

Chemical Profiling and Bioactivities of *Pelargonium graveolens* Essential Oil against *Spodoptera littoralis* (Lepidoptera: Noctuidae)¹

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Abstract Scientists are searching for safer substitutes for several hazardous chemicals because of environmental concerns and the development of arthropod resistance to synthetic pesticides. Essential oils pose fewer risks to the environment and human health and are potential alternatives for crop protection; therefore, this study assessed the toxicity and biochemical activity of essential oils from geranium (*Pelargonium graveolens* L'Hérit) against larvae of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). In addition, molecular docking was used to determine the binding pattern of the detoxifying enzymes glutathione S-transferase (GST), cytochrome P450, and α -esterase with citronellol, the main ingredient (20.91%) of the *P. graveolens* extract. Concentration–mortality response assays determined lethal concentrations (LCs) of the essential oil as LC₁₅ = 608.52 mg/L and LC₅₀ = 1,820.77 mg/L against second-instar larvae. Additional sublethal studies showed that, compared with the control, exposure of the second-instar larvae with LC₁₅ or LC₅₀ levels of *P. graveolens* essential oil significantly increased the duration of the larval and pupal stages. Considerable biochemical alterations were found in relation to *P. graveolens* essential oil biochemical impact on *S. littoralis* larvae. According to the activity of detoxifying enzymes and the results of the molecular docking study, the citronellol molecule of *P. graveolens* essential oil exhibited a binding affinity of GST > cytochrome P450 > α -esterase, with an energy score of –5.346, –5.295, and –5.4278 kcal/mol, respectively. The findings confirm the potential of using essential oils in sustainable pest management.

Key Words geranium essential oil, detoxification enzymes, molecular docking

Essential oils derived from aromatic and therapeutic plants are rich in biomolecules that have the potential to be used as insecticides and are known to pose little danger to the environment or humans (Regnault-Roger et al. 2012). These chemicals possess distinct chemical characteristics, modes of action, and impacts on insects. They can be classified as insect repellents, toxins, feeding deterrents or inhibitors, and oviposition deterrents (Kendra et al. 2014, Mostafiz et al. 2019). Most essential oils are composed of monoterpenes and terpenoids that are considered ecologically friendly pesticides that can be used against a variety of insect species (Isman 2000). As a result, many pest management programs use

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essential oils as alternatives to synthetic pesticides (Hikal et al. 2023). Although they are often used to manage pests on a variety of crops because of their fast action and efficacy, synthetic insecticides have negative consequences, including resistance development in the targeted pest populations, death to nontarget organisms, environmental residues and impacts, and pest population resurgence (Dayan et al. 2009).

Pelargonium graveolens L'Herit (Geraniaceae), the rose-scented geranium, is a fragrant medicinal plant indigenous to South Africa (Rana 2002) and is commonly grown in Saudi Arabia. It is regarded as an important source of biologically active chemicals, including promising plant-derived insecticides (Machalova et al. 2015). Studies have shown that its essential oil possesses pharmacological, insecticidal, antioxidant, and antibacterial properties (Saraswathi et al. 2011). Use of crude geranium essential oil can also resist infestations of *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.) (Abouelatta et al. 2020). The essential oil is reported as an ovipositional deterrent to *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) (Abd El-Aziz 1998) and a repellent to *Leptotrombidium* sp. (Acari: Trombiculidae) (Eamsobhana et al. 2009), *Ixodes ricinus* L. (Acari: Ixodidae) (Jaenson et al. 2006), common flies (Diptera) (Yusufoglu and Hasdemir 1996), and *Aedes aegypti* (L.) (Diptera: Culicidae) (Trongtokit et al. 2005).

Various biological components or phytochemicals with varied polarity are commonly combined to form essential oils (Sasidharan et al. 2011). Insects have evolved a variety of defense mechanisms to combat the possible toxicity of these xenobiotics (El-Sayed et al. 2023). Before insecticides reach their target locations to produce their harmful effects, they go through three phases in the insect detoxifying enzyme system: biotransformation, metabolism, and secretion (Awad et al. 2024). Evaluations of insecticidal activity of essential oils against insect pest species have been conducted regularly (Benelli et al. 2018). Essential oils, however, have been shown to block several enzymes, including detoxification enzymes (Arokiyaraj et al. 2022). Our knowledge of the mode of action of essential oils however remains limited, and further research is required (Hashem et al. 2020).

This study was implemented to assess the toxicity and biochemical effectiveness of *P. graveolens* essential oils against *S. littoralis* to lessen some of the negative effects of using essential oils in pest management programs. Furthermore, we performed a molecular docking analysis to obtain a thorough understanding of the binding pattern between detoxifying enzymes such as cytochrome P450, α -esterase, and glutathione S-transferase (GST) with citronellol, the main ingredient of *P. graveolens* essential oil.

Materials and Methods

Extraction and chemical characterization of *P. graveolens* essential oil.

Geranium oil samples were obtained from the Qassim governorate (25°48'23"N, 42°52'24"E), Saudi Arabia. Using Clevenger-type equipment (Clevenger 1928), *P. graveolens* extract was obtained by hydrodistillation from 100 g of fresh plant samples for 3.0 h after heating in water at 75°C until no additional extract was seen. This process yielded an essential oil 3.5% (based on fresh weight) with fresh geranium leaves. The chemical composition of the essential oil was then identified using chromatography–mass spectrometry (QP2020 NX system, Shimadzu,

Tokyo, Japan) equipped with a 50-cm HP capillary column and a flame ionization detector. The retention time of each peak was compared with the information in the Tutor and Wiley Registry/NIST mass spectral libraries (Beckley et al. 2014) to determine chemical components.

Bioassay. An *S. littoralis* colony was maintained in sterile plastic containers (17 × 25 × 8 cm) under a photoperiod of 16:8 (L:D) h at 25°C and 60% relative humidity. Five essential oil concentrations—6,000, 4,000, 2,000, 1,000, and 500 mg/L—were used to determine the lethal and sublethal concentrations of the essential oils. Each concentration was replicated five times with 20 larvae per replicate ($n = 100$). Castor bean (*Ricinus communis* L.; Euphorbiaceae) leaves in the control group were immersed in water, whereas leaves in the treatments were submerged for 10 s in the appropriate concentration. All leaves were then allowed to air dry. Second-instar larvae of *S. littoralis* were placed on the leaves, and larval death was recorded every day for 4 d. Cumulative mortality was used to determine sublethal (LC₁₅) and median lethal (LC₅₀) concentrations with probit analysis (Finney 1971). Abbott's formula (Abbott 1925) was used to correct for control mortality before analysis. Two iterations of the assay were conducted.

Lethal and sublethal effects. The LC₁₅ and LC₅₀ values were used to evaluate the effect of *P. graveolens* essential oil on the developmental parameters of *S. littoralis* larvae. For every concentration, three replicates (50 larvae per replicate) were used. Fresh, untreated castor bean leaves were provided every day to the larvae, which were housed individually in sterile cups. Daily evaluations of developmental changes were conducted using the following variables: sex ratio, adult emergence rate, pupation percentage, pupal weight (in grams), and duration of larval and pupal stages.

Biochemical assay. Second-instar *S. littoralis* larvae were treated with the LC₁₅ and LC₅₀ values per 0.1 g of fresh body weight for 24 and 96 h to assess the activity of detoxification enzymes response to the *P. graveolens* essential oil. Five replicates were used for each concentration. Phosphate buffer (0.1 M) was used to homogenize the larvae. The pH value was 7.4 for cytochrome P450, 7.0 for α -esterase, and 6.5 for GST. A 15-min centrifugation at 10,000 rpm produced the supernatants from the homogenates that were then poured into 1.5-ml sterile tubes. For cytochrome P450 analysis, p-nitro anisole (PN) was used to test cytochrome P450 activity in accordance with Hansen and Hodgson (1971). After 2 min of incubation at 27°C, a combination of 2 mM PN and homogenate sample was added, along with 9.6 mM NADPH. The optical density was measured at 405 nm. In addition, the techniques described by Van Asperen (1962) were used to measure the activity of α -esterase. The homogenate sample was mixed with α -naphthyl acetate and allowed to sit at 25°C for 15 min. To halt the process, sodium dodecyl sulfate (5%) and Fast Blue B (2%) were added. The optical density was determined at 550 nm by using a Jenway UV/Vis spectrophotometer (Agilent, Santa Clara, CA, USA). The protocol of Habig et al. (1974) with 1-chloro-2,4-dinitrobenzene (CDNB) was used to measure GST activity. The homogenate of the sample, 30 mM CDNB, and 50 mM glutathione (GSH) made up the sample solution. The UV/Vis spectrophotometer was used to measure the GST activity at 340 nm for 5 min at 1-min intervals. Finally, the total protein content was determined using the Coomassie brilliant blue test (Bradford 1976).

Molecular docking analysis. The binding mechanism and interactions between citronellol complexes and the enzymes α -esterase (PDB ID: 4fnm), cytochrome P450 (PDB ID: 4h24), and GST (PDB ID: 5zwp) were examined by molecular docking studies using MOE 2015 software (Chemical Computing Group, Montreal, Canada). The crystal structures of α -esterase (PDB ID: 4fnm), cytochrome P450 (PDB ID: 4h24), and GST (PDB ID: 5zwp) were downloaded from the RCSB Protein Data Bank (<http://www.rcsb.org>). The three-dimensional structures of the most potent complexes, known as ligands, were created using Chem Draws 18.0 (PerkinElmer, Waltham, MA, USA) and stored as MDL Molfiles. In comparison, the greatest score obtained for a molecule indicated the lowest binding affinity.

Statistical analysis. The normality of continuous variables was assessed using the Shapiro–Wilk and Kolmogorov–Smirnov tests (Ahmad and Khan 2015). To normalize probability and percentile data, an arcsine square root transformation was applied (Zimmerman and Zumbo 2005). One-way analysis of variance was conducted to compare the means between the treatments and control groups, followed by Tukey's post hoc test for pairwise comparisons, with a $P < 0.05$ considered as statistically significant (Agbangba et al. 2024). The sex ratio was analyzed using the chi-square test with MINITAB 14 (Minitab, State College, PA).

Results

Chemical characterization of the *P. graveolens* essential oil. The primary chemical components of *P. graveolens* essential oil are listed in Table 1 and include geraniol (13.03%) and citronellol (20.91%) as the major constituents. In addition, *P. graveolens* essential oil is rich in monoterpenes (26.57%; Table 1).

Toxicity of *P. graveolens* essential oil to *S. littoralis* larvae. After 96 h of treatment, the LC₁₅ and LC₅₀ value of *P. graveolens* essential oil to second-instar *S. littoralis* larvae was 608.52 and 1,820.77 mg/L, respectively (Table 2).

Impact of *P. graveolens* essential oil *S. littoralis* development. Significant differences in the duration of the larval and pupal stages of *S. littoralis* were observed in response to exposure to the essential oil ($F = 3.45$; $df = 2, 230$; $P = 0.033$ and $F = 11.01$; $df = 2, 205$; $P = 0.0001$, respectively; Table 3). Each stage was significantly longer when exposed to the essential oil in comparison with the control group. Significant decreases in percentage pupation occurred only when second instars were treated with the LC₅₀ value ($F = 7.65$; $df = 2, 6$; $P = 0.022$). Neither gender ratio nor adult emergence varied significantly after exposure to the essential oil ($F = 1.05$; $df = 2, 6$; $P = 0.357$; Table 4).

Interactions of *P. graveolens* essential oils with detoxifying enzymes. After treating second-instar *Agrotis ipsilon* (Hufnagel) and *S. littoralis* with *P. graveolens* essential oil for 24 and 96 h, cytochrome P450 activity increased significantly ($F = 28.53$; $df = 2, 6$; $P = 0.001$) in *S. littoralis* after treatment with LC₁₅ of *P. graveolens* essential oil (Table 5), whereas the α -esterase activity decreased significantly ($F = 11.79$; $df = 2, 6$; $P = 0.008$). By contrast, no significant differences were observed in GST activity after 24 h ($F = 1.86$; $df = 2, 6$; $P = 0.236$) and 96 h ($F = 3.98$; $df = 2, 6$; $P = 0.079$) of exposure to the essential oil (Table 5).

Docking on the receptor of cytochrome P450, α -esterase, and GST. Molecular docking was used on citronellol molecules by applying the target molecules against cytochrome P450 (PDB ID: 4h24), α -esterase (PDB ID: 4fnm), and GST

Table 1. Chemical compounds identified in the essential oil from *Pelargonium graveolens*.

Retention Time	Area %	Compound	Match Factor
3.67	0.85	α -Pinene	924
7.04	6.48	Linalool	938
7.24	0.56	(2 <i>R</i> ,4 <i>R</i>)-4-Methyl-2-(2-methylprop-1-en-1-yl) tetrahydro-2 <i>H</i> -pyran	938
8.60	6.52	l-Menthone	940
9.45	0.54	L- α -Terpineol	893
10.29	20.91	Citronellol	922
10.51	0.50	2,6-Octadienal, 3,7-dimethyl-, (<i>Z</i> -)	871
10.88	13.03	Geraniol	933
11.27	1.69	2,6-Octadienal, 3,7-dimethyl-, (<i>E</i> -)	917
11.38	9.98	3,7-Dimethyloct-6-enyl ethylcarbonate	901
12.02	5.53	Geranyl formate	880
13.33	0.37	2,6-Octadiene, 2,6-dimethyl-, acetate	888
13.98	0.84	Copaene	913
14.05	0.72	Geranyl acetate	913
14.18	1.61	(-)- α -Bourbonene	921
15.06	1.20	Caryophyllene	925
15.58	0.72	Patchoulene	807
15.77	0.40	Isoledene	884
16.04	0.34	Alloaromadendrene	907
16.29	0.94	Geranyl propionate	899
16.99	0.34	α -Muurolene	922
17.21	0.31	Farnesyl butanoate	840
17.33	0.34	ε -Muurolene	917
17.46	0.50	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a-hexahydronaphthalene	916
17.53	0.78	<i>cis</i> -Calamenene	888
17.63	0.36	Citronellyl butyrate	914
18.12	0.68	α -Vetivol	797
18.34	1.25	3,7-Dimethyl-2,6-octadienyl ester, (<i>E</i> -)	889

Table 1. Continued.

Retention Time	Area %	Compound	Match Factor
18.85	0.84	(–)-Spathulenol	896
18.98	2.20	2-Phenylethyl tiglate	907
19.29	0.44	<i>trans</i> -Geranylgeraniol	773
19.73	0.42	Cubenol	895
19.86	10.25	2-((2 <i>S</i> ,4 <i>aR</i>)-4 <i>a</i> ,8-Dimethyl-1,2,3,4,4 <i>a</i> ,5,6,7-octahydronaphthalen-2-yl)propan-2-ol	940
20.12	0.37	α -Guaiene	877
20.20	0.51	Agarospinol	960
20.37	0.63	α -Acorenol	833
20.42	0.45	(–)-Aristolene	840
20.60	1.46	2-Naphthalenemethanol, decahydro- α , α ,4 <i>a</i> -trimethyl-8-methylene-, [2 <i>R</i> -(2 <i>a</i> ,4 <i>a</i> ,8 <i>a</i>)]-	889
20.68	0.44	Benz[<i>A</i>]Azulene-1,4-Dione, 10-Methoxy-	976
21.52	3.03	Geranyl tiglate	911
21.89	0.32	Hexanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (<i>Z</i> -)	916
29.95	0.38	Citronellyl oleate	768

(PDB ID: 5zwp) through MOE 2015 software. The energy score of citronellol molecules (Table 6) was determined to be -5.346 , -5.295 , and -5.4278 kcal/mol, respectively, values that were higher than the control ligand (diethyl hydrogen phosphate, -4.5809), protoporphyrin IX containing Fe, -5.1968 ; and glutathione, -4.5697 (Table 6). The negative binding energy increases with the strength of contact. Fig. 1 shows the overall bonding connections via $-OH-$ bonds of the amino acid residue in question against the docked molecule.

Discussion

Synthetic chemical insecticides have many unfavorable attributes (e.g., environmental impacts, human exposure hazards, development of resistance in target populations), thereby triggering efforts to find effective alternatives. Some plant extracts have proven safe and powerful against target pests and are a potential source of insecticides. *Pelargonium graveolens* essential oil is widely used in the food and fragrance industries as an antiseptic, antioxidant, antibacterial, and antifungal substance (Džamić et al. 2014, Ibrahim et al. 2022).

Citronellol (20.91%) and geraniol (13.03%) are the main chemical constituents of the *P. graveolens* extract; however, citronellol and geraniol percentages in *P.*

Table 2. Concentration–mortality response of second-instar *Spodoptera littoralis* to *Pelargonium graveolens* essential oil.

Response	Concentration (mg/L)	95% Confidence Limit	Regression Line (Slope ± SE)	χ^2
LC ₁₅	608.52	357.21–850.36	2.17 ± 0.31	3.17
LC ₅₀	1,820.77	1,404.26–2,342.42		

graveolens essential oil vary with cultivation site, harvest time, soil characteristics, and plant age (Abd El-Wahab et al. 2016). The proportion of citronellol and geraniol in *P. graveolens* essential oil in this study differed from the results reported in Egypt by Ibrahim et al. (2022), who indicated that the principal constituents were citronellol (14.44%) and geraniol (11.08%). Regardless, citronellol and geraniol are important constituents that have insect-repellent properties and their modes of action are food deterrence, contact repellency, and toxicity (Subramanya et al. 2022).

The LC₁₅ and LC₅₀ value at 96 h after treatment with *P. graveolens* essential oil was 608.52 and 1,820.77 mg/L, respectively, against second-instar *S. littoralis* larvae. These findings are consistent with those of Mesbah et al. (2023), who determined an LC₅₀ of 67.66 ppm/cm² of *P. graveolens* essential oil against *Sitophilus oryzae* (L.) adults at 72 h posttreatment.

Furthermore, exposure of insects to chemical concentrations can be fatal or sublethal in their natural habitat (xenobiotics) (Kinareikina and Silivanova 2023), causing a significant reduction in insect vitality, fecundity, and reproduction (Hategekimana and Erlor 2020, Pavela et al. 2021). Moreover, it may result in pertinent behavioral alterations (Benelli et al. 2021) that reduce insect populations in crops in subsequent generations by interfering with biological processes. I therefore examined the effects of exposure to *P. graveolens* essential oil at the LC₁₅ and

Table 3. Mean ± SE response of *Spodoptera littoralis* development after exposure of second-instar larvae to *Pelargonium graveolens* essential oil at the LC₁₅ and LC₅₀ levels.*

Developmental Parameter	<i>P. graveolens</i> Essential Oil (mg/L)		
	0 (Control)	608.52 (LC ₁₅)	1,820.77 (LC ₅₀)
Larval duration (d)	16.27 ± 0.963b	16.45 ± 2.062ab	16.91 ± 1.512a
Pupal duration (d)	10.21 ± 1.126b	10.71 ± 1.608b	11.34 ± 1.568a
Pupation (%)	95.53 ± 5.08a	96.53 ± 6a	82.23 ± 3.62b
Male pupal weight (g)	0.27 ± 0.035a	0.278 ± 0.048a	0.262 ± 0.041a
Female pupal weight (g)	0.283 ± 0.039a	0.293 ± 0.047a	0.284 ± 0.037a
Emergence (%)	100 ± 0a	100 ± 0a	100 ± 0a

* Means within a row that are not followed by the same lowercase letter are not significantly different according to Tukey's honestly significant difference test ($P < 0.05$).

Table 4. Sex ratio of the emerged adults of *Spodoptera littoralis* after treating the second-instar larvae with LC₁₅ and LC₅₀ levels of *Pelargonium graveolens* essential oil.

	Male	Female	χ^2	P
Control	46.9	53.03	0.3764	0.54
LC ₁₅	37.67	62.27	6.0501	0.014
LC ₅₀	45.9	54.08	0.6687	0.413

LC₅₀ levels on selected developmental characteristics of *S. littoralis*. Compared with the control, exposure to LC₅₀ levels of *P. graveolens* essential oil significantly extended the duration of the larval and pupal stages of *S. littoralis*. When treated with the LC₅₀ value, the percentage of pupation was dramatically reduced by 1.1-fold. By contrast, the emergence rate, sex ratio, and adult emergence from pupae after exposure to the LC₁₅ and LC₅₀ levels of *P. graveolens* essential oil were not significantly impacted.

Interestingly, essential oils have strong insecticidal effects by controlling the neuroendocrine system and metabolism of the target insect (Arokiyaraj et al. 2022). The metabolic alterations of target insects exposed to essential oils have therefore been demonstrated in several investigations (Shekari et al. 2008). By

Table 5. Activity of cytochrome P450, α -esterase, and GST after 24 and 96 h of treating second-instar *S. littoralis* with LC₁₅ and LC₅₀ levels of *P. graveolens* essential oil.

Enzyme	Treatment	Activity (Mean \pm SE)*	
		24 h	96 h
Cytochrome P450 (nmol/mg protein/min)	Control	8.39 \pm 0.51b	10.02 \pm 0.29a
	LC ₁₅	12.59 \pm 0.85a	11.25 \pm 3.3a
	LC ₅₀	15.32 \pm 1.25a	15.34 \pm 2.28a
α -Esterase (μ mol/mg protein/min)	Control	0.28 \pm 0.009a	0.23 \pm 0.04a
	LC ₁₅	0.192 \pm 0.026b	0.158 \pm 0.033a
	LC ₅₀	0.198 \pm 0.025b	0.196 \pm 0.025a
GST (μ mol/mg protein/ml)	Control	22.82 \pm 5.38a	22.9 \pm 6.81a
	LC ₁₅	19.45 \pm 1.62a	29.65 \pm 54a
	LC ₅₀	33.15 \pm 11.53a	37.8 \pm 2.87a

GST = glutathione S-transferase.

* Means within a row that are not followed by the same lowercase letter are not significantly different according to Tukey's honestly significant difference test ($P < 0.05$).

Table 6. Docking results inside α -esterase, cytochrome P450, and GST active spots, arranged from the negatively highest to the lowest score.

Enzyme	Molecule	Ligand	Receptor	Interaction	Distance (in Åo from Main Residue)	Binding Energy (kCal/mol)	Entropy (kCal/mol)
α -Esterase (PDB ID: 4fsm)	Citronellol	O 22	PHE 3	H-acceptor	3.08	-2.1	-5.295
	Control ligand (diethyl hydrogen phosphate)	O 3	ASN 2	H-donor	3.27	-1.1	-4.5809
	Citronellol	O 3	PHE 3	H-acceptor	3.19	-0.8	
Cytochrome P450 (PDB ID: 4h24)	Citronellol	O 22	SER 72	H-acceptor	3.15	-0.6	-5.346
	Control ligand (protoporphyrin IX containing Fe)	C 3	MET 185	H-donor	3.82	-0.8	-5.1968
GST (PDB ID: 5zwp)	Citronellol	O 52	ALA 330	H-acceptor	2.94	-2.2	
	Citronellol	O 22	SER 9	H-donor	2.94	-1.1	-5.4278
	Control ligand (glutathione)	O 34	HIS 50	H-donor	3.13	-2	-4.5697
		O 37	TYR 113	H-acceptor	2.5	2.9	

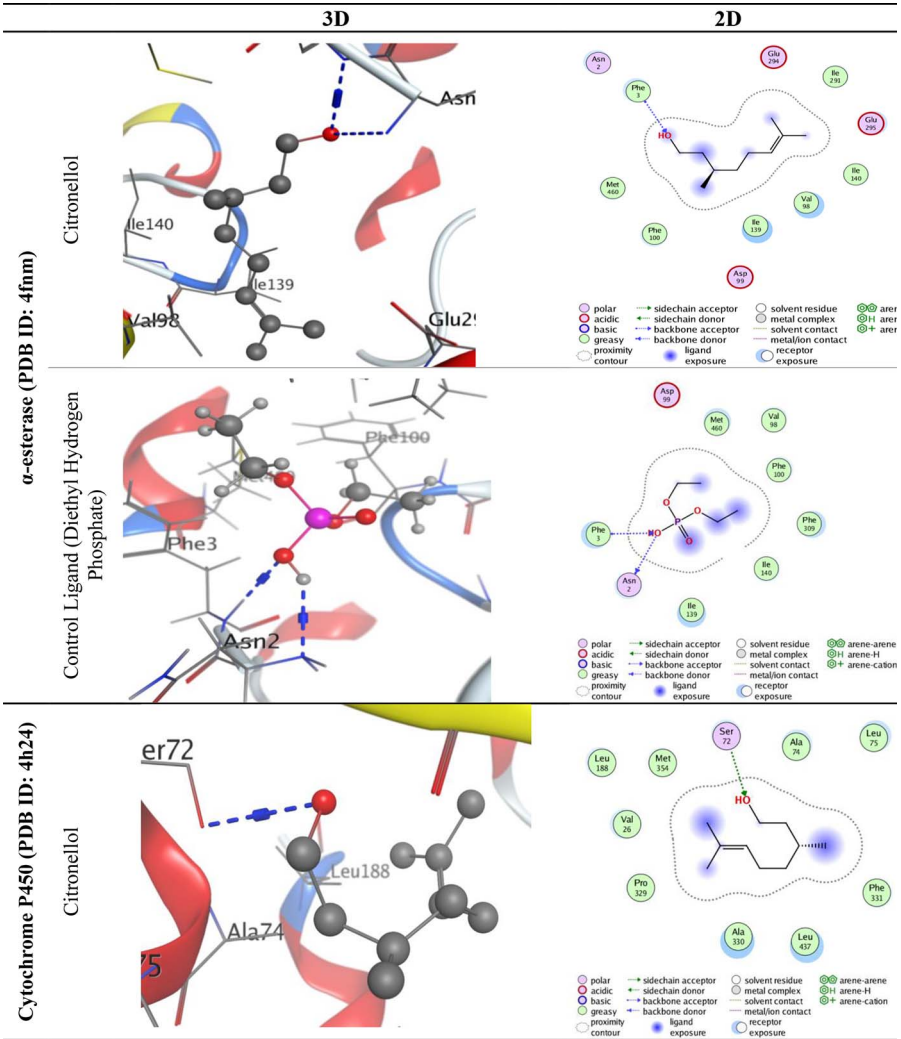


Fig. 1. Two- and three-dimensional representations of citronellol and control ligand complexes against the active site of α -esterase (PDB ID: 4fnm), cytochrome P450 (PDB ID: 4h24), and GST (PDB ID: 5zwp). Hydrogen bonds are displayed in cyan, and H- π -bonds are displayed in dark magenta.

eliminating toxic substances (xenobiotics), such as pesticides and hazardous secondary metabolites, prominent detoxification enzymes such as carboxyl esterase, cytochrome P450 monooxygenases, and GST allow insect pests to maintain biological functions (Feyereisen 2012). When assessing the possible use of novel compounds generated from plants that have insecticidal action, it is critical to regularly check the levels of these enzymes (Pereira Filho et al. 2024).

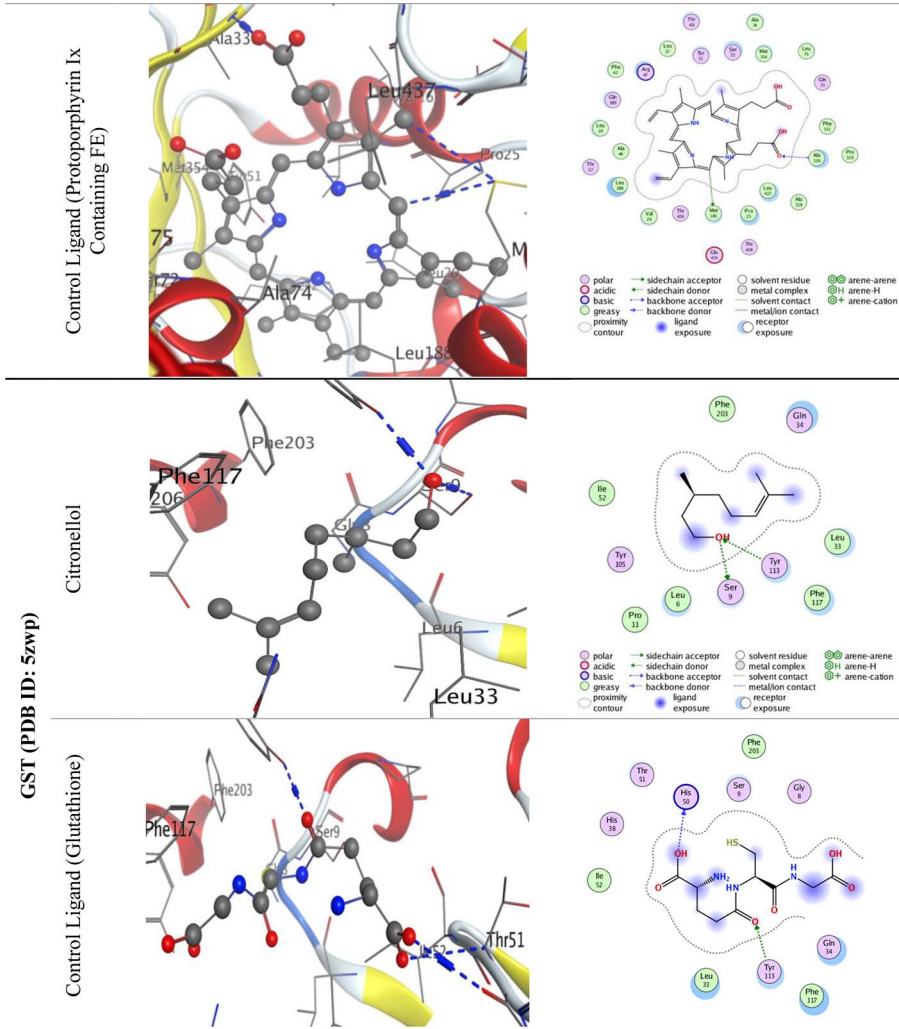


Fig. 1. Continued.

Based on the data obtained from the biochemical changes in the tested detoxification enzymes, I found a significant increase in the activity of cytochrome P450 after 24 h posttreatment in *S. littoralis* larvae treated with the LC₁₅ and LC₅₀ levels of *P. graveolens* essential oil, reaching a peak at the LC₅₀ value, confirming engagement of defensive systems against substances (Radwan et al. 2023), whereas α -esterase was significantly inhibited after treatment of *S. littoralis* larvae with LC₁₅ and LC₅₀ levels. In general, this decline in detoxifying enzyme activity is regarded as a favorable indicator of delayed tolerance to harmful substances (Pengsook et al. 2022). Likewise, no considerable differences were observed in GST activity after 24 and 96 h of treatment.

Using a molecular docking study, I compared the tested compound to the active site of the tested detoxification enzymes, cytochrome P450 (PDB ID: 4h24), α -esterase (PDB ID: 4fnm), and GST (PDB ID: 5zwp), to better understand the interactions between the citronellol molecules and the key amino acids of these well-known detoxification enzymes and their binding modes. Remarkably, the findings demonstrated that citronellol and the detoxifying enzymes had a strong binding tendency, particularly for the GST enzyme. The fact that citronellol, the principal ingredient in *P. graveolens* essential oil, binds strongly to the GST receptor raises the possibility that *P. graveolens* essential oil primarily targets GST. Additional studies are needed to further define these interactions.

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References Cited

- Abbott, W.S. 1925.** A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265–267.
- Abd El-Aziz, S. 1998.** Essential oil of geranium *Pelargonium graveolens* (Linn.) as a feeding deterrent, growth retardant and oviposition repellent for the cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). *J. Egypt. Soc. Zool.* 25: 47–58.
- Abd El-Wahab, M.A., W.I.M. Toaima and E.S. Hamed. 2016.** Effect of different planting locations in Egypt on volatile oil of geranium (*Pelargonium graveolens* L.) plant. *J. Basic Appl. Res.* 2: 522–533.
- Abouelatta, A.M., A.Y. Keratum, S.I. Ahmed and H.M. El-Zun. 2020.** Repellent, contact and fumigant activities of geranium (*Pelargonium graveolens* L.'Hér) essential oils against *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.). *Int. J. Trop. Insect Sci.* 40: 1021–1030.
- Agbangba, C.E., E.S. Aide, H. Honfo and R.G. Kakai. 2024.** On the use of post-hoc tests in environmental and biological sciences: A critical review. *Heliyon* 10: e25131.
- Ahmad, F. and R.A. Khan. 2015.** A power comparison of various normality tests. *Pak. J. Stat. Oper. Res.* 11: 331–345.
- Arakiyaraj, C., K. Bhattacharyya and S.G.E. Reddy. 2022.** Toxicity and synergistic activity of compounds from essential oils and their effect on detoxification enzymes against *Platanococcus lilacinus*. *Front. Plant Sci.* 13: 1016737.
- Awad, M., A.H. El Kenawy, N.A. Alfuhaid, E.S. Ibrahim, J.K. J6svai, A. F6nagy and M.A.M. Moustafa. 2024.** Lethal and sublethal effects of cyantraniliprole on the biology and metabolic enzyme activities of two lepidopteran pests, *Spodoptera littoralis* and *Agrotis ipsilon*, and a generalist predator, *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Insects* 15: 450.
- Beckley, L., K. Gorder, E. Dettenmaier, I. Rivera-Durate and T. McHugh. 2014.** On site gas chromatography mass spectrometry (GC-MS) analysis to stream line vapor intrusion investigations. *Environ. Forensics* 15: 2234–2243.
- Benelli, G., R. Pavela, C. Giordani, L. Casettari, G. Curzi, L. Cappellacci, R. Petrelli and F. Maggi. 2018.** Acute and sub-lethal toxicity of eight essential oils of commercial interest against the filariasis mosquito *Culex quinquefasciatus* and the housefly *Musca domestica*. *Ind. Crop Prod.* 112: 668–680.
- Benelli, G., R. Rizzo, V. Zeni, A. Govigli, A. Samková and M. Sinacori. 2021.** *Carlina acaulis* and *Trachyspermum ammi* essential oils formulated in protein baits are highly

- toxic and reduce aggressiveness in the medfly, *Ceratitis capitata*. Ind. Crops Prod. 161: 113191.
- Bradford, M.M.A. 1976.** Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254.
- Clevenger, J.F. 1928.** Apparatus for the determination of volatile oil. J. Am. Pharm. Assoc. 17: 345–349.
- Dayan, F.E., C.L. Cantrell and S.O. Duke. 2009.** Natural products in crop protection Bioorg. Med. Chem. 17: 4022–4034.
- Džamić, A.M., M.D. Soković, M.S. Ristić, S.M. Grujić, K.S. Mileski and P.D. Marin. 2014.** Chemical composition, antifungal and antioxidant activity of *Pelargonium graveolens* essential oil. J. Appl. Pharm. Sci. 4: 001–005.
- Eamsobhana, P., A. Yoolek, W. Kongkaew, K. Lerdthusnee, N. Khlaimanee, A. Parsartvit, N. Malainul and H.S. Yong. 2009.** Laboratory evaluation of aromatic essential oils from thirteen plant species as candidate repellents against *Leptotrombidium* chiggers (Acari: Trombiculidae), the vector of scrub typhus. Exp. Appl. Acarol. 47: 257–262.
- El-Sayed, M.H., M.M. Ibrahim, A.E. Elsobki and A.A. Aioub. 2023.** Enhancing the toxicity of cypermethrin and spinosad against *Spodoptera littoralis* (Lepidoptera: Noctuidae) by inhibition of detoxification enzymes. Toxics 11: 215.
- Feyereisen, R. 2012.** Insect CYP genes and P450 enzymes, Pp. 236–316. In Gilbert, L.I. (ed.), Insect Molecular Biology and Biochemistry. Academic Press, London.
- Finney, D.J. 1971.** Probit Analysis. 3rd Ed. Cambridge University Press, Cambridge, U.K.
- Habig, W.H., M.J. Pabst and W.B. Jakoby. 1974.** Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249: 7130–7139.
- Hansen, L.G. and E. Hodgson. 1971.** Biochemical characteristics of insect microsomes: N- and O-demethylation. Biochem. Pharmacol. 20: 1569–1578.
- Hashem, A.S., M.M. Ramadan, A.A.A. Abdel-Hady, S. Sut, F. Maggi and D. Acqua. 2020.** *Pimpinella anisum* essential oil nanoemulsion toxicity against *Tribolium castaneum*? Shedding light on its interactions with aspartate aminotransferase and alanine aminotransferase by molecular docking. Molecules 25: 4841.
- Hategekimana, A. and F. Erler. 2020.** Fecundity and fertility inhibition effects of some plant essential oils and their major components against *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae). J. Plant Dis. Prot. 5: 615–623.
- Hikal, W.M., M.M. Baz, M.A. Alshehri, O. Bahattab, R.S. Baeshen, A.M. Selim, L. Alhwity, R. Bousbih, M.S. Alshourbaji and H.A.H.S. Ahi. 2023.** Sustainable pest management using novel nanoemulsions of honeysuckle and patchouli essential oils against the West Nile virus vector, *Culex pipiens*, under laboratory and field conditions. Plants 12: 3682.
- Ibrahim, S.M., S.M. Aboelhadid, A.A. Wahba, A.A. Farghali, R.J. Miller, A.S. Abdel-Baki and S. Al-Quraishy. 2022.** Preparation of geranium oil formulations effective for control of phenotypic resistant cattle tick *Rhipicephalus annulatus*. Sci. Rep. 12: 11693.
- Isman, M.B. 2000.** Plant essential oils for pest and disease management. Crop Prot. 9: 603–608.
- Jaenson, T.G.T., S. Carboui and K. Palsson. 2006.** Repellency of oils of lemon, eucalyptus, geranium, and lavender and the mosquito repellent MyggA natural to *Ixodes ricinus* (Acari: Ixodidae) in the laboratory and field. J. Med. Entomol. 43: 731–736.
- Kendra, P.E., W.S. Montgomery, J. Niogret, E.Q. Schnell, M.A. Deyrup and N.D. Epsky. 2014.** Evaluation of seven essential oils identifies cubeb oil as most effective attractant for detection of *Xyleborus glabratus*. J. Pest Sci. 87: 681–689.
- Kinareikina, A. and E. Silivanova. 2023.** Impact of insecticides at sublethal concentrations on the enzyme activities in adult *Musca domestica* L. Toxics 11: 47.
- Machalova, Z., M. Sajfirtova, R. Pavela and M. Topiar. 2015.** Extraction of botanical pesticides from *Pelargonium graveolens* using supercritical carbon dioxide. Ind. Crops Prod. 67: 310–317.

- Mesbah, A., M.A. Massoud, M.S. Aajel, N.R. Abdelsalam and M.M. Adel. 2023.** Nano-formulation of *Pelargonium graveolens* essential oil: Physicochemical characterization and its bioactivity against rice weevil *Sitophilus oryzae* on stored wheat grain. *Egypt. J. Chem.* 66: 459–469.
- Mostafiz, M.M., E. Hassan, J.K. Shim and K.Y. Lee. 2019.** Insecticidal efficacy of three benzoate derivatives against *Aphis gossypii* and its predator *Chrysoperla carnea*. *Ecotoxicol. Environ. Saf.* 184: 109653.
- Pavela, R., F. Maggi, E. Mazzara, J. Torresi, K. Cianfaglione, G. Benelli and A. Canale. 2021.** Prolonged sublethal effects of essential oils from non-wood parts of nine conifers on key insect pests and vectors. *Ind. Crops Prod.* 168: 113590.
- Pengsook, A., S. Tharamak, K. Keosaeng, O. Koul, V. Bullangpoti, N. Kumrungsee and W. Pluempanupat. 2022.** Insecticidal and growth inhibitory effects of some thymol derivatives on the beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae) and their impact on detoxification enzymes. *Pest Manag. Sci.* 78: 684–691.
- Pereira Filho, A.A., V.F. do Vale, M.C.M. de Oliveira, M.M. Barrozo, M.A. Stanton, L.F. Yamaguchi, M.J. Kato and R.N. Araújo. 2024.** Effects of *Piper aduncum* (Piperales: Piperaceae) essential oil and its main component dillapiole on detoxifying enzymes and acetylcholinesterase activity of *Amblyomma sculptum* (Acari: Ixodidae). *Int. J. Mol. Sci.* 25: 5420.
- Radwan, I.T., A.M.Z. Sayed, N.A. Ghazawy, S.S. Alqahtani, S. Ahmad, N. Alam, A.M. Alkhaibari, M.S. Ali, A. Selim and E.A. AbdelFattah. 2023.** Effect of nanostructure lipid carrier of methylene blue and monoterpenes as enzymes inhibitor for *Culex pipiens*. *Sci. Rep.* 13: 12522.
- Rana, V.S. 2002.** Chemical constituents of essential oil of *Pelargonium graveolens* leaves. *Int. J. Aromather.* 12: 216–218.
- Regnault-Roger, C., C. Vincent and J.T. Arnason. 2012.** Essential oils in insect control: Low-risk products in a high-stakes world. *Annu. Rev. Entomol.* 57: 405–424.
- Saraswathi, J., K. Venkatesh, N. Baburao, M.H. Hilal and A.R. Rani. 2011.** Phytopharmaceutical importance of *Pelargonium* species. *J. Med. Plants Res.* 5: 2587–2598.
- Sasidharan, S., Y. Chen, D. Saravanan, K.M. Sundram and L.Y. Latha. 2011.** Extraction, isolation and characterization of bioactive compounds from plants' extracts. *Afr. J. Trad. Compl. Alt. Med.* 8: 1–10.
- Shekari, M., J.J. Sendi, K. Etebari, A. Zibae and A. Shadparvar. 2008.** Effects of *Artemisia annua* L. (Asteraceae) on nutritional physiology and enzyme activities of elm leaf beetle, *Xanthogaleruca luteola* Mull. (Coleoptera: Chrysomellidae). *Pestic. Biochem. Physiol.* 91: 66–74.
- Subramanya, S., K. Sumanth, P.K. Gupta, V. Chayapathy, E. Keshamma and K. Murugan. 2022.** Formulation of green nanoemulsions for controlling agriculture insects, Pp. 165–176. *In* Abd-Elsalam, K.A. and Murugan, K. (eds.), *Nanobiotechnology for Plant Protection, Bio-Based Nanoemulsions for Agri-Food Applications*. Elsevier. (Amsterdam, The Netherlands)
- Trongtokit, Y., Y. Rongsriyam, N. Komalamisra and C. Apiwathnasorn. 2005.** Comparative repellency of 38 essential oils against mosquito bites. *Phytother. Res.* 19: 303–309.
- Van Asperen, K. 1962.** A study of housefly esterases by means of a sensitive colorimetric method. *J. Insect Physiol.* 8: 401–416.
- Yusufoglu, A. and B. Hasdemir. 1996.** Essential oil from *Pelargonium graveolens* L'Herit as a natural mosquito repellent. *Chim. Acta Tur.* 24: 105–109.
- Zimmerman, D.W. and B.D. Zumbo. 2005.** Can Percentiles replace raw scores in the statistical analysis of test data? *Edu. Psychol. Measur.* 65: 616–638.