



Assessment of detoxification enzyme activities and hemocyte examination of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) in response to *Mentha pulegium* and *Artemisia camphorata* extracts

Sahar E. Eldesouky · Abir A. Gad ·
Mohamed Z. M. Salem

Received: 24 September 2024 / Accepted: 19 March 2025 / Published online: 15 April 2025
© The Author(s) 2025

Abstract The interest in botanical insecticides has surged due to environmental apprehensions and insect resistance to synthetic insecticides. In this study, we explored the insecticidal, biochemical, and physiological effects of *Mentha pulegium* essential oil (EO) and *Artemisia camphorata* methanol extract (ME) on the fourth-instar larvae of *Spodoptera littoralis*. By GC–MS analysis, the main compounds identified in *M. pulegium* EO were menthone (22.45%), 1,8-cineole or eucalyptol (19.37%), and pulegone (8.67%). The abundant flavonoid compounds were apigenin, luteolin, quercetin, and naringin, whereas the abundant phenolic compounds were 4-hydroxybenzoic,

ferulic, and chlorogenic acids in *A. camphorata* ME after analysis by HPLC. The LC₅₀ values after 24 h of *S. littoralis* treatment with *M. pulegium* EO, *A. camphorata*, and novaluron were 4458.3, 7349.9, and 9.8 mg/L, whereas the LC₅₀ values after 48 h of treatment were 2495.3, 4024.5, and 7.7 mg/L, respectively. The activities of mixed-function oxidase, α and β -esterases, and glutathione *S*-transferase were significantly increased in *S. littoralis* larvae after 24 and 48 h of treatment with LC₂₅ and LC₅₀ concentrations of novaluron, *M. pulegium* EO, and *A. camphorata* ME. The mean number of prohemocytes, granulocytes, and plasmatocyte cells was significantly decreased at 24, 48 and 72 h of treatment with LC₂₅ of *M. pulegium* EO and *A. camphorata* ME. In contrast, the number of oenocytes and spherulocytes was increased compared to the control. On the other hand, novaluron caused a significant decrease in all hemocyte numbers. The hemocyte surface areas increased dramatically in all hemocyte types after treatment with *M. pulegium* EO and *A. camphorata* ME. In contrast, hemocyte surface areas significantly decreased after novaluron treatment compared to the control. These findings suggest that *M. pulegium* and *A. camphorata* may be eco-friendly alternatives for synthetic insecticides in *S. littoralis* control.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s12600-025-01265-8>.

S. E. Eldesouky
Cotton Pesticides Evaluation Department, Plant Protection
Research Institute, Agricultural Research Center,
El-Sabha, Alexandria 21616, Egypt
e-mail: elsayedssahar@gmail.com

A. A. Gad
Applied Entomology and Zoology Department,
Faculty of Agriculture, Alexandria University,
El-Shatby, Alexandria 21545, Egypt
e-mail: abeer.gad@alexu.edu.eg

M. Z. M. Salem (✉)
Forestry and Wood Technology Department, Faculty
of Agriculture (El-Shatby), Alexandria University,
Alexandria 21545, Egypt
e-mail: mohamed-salem@alexu.edu.eg

Keywords Cotton leafworm · *Mentha* · *Artemisia* ·
Chemical composition · Detoxification enzymes ·
Hemocytes

Introduction

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is among the most devastating polyphagous insect pests of many cultivated crops in subtropical and tropical zones. It attacks over 87 plant species belonging to 40 plant families of economically important crops such as cotton, maize, potatoes, vegetables, and ornamental plants (CABI, 2022; EPPO, 2022). Based on data retrieved from the Arthropod Pesticide Resistance Database (<http://www.pesticideresistance.org>, accessed on 18 May 2021), it has been shown that *S. littoralis* ranks in the top 30 species globally in terms of resistance to pesticides. The excessive and continuous use of conventional insecticides to control *S. littoralis* has led to the development of resistance to major classes of pesticides (Fouad et al., 2022; Hilliou et al., 2021; Moustafa et al., 2023b). Thus, it is necessary to search for alternative pest management methods that are more cost-effective and sustainable than conventional insecticides (Hussein et al., 2023).

Botanical insecticides have long been boosted as alternatives to synthetic insecticides for pest control because their natural sources are biodegradable, and they do not leave toxic residues or by-products to pollute the environment (Abdelkhalek et al., 2020; Isman, 2020; Moustafa et al., 2023a; Awad et al., 2024). From among these botanical substances, *Mentha pulegium* L. is one of the *Mentha* species commonly known as pennyroyal. It is a native species of Europe, North Africa, Asia Minor, and the Near East (Chalchat et al., 2000). The flowering aerial parts of *M. pulegium* have been traditionally used as its antiseptic for the treatment of colds, sinusitis, cholera, food poisoning, bronchitis, and tuberculosis (Boukhebt et al., 2011), and as antifatulent, carminative, expectorant, diuretic, antitussive, and menstuate (Ghalamkari et al., 2012). Some pharmacological effects of *M. pulegium* essential oil (EO) such as the abortifacient effect in rat myometrium have been reported (Soares et al., 2012). *M. pulegium* has been shown to have anticholinesterase and antioxidant (Yakoubi et al., 2021), and anthelmintic (Sebai et al., 2020), as well as insecticidal effects (Ahmed et al., 2018; Domingues & Santos, 2019). The aerial components of several *Mentha* species have been utilized for an extended period as tonics, carminatives, digestives, stomachic, antispasmodics, and

anti-inflammatory drugs (Benabdallah et al., 2018). Furthermore, *Artemisia*, a genus of tiny herbs and shrubs, is a family of Asteraceae native primarily to the northern temperate parts of the world. Approximately 500 species of *Artemisia* have been examined, and it has been discovered that they possess a wide range of secondary metabolites, such as terpenoids, flavonoids, coumarins, glycosides, and polyacetylenes (Bisht et al., 2021). *A. camphorata* (synonym of *Artemisia alba* subsp. *alba*) EO showed potential insecticidal activity against *Sitophilus oryzae* L. (Tawfeek et al., 2021).

Chitin synthesis inhibitors are insect growth regulators that affect insect chitin synthesis (Abbas & Hafez, 2021). Novaluron is a benzoylphenylurea insecticide that interferes with developmental processes in immature insects (Chang et al., 2022). Moreover, it has low toxicity to mammals and several important natural enemies (Xu et al., 2017).

The detoxifying enzymes are involved in the detoxification of various plant xenobiotics in insects by removing harmful substances through different detoxification mechanisms (Gong et al., 2021). Induction of detoxification enzymes, such as carboxylesterases (CarEs), mixed function oxidase (MFO), and glutathione S-transferase (GST), is one of the defense physiological responses that play important roles in plant extract metabolism, through increasing their solubility and promoting rapid excretion or facilitating degradation (Hilliou et al., 2021). Additionally, hemocytes play a crucial role in an insect's immune response, safeguarding the insect against pathogens and foreign agents, such as plant extracts and pesticides. Upon exposure to pesticides or toxic substances, hemocytes can identify the chemicals as foreign entities and initiate immune responses to neutralize them. Furthermore, certain hemocyte types possess enzymes that aid in detoxifying harmful substances. In addition to many detoxification enzymes in insect hemolymph that may metabolize and decompose hazardous substances, including pesticides. These enzymes can diminish pesticides' toxicity, enhancing the insect's survival (Gad, 2020). Moreover, insects can enhance their resistance to certain pesticides over time via the adaptive responses of their immune system, known as "immune memory," enabling them to mount a more effective reaction upon reapplication of the pesticide (Belial and Gad, 2023).

Therefore, this study aimed to characterize the chemical compositions and evaluate the toxicity of *M. pulegium* EO and *A. camphorata* ME compared with the insecticide novaluron against the fourth-instar larvae of *S. littoralis*. Their effects on MFO, CarEs, GST enzyme activities, hemocyte counts, and hemocyte surface areas of *S. littoralis* were also estimated.

Materials and methods

Chemicals

The chemicals used for enzyme activity analysis included 4-nitroanisole, *p*-nitrophenol, fast blue B salt, α - and β -naphthol, glutathione (GSH), sodium dodecyl sulfate (SDS) and 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from Sigma-Aldrich (Germany). Nicotinamide adenine dinucleotide phosphate (NADPH) was acquired from Sorachim (Switzerland), and α - and β -naphthyl acetate was purchased from MP Biomedicals (India). Novaluron (Equo® 10% EC; Isagro Co., Italy) was a positive control.

Insect rearing

The strain of *Spodoptera littoralis* was originally collected from the infested cotton fields in Abees district, Alexandria, Egypt, and reared for several generations without exposing it to insecticides. The larvae were fed on fresh leaves from the *Ricinus communis* L., which belongs to the Euphorbiaceae family, while moths were fed on a 15% sugar solution. The culture was kept in an incubator at a temperature of 25 ± 2 °C, relative humidity of $65 \pm 5\%$ with 8:16 h dark: light intervals, as described previously (Hussein & Eldesouky, 2019).

Mentha pulegium aerial parts and essential oil extraction

Mentha pulegium, family Labiatae with the local name Felaiyya, habitat in Egypt, was collected during the summer of 2022 from growing wild plants at the canal banks and moist ground in Abees district, Alexandria, Egypt. The aerial parts (stem and leaves) were cut from the plants and used for the extraction of the essential oil (EO). Aerial parts of about 100 g were cut into small pieces using scissors and put into a 2-L

flask containing 1500 mL of distilled water (DW) and extracted by the hydrodistillation method using a Clevenger-type apparatus for 3 h (Chakravarty et al., 2021). After obtaining the EO, they were dried over anhydrous sodium sulfate and kept dry in sealed Eppendorf tubes stored at 4 °C until chemical and bioassay analyses. The yield of EO was 2.5 mL/100 g plant fresh aerial parts.

GC–MS analysis of the essential oil

The chemical compositions of the EO were performed using a Trace GC Ultra-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG–5MS (30 m $\times$ 0.25 mm $\times$ 0.25 μ m film thickness). The column oven temperature was initially held at 70 °C and then increased by 5 °C/min to 280 °C with hold 5 min then increased to 300 with 5 °C/min. The injector and MS transfer line temperatures were kept at 250 °C. Helium was used as a carrier gas at a constant 1 mL/min flow rate. The solvent delay was 2 min and diluted samples of 1 μ L were injected automatically using Autosampler AS1310 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of *m/z* 40–600 in full scan mode. The ion source was set at 200 °C. The components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 14 mass spectral databases.

Artemisia camphorata methanol extract and HPLC analysis

Artemisia camphorata aerial parts (stem and leaves) supplied by a commercial horticultural nursery, in Alexandria, Egypt (Tawfeek et al., 2021) was used for the extraction. The plant material was air-dried at room temperature for about 2 weeks and then ground to powder using a small laboratory mill. Approximately 100 g of the powdered material was extracted with 150 mL of methanol by the soaking method for 1 week at room temperature (Elbanoby et al., 2024). The extract was filtered through filter paper (Whatman no. 1) and then with a cotton plug. The methanol extract (ME) was concentrated and stored in brown vials in the refrigerator before use. The yield of the ME of *A. camphorata* was 3.04%.

HPLC (Agilent 1100, USA) was used to classify the phenolic components from the ME of *A. camphorata*. This device consists of a C18 column (125 mm, 4.60 mm, with 5 μ m film thickness), a binary LC pump, and a UV/Vis detector. Chromatograms were obtained and examined using the Agilent ChemStation. Phenolic acids were separated using a gradient mobile phase consisting of two solvents: Solvent A (MeOH) and Solvent B [Acetic acid in H₂O (1:25)]. For three minutes, the gradient program stayed at 100% B. Five minutes of 50% eluent A, two minutes of 80% eluent A, five minutes of 50% eluent A, two minutes of 80% eluent A, five minutes of 50% eluent A, five minutes of 50% eluent A, and five minutes of detection wavelength at 250 nm are the next steps. Consequently, this mobile phase ordered the phenolic components to authenticate standard components. HPLC (Agilent 1100), which consists of a C18 column (250 mm $\times$ 4.6 mm, with 5 μ m film thickness), two LC pumps, and a UV/Vis detector, was utilized to analyze the flavonoid compounds. Acetonitrile (A) and 0.2% (v/v) aqueous formic acid (B) with an isocratic elution (70:30) protocol served as the mobile phase. 360 nm was the chosen wavelength for the detection of flavonoid compounds (EL-Hefny et al., 2023; Alkharpotly et al., 2024; Eldesouky et al., 2024).

Bioassays

The toxicity of *M. pulegium* EO and *A. camphorata* ME compared to the insecticide novaluron against the fourth-instar larvae of *S. littoralis* was evaluated using a leaf-dipping technique (Moustafa et al., 2023a). Preliminary experiments were conducted to ascertain the best concentrations for testing. Afterward, six concentrations of each plant material (250, 500, 1000, 2000, 4000, and 8000 mg/L) were diluted with distilled water containing 0.1% Triton X-100 as an emulsifier. Six concentrations of the insecticide novaluron (2.5, 5, 10, 15, 20, and 30 mg/L) were prepared in distilled water. There were two controls: one with distilled water only, and the other with distilled water in addition to 0.1% Triton X-100. Castor bean leaves of the same size were dipped in each concentration for 20 s and then air-dried at room temperature. Five replicates were performed for each treatment, with twenty larvae in each replication. The experiment was kept in an incubator at a temperature

of 25 ± 2 °C, relative humidity of $65 \pm 5\%$ with 8:16 h dark: light intervals. The number of dead larvae and the mortality percentages were recorded after 24 and 48 h of treatment.

Detoxification enzyme activities assessment

Sample preparation

The fourth-instar larvae of *S. littoralis* were treated with the LC₂₅ and LC₅₀ values of *M. pulegium* EO, *A. camphorata* ME, and the insecticide novaluron. After 24 and 48 h of treatment, 100 mg of the fresh body weight of the surviving larvae were homogenized in 9 volumes (w/v) of 0.1 M phosphate buffer with a pH of 7.4 for mixed-function oxidases (MFO), a pH of 7.0 for carboxylesterase (CarE), and a pH of 6.5 for glutathione *S*-transferase (GST). The weight of an adult (mg): the volume of buffer (mL) was kept in a ratio of 1:9 (Chauhan et al., 2022). The homogenates were centrifuged at 12,000 rpm for 30 min at 4°C using Cryofuge 20–3, Heraeus Christ centrifuge. The clear supernatants were stored at –20°C to measure the protein content and enzyme activity. For each enzyme assay, five replicates per treatment were used. The protein content was determined using the Coomassie brilliant blue assay (Bradford, 1976).

Mixed-function oxidases (MFO) activity assay

The MFO activity was determined using the methodology of Hansen and Hodgson (1971), with slight modification. The overall volume of the reaction was 200 μ L, comprising 100 μ L of a 2 mM *p*-nitroanisole (*p*-NA) solution. Subsequently, 90 μ L of the supernatant was introduced into the reaction mixture and incubated at 27 °C for 2 min. Then, 10 μ L of a 9.6 mM NADPH solution was introduced into the reaction. The MFO activity was measured for 15 min using a V-530 UV/Vis spectrophotometer at a wavelength of 405 nm. The MFO activity was determined by utilizing a standard curve of *p*-nitrophenol. The specific activity was denoted as μ mol of *p*-nitrophenol produced per minute per mg protein.

Carboxylesterases (CarEs) activity assay

The CarEs activity, which encompasses both α - and β - esterases, was assessed using the method of Van

Asperen (1962) modified by Cao et al. (2008). To assess the activity of α - and β - esterases, a 50 μ L portion of the supernatant was subjected to incubation with 50 μ L of (30 mM) alpha (α) or beta (β)-naphthyl acetate at a temperature of 30 °C for 15 min. The reaction was halted by the addition of 50 μ L of a stop solution composed of a mixture of 5% sodium dodecyl sulfate and 1% fast blue B salt solution, with a volume ratio of 5:2. The measurement of color change was conducted at a wavelength of 600 nm for the hydrolysis of α -naphthyl acetate, and at a wavelength of 550 nm for the hydrolysis of β -naphthyl acetate, using the V-530 UV/Vis Spectrophotometer. The mean enzyme activity levels were determined using the standard curves of α - and β -naphthol. The specific activity was denoted as μ mol of α - and β - naphthol produced per minute per mg protein.

Glutathione S-Transferase (GST) activity assay

The GST activity was conducted by using 1-chloro-2, 4-dinitrobenzene (CDNB), and reduced glutathione (GSH) as substrates, following the methodology described by Habig et al. (1974). The experimental setup involved a reaction solution including 100 μ L of enzyme solution, 10 μ L of 30 mM CDNB, and 10 μ L of 50 mM GSH. This solution was subjected to measurement at 340 nm using a V-530 UV/Vis spectrophotometer, with the temperature maintained at 25 °C. The measurement duration was set to 3 min. The enzyme activity was determined using a molar absorptivity of 9.6 mM cm⁻¹ for CDNB. The specific activity was denoted as μ mol of CDNB conjugate produced per minute per mg protein.

Hemocytes study

For hemocyte examination, the hemolymph samples of the fourth-instar larvae of *S. littoralis* were taken after 24, 48, and 72 h of treatment with the LC₂₅ values of *M. pulegium* EO, *A. camphorata* ME, and the insecticide novaluron, in addition, the negative control. The LC₂₅ concentration (Sublethal dose) affected the treated larvae. Still, the percentage of mortality was low, which allowed the examination of the changes and deformities that occurred to the hemocytes as a result of the treatments (Khamis et al., 2016). One drop of larval hemolymph from each treatment was smeared on a glass slide. The slides

were kept to dry for 5 min and then stained with 10% Wright's stain for 10 min. Each slide was scanned under a light microscope $\times 100$ magnification. A hundred hemocytes in the microscopic field were identified. In addition, the surface area of each hemocyte type was measured by a format micrometric slide in all treatments and the control.

Statistical analysis

The LC₂₅, LC₅₀, and LC₉₀ values were calculated using the probit analysis (Finney, 1971). One-way analysis of variance (ANOVA) was used to compare the differences between the means of the treatments with Tukey's honest significant difference (HSD) test (Cohort Software Inc., 1985); $P < 0.05$ was considered significant.

Results

Chemical compounds of *M. pulegium* EO

The chemical composition of *M. pulegium* EO by GC–MS is shown in Table 1, and the mass spectral fragmentations of each compound are presented in Table S1. The GC–MS chromatograms of the identified compounds in *M. pulegium* EO are shown in Fig. 1. The abundant compounds were menthone (22.45%), 1,8-cineole or eucalyptol (19.37%), pulegone (8.67%), endo-borneol (7.90%). Furthermore, other compounds like β -caryophyllene oxide (3.87%), 5-isopropyl-6-methyl-5-hepten-3-yn-2-ol (3.64%), isomenthone (3.38%), 6-hydroxy-5-methyl-6-vinylbicyclo[3.2.0]heptan-2-one (3.26%), α -terpineol (2.81%), dimethyl anthranilate (2.11%), β -pinene (2.02%), 2-methyl-2-(3-methyl-2-oxobutyl)cyclohexanone (1.99%), *trans,trans*-5-caranol (1.78%), *p*-cymene (1.56%), piperitone (1.54%), and camphor (1.48%) were found in low percentages.

Phenolic and flavonoid compounds in *A. camphorata* methanol extract

The HPLC chromatograms of the separation and identification of phenolic and flavonoid compounds in *A. camphorata* ME are shown Fig. 2 (a and b), respectively.

Table 1 Chemical constituents of *M. pulegium* essential oil by gas chromatography-mass spectrometry

Retention time (min)	Area (%)	Compound	Match factor
4.29	0.80	Sabinene	951
4.39	2.02	β -Pinene	938
5.28	1.56	<i>p</i> -Cymene	938
5.45	19.37	1,8-Cineole (Eucalyptol)	930
5.99	0.85	γ -Terpinene	942
6.27	0.40	cis- β -Terpineol	937
7.95	0.81	laevo-Pinocarveol	938
8.07	1.48	Camphor	923
8.35	22.45	Menthone	933
8.53	3.38	Isomenthone	949
8.74	7.90	endo-Borneol	936
9.31	2.81	α -Terpineol	926
9.59	0.75	Levoverbenone	916
9.80	1.28	6,6,10-Trimethyl-3,7-undecanedione	720
10.12	1.78	<i>trans,trans</i> -5-Caranol	750
10.38	8.67	Pulegone	920
10.78	1.54	Piperitone	930
10.94	1.99	2-Methyl-2-(3-methyl-2-oxobutyl)cyclohexanone	763
11.56	3.64	5-Isopropyl-6-methyl-5-hepten-3-yn-2-ol	807
11.66	3.26	6-Hydroxy-5-methyl-6-vinylbicyclo[3.2.0]heptan-2-one	771
12.00	0.88	(3E)-3-Methyl-3-decen-2-one	745
12.94	0.62	Verbenone	811
13.16	1.06	Menthofurolactone	830
14.65	2.11	Dimethyl anthranilate	935
15.22	0.51	8-Hydroxycarvotanacetone	907
15.37	0.80	5-Methyl-2-(1-methylethylidene)cyclohexanone	832
15.87	0.97	Carvenone oxide	695
18.38	1.22	7a-Hydroxymintlactone	927
18.83	3.87	β -Caryophyllene oxide	952
20.21	0.80	. <i>tau</i> .-Cadinol	911
20.85	0.43	Isoaromadendrene epoxide	850

Phenolic compounds in *A. camphorata* ME

The abundant phenolic compounds were 4-hydroxybenzoic acid (12.33 mg/mL) followed by ferulic acid (9.05 mg/mL) and chlorogenic acid (6.36 mg/mL) (Table 2).

Flavonoid compounds in *A. camphorata* ME

The abundant flavonoid compounds were apigenin (6.71 μ g/mL), luteolin (6.65 μ g/mL), quercetin (4.40 μ g/mL), and naringin (4.01 μ g/mL) (Table 3).

Toxicity of tested compounds to *S. littoralis* larvae

The mortality percentages, LC₂₅, and LC₅₀ values of *M. pulegium* EO and *A. camphorata* ME compared to the insecticide novaluron against the fourth-instar larvae of *S. littoralis* are shown in Tables 4 and 5. The LC₂₅ values after 24 h of treatment with *M. pulegium* EO, *A. camphorata*, and novaluron were 1066.8, 1793.8, and 4.2 mg/L, while the LC₅₀ values were 4458.3, 7349.9, and 9.8 mg/L, respectively. The LC₂₅ values after 48 h of treatment were 642.2, 1042.4, and 3.0 mg/L, whereas the LC₅₀ values were 2495.3, 4024.5, and 7.7 mg/L, respectively (Table 4).

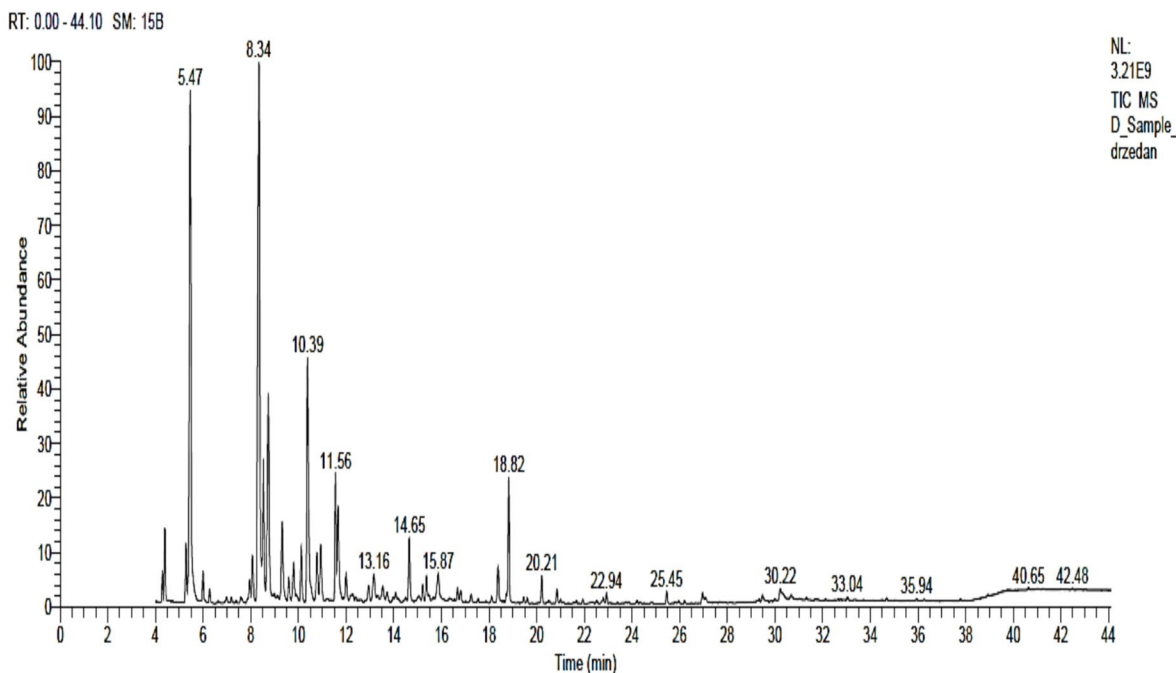


Fig. 1 Gas chromatography–mass spectrometry chromatograms of the identified compounds in *M. pulegium* essential oil

In vivo effect of tested compounds on detoxification enzyme activities of *S. littoralis*

Detoxifying enzyme activities of *M. pulegium* EO and *A. camphorata* ME in comparison with the insecticide novaluron are performed against *S. littoralis* after 24 and 48 h of treatment and presented in Figs. 3, 4, and 5. The enzyme activities in larvae treated with LC₂₅ and LC₅₀ concentrations were dose and time-dependent. Data showed that all the concentrations of *M. pulegium* EO, *A. camphorata* ME, and novaluron significantly induced the enzyme activities of MFO, α -esterase, β -esterase, and GST in *S. littoralis* after 24 h of treatment ($F=7.583$, $df=6$, $p<0.001$; $F=34.518$, $df=6$, $p<0.001$; $F=42.364$, $df=6$, $p<0.001$; $F=23.789$, $df=6$, $p<0.001$), whereas, after 48 h of treatment ($F=3.653$, $df=6$, $p<0.001$; $F=51.607$, $df=6$, $p<0.001$; $F=56.297$, $df=6$, $p<0.001$; $F=15.086$, $df=6$, $p<0.001$), as compared to the controls.

Hemocyte counts

Five basic types of hemocytes have been identified as prohemocytes (Pr), plasmatocytes (Pl),

granulocytes (Gr), oenocytes (Oe), and spherule cells (Sp) (Fig. 6). Table 6 shows changes in the mean number of different hemocytes in the hemolymph of the fourth-instar larvae of *S. littoralis* after treatment with LC₂₅ of *M. pulegium* EO, *A. camphorata* ME, and the insecticide novaluron after 24, 48, and 72 h of treatment. All tested compounds were markedly affected by different hemocyte counts. For the *M. pulegium* EO treatment, the maximum decrease in the number of prohemocytes was observed after 72 h of treatment about 5.6 cells compared to the control 12 cells. A similar decrease was seen with *A. camphorata* ME treatment, where the prohemocyte count reduced significantly to 4.4 cells at 72 h of treatment. There was a significant reduction in the number of granulocytes, especially 72 h after LC₂₅ *M. pulegium* EO and *A. camphorata* ME of treatment, about 28.4 and 20.3 cells, respectively, compared to the control (35 cells). The same trend was observed in the number of plasmatocytes; both *M. pulegium* EO and *A. camphorata* ME significantly decreased the number of (Pl) to 25.4 and 19.3 cells, respectively after 72 h of treatment while the control was 34.1 cells.

In contrast, Oenocytes significantly increased after 72 h of treatment with *M. pulegium* EO and

Fig. 2 HPLC analysis of phenolic (a) and flavonoid (b) compounds in *A. camphorata* aerial parts

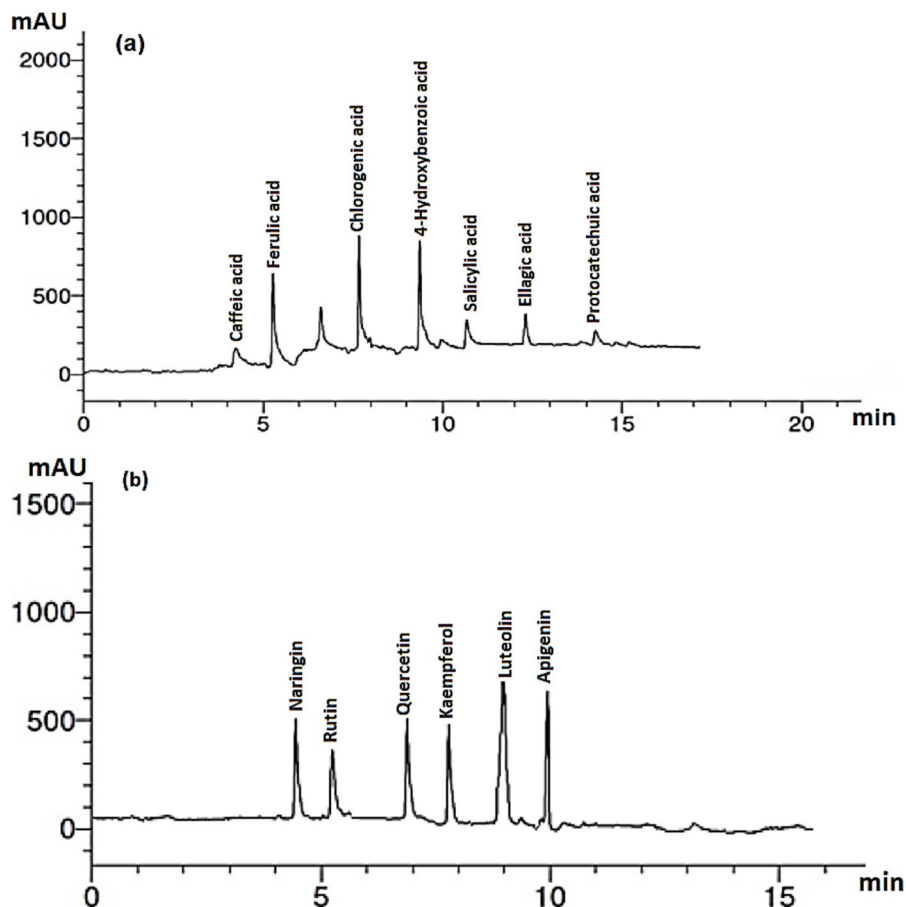


Table 2 Phenolic compounds from *A. camphorata* methanol extract by high-performance liquid chromatography analysis

RT* (min)	Phenolic compound	Concentration (mg/mL)
4.4	Caffeic acid	0.56
5.0	Ferulic acid	9.05
7.89	Chlorogenic acid	6.36
9.2	4-Hydroxybenzoic acid	12.33
11.0	Salicylic acid	0.78
12.2	Ellagic acid	1.09
14.2	Protocatechuic acid	0.09

*RT: Retention time

Table 3 Flavonoid compounds from *A. camphorata* methanol extract by high-performance liquid chromatography analysis

RT* (min)	Flavonoid compound	Concentration (μg/mL)
4.4	Naringin	4.01
5.0	Rutin	2.11
7.0	Quercetin	4.40
8.0	Kaempferol	3.89
9.0	Luteolin	6.65
10.0	Apigenin	6.71

*RT: Retention time

A. camphoratae ME to 17.5 and 12.6, respectively, while the control was 9.5 cells (Table 6).

Hemocyte surface area

Table 7 shows the variations in the surface area of each hemocyte type in treatments. The results

A. camphorata ME LC₂₅ to 27.3 and 22.1, respectively, while the control was 15 cells. Furthermore, the number of spherule cells significantly increased after 72 h of treatment with *M. pulegium* EO and

Table 4 Mortality percentages of the fourth-instar larvae of *S. littoralis* after 24 and 48 h of treatment with *M. pulegium* EO, *A. camphorata* ME compared to the insecticide novaluron

Treatment	Conc (mg/L)	Mortality percentages (mean $\pm$ SE)	
		24 h	48 h
<i>M. pulegium</i> EO	250	8 $\pm$ 1.4 ⁱ	12 $\pm$ 1.8 ^k
	500	15 $\pm$ 1.3 ^h	22 $\pm$ 1.1 ⁱ
	1000	25 $\pm$ 1.8 ^g	34 $\pm$ 1.3 ^h
	2000	36 $\pm$ 2.1 ^e	45 $\pm$ 2.6 ^f
	4000	48 $\pm$ 1.6 ^d	56 $\pm$ 2.4 ^d
	8000	60 $\pm$ 1.3 ^c	74 $\pm$ 3.2 ^b
<i>A. camphorata</i> ME	250	5 $\pm$ 0.9 ^j	8 $\pm$ 0.6 ^l
	500	10 $\pm$ 1.1 ⁱ	16 $\pm$ 1.5 ^j
	1000	18 $\pm$ 0.7 ^h	24 $\pm$ 1.3 ⁱ
	2000	26 $\pm$ 1.8 ^g	36 $\pm$ 1.1 ^g
	4000	38 $\pm$ 2.3 ^e	48 $\pm$ 2.8 ^e
	8000	52 $\pm$ 1.5 ^d	65 $\pm$ 3.0 ^c
Novaluron	2.5	15 $\pm$ 1.2 ^h	22 $\pm$ 0.7 ⁱ
	5	32 $\pm$ 0.8 ^f	38 $\pm$ 1.9 ^g
	10	48 $\pm$ 1.5 ^d	54 $\pm$ 1.3 ^d
	15	60 $\pm$ 2.1 ^c	68 $\pm$ 1.2 ^c
	20	72 $\pm$ 2.2 ^b	76 $\pm$ 1.7 ^b
	30	80 $\pm$ 1.3 ^a	85 $\pm$ 2.1 ^a

Five replicates were performed for each treatment, with twenty-fourth instar larvae in each replication. Means within a column followed by the same letter are not significantly different by Tukey's honestly significant difference test ($p < 0.05$).

revealed that all hemocyte surface areas were markedly increased after treatment with *M. pulegium* EO and *A. camphorata* ME at LC₂₅, especially after 72 h of treatment compared with the control. On the other hand, treatment with novaluron at LC₂₅ significantly decreased all hemocyte surface areas, especially after 72 h of treatment compared to the control (Table 7). The maximum values of Pr, Gr, Pl, Oe, and Sp were observed after treatment with *M. pulegium* EO LC₂₅ at 72 h to 4.9, 9.9, 13.6, 26.1, and 17.8 μm^2 , respectively while control was 3, 5.9, 6.7, 15.0 and 9.7 μm^2 , respectively. The same trend was observed in *A. camphorata* ME treatment but the increase in the hemocyte surface areas was lower than *M. pulegium* EO treatment. The highest effect was observed after 72 h of treatment, where the surface areas of Pr, Gr, Pl, Oe, and Sp were 4.6, 8.5, 10.4, 25.6, and 17.1 μm^2 , respectively, compared with the control. On the contrary, novaluron insecticide significantly decreased the surface area of all hemocytes of the fourth-instar larvae of *S. littoralis*. The highest effect was observed at 72 h of treatment, where the surface areas of Pr, Gr, Pl, Oe, and Sp were 2.1, 3.6, 4.0, 12.7, and 5.8 μm^2 , respectively, compared with the control (Table 7).

As shown in Figs. 7 and 8, treatment with *M. pulegium* EO, *A. camphorata* ME, and novaluron insecticide at LC₂₅ caused different levels of deformation on all hemocyte types, especially after 72 h of treatment.

Treatments with these tested compounds caused a reduction in the cytoplasm (Fig. 7a) destroyed cell membrane (Fig. 7b) irregular cell membrane

Table 5 Toxicity of *M. pulegium* EO and *A. camphorata* ME compared to the insecticide novaluron against the fourth-instar larvae of *S. littoralis* after 24 and 48 h of treatment

Treatment	Time (h)	LC ₂₅ (mg/L) (95% CL)	LC ₅₀ (mg/L) (95% CL)	LC ₉₀ (mg/L) (95% CL)	Slope $\pm$ SE	χ^2
<i>M. pulegium</i> EO	24	1066.8 (797.5–1359.3)	4458.3 (3403.5–6349.3)	67,492.5 (34,725.8–18339E+1)	1.086 $\pm$ 0.117	0.167
	48	642.2 (464.4–826.9)	2495.3 (2000.7–3218.9)	32,892.6 (19,416.3–70,394.3)	1.144 $\pm$ 0.113	0.864
<i>A. camphorata</i> ME	24	1793.8 (1386.1–2291.9)	7349.9 (5313.8–11,624.6)	107,180 (50,381.7–34458E+1)	1.101 $\pm$ 0.125	0.132
	48	1042.4 (789.7–1313.8)	4024.5 (3140.2–5513.6)	52,415.6 (28,847.1–12616E+1)	1.15 $\pm$ 0.117	0.370
Novaluron	24	4.2 (3.1–4.9)	9.8 (8.6–11.5)	55.2 (41.4–82.3)	1.723 $\pm$ 0.16	0.713
	48	3.0 (2.2–3.8)	7.7 (6.5–9.0)	45.7 (34.6–67.2)	1.659 $\pm$ 0.157	0.730

LC: Lethal concentration; CL: Confidence limit; SE: Standard error; χ^2 : Chi-square; df = 5; $p < 0.05$.

Fig. 3 MFO activity in the fourth-instar larvae of *S. littoralis* after 24 and 48 h of treatment with LC₂₅ and LC₅₀ concentrations of *M. pulegium* EO, *A. camphorata* ME, and novaluron in comparison with the control. The different letters between treatments indicate significant differences by Tukey's honestly significant difference test ($p < 0.05$)

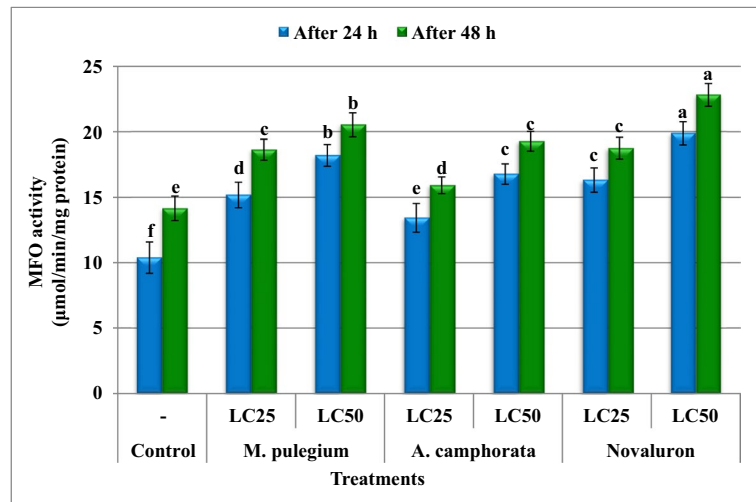
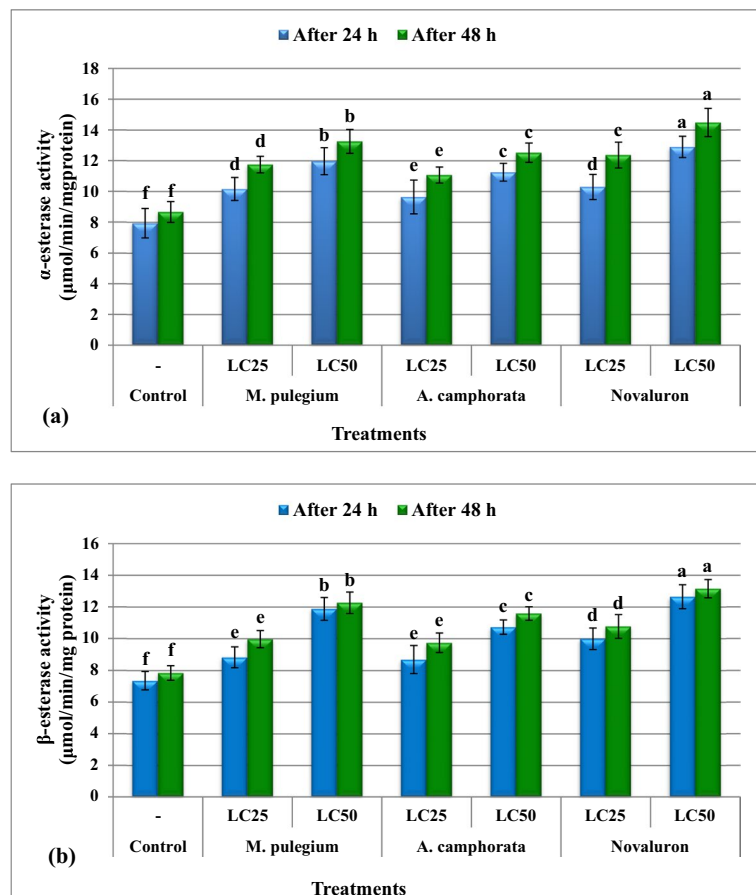


Fig. 4 α -(a) and β -(b) Esterases activities in the fourth-instar larvae of *S. littoralis* after 24 and 48 h of treatment with LC₂₅ and LC₅₀ concentrations of *M. pulegium* EO, *A. camphorata* ME, and novaluron in comparison with the control. The different letters between treatments indicate significant differences by Tukey's honestly significant difference test ($p < 0.05$)



(Fig. 7c) extruded cytoplasmic constituents (Fig. 7d). Moreover, increase in cell surface area of PI and Gr (Fig. 7e), apoptotic cell (Fig. 7f). On the other hand,

treatment of the fourth-instar larvae of *S. littoralis* after 72 h with novaluron insecticide at LC₂₅ caused a reduction in hemocyte surface areas (Fig. 8a, b, c,

Fig. 5 GST activity in the fourth-instar larvae of *S. littoralis* after 24 and 48 h of treatment with LC₂₅ and LC₅₀ concentrations of *M. pulegium* EO, *A. camphorata* ME, and novaluron compared with the control. The different letters between treatments indicate significant differences by Tukey's honestly significant difference test ($p < 0.05$)

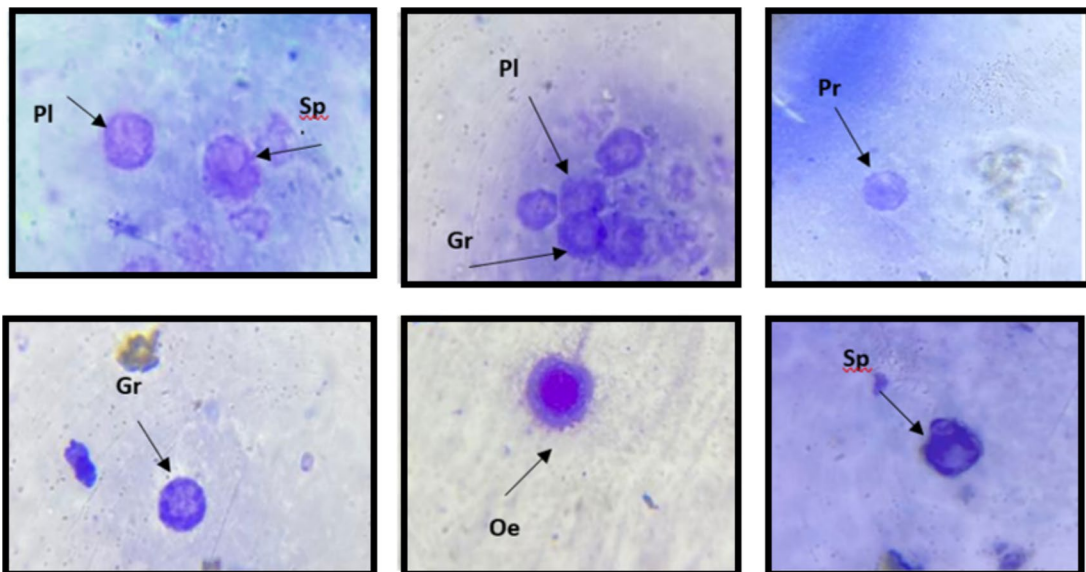
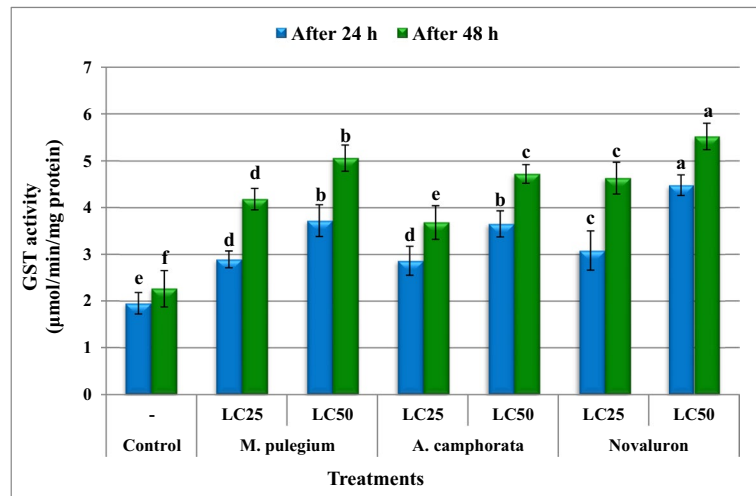


Fig. 6 Light microscopy of the fourth-instar larvae of *S. littoralis* (X100- oil, bar 5 μ m) Normal hemocytes. Pr: Prohemocytes, Gr: Granulocytes, PI: Plasmatocytes, Oe: Oenocytoides, Sp: Spherulocytes

and d), a reduction in the cytoplasm (Fig. 7e) vacuolization of granulocytes (Fig. 8f) forcing the nucleus through the cell membrane of plasmatocytes (Fig. 8g) and dead cells (Fig. 8h).

Discussion

Essential oils (EOs) and plant extracts are among the most promising botanicals to be used as alternatives

for insect control. The studies on the insecticidal activity of EOs and plant extracts have increased because these substances are nontoxic to mammals and biodegradable in the environment (Hussein et al., 2023; Awad et al., 2024; Ibrahim & Sammour, 2024). Common management practices focus on applying EOs and plant extract-based biopesticides without fully comprehending their mode of action (Álvarez-Martínez et al., 2021). Recent research focusing on pest management using EOs and plant extracts

Table 6 Effect of LC₂₅ of *M. pulegium* EO and *A. camphorata* ME compared with the insecticide novaluron on the number of different hemocytes in the fourth-instar larvae of *S. littoralis* after 24, 48 and 72 h of treatment

Treatment	Time (h)	Hemocyte number (cell/mm ³)					Abnormal cells	Apoptotic cells
		Pr	Gr	Pl	Oe	Sp		
<i>M. pulegium</i> EO (1066.8 mg/L)	24	11.6 ± 1.3 ^b	33.7 ± 1.3 ^b	29.2 ± 0.7 ^b	20.8 ± 1.1 ^c	13.5 ± 0.7 ^b	–	–
	48	7.3 ± 1.1 ^d	31.1 ± 0.6 ^b	27.0 ± 0.9 ^b	23.5 ± 1.5 ^b	15.3 ± 0.8 ^a	10	8
	72	5.6 ± 1.5 ^e	28.4 ± 0.8 ^c	25.4 ± 1.2 ^c	27.3 ± 1.1 ^a	17.5 ± 1.0 ^a	52	11
<i>A. camphorata</i> ME (1793.8 mg/L)	24	9.3 ± 0.1 ^c	26.5 ± 1.1 ^c	23.6 ± 1.2 ^c	17.7 ± 1.4 ^d	9.9 ± 0.6 ^d	–	–
	48	5.1 ± 0.2 ^e	22.5 ± 1.5 ^d	20.2 ± 1.6 ^d	19.0 ± 1.5 ^c	11.4 ± 0.8 ^c	16	10
	72	4.4 ± 0.7 ^e	20.3 ± 1.5 ^d	19.3 ± 1.2 ^d	22.1 ± 1.6 ^b	12.6 ± 0.2 ^b	67	19
Novaluron (4.2 mg/L)	24	4.6 ± 0.1 ^e	18.4 ± 1.4 ^e	19.0 ± 1.2 ^d	14.1 ± 1.6 ^e	8.0 ± 0.4 ^e	20	14
	48	3.5 ± 0.1 ^f	16.5 ± 1.2 ^e	18.1 ± 1.4 ^d	12.3 ± 1.5 ^e	8.7 ± 0.5 ^e	45	37
	72	2.9 ± 0.1 ^f	13.2 ± 0.5 ^f	15.7 ± 0.8 ^f	11.8 ± 1.1 ^f	6.2 ± 0.8 ^f	53	45
Control	–	12.0 ± 1.3 ^a	35.0 ± 1.5 ^a	34.1 ± 1.5 ^a	15.0 ± 0.2 ^d	9.5 ± 0.1 ^d	–	–

Pr: Prohemocytes, Gr: Granulocytes, Pl: Plasmacytes, Oe: Oenocytoides, Sp: Spherulocytes.

Each value represents the mean ± SE. Means in the same column followed by the same letters are not significant.

Table 7 Effect of LC₂₅ of *M. pulegium* EO and *A. camphorata* ME compared with the insecticide novaluron on the surface area of different hemocytes in the fourth-instar larvae of *S. littoralis* after 24, 48 and 72 h of treatment

Treatment	Time (h)	Hemocyte surface area (μm ²)				
		Pr	Gr	Pl	Oe	Sp
<i>M. pulegium</i> EO (1066.8 mg/L)	24	2.7 ± 0.5 ^{bc}	8.9 ± 0.5 ^{ab}	12.2 ± 0.9 ^a	22.7 ± 1.2 ^a	13.3 ± 0.3 ^c
	48	3.5 ± 0.4 ^b	9.5 ± 0.6 ^a	12.8 ± 0.9 ^a	24.5 ± 1.7 ^a	15.8 ± 0.5 ^b
	72	4.9 ± 0.7 ^a	9.9 ± 0.4 ^a	13.6 ± 1.2 ^a	26.1 ± 1.5 ^a	17.8 ± 1.2 ^a
<i>A. camphorata</i> ME (1793.8 mg/L)	24	2.5 ± 0.1 ^c	6.2 ± 1.6 ^{cd}	8.6 ± 1.6 ^c	23.0 ± 1.4 ^b	14.5 ± 0.6 ^b
	48	3.2 ± 0.2 ^b	7.3 ± 1.6 ^c	9.0 ± 1.6 ^{bc}	24.0 ± 1.7 ^{ab}	15.4 ± 0.8 ^b
	72	4.6 ± 0.6 ^a	8.5 ± 1.5 ^b	10.4 ± 1.2 ^b	25.6 ± 1.6 ^a	17.1 ± 0.9 ^a
Novaluron (4.2 mg/L)	24	2.6 ± 0.3 ^c	4.5 ± 0.2 ^d	5.3 ± 1.4 ^d	14.2 ± 0.5 ^c	8.2 ± 0.6 ^d
	48	2.4 ± 0.3 ^c	4.5 ± 0.2 ^d	4.6 ± 1.4 ^d	13.5 ± 0.7 ^c	7.0 ± 0.4 ^d
	72	2.1 ± 0.2 ^c	3.6 ± 0.5 ^d	4.0 ± 0.8 ^d	12.7 ± 0.5 ^d	5.8 ± 0.8 ^e
Control	–	3.0 ± 1.3 ^b	5.9 ± 0.8 ^d	6.7 ± 1.5 ^c	15.0 ± 0.6 ^b	9.7 ± 0.6 ^d

Pr: Prohemocytes, Gr: Granulocytes, Pl: Plasmacytes, Oe: Oenocytoides, Sp: Spherulocytes.

Each value represents the mean ± SE. Means in the same column followed by the same letters are not significant.

revealed several mechanisms involved in the insecticidal effects of EOs on targeted organisms (Ni et al., 2021). Accordingly, it is crucial to identify the active compounds of *M. pulegium* EO and *A. camphorata* ME to study their mode of action and thus develop effective biopesticide products.

The present study found that menthone, 1,8-cineole or eucalyptol, pulegone, and endo-borneol had the highest percentage of compounds in the essential oil (EO) of *M. pulegium*, with respective values of 22.45, 19.37, 8.67, and 7.90%. These chemical compounds were identified in the EOs with different percentages from the plants growing worldwide.

The difference in chemical constituents was investigated from the EOs of *M. pulegium* depending on the regions of cultivation and from other countries. For example, the abundant compounds from the Uruguayan plants were pulegone (73.4%), and isomenthone (12.9%) (Lorenzo et al., 2002), while from the Tunisian plants were pulegone (41.8%), and isomenthone (11.3%) (Mkaddem et al., 2007). The principal compounds found in the EO of wild-growing *M. pulegium* from the Yugoslavian plants were menthone (30.9%), and pulegone (14.1%) (Chalchat et al., 2000). *M. pulegium* EO contains pulegone (73.44%), piperitenone (5.49%), decane

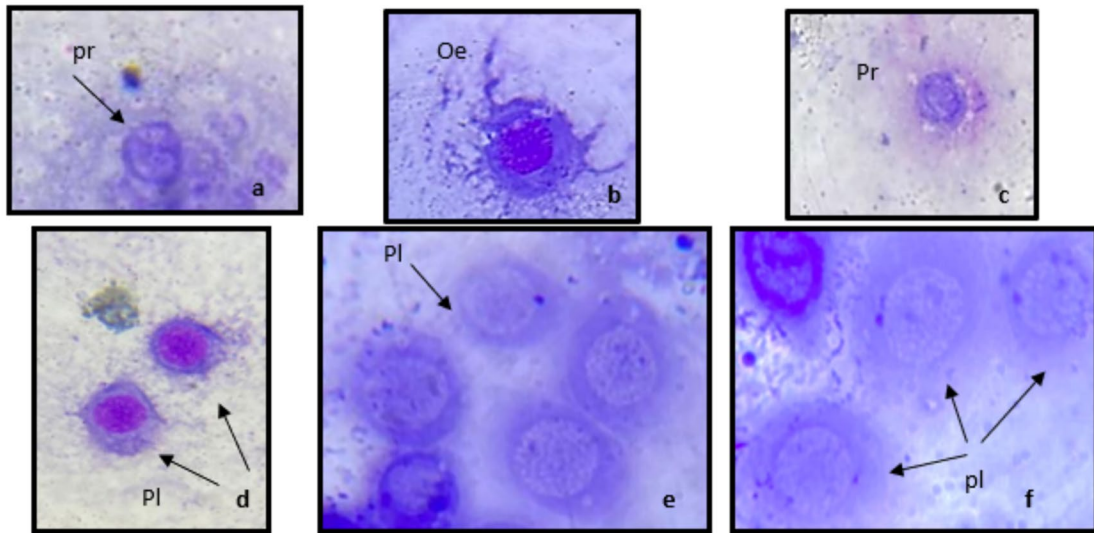


Fig. 7 The effect of LC₂₅ concentration of *M. pulegium* EO and *A. camphorata* ME on the morphology of different hemocytes of the fourth-instar larvae of *S. littoralis* after 72 h of treatment. (a) reducing in cytoplasm (b) destroyed cell mem-

brane (c) irregular cell membrane (d) extruded cytoplasmic constituents (e) increase in cell surface area in Pl and Gr (f) apoptotic cell. (X100- oil, bar 5 μ m). Gr: Granulocytes, Pl: Plasmatocytes, Oe: Oenocytoides, Sp: Spherulocytes

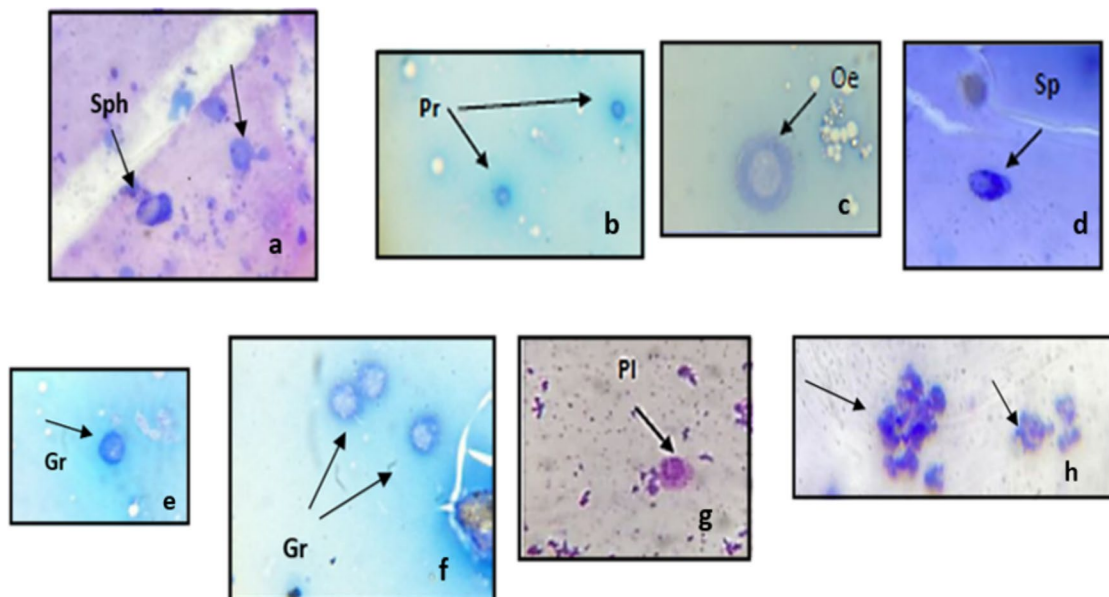


Fig. 8 The effect of LC₂₅ concentration of the insecticide novaluron on the morphology of different hemocytes of the fourth-instar larvae of *S. littoralis* after 72 h of treatment. (a, b, c, d) reducing in hemocyte surface areas, (e) reducing in

the cytoplasm (f) vacuolization of granulocytes (g) forcing the nucleus through the cell membrane of plasmacytes (h) dead cells (X100- oil, bar 5 μ m). Gr: Granulocytes, Pl: Plasmatocytes, Oe: Oenocytoides, Sp: Spherulocytes

(4.99%), and limonene (3.07%) (Ebadollahi et al., 2017).

The EO from aerial parts of Moroccan *M. pulegium* showed the presence of pulegone (57.8–62.8%), and menthone (9.5–15.0%) were found to be the main constituents (Ahmed et al., 2018). The main EO compound in plants grown in Bulgaria was pulegone (42.9–45.4%) (Stoyanova et al., 2005). The major component of the EO from *M. pulegium* (North-East Algeria) was pulegone (38.81%), with other components including menthone (19.24%) (Boukhebt et al., 2011). Menthone, pulegone, and neo-menthol were the main constituents in the EO from the plant grown in Portugal (Teixeira et al., 2012). Algerian *M. pulegium* leaves revealed that EO contains pulegone (70.66%) and neo-menthol (11.21%), as the major compounds (Abdelli et al., 2016).

Some phenolic and flavonoid compounds, including caffeic acid, ferulic acid, chlorogenic acid, 4-hydroxybenzoic acid, salicylic acid, ellagic acid, protocatechuic acid, naringin, rutin, quercetin, kaempferol, luteolin, and apigenin, were identified from ME from *A. camphorata* (synonym of *Artemisia alba* Turra) (Trendafilova et al., 2018).

Many researchers have previously studied the chemical compositions of plant extracts of the genus *Artemisia* (Lopes-Lutz et al., 2008; Pandey & Singh, 2017). Chlorogenic acid, rutin, luteolin, and apigenin were reported as main compounds in the ethanolic extract of *A. alba* from Serbia (Đorđević et al., 2013). Chlorogenic acid, 3,5- and 4,5-dicaffeoylquinic acids, scopoletin, umbeliferone, luteolin, nepetin, quercetin 3-methyl ether, axillarin, apigenin, hispidulin, diosmetin, chrysoeriol, desmethoxycentaureidin, jaceosidin, quercetin 3,3'-dimethyl ether, centaureidin, and rutin were isolated from the aerial parts (leaves and flowers) of *A. alba* from Bulgaria (Trendafilova et al., 2018).

Flavonoids (rutin, myricetin, luteolin, quercetin, and apigenin) and gallic, *p*-coumaric, vanillic, and ferulic acids were extracted from the plant's aerial portions using ethyl acetate (Jakovljević et al., 2019). The methanol extract of the same plant parts contained the following compounds: ferulic acid, quercetin, 3,5-dihydroxybenzoic acid, rutin, syringin, protocatechuic acid, vanillic acid, fisetin, trans-cinnamic, trans-coumaric, chlorogenic acid, and quercetin-3-O-glucopyranoside (Jakovljević et al., 2020). A probable reason for the cytotoxic activity of the acetone extract

from *A. alba* is the higher levels of trans-cinnamic acid, chlorogenic acid, and quercetin-3-O-glucopyranoside that were validated by HPLC–PDA analysis (Jakovljević et al., 2022). Rutin, apigenin glucoside, quercetin, luteolin, kaempferol, and apigenin were among the flavonoid components discovered in two samples from Serbia. Phenolic acids such as chlorogenic acid, *p*-coumaric acid, cynarine, and rosmarinic acid were also observed (Ickovski et al., 2020). The plant from the northeast of Italy contains several polyphenolic compounds like chlorogenic acid, rutin, and kaempferol (Peron et al., 2017).

In the present study, novaluron was the most toxic than *M. pulegium* EO and *A. camphorata* ME against the fourth-instar larvae of *S. littoralis* after 24 and 48 h of treatment. The insecticidal effect of *M. pulegium* EOs was mainly due to active compounds including menthone, 1,8-cineole or eucalyptol, and pulegone. The biological activity of the EO typically arises from its intricate blend of volatiles, which may exhibit synergistic interactions, where the EO's activity surpasses the cumulative effects of its volatiles; antagonistic interactions, where certain compounds detrimentally affect the efficacy of bioactive constituents; and additive interactions, where the EO's biological activity is simply the aggregate of its components' activities. Synergistic interactions among substances frequently arise from an augmented permeability of the plasma membrane to the bioactive EO compound or an improved binding to transmembrane proteins, however, the precise mechanism of action is sometimes challenging to ascertain (Bassolé & Juliani, 2012). Research on the interactions of several distinctive EO compounds against *Spodoptera littoralis* larvae revealed that most synergistic interactions were ascribed to six compounds, including *trans*-anethole, *g*-terpinene, and *p*-cymene (Pavela, 2014). Several plant species from the *Mentha* genus have shown remarkable efficiency against stored product insects (Abdelli et al., 2016; Aimad et al., 2021), and as larvicidal and repellent agents against some mosquitoes (Conti et al., 2010; Kazempour et al., 2021; Rocha et al., 2015; Tine-Djebbar et al., 2018). Also, the contact toxicity of *M. pulegium* EO induced mortality of 100% for the second and the third instar larvae of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Boulamtat et al., 2020). *M. pulegium* and its primary monoterpenes exhibited a superior insecticidal activity compared to

the chemical pesticide against *Culex pipiens* after 24 and 48 h of treatment (Ramzi et al., 2022). Moullamri et al., (2024) also showed that *M. pulegium* EO contact toxicity against *Plodia interpunctella* (Lepidoptera: Pyralidae).

Several studies have documented the insecticidal effects of the plant extracts from *Artemisia* species against stored-product insects. The EOs of three *Artemisia* species (*A. absinthium*, *A. santonicum*, and *A. spicigera*) and their main components, 1,8-cineole, and terpinen-4-ol, can control *Sitophilus granarius* (L.) (Kordali et al., 2006). The aerial parts of *A. lavandulaefolia* and *A. sieversiana* showed contact toxicity against *Sitophilus zeamais* Motsch adults (Liu et al., 2010). Another study found that *A. arborescens* EO exhibited efficacy against the stored-grain pest *Rhyzopertha dominica* (Bouzenna & Krichen, 2013). The EO of *A. rupestris* aerial parts has an insecticidal effect against the booklice *Liposcelis bostrychophila* (Liu et al., 2013). The EO of *A. camphorata* was the most active in the screening bioassay against *Aedes aegypti* L. and presented LC₅₀ and LC₉₅ of 64.95 and 74.18 µg/mL, respectively (Seixas et al., 2023). In addition, few studies demonstrated the insecticidal activity of *Artemisia* species against the lepidopteran insects. The toxicity of *A. annua* L. crude leaf extract against *Pieris rapae* L. (Lepidoptera: Pieridae) was observed (Hasheminia et al., 2011).

Various studies have demonstrated that plant phenolic metabolites negatively affect insect feeding behavior, growth, development, and reproduction, and they may have lethal effects on specific insects. Ferulic acid, the most abundant phenolic compound from *A. camphorata* ME decreased adult emergence, delayed the developmental period and reduced the nutritional indices of *Spodoptera litura* (Fabricius) larvae (Punia et al., 2022). The various concentrations of purified phenolic compounds like chlorogenic acid affected the larval growth, survival, adult emergence, pupal weight, and different dietary indices of *S. litura* (Fab.) (Gautam et al., 2021). The lethal effect of chlorogenic acid on *Mythimna separata* (Walker) (Lepidoptera: Noctuidae) and that a sublethal concentration harmed larval growth and development (Lin et al., 2022).

The results indicated that larvae treatment with *M. pulegium* EO and *A. camphorata* ME elevated MFO, CarEs, and GST enzyme activities compared with the control group. Plant extracts enter

the tissues and organs of target insects and affect the activity of various detoxifying enzymes to cope with xenobiotic compounds. The lethal effects of secondary plant metabolites on insects are related to the induction or inhibition of detoxification enzymes (Gong et al., 2021). Shojaei et al., (2017) showed higher GST activity in *Tribolium confusum* adults treated with three different concentrations (160.0, 178.4, and 199.2 µL/L air) of *A. dracunculus* EO. *M. pulegium* EO with LC₅₀ value (7.939 µL/ mL) increased the GST activity of *T. castaneum* but decreased the general esterase (Heydarzade et al., 2019). Also, CarEs and MFO dramatically increased in *T. castaneum* larvae when exposed to *A. vulgaris* EO at 5% concentration (LC₅₀) (Gao et al., 2020). Moreover, after applying *A. annua* extract on *Glyphodes pyloalis*, the activity of detoxifying enzymes increased significantly after 48 h of treatment with LC₁₀, LC₃₀, and LC₅₀ values, which were estimated at 12.82%, 20.6%, and 27.35%, respectively (Afraze & Sendi, 2021). In contrast, higher concentrations of *A. maritima* EO (8 and 6 mg/L) in *Callosobruchus maculatus* showed significant inhibition of the GST activity (7.14 and 5.61 nmol/min/mL, respectively) (Chauhan et al., 2022).

The principal activities of insect hemocytes include coagulation, phagocytosis, encapsulation, detoxification, and the storage and distribution of nutritional substances. Knowledge of normal hemocytes of an insect is necessary to physiologists, toxicologists, and biochemists, as alterations in structure, types, and numbers of cells reflect changes in physiological and biochemical processes (Eldesouky et al., 2018; Ghoneim et al., 2015). The findings on hemocyte counts and surface areas were consistent with those made by Khamis et al., (2016), who found that hexane apricot kernel extract dramatically reduced the various hemocyte counts of the fourth-instar larvae of *S. littoralis* at LC₂₅ and LC₅₀ values after 72 h of exposure. Furthermore, the fourth-instar larvae of *S. littoralis* treated with LC₁₀ of castor and camphor oils showed that both oils reduced all types of hemocytes except plasmatocytes, which were reduced only by castor oil (Ali & Ibrahim, 2018). The hemocyte count in *Galleria mellonella* (Lepidoptera: Pyralidae) showed a statistically significant decrease across all given doses of the *A. annua* extract when compared to the control groups (Öztürk & Kaya, 2024).

Conclusions

The findings of the present study revealed that *M. pulegium* EO and *A. camphorata* ME toxicity against *S. littoralis* could be associated with its content in bioactive molecules such as phenolic and flavonoid compounds, which elevated the activities of MFO, CarEs, and GST enzymes in the treated larvae. Furthermore, tested compounds significantly decreased the different hemocyte counts of *S. littoralis* but significantly increased the hemocyte surface areas except for the insecticide novaluron the surface areas decreased. *M. pulegium* EO and *A. camphorata* ME may be recommended as an efficient source of promising phytochemicals compounds exhibiting high insecticidal activity for pest management.

Acknowledgements The authors are grateful to Dr. Mamoun S. M. Abd El-Kareem, Atomic and Molecular Physics Unit, Experimental Nuclear Physics Department, Nuclear Research Centre, Egyptian Atomic Energy Authority, Inshas, Cairo for his assistance in the GC-MS and HPLC measurements.

Author contributions Conceptualization, S.E.E. and A.A.G.; methodology, S.E.E., A.A.G. and M.Z.M.S.; software, M.Z.M.S.; validation, S.E.E., A.A.G. and M.Z.M.S.; formal analysis, S.E.E., A.A.G. and M.Z.M.S.; investigation, A.A.G.; resources, S.E.E., A.A.G. and M.Z.M.S.; data curation, S.E.E., A.A.G. and M.Z.M.S.; writing—original draft preparation, S.E.E., A.A.G. and M.Z.M.S.; writing—review and editing, S.E.E., A.A.G. and M.Z.M.S.; visualization, S.E.E., A.A.G. and M.Z.M.S. All authors have read and agreed to the published version of the manuscript.

Funding Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethical approval Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits

use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Abbas, N., & Hafez, A. M. (2021). Resistance to insect growth regulators and age-stage, two-sex life table in *Musca domestica* from different dairy facilities. *PLoS ONE*, 16(4), e0248693. <https://doi.org/10.1371/journal.pone.0248693>
- Abdelkhalek, A., Salem, M. Z. M., Kordy, A. M., Salem, A. Z. M., & Behiry, S. I. (2020). Antiviral, antifungal, and insecticidal activities of Eucalyptus bark extract: HPLC analysis of polyphenolic compounds. *Microbial Pathogenesis*, 147, 104383. <https://doi.org/10.1016/j.micpath.2020.104383>
- Abdelli, M., Moghrani, H., Aboun, A., & Maachi, R. (2016). Algerian *Mentha pulegium* L. leaves essential oil: Chemical composition, antimicrobial, insecticidal and antioxidant activities. *Industrial Crops and Products*, 94, 197–205. <https://doi.org/10.1016/j.indcrop.2016.08.042>
- Afrazee, Z., & Sendi, J. J. (2021). Immunological and oxidative responses of the lesser mulberry pyralid, *Glyphodes pyloalis* by an aqueous extract of *Artemisia annua* L. *ISJ-Invertebrate Survival Journal*, 18, 75–85. <https://doi.org/10.25431/1824-307X/isj.v18i1.75-85>
- Ahmed, A., Ayoub, K., Chaima, A. J., Hanaa, L., & Abdelaziz, C. (2018). Effect of drying methods on yield, chemical composition and bioactivities of essential oil obtained from Moroccan *Mentha pulegium* L. *Biocatalysis and Agricultural Biotechnology*, 16, 638–643. <https://doi.org/10.1016/j.bcab.2018.10.016>
- Aimad, A., Sanae, R., Anas, F., Abdelfattah, E. M., Bourhia, M., Mohammad Salamatullah, A., Alzahrani, A., Alyahya, H. K., Albadr, N. A., Abdelkrim, A., El Bar-nossi, A., & Noureddine, E. (2021). Chemical characterization and antioxidant, antimicrobial, and insecticidal properties of essential oil from *Mentha pulegium* L. *Evidence-Based Complementary and Alternative Medicine*, 2021, 1108133. <https://doi.org/10.1155/2021/1108133>
- Ali, A. M., & Ibrahim, A. M. A. (2018). Castor and camphor essential oils alter hemocyte populations and induce biochemical changes in larvae of *Spodoptera littoralis*

- (Boisduval) (Lepidoptera: Noctuidae). *Journal of Asia-Pacific Entomology*, 21(2), 631–637. <https://doi.org/10.1016/j.aspen.2018.04.005>
- Alkharpotly, A. A., Abd-Elkader, D. Y., Salem, M. Z. M., & Hassan, H. S. (2024). Growth, productivity and phytochemicals of coriander in responses to foliar application of *Acacia saligna* fruit extract as a biostimulant under field conditions. *Scientific Reports*, 14(1), 2921. <https://doi.org/10.1038/s41598-024-53378-5>
- Álvarez-Martínez, F. J., Barrajón-Catalán, E., Herranz-López, M., & Micol, V. (2021). Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action. *Phytomedicine*, 90, 153626. <https://doi.org/10.1016/j.phymed.2021.153626>
- Awad, M., Hassan, N. N., Alfuhaid, N. A., Amer, A., Salem, M. Z. M., Fónagy, A., & Moustafa, M. A. M. (2024). Insecticidal and biochemical impacts with molecular docking analysis of three essential oils against *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Crop Protection*, 180, 106659. <https://doi.org/10.1016/j.cropro.2024.106659>
- Bassolé, I. H. N., & Juliani, H. R. (2012). Essential oils in combination and their antimicrobial properties. *Molecules*, 17, 3989–4006. <https://doi.org/10.3390/molecules17043989>
- Belal, R., & Gad, A. (2023). Zinc oxide nanoparticles induce oxidative stress, genotoxicity, and apoptosis in the hemocytes of *Bombyx mori* larvae. *Scientific Reports*, 13, 3520. <https://doi.org/10.1038/s41598-023-30444-y>
- Benabdallah, A., Boumendjel, M., Aissi, O., Rahmoune, C., Boussaid, M., & Messaoud, C. (2018). Chemical composition, antioxidant activity and acetylcholinesterase inhibitory of wild *Mentha* species from northeastern Algeria. *South African Journal of Botany*, 116, 131–139.
- Bisht, D., Kumar, D., Kumar, D., Dua, K., & Chellappan, D. K. (2021). Phytochemistry and pharmacological activity of the genus *Artemisia*. *Archives of Pharmacological Research*, 44(5), 439–474. <https://doi.org/10.1007/s12272-021-01328-4>
- Boukhebt, H., Chaker, A. N., Belhadj, H., Sahli, F., Ramdhani, M., Laouer, H., & Harzallah, D. (2011). Chemical composition and antibacterial activity of *Mentha pulegium* L. and *Mentha spicata* L. essential oils. *Der Pharmacia Lettre*, 3(4), 267–275.
- Boulamtat, R., Lhaloui, S., Sabraoui, A., El-Fakhouri, K., Oubayoucef, A., Mesfioui, A., & El-Bouhssini, M. (2020). Antifeedant and larvicidal activities of *Mentha pulegium* on chickpea pod borer *Helicoverpa armigera* (Lepidoptera: Noctuidae). *International Journal of Tropical Insect Science*, 40, 151–156. <https://doi.org/10.1007/s42690-019-00064-z>
- Bouzenna, H., & Krichen, L. (2013). *Pelargonium graveolens* L'Her. and *Artemisia arborescens* L. essential oils: Chemical composition, antifungal activity against *Rhizoctonia solani* and insecticidal activity against *Rhyssopertha dominica*. *Natural Product Research*, 27(9), 841–846. <https://doi.org/10.1080/14786419.2012.711325>
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72(1–2), 248–254. <https://doi.org/10.1006/abio.1976.9999>
- CABI (2022). *Spodoptera littoralis*. In: *Invasive Species Compendium*. <https://doi.org/10.1079/cabicompendium.51070>
- Cao, C.-W., Zhang, J., Gao, X.-W., Liang, P., & Guo, H.-L. (2008). Overexpression of carboxylesterase gene associated with organophosphorous insecticide resistance in cotton aphids, *Aphis gossypii* (Glover). *Pesticide Biochemistry and Physiology*, 90(3), 175–180. <https://doi.org/10.1016/j.pestbp.2007.11.004>
- Chakravarty, I., Parmar, V. M., & Mandavgane, S. A. (2021). Current trends in essential oil (EO) production. *Biomass Conversion and Biorefinery*, 13, 15311–15334. <https://doi.org/10.1007/s13399-021-01963-3>
- Chalchat, J.-C., Gorunovic, M. S., Maksimovic, Z. A., & Petrovic, S. D. (2000). Essential oil of wild growing *Mentha pulegium* L. from Yugoslavia. *Journal of Essential Oil Research*, 12(5), 598–600. <https://doi.org/10.1080/10412905.2000.9712166>
- Chang, Y.-W., Wang, Y.-C., Yan, Y.-Q., Wu, C.-D., Xie, H.-F., Gong, W.-R., & Du, Y.-Z. (2022). Insect hormones affect the toxicity of the insecticidal growth regulator cyromazine in *Liriomyza trifolii* (Diptera: Agromyzidae). *Pesticide Biochemistry and Physiology*, 188, 105263. <https://doi.org/10.1016/j.pestbp.2022.105263>
- Chauhan, N., Kashyap, U., Dolma, S. K., & Reddy, S. G. E. (2022). Chemical composition, insecticidal, persistence and detoxification enzyme inhibition activities of essential oil of *Artemisia maritima* against the pulse beetle. *Molecules*, 27(5), 1547. <https://doi.org/10.3390/molecules27051547>
- Cohort Software Inc. (1985). Costat User's Manual. Version 3. Tucson, AZ: Cohort.
- Conti, B., Canale, A., Bertoli, A., Gozzini, F., & Pistelli, L. (2010). Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito *Aedes albopictus* (Diptera: Culicidae). *Parasitology Research*, 107(6), 1455–1461. <https://doi.org/10.1007/s00436-010-2018-4>
- Domingues, M. P., & Santos, L. (2019). Essential oil of pennyroyal (*Mentha pulegium*): Composition and applications as alternatives to pesticides—New tendencies. *Industrial Crops and Products*, 139, 111534. <https://doi.org/10.1016/j.indcrop.2019.111534>
- Đorđević, S., Stanisavljević, D., Ristić, M., Milenković, M., Veličković, D., Stojičević, S. S., & Zlatković, B. (2013). Chemical, antioxidant and antimicrobial analysis of the essential oil and extract of *Artemisia alba* Tura. *Digest Journal of Nanomaterials and Biostructures*, 8(4), 1377–1388.
- Ebadollahi, A., Davari, M., Razmjou, J., & Naseri, B. (2017). Separate and combined effects of *Mentha piperata* and *Mentha pulegium* essential oils and a pathogenic fungus *Lecanicillium muscarium* against *Aphis gossypii* (Hemiptera: Aphididae). *Journal of Economic Entomology*, 110(3), 1025–1030.
- Elbanoby, N. E., El-Settawy, A. A. A., Mohamed, A. A., & Salem, M. Z. M. (2024). Phytochemicals derived from

- Leucaena leucocephala* (Lam.) de Wit (Fabaceae) biomass and their antimicrobial and antioxidant activities: HPLC analysis of extracts. *Biomass Conversion and Biorefinery*, 14, 14593–14609. <https://doi.org/10.1007/s13399-022-03420-1>
- Eldesouky, S. E., Hassan, S. M., & Farag, D. A. (2018). Toxicity of certain IGRs and conventional insecticides against cotton leafworm and their effects on the development and haemocyte counts. *Alexandria Journal of Agricultural Sciences*, 63(2), 93–103. <https://doi.org/10.21608/alexja.2019.28571>
- Eldesouky, S. E., Tawfeek, M. E., & Salem, M. Z. M. (2024). The toxicity, repellent, and biochemical effects of four wild plant extracts against *Aphis gossypii* Glover and *Phenacoccus solenopsis* Tinsley: HPLC analysis of phenolic compounds. *Phytoparasitica*, 52(98), 1–20. <https://doi.org/10.1007/s12600-024-01212-z>
- EL-Hefny, M., Mohamed, A. A., Abdelkhalek, A., & Salem, M. Z. M. (2023). Productivity and phytochemicals of *Asclepias curassavica* in response to compost and silver nanoparticles application: HPLC analysis and antibacterial activity of extracts. *Plants*, 12(12), 2274. <https://doi.org/10.3390/plants12122274>
- EPPO (2022) *Spodoptera littoralis*. EPPO Datasheets on Pests Recommended for Regulation. <https://gd.eppo.int>
- Finney, D. J. (1971). *Probit analysis*. Cambridge University Press.
- Fouad, E. A., Ahmed, F. S., & Moustafa, M. A. (2022). Monitoring and biochemical impact of insecticides resistance on field populations of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) in Egypt. *Polish Journal of Entomology*, 91(3), 109–118. <https://doi.org/10.5604/01.3001.0015.9707>
- Gad, A. A. (2020). Toxicity effect of silver nanoparticles to the haemocytes and antioxidant activity of silkworm *Bombyx mori*. *Physiological Entomology*, 45(4), 154–160. <https://doi.org/10.1111/phen.12333>
- Gao, S., Zhang, K., Wei, L., Wei, G., Xiong, W., Lu, Y., Zhang, Y., Gao, A., & Li, B. (2020). Insecticidal activity of *Artemisia vulgaris* essential oil and transcriptome analysis of *Tribolium castaneum* in response to oil exposure. *Frontiers in Genetics*, 11, 589. <https://doi.org/10.3389/fgene.2020.00589>
- Gautam, S., Samiksha, C. S. S., Arora, S., & Sohal, S. K. (2021). Toxic effects of purified phenolic compounds from *Acacia nilotica* against common cutworm. *Toxicon*, 203, 22–29. <https://doi.org/10.1016/j.toxicon.2021.09.017>
- Ghalamkari, G., Toghyani, M., Landy, N., & Tavalaeian, E. (2012). Investigation the effects using different levels of *Mentha pulegium* L. (pennyroyal) in comparison with an antibiotic growth promoter on performance, carcass traits and immune responses in broiler chickens. *Asian Pacific Journal of Tropical Biomedicine*, 2(3, Supplement), S1396–S1399. [https://doi.org/10.1016/S2221-1691\(12\)60424-9](https://doi.org/10.1016/S2221-1691(12)60424-9)
- Ghoneim, K., Tanani, M., Hamadah, K., Basiouny, A., & Waheeb, H. (2015). Effects of Novaluron and Cyromazine, chitin synthesis inhibitors, on the larval haemogram of *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *International Journal of Advanced Research*, 3(1), 554–576.
- Gong, P., Chen, D., Wang, C., Li, M., Li, X. & Zhu X. (2021). Susceptibility of four species of aphids in wheat to seven insecticides and its relationship to detoxifying enzymes. *Frontiers in Physiology*, 11, 623612. <https://doi.org/10.3389/fphys.2020.623612>
- Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-Transferases: The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry*, 249(22), 7130–7139. [https://doi.org/10.1016/S0021-9258\(19\)42083-8](https://doi.org/10.1016/S0021-9258(19)42083-8)
- Hansen, L. G., & Hodgson, E. (1971). Biochemical characteristics of insect microsomes: N- and O-demethylation. *Biochemical Pharmacology*, 20(7), 1569–1578. [https://doi.org/10.1016/0006-2952\(71\)90285-1](https://doi.org/10.1016/0006-2952(71)90285-1)
- Hashemina, S. M., Sendi, J. J., Jahromi, K. T., & Moharrami-pour, S. (2011). The effects of *Artemisia annua* L. and *Achillea millefolium* L. crude leaf extracts on the toxicity, development, feeding efficiency and chemical activities of small cabbage *Pieris rapae* L. (Lepidoptera: Pieridae). *Pesticide Biochemistry and Physiology*, 99(3), 244–249. <https://doi.org/10.1016/j.pestbp.2010.12.009>
- Heydarzade, A., Valizadegan, O., Negahban, M., & Mehrkhou, F. (2019). Efficacy of *Mentha spicata* and *Mentha pulegium* essential oil nanoformulation on mortality and physiology of *Tribolium castaneum* (Col.: Tenebrionidae). *Journal of Crop Protection*, 8(4), 501–520. <http://jcp.modares.ac.ir/article-3-34193-en.html>
- Hilliou, F., Chertemps, T., Maibèche, M., & Le Goff, G. (2021). Resistance in the genus *Spodoptera*: Key insect detoxification genes. *Insects*, 12(6), 544. <https://doi.org/10.3390/insects12060544>
- Hussein, H. S., & Eldesouky, S. E. (2019). Insecticidal, behavioral and biological effects of chlorantraniliprole and chlorfluzuron on cotton leafworm (*Spodoptera littoralis*). *Pakistan Journal of Biological Sciences*, 22(8), 372–382. <https://doi.org/10.3923/pjbs.2019.372.382>
- Hussein, H. S., Salem, M. Z. M., Soliman, A. M., & Eldesouky, S. E. (2023). Comparative study of three plant-derived extracts as new management strategies against *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *Scientific Reports*, 13(1), 3542. <https://doi.org/10.1038/s41598-023-30588-x>
- Ibrahim, S. S., & Sammour, E. A. (2024). Evaluating the persistence and insecticidal effects of emulsifiable concentrate containing *Cananga odorata* essential oil on survival and enzymatic activity of *Spodoptera littoralis*. *Phytoparasitica*, 52, 85. <https://doi.org/10.1007/s12600-024-01199-7>
- Ickovski, J. D., Mitić, M., Stojković, M., & Stojanović, G. (2020). Comparative analysis of HPLC profiles and antioxidant activity of *Artemisia alba* Turra from two habitats in Serbia. *Chemia Naissensis*, 3(2), 89–95.
- Isman, M. B. (2020). Botanical insecticides in the twenty-first century—fulfilling their promise? *Annual Review of Entomology*, 65(1), 233–249. <https://doi.org/10.1146/annurev-ento-011019-025010>
- Jakovljević, M. R., Grujičić, D., Vukajlović, J. T., Marković, A., Milutinović, M., Stanković, M., Vuković, N., Vukić, M., & Milošević-Djordjević, O. (2020). *In vitro* study of genotoxic and cytotoxic activities of methanol extracts of *Artemisia vulgaris* L. and *Artemisia alba* Turra. *South*

- African Journal of Botany, 132, 117–126. <https://doi.org/10.1016/j.sajb.2020.04.016>
- Jakovljević, M. R., Grujičić, D., Živanović, M., Stanković, M., Ćirić, A., Djurdjević, P., Todorović, Ž., Živančević-Simonović, S., Mihaljević, O., & Milošević-Djordjević, O. (2019). Ethyl acetate extracts of two *Artemisia* species: Analyses of phenolic profile and anticancer activities against SW-480 colon cancer cells. *Natural Product Communications*, 14, 1–6. <https://doi.org/10.1177/1934578X19843011>
- Jakovljević, M. R., Stanković, M., Vuković, N., Vukić, M., Grujičić, D., & Milošević-Djordjević, O. (2022). Comparative study of the genotoxic activity of *Artemisia vulgaris* L. and *Artemisia alba* Turra extracts in vitro. *Drug and Chemical Toxicology*, 45(4), 1915–1922. <https://doi.org/10.1080/01480545.2021.2007025>
- Kazempour, S., Shayeghi, M., Abai, M. R., Vatandoost, H., & Pirmohammadi, M. (2021). Larvicidal activities of essential oils of indigenous medicinal plants, *Mentha pulegium* L., *Satureja hortensis* L., and *Thymus vulgaris* L. against malaria vector, *Anopheles stephensi*. *South African Journal of Botany*, 139, 38–41. <https://doi.org/10.1016/j.sajb.2021.01.029>
- Khamis, W. M., El-Desouky, S. E., & Gad, A. A. (2016). Toxicity and antifeedant effects of apricot kernel extract and its main components against cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae) larvae with reference to some physiological effects. *Alexandria Science Exchange Journal*, 37(October-December), 637–646. <https://doi.org/10.21608/asejaiqsae.2016.2542>
- Kordali, S., Aslan, I., Çalmaşur, O., & Cakir, A. (2006). Toxicity of essential oils isolated from three *Artemisia* species and some of their major components to granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). *Industrial Crops and Products*, 23(2), 162–170. <https://doi.org/10.1016/j.indcrop.2005.05.005>
- Lin, D.-j., Fang, Y., Li, L.-y., Zhang, L.-z., Gao, S.-j., Wang, R., & Wang, J.-d. (2022). The insecticidal effect of the botanical insecticide chlorogenic acid on *Mythimna separata* (Walker) is related to changes in MsCYP450 gene expression. *Frontiers in Plant Science*, 13, 1015095. <https://doi.org/10.3389/fpls.2022>
- Liu, X. C., Li, Y. P., Li, H. Q., Deng, Z. W., Zhou, L., Liu, Z. L., & Du, S. S. (2013). Identification of repellent and insecticidal constituents of the essential oil of *Artemisia rupestris* L. aerial parts against *Liposcelis bostrychophila* Badonnel. *Molecules*, 18(9), 10733–10746. <https://doi.org/10.3390/molecules180910733>
- Liu, Z. L., Liu, Q. R., Chu, S. S., & Jiang, G. H. (2010). Insecticidal activity and chemical composition of the essential oils of *Artemisia lavandulaefolia* and *Artemisia sieversiana* from China. *Chemistry & Biodiversity*, 7(8), 2040–2045. <https://doi.org/10.1002/cbdv.200900410>
- Lopes-Lutz, D., Alviano, D. S., Alviano, C. S., & Kolodziejczyk, P. P. (2008). Screening of chemical composition, antimicrobial and antioxidant activities of *Artemisia* essential oils. *Phytochemistry*, 69(8), 1732–1738. <https://doi.org/10.1016/j.phytochem.2008.02.014>
- Lorenzo, D., Paz, D., Dellacassa, E., Davies, P., Vila, R., & Cañigueral, S. (2002). Essential oils of *Mentha pulegium* and *Mentha rotundifolia* from Uruguay. *Brazilian Archives of Biology and Technology*, 45. <https://doi.org/10.1590/S1516-89132002000600016>
- Mkaddem, M., Boussaid, M., & Fadhel, N. B. (2007). Variability of volatiles in Tunisian *Mentha pulegium* L. (Lamiaceae). *Journal of Essential Oil Research*, 19(3), 211–214. <https://doi.org/10.1080/10412905.2007.9699263>
- Moullamri, M., Rharrabe, K., Annaz, H., Laglaoui, A., Alibrando, F., Cacciola, F., Mondello, L., Bouayad, N., Zantari, S., & Bakkali, M. (2024). *Salvia officinalis*, *Lavandula angustifolia*, and *Mentha pulegium* essential oils: Insecticidal activities and feeding deterrence against *Plochia interpunctella* (Lepidoptera: Pyralidae). *Journal of Essential Oil Bearing Plants*, 27(1), 16–33. <https://doi.org/10.1080/0972060X.2023.2301699>
- Moustafa, M. A., Hassan, N. N., Alfuhaid, N. A., Amer, A., & Awad, M. (2023a). Insights into the toxicity, biochemical activity, and molecular docking of *Cymbopogon citratus* essential oils and citral on *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, 116(4), 1185–1195. <https://doi.org/10.1093/jee/toad093>
- Moustafa, M. A., Moteleb, R. I., Ghoneim, Y. F., Hafez, S. S., Ali, R. E., Eweis, E. E., & Hassan, N. N. (2023b). Monitoring resistance and biochemical studies of three Egyptian field strains of *Spodoptera littoralis* (Lepidoptera: Noctuidae) to six insecticides. *Toxics*, 11(3), 211. <https://doi.org/10.3390/toxics11030211>
- Ni, Z.-J., Wang, X., Shen, Y., Thakur, K., Han, J., Zhang, J.-G., Hu, F. & Wei, Z.-J. (2021). Recent updates on the chemistry, bioactivities, mode of action, and industrial applications of plant essential oils. *Trends in Food Science and Technology*, 110, 78–89. <https://doi.org/10.1016/j.tifs.2021.01.070>
- Öztürk, R., & Kaya, S. (2024). Influence of *Artemisia annua* (Asteraceae) leaf extract on immunity in *Galleria mellonella* (Lepidoptera: Pyralidae). *Biologia*, 79, 1827–1840. <https://doi.org/10.1007/s11756-024-01677-7>
- Pandey, A. K., & Singh, P. (2017). The Genus *Artemisia*: A 2012–2017 literature review on chemical composition, antimicrobial, insecticidal and antioxidant activities of essential oils. *Medicines*, 4(3), 68. <https://doi.org/10.3390/medicines4030068>
- Pavela, R. (2014). Acute, synergistic and antagonistic effects of some aromatic compounds on the *Spodoptera littoralis* Bois. (Lep., Noctuidae) larvae. *Industrial Crops and Products*, 60, 247–258. <https://doi.org/10.1016/j.indcrop.2014.06.030>
- Peron, G., Baldan, V., Sut, S., Faggian, M., Roccabruna, L., Zanini, D., Manzini, P., Maggi, F., & Dall'Acqua, S. (2017). Phytochemical investigations on *Artemisia alba* Turra growing in the North-East of Italy. *Natural Product Research*, 31(16), 1861–1868. <https://doi.org/10.1080/14786419.2016.1263845>
- Punia, A., Chauhan, N., Singh, R., Kaur, S. & Sohal, S. (2022). Growth disruptive effects of ferulic acid against *Spodoptera litura* (Fabricius) and its parasitoid *Bracon hebetor* (Say). *Allelopathy Journal*, 55, 79–92. <https://doi.org/10.26651/allelo.j/2022-55-1-1372>
- Ramzi, A., El Ouali Lalami, A., Ez Zoubi, Y., Assouguem, A., Almeer, R., Najda, A., Ullah, R., Ercisli, S., & Farah, A. (2022). Insecticidal effect of wild-grown *Mentha pulegium* and *Rosmarinus officinalis* essential oils and their

- main monoterpenes against *Culex pipiens* (Diptera: Culicidae). *Plants (Basel)*, 11(9), 1193. <https://doi.org/10.3390/plants11091193>
- Rocha, D., Novo, M., Matos, O., Figueiredo, A. C., Delgado, M., Cabral, M. D., Liberato, M., & Moiteiro, C. (2015). Potential of *Mentha pulegium* for mosquito control. *Revista de Ciências Agrárias*, 38(2), 155–165. <https://doi.org/10.19084/rca.16908>
- Sebai, E., Serairi, R., Saratsi, K., Abidi, A., Sendi, N., Darghouth, M. A., Wilson, M. S., Sotiraki, S., & Akkari, H. (2020). Hydro-ethanolic extract of *Mentha pulegium* exhibit anthelmintic and antioxidant proprieties in vitro and in vivo. *Acta Parasitologica*, 65(2), 375–387. <https://doi.org/10.2478/s11686-020-00169-3>
- Seixas, P. T. L., Demuner, A. J., Barbosa, L. C. A., Cerceau, C. I., Blank, D. E., Santos, M. H. D., Farias, E., & d. S., & Picanço, M. C. (2023). Chemical composition and larvicidal activity of essential oils of three *Artemisia* species. *Journal of Applied Entomology*, 147(2), 116–125. <https://doi.org/10.1111/jen.13094>
- Shojaei, A., Talebi, K., Sharifian, I., & Ahsaei, S. M. (2017). Evaluation of detoxifying enzymes of *Tribolium castaneum* and *Tribolium confusum* (Col.: Tenebrionidae) exposed to essential oil of *Artemisia dracunculus* L. *Biharean Biologist*, 11(1), 5–9.
- Soares, P. M. G., de Freitas Pires, A., de Souza, E. P., Assreuy, A. M. S., & Criddle, D. N. (2012). Relaxant effects of the essential oil of *Mentha pulegium* L. in rat isolated trachea and urinary bladder. *Journal of Pharmacy and Pharmacology*, 64(12), 1777–1784. <https://doi.org/10.1111/j.2042-7158.2012.01558.x>
- Stoyanova, A., Georgiev, E., Kula, J., & Majda, T. (2005). Chemical composition of the essential oil of *Mentha pulegium* L. from Bulgaria. *Journal of Essential Oil Research*, 17(5), 475–476. <https://doi.org/10.1080/10412905.2005.9698968>
- Tawfeek, M. E., Ali, H. M., Akrami, M., & Salem, M. Z. M. (2021). Potential insecticidal activity of four essential oils against the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). *BioResources*, 16(4), 7767–7783. <https://doi.org/10.15376/biores.16.4.7767-7783>
- Teixeira, B., Marques, A., Ramos, C., Batista, I., Serrano, C., Matos, O., Neng, N. R., Nogueira, J. M. F., Saraiva, J. A., & Nunes, M. L. (2012). European pennyroyal (*Mentha pulegium*) from Portugal: Chemical composition of essential oil and antioxidant and antimicrobial properties of extracts and essential oil. *Industrial Crops and Products*, 36(1), 81–87. <https://doi.org/10.1016/j.indcrop.2011.08.011>
- Tine-Djebbar, F., Guenez, R., spsampsps Soltani, N. (2018). Chemical Composition and Insecticide Properties of the Essential Oils from *Mentha pulegium* against *Aedes caspius* (Diptera: Culicidae). Recent Advances in Environmental Science from the Euro-Mediterranean and Surrounding Regions, Cham.
- Trendafilova, A., Todorova, M., Genova, V., Peter, S., Wolf-ram, E., Danova, K., & Evstatieva, L. (2018). Phenolic Profile of *Artemisia alba* Turra. *Chemistry & Biodiversity*, 15(7), e1800109. <https://doi.org/10.1002/cbdv.201800109>
- van Asperen, K. (1962). A study of housefly esterases by means of a sensitive colorimetric method. *Journal of Insect Physiology*, 8(4), 401–416. [https://doi.org/10.1016/0022-1910\(62\)90074-4](https://doi.org/10.1016/0022-1910(62)90074-4)
- Xu, Q.-Y., Meng, Q.-W., Shi, J.-F., Deng, P., Guo, W.-C., & Li, G.-Q. (2017). Novaluron ingestion causes larval lethality and inhibits chitin content in *Leptinotarsa decemlineata* fourth-instar larvae. *Pesticide Biochemistry and Physiology*, 143, 173–180. <https://doi.org/10.1016/j.pestbp.2017.07.010>
- Yakoubi, R., Megateli, S., Hadj Sadok, T., Bensouici, C., & Bağci, E. (2021). A synergistic interactions of Algerian essential oils of *Laurus nobilis* L., *Lavandula stoechas* L. and *Mentha pulegium* L. on anticholinesterase and antioxidant activities. *Biocatalysis and Agricultural Biotechnology*, 31, 101891. <https://doi.org/10.1016/j.bcab.2020.101891>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.