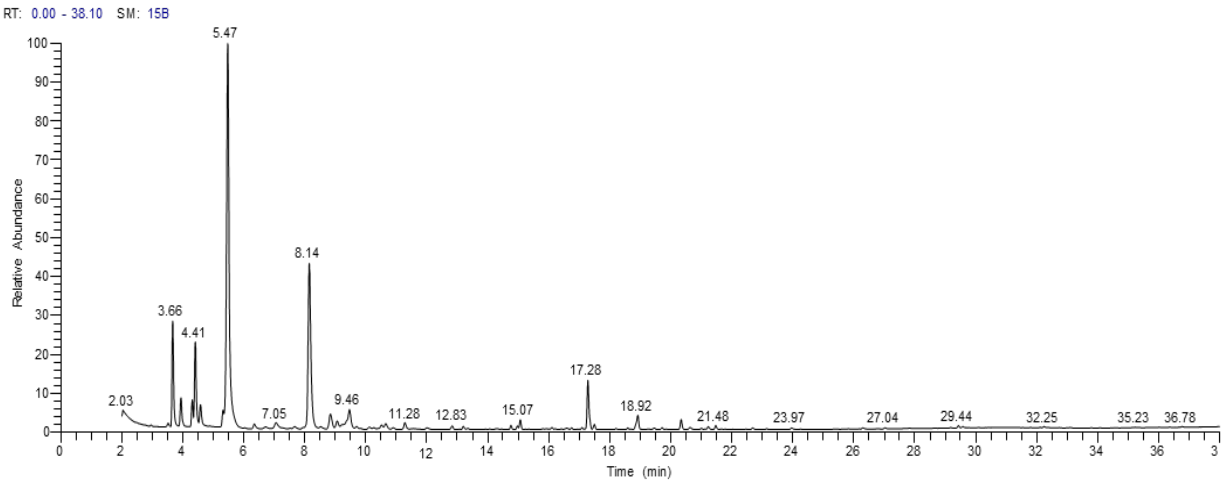

Fig 1. GC-MS chromatogram of the identified chemical compounds in the essential oil of lavender (*Lavandula multifida*)

Table 2. Toxicity of lavender (*Lavandula multifida*) EO to the 2nd instar larvae of *Spodoptera littoralis* and *Agrotis ipsilon* 96 hours post-treatment

Species	LC_{15} [mg · ml^{-1}] (95% confidence limits)	LC_{50} [mg · ml^{-1}] (95% confidence limits)	Slope ± SE	χ^2
S. littoralis	0.401 (0.157–0.652)	2.350 (1.715–3.451)	1.34 ± 0.24	0.66
A. ipsilon	0.829 (0.464–1.182)	2.991 (2.260–4.172)	1.86 ± 0.29	2.73

Relative potency – LC_{50} of EO on *A. ipsilon*/ LC_{50} of EO on *A. ipsilon*

Table 3. The latent effect of LC_{15} and LC_{50} of the EO of lavender (*Lavandula multifida*) on the larvae, pupae, and adults of *Spodoptera littoralis* and *Agrotis ipsilon*

Parameter	Treatments	*S. littoralis*	*A. ipsilon*
Larval duration [days]	control	16.71 ± 1.22 a	19.7 ± 1.3 b
	LC_{15}	15.2 ± 1.54 b	20.1 ± 2.84 ab
	LC_{50}	14.2 ± 1.45 b	21.0 ± 2.60 b
Pupal duration [days]	control	13.1 ± 1.44 a	17.5 ± 1.69 a
	LC_{15}	11.2 ± 2.47 b	17.6 ± 1.25 a
	LC_{50}	10.5 ± 1.66 b	18.0 ± 1.56 a
Pupation [%]	control	95.5 ± 4.15 a	100 ± 0.00 a
	LC_{15}	80.1 ± 7.76 a	97.2 ± 3.93 a
	LC_{50}	66.5 ± 22.6 a	91.8 ± 4.31 a
Male pupal weight [g]	control	0.27 ± 0.03 a	0.35 ± 0.07 a
	LC_{15}	0.27 ± 0.04 a	0.34 ± 0.05 a
	LC_{50}	0.27 ± 0.10 a	0.34 ± 0.06 a
Female pupal weight [g]	control	0.28 ± 0.03 a	0.36 ± 0.08 a
	LC_{15}	0.29 ± 0.05 a	0.32 ± 0.06 a
	LC_{50}	0.28 ± 0.04 a	0.32 ± 0.06 a
Emergency [%]	control	100 ± 0.00 a	98.6 ± 1.96 a
	LC_{15}	96.2 ± 2.68 a	100 ± 0.00 a
	LC_{50}	98.1 ± 2.63 a	98.1 ± 2.62 a

For each parameter, means in a column that share the same letter are not significantly different ($p > 0.05$)

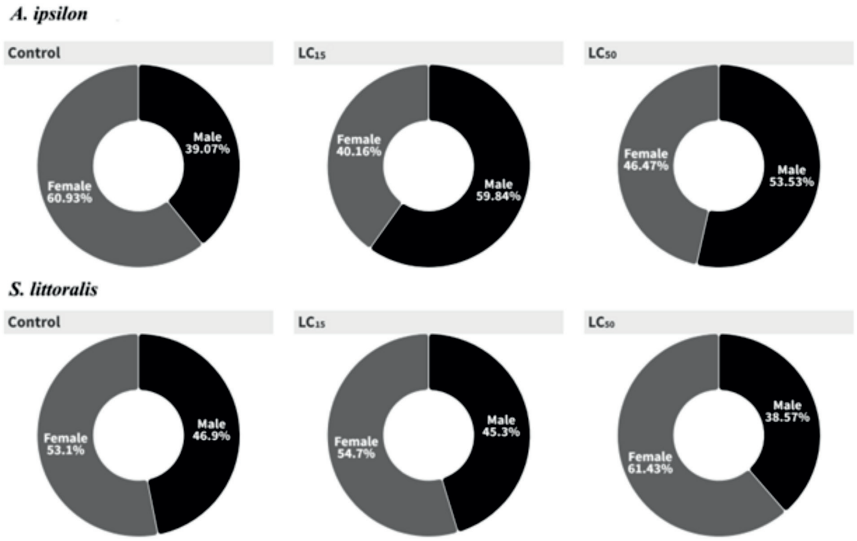

Fig 2. Impact of LC_{15} and LC_{50} of the EO of lavender (*Lavandula multifida*) on the sex ratio of *Spodoptera littoralis* and *Agrotis ipsilon*

respectively. However, these concentrations did not significantly influence the biological parameters of *A. ipsilon*. On the other hand, a significant change ($p < 0.05$) in the sex ratio of *S. littoralis* was observed with the LC_{50}, compared to the control group.

Biochemical impact of lavender EO on *Spodoptera littoralis* and *Agrotis ipsilon*

Data in Table 4 represent the effect of LC_{15} and LC_{50} of lavender EO on the activities of α-esterase, glutathione

Table 4. Biochemical impact of LC_{15} and LC_{50} of the EO of lavender (*Lavandula multifida*) on the activities of α-esterase, glutathione S-transferase (GST), and cytochromes P450 in *Spodoptera littoralis* and *Agrotis ipsilon* larvae 24-, 48-, 72-, and 96 hours post-treatment

Enzyme	Species	Treatment	24 h	48 h	72 h	96 h
α-esterase [mmol · min^{-1} · mg^{-1} of protein]	*S. littoralis*	control	0.30 ± 0.03 a	0.14 ± 0.00 b	0.07 ± 0.01 b	0.22 ± 0.05 a
		LC_{15}	0.15 ± 0.02 b	0.15 ± 0.01 b	0.21 ± 0.00 a	0.17 ± 0.01 a
		LC_{50}	0.26 ± 0.03 b	0.23 ± 0.03 a	0.03 ± 0.00 c	0.06 ± 0.01 b
	A. ipsilon	control	0.08 ± 0.01 c	0.13 ± 0.02 b	0.10 ± 0.02 a	0.19 ± 0.01 a
		LC_{15}	0.33 ± 0.04 a	0.24 ± 0.02 a	0.14 ± 0.02 a	0.20 ± 0.01 a
		LC_{50}	0.20 ± 0.04 b	0.25 ± 0.04 a	0.13 ± 0.02 a	0.12 ± 0.02 b
GST [mmol · min^{-1} · mg^{-1} of protein]	*S. littoralis*	control	22.8 ± 5.38 a	11.7 ± 1.57 a	17.4 ± 1.83 a	22.9 ± 6.81 a
		LC_{15}	7.98 ± 1.53 b	7.95 ± 4.30 a	8.60 ± 3.66 b	9.47 ± 2.78 b
		LC_{50}	4.03 ± 0.43 b	5.34 ± 0.50a	4.97 ± 0.78 b	2.97 ± 0.42 b
	A. ipsilon	control	20.5 ± 5.04 a	21.2 ± 6.15 b	35.8 ± 1.80 a	47.3 ± 5.39 a
		LC_{15}	43.3 ± 11.7 a	56.3 ± 17.9 a	34.4 ± 9.80 a	42.8 ± 16.3 a
		LC_{50}	38.3 ± 6.95 a	46.6 ± 1.52 ab	48.4 ± 0.15 a	63.3 ± 17.5 a
P450 [mmol · min^{-1} · mg^{-1} of protein]	*S. littoralis*	control	7.71 ± 0.51 b	6.29 ± 0.21b	3.78 ± 0.16 b	7.49 ± 0.29 b
		LC_{15}	7.75 ± 0.62 b	10.7 ± 1.42 a	10.0 ± 1.90 a	16.2 ± 0.87 a
		LC_{50}	11.1 ± 1.10 a	12.4 ± 1.03 a	8.52 ± 1.51 a	9.75 ± 2.74 b
	A. ipsilon	control	7.05 ± 0.46 b	8.52 ± 0.31 a	5.58 ± 0.55 b	11.0 ± 1.10 a
		LC_{15}	10.6 ± 1.37 ab	12.5 ± 2.09 a	9.53 ± 1.63 a	17.0 ± 2.87 a
		LC_{50}	12.7 ± 1.98 b	14.5 ± 3.16 a	8.80 ± 1.12 ab	10.8 ± 2.87 a

For each parameter, means in a column that share the same letter are not significantly different ($p > 0.05$)

S transferase (GST), and cytochrome P450 of *S. littoralis* and *A. ipsilon* 24-, 48-, 72- and 96 hours post-treatment. The LC_{50} significantly inhibited the activity of α-esterase at 72- and 96 hours post-treatment in *S. littoralis*, while in *A. ipsilon*, the inhibition was observed only at 96 hours post-treatment. The corresponding activities of α-esterase were 0.031 and 0.063 mmol · min^{-1} · mg^{-1} protein in *S. littoralis*, respectively, and 0.129 mmol · min^{-1} · mg^{-1} protein in *A. ipsilon*.

At 72- and 96 hours post-treatment, the GST activity in *S. littoralis* was inhibited with both tested concentrations. The activity declined to 8.6 and 9.47 mmol · min^{-1} · mg^{-1} of protein with LC_{15} and to 4.9 and 2.9 mmol · min^{-1} · $mg^{-1}$of protein with LC_{50}, respectively.

At 72 hours post-treatment, the P450 activity in *S. littoralis* was increased with both tested concentrations. The activity increased to 10.0 and 8.52 mmol · min^{-1} · mg^{-1} of protein with LC_{15} and LC_{50}, respectively, compared to 3.78 mmol · min^{-1} · mg^{-1} of protein in the control group.

Discussion

The use of EOs as pest control agents has gained increasing interest in the past decades (El-Shourbagy *et al.* 2023). EOs are environmentally friendly and have low toxicity to mammals. The cotton leafworm, *S. littoralis*, a major pest in Egypt, has developed significant resistance to a range of synthetic insecticides (Swelam *et al.* 2022) including organophosphates, pyrethroids, and insect growth regulators (Ismail 2023). Field strains have even shown resistance to newer insecticides like chlorantraniliprole, spinetoram, and emamectin benzoate (Fouad *et al.* 2022; Moustafa *et al.* 2024). Additionally, the black cutworm, *A. ipsilon*, exhibited similar resistance patterns due to intensive insecticide use (Moustafa *et al.* 2022). These challenges underscore the need for alternative pest management strategies, and plant essential oils with their unique modes of action could offer potential solutions. In this respect, lavender essential oil, in particular, has shown potential as a botanical insecticide. Nevertheless, the effectiveness of the essential oil's insect-killing properties hinges on several factors: the presence of key components, how it is applied, its concentration, and the specific stage and species of the insect. In the present study, lavender proved to have a toxic effect on the second-instar larvae of both *S. littoralis* and *A. ipsilon*. Although the relative potency indicated that EO was 1.27 times more toxic to *S. littoralis* than *A. ipsilon,* the overlapping confidence intervals for both insects suggest that the EO's toxic effect was not significantly different for the two species 96 hours post-treatment. Eesa *et al.* (2017) also found that lavender EO was highly toxic to the 2nd and 4th instar larvae of *S. littoralis*. However, a study by Lee and Potter (2013) found that applying 2000 ppm of lavender oil did not significantly affect the survival rate or feeding damage of *A. ipsilon* 6 days post-treatment, compared to untreated checks.

Traditionally, insect toxicity studies have focused on acute median lethal doses of chemicals (Swelam *et al.* 2022). However, it is crucial to also consider the latent effects of these doses, which can alter an insect's physiological and behavioral patterns, thereby affecting its performance. These doses can also provide insights into the overall efficacy of insecticides and their selectivity towards non-target organisms. In our study, we evaluated the impact of sub-lethal concentrations (LC_{15} and LC_{50}) of lavender essential oil on the 2nd instar larvae of *S. littoralis* and *A. ipsilon*. Our findings revealed a significant decrease in both larval and pupal durations of *S. littoralis* in response to both tested concentrations, compared to the control group. However, *A. ipsilon* larval and pupal durations showed no significant changes. Furthermore, when subjected to the lethal concentration (LC_{50}) of lavender EO (EO), the adult sex ratio of *S. littoralis* underwent a significant ($p < 0.05$) shift, with females accounting for 61.4% and males 38.5% of the population. This skewed distribution towards females suggests a more pronounced impact of lavender EO on *S. littoralis* males than on females. A similar pattern was observed in a study by Moustafa *et al.* (2023), where exposure to the LC_{50} of citral, a major component of lemongrass EO (*Cymbopogon citratus* L.), resulted in an increased proportion of females (60%) compared to males (40%) in the population of *S. littoralis*. Conversely, *A. ipsilon* did not display any significant alteration in the male/female sex ratio when exposed to either the sublethal concentration (LC_{15}) or the lethal concentration (LC_{50}) of lavender EO. This suggests that lavender EO does not induce a biased delayed effect towards either females or males of *A. ipsilon*.

Grasping how plant essential oils impact insects through their toxic properties is crucial for enhancing sustainable pest management strategies. Our findings indicated that the primary components of lavender essential oil, namely 1,3,3-trimethyl-2-oxabicyclo [2.2.2] octane (eucalyptol), (1*S*,4*S*)-1,7,7-trimethylbicyclo [2.2.1] heptan-2-one (Camphor), 2,6,6-trimethylbicyclo [3.1.1] hept-2-ene (alpha-pinene), and 6,6-dimethyl-2-methylidenebicyclo [3.1.1] heptane (beta-pinene), are all terpenoids. Eucalyptol and camphor have been consistently identified as the primary compounds of interest in *Lavandula angustifolia* L.

essential oil (commonly known as English lavender) in recent literature (Beale *et al*. 2017; Danila *et al*. 2018; Wainer *et al*. 2022). Additionally, linalool and linalyl acetate are also recognized as major components in *L. angustifolia*. However, since our current study focuses on a different lavender species, *L. multifida*, it is logical to expect variations in the percentage of these components.

The insecticidal activity of EOs or crude plant extracts relies on the high concentrations of major compounds belonging to classes such as terpenes, phenolics, and alkaloids (Ootani *et al*. 2013). As stated by Bassolé and Juliani (2012), terpenes and terpenoids constitute the main components of EOs. Preliminary research suggested that these terpene compounds may interfere with cellular ATP production, ion channel functions, or oxidative homeostasis in insects, similar to conventional insecticides (Regnault-Roger *et al.* 2012; Tong and Bloomquist 2013). Further research employing advanced methods such as RNA interference could reveal the modes of action of essential oils and potential synergies with other control strategies like biological control agents.

In arthropods, the role of cytochrome P450 (P450), carboxylesterases (CarEs), and glutathione S-transferases (GSTs) in the biosynthesis of many endogenous compounds is well-established. Additionally, these enzymes eliminate a wide range of toxic compounds and can also detoxify essential oils (Farahani *et al.* 2020; Fergani *et al.* 2020). Our study investigated the toxicity of lavender essential oil by examining its inhibitory effect on these detoxification enzyme systems.

Our data indicated that lavender essential oil has an inhibitory effect on the glutathione S-transferase (GST) activities of *S. littoralis*. At 72- and 96 hours post-treatment, the GST activities were 8.6 and 9.47 mmol · min^{-1} · mg^{-1} of protein for LC_{15} and 4.9 and 2.9 mmol · min^{-1} · mg^{-1} of protein for LC_{50}, respectively. This suggests that lavender EO has a dose-dependent effect on the GST activities in *S. littoralis*, i.e., higher concentrations cause greater inhibition. This is an interesting finding that could have implications for the use of lavender EO in pest control strategies. The LC_{50} of lavender essential oil also demonstrated an inhibitory effect on the α-esterase activity in *S. littoralis* 72- and 96 hours post-treatment. The inhibition of α-esterase and GST activities by lavender EO suggest that these enzymes could play a role in detoxifying lavender essential oil in *S. littoralis*. This observation aligns with the findings of Döker *et al.* (2021), who noted lower detoxification enzyme activities in more sensitive populations. Similarly, essential oils from dill, crane's-bills, basil, and citronella were found to significantly reduce the GST activity in the 3rd instar larvae of *S. littoralis*, compared to control larvae (Fergani *et al.* 2020).

Interestingly, compared to the control group, *A. ipsilon* exhibited high levels of α-esterase, GST, and P450, especially with LC_{15}, 24-, 48-,72-, and 96 hours post-treatment. This could explain why lavender essential oil did not affect the biological parameters of *A. ipsilon* larvae and pupae in our study. In this context, the diminished toxicity of certain essential oils to specific pest species has been attributed partially to increased levels of detoxification enzymes that effectively metabolize the toxic components of essential oils. This is also supported by the evidence of shared degradation mechanisms such as heightened activities of P450s, GSTs, and carboxylesterases (CarEs) in treated populations. The results obtained by Farahani *et al.* (2020) also implied that GSTs, and, to a lesser extent, carboxylesterases, are the major enzymes involved in the metabolic resistance to plant essential oils. These findings suggest a complex interaction between essential oil toxicity and pest species' detoxification capabilities.

This study has established a correlation between the insecticidal activity of lavender essential oil and the inhibition of detoxification enzyme activities. However, the specific mechanism remains unclear. Our findings indicate that lavender essential oil effectively inhibits the glutathione S-transferases (GSTs) and carboxylesterases (CarEs) in *S. littoralis*, but further research is needed to determine its inhibitory potential on the detoxification enzymes in *A. ipsilon*. By integrating biomarker assays, we can conduct a comprehensive analysis of the impact of plant-derived chemicals, such as lavender essential oil, on the target organism. The observed correlation between comet assays, biomarkers, and toxicological responses provides a method for assessing the insect's response to essential oil application. This could be instrumental in risk assessment and decision-making regarding its future incorporation into Integrated Pest Management (IPM) programs.

Conclusions

Our study provides definitive evidence of the significant bioactivity of lavender essential oil against all developmental stages of *S. littoralis*, suggesting its potential for formulation development. However, comprehensive investigations into its genotoxic and ecotoxic impacts are imperative before its integration into *S. littoralis* pest management strategies. Stability studies are also required to ascertain safety and efficacy, thereby facilitating a safer and more sustainable pest management approach. We recommend further research to assess the field efficacy of these

essential oils and their safety towards non-target organisms.

Acknowledgements

Special thanks are due to Dr. Hamed Khairullah Said for revising and editing the manuscript for grammatical correctness, coherence, and sense. Dr, Said is an Associate Professor of pesticide toxicology at the Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Egypt, and a holder of a BA in English Linguistics and Translations.

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