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Article

Open Access 

J. Mex. Chem. Soc. **2026**, 70(1):e2451

Received March 6th, 2025

Accepted June 12th, 2026

<http://dx.doi.org/10.29356/jmcs.v70i1.2451>
e-location ID: 2451

Keywords:

Pelargonium graveolens, insecticidal activity, *Ephestia kuehniella*, molecular docking

Palabras clave:

Pelargonium graveolens, actividad insecticida, *Ephestia kuehniella*, acoplamiento molecular

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Chemical Composition, Insecticidal Effects against the Mediterranean Flour Moth, and *in silico* Molecular Docking Study of the Essential Oil of Algerian Rose-Scented Geranium ‘*Pelargonium graveolens* L’Her’

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Abstract. In the context of exploring alternative strategies for managing pests in stored products, the insecticidal potential of *Pelargonium graveolens* L’Hér. (Geraniaceae) An essential oil (EO) was evaluated against *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), a major stored-product pest. Firstly, its chemical composition was determined by GC-MS, and its toxicity was assessed by topical application to newly molted pupae. Next, the estimated median lethal dose (LD50) was used to assess developmental and reproductive parameters in adult females.

In addition, an *in silico* study was conducted using molecular docking to illustrate interactions between the EO's main components and Juvenile hormone-binding protein (JHBP), which regulates the availability of Juvenile hormone, the key hormonal regulator of insect development and reproduction.

A total of 54 compounds were identified by GC-MS, representing 96.40 % of the total peak area, with β -citronellol (28.67 %), geraniol (14.57 %), and linalool (6.09 %) as the main compounds. Lethal doses (LD₅₀ and LD₉₀) were estimated at 13.18 $\mu\text{L mL}^{-1}$ and 85.11 $\mu\text{L mL}^{-1}$, respectively. In addition, the LD₅₀ administered to *E. kuehniella* female pupae caused disturbances in development and reproductive potential. The duration of pupal development and the fecundity were significantly reduced. Morphometric and biochemical studies showed that EO also reduced ovary size, volume, and vitellin content in adult females. *In silico* results show that the compounds engaged in hydrophobic interactions and formed hydrogen bonds with the active site of JHBP, explaining their strong interaction with the protein target. Our findings confirm the effectiveness of *P. graveolens* EO as a bioinsecticide, supporting its potential as an alternative to chemical insecticides in stored-product pest control.

Resumen. En el contexto de la exploración de estrategias alternativas para la gestión de plagas en productos almacenados, se evaluó el potencial insecticida del aceite esencial (AE) de *Pelargonium graveolens* L'Hér. (Geraniaceae) contra *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), una plaga importante de productos almacenados. En primer lugar, se determinó su composición química mediante GC-MS y se evaluó su toxicidad mediante su aplicación tópica en pupas recién mudadas. A continuación, se aplicó la dosis letal media estimada (DL₅₀) para determinar ciertos parámetros de desarrollo y de reproducción en hembras adultas. Además, se llevó a cabo un estudio *in silico* mediante acoplamiento molecular para ilustrar la interacción entre los componentes principales del aceite esencial y la proteína de unión a la hormona juvenil (JHBP), que regula la disponibilidad de la hormona juvenil, principal regulador hormonal del desarrollo y la reproducción de los insectos. Mediante GC-MS se identificaron un total de 54 compuestos, que representaban el 96,40 % del área total de los picos, siendo el β -citronelol (28.67 %), el geraniol (14.57 %) y el linalool (6.09 %) los compuestos principales. Las dosis letales (LD₅₀ y LD₉₀) se estimaron en 13.18 $\mu\text{L mL}^{-1}$ y 85.11 $\mu\text{L mL}^{-1}$, respectivamente. Además, la DL₅₀ administrada a las pupas hembras de *E. kuehniella* provocó alteraciones en el desarrollo y en el potencial reproductivo. La duración del desarrollo pupal y la fecundidad se redujeron significativamente. Los estudios morfológicos y bioquímicos mostraron que el AE también reducía el tamaño, el volumen y el contenido de vitelina en los ovarios de las hembras adultas. Los resultados del estudio *in silico* muestran que los compuestos establecieron interacciones hidrofóbicas y formaron enlaces de hidrógeno con el sitio activo de la JHBP, lo que explica su buena interacción con la proteína diana. Nuestros resultados confirman la eficacia del AE de *P. graveolens* como bioinsecticida y respaldan su potencial como alternativa a los insecticidas químicos para el control de plagas en productos almacenados.

Introduction

For years, implementing effective pest management strategies for stored food has been crucial to achieving sustainable food security. Various curative and preventive methods, incorporating physical and biological techniques, have been involved [1]. However, synthetic chemistry has shifted the focus from traditional approaches to fast-acting pesticides, resulting in complete dependence on them. Although chemical insecticides are highly effective, their excessive use raises significant concerns, including harm to biodiversity, ecosystem disruption, and risks to human health [2-4]. According to the FAO, most synthetic products used for insect pest management have become a serious issue. Many non-target species are also affected, often suffering lethal effects and significant toxicity [5-11]. On the other hand, the population is growing at an alarming rate, rising from 2 billion in 1950 to over 8 billion in 2024. At this rate, food production must increase proportionally to meet demands and enhance nutrition. In addition, pest infestations in stored foodstuffs can reduce their nutritional value. It can also lead to mold growth, which further accelerates the loss of global production, ultimately affecting food security.

Lepidoptera is the second-largest insect order after Coleoptera, comprising approximately 150,000 species [12]. Furthermore, it encompasses several significant insect pests, many constantly expanding their geographical range [13,14]. Some species have become widespread, requiring stringent control measures, particularly in temperate, subtropical, and tropical regions [15-17]. Several Lepidoptera species have already caused serious economic damage. *E. kuehniella* moth is regarded as a highly significant pest, causing severe losses in a wide range of products, particularly during storage, by their feeding behavior [18-20].

The above considerations have posed a serious challenge to the search for new molecules that are effective against pests and have fewer harmful effects. Moreover, control strategies tend to emphasize the non-chemical aspects of pesticides [21]. In this respect, the proven effectiveness of plant-based pesticides against various stored-product pests [6,22-25] and their biodegradability, environmental friendliness, and safety for non-target organisms [26] have increasingly become a focus of interest. Furthermore, their use aligns with green and sustainable development principles [27].

Essential oils and plants' main secondary metabolites exhibit a wide variety of bioactive components that reflect their ability to act in multiple ways against pests [28-31]. Moreover, their lipophilic characteristics make them more likely to interfere with metabolic, biochemical, and behavioral functions essential for insect survival [32,33]. Accordingly, essential oils have been classified as repellent [6,34], reprotoxic, neurotoxic [35,36], and even anti-appetent agents [37,24].

Reproductive events are a privileged target of insecticides for controlling insect pest infestations. In addition, data on the effects of EOs on the reproductive events of stored pests are scarce. Therefore, based on the above considerations, the present study aims to determine the chemical composition of the essential oil from the aromatic plant *Pelargonium graveolens* L'Hér. growing in Algeria, and to assess its insecticidal potential against the Mediterranean flour moth *E. kuehniella* by evaluating its effect on development and reproduction; and by determining, through a computational chemistry approach, molecular docking, the mechanism of action of the main compounds with the biological target.

Materials and methods

Insect rearing

The cosmopolitan insect pest *E. kuehniella* is the biological model studied. The insect was reared on white flour in the laboratory of applied animal biology under optimal temperature ($27 \pm 2^\circ\text{C}$), relative humidity of 70 %, and a photoperiod of 8:16 (L/D) h, as previously described [38].

Plant material and essential oil extraction

Aerial parts (leaves, stems, and flowers) of *Pelargonium graveolens* were collected during the flowering period between May and June (2023), in Annaba ($36^\circ 53' 60.00'' \text{ N } 7^\circ 46' 0.01'' \text{ E}$) (Northeast Algeria). After identification in the Botany Department (Faculty of Science, Badji Mokhtar Annaba University), the collected samples were dried in the shade at room temperature for approximately two weeks. Then, 100 g of the dried collection was hydrodistilled to extract the essential oil using a Clevenger-type apparatus. Extraction was carried out for up to 3 hours to maximize the amount of active ingredients and avoid altering the chemical composition of the extracted EO. The obtained EO was placed in a dark bottle and stored at 4°C until use. The yield was calculated using the French standardization association (AFNOR) formula:

$$\text{EO yield (\%)} = [\text{essential oil weight (g)} / \text{plant weight (g)}] \times 100$$

Essential oil quality was assessed based on its physicochemical and organoleptic properties, in accordance with international standards set by the French standards association AFNOR.

Chemical analysis by Gas Chromatography-Mass Spectrometry

The extracted essential oil was subjected to detailed chemical profiling by gas chromatography-mass spectrometry (GC-MS) to identify its constituents, using an HP Agilent 6890 chromatograph interfaced with an HP Agilent 5973 mass spectrometer. An EO quantity of 0.2 μL was introduced into the injector, which was set

to 250 °C. The compounds were transported using a carrier gas, pure helium 6.0, through an HP5MS column with dimensions of 30 m in length, 0.25 µm in thickness, and an internal diameter of 0.25 mm. The analysis lasted 113 min at 45 °C for the first 8 minutes, then increased at 2 °C min⁻¹ until 250 °C was reached. The mass spectrometry analysis was carried out in electron-impact ionization mode at an intensity of 70 eV. The constituents were identified by comparing retention times and indices with reference standards from the NIST-02 database and published data; no authentic chemical standards were used.

Treatment of pupae and insecticidal bioassays

P. graveolens essential oil was administered *via* topical application (contact) on the abdominal sternites of newly molted pupae females of *E. kuehniella* (age < 8 hours). Females were preferred over males because they drive population growth and invest more energy into reproduction, making them key targets for pest control [39-42]. Newly molted pupae were chosen for their soft cuticle, which allows better product diffusion [38], and topical application was used to ensure precise, uniform dosing and reproducibility [43]. The EO was administered at 10, 15, and 20 µL/mL of acetone. The EO was administered at 10, 15, and 20 µL mL⁻¹ of acetone. Four groups of 10 pupae per dose were used. Control series were treated with acetone alone (2 µL pupa⁻¹). This solvent was chosen because it permits a better diffusion of the EO throughout the cuticle. Mortality rates and pupal development were evaluated, and dead insects were counted daily until the adult emergence. The observed mortalities (OM) were corrected [44], thereby enabling the establishment of a probit curve based on the decimal logarithms of doses [45]. According to Finney's mathematical method, the decimal logarithms of the lethal doses LD₅₀ and LD₉₀ were determined for essential oil from the regression lines [46]. The confidence intervals for LD₅₀ and LD₉₀ were calculated using the Swaroop method [47] at the 95 % confidence level.

Pupal development duration and reproductive events

To assess the effects of EO on development and reproductive events, pupae were treated with LD₅₀ as described above. Thus, the insecticidal activity of *P. graveolens* EO was first estimated over the duration of pupal development (separating nymph exuviation from adult emergence), expressed in days.

The parameters determining the potential effect of the EO on female reproduction are measured on newly emerged females that resulted from treated pupae. At emergence (0 days), a treated female and an untreated male of the same age are paired up, and the reproductive process is monitored. The pre-oviposition period (the number of days between emergence and the onset of oviposition), the oviposition period (the number of days the female lays eggs), and fecundity (the number of eggs per female throughout its lifespan) were determined. Untreated couples served as a control, and the experiments were carried out with five replicates of 12 samples each.

Ovarian morphometric measurements

Morphometric measurements of ovaries were carried out on a control and treated series with LD₅₀ as mentioned above; 30 females were used for each series. Ovaries were dissected using a binocular magnifying glass as soon as the adult females emerged (< 8 h). Subsequently, the fresh weight of the ovaries and the number of oocytes in each pair were determined. The length (L) and width (l) of the basal oocyte were measured using an ocular micrometer, and the volume (V), expressed in mm³, was calculated using the following formula [48]:

$$V = 4/3\pi (L/2) (l/2)^2$$

Vitellin quantification

Ovarian extraction was made in 0-day-old female adults, according to Postlethwait *et al.* [49]. Ovaries from control and treated females were preserved in 0.5 mL Tris-HCl-NaCl buffer at pH 7.4. Samples were homogenized by ultrasound, and the resulting homogenate was centrifuged for 10 min at 5000 rpm. Three layers were distinguished: the supernatant, the pellet, and the intermediate containing the vitellin. The latter was recovered and stored at -20 °C.

The vitellin quantification was performed according to the protocol of Bradford *et al.* [50], using Coomassie Brilliant Blue (CBB) as the reagent and bovine serum albumin as the standard. Absorbance readings were taken using a spectrophotometer at 595 nm, and results are expressed in µg mg⁻¹.

Molecular docking study

The molecular docking study was performed to investigate the potential interactions between the major constituents of *P. graveolens* essential oil and the mosquito Juvenile Hormone-Binding Protein (mJHBP) [51,52]. mJHBP plays a vital role in regulating juvenile hormones (JHs), which govern insect growth, development, and reproduction by stabilizing and transporting JH II and JH III. Targeting mJHBP provides a precise way to disrupt these processes, leading to developmental and reproductive failures in pests. By mimicking or blocking mJHBP activity, inhibitors can serve as eco-friendly and species-specific insecticides, offering a promising strategy to combat insect resistance while minimizing environmental impact [53,54]

Target selection and ligand preparation

The crystal structure of the mosquito JHBP (mJHBP, PDB ID: 5V13) was selected as the target receptor based on three criteria: a high-resolution (1.84 Å) X-ray crystal structure [52]; a highly conserved JHBP binding pocket across insect orders, including Lepidoptera; and, the absence of any specific *E. kuehniella* JHBP crystal structure available in the Protein Data Bank, making 5V13 the most reliable and widely validated surrogate target. The ligands selected for docking were the seven major constituents of the *P. graveolens* EO identified by GC-MS with a relative abundance greater than 3 %: β -citronellol, geraniol, linalool, geranyl formate, citronellyl formate, geranyl tiglate, and citronellyl propionate.

Pre-processing and docking protocol

Pre-processing is a critical step in preparing both the macromolecule and the ligands for accurate energy calculations. The protein structure was processed using the Protein Preparation Wizard (Schrödinger Suite) [55,56], which involved removing non-essential crystallographic water molecules, adding missing hydrogen atoms, assigning correct bond orders, and optimizing the hydrogen-bonding network. The 3D ligand structures were generated, energy-minimized, and assigned Gasteiger partial atomic charges using the LigPrep tool. Molecular docking was performed using AutoDock 4.2. Each compound underwent 200 independent docking runs utilizing the Lamarckian Genetic Algorithm to explore multiple conformations. Docking poses were selected based on a combination of criteria: the lowest binding free energy (kcal mol⁻¹), membership in the most populated conformational cluster, and structural validation, ensuring that the pose maintained key interactions analogous to those of the co-crystallized native ligand [55].

Data analysis

The toxic effects of *P. graveolens* essential oil on the biological parameters of *E. kuehniella* between groups were compared using Mann-Whitney and Student's t-tests. Probit analysis was used to determine the LD50 and LD90. Each result is expressed as the mean and standard deviation (mean $\pm$ SD). The significance level is defined as $P < 0.05$.

Results

Yield, characteristics, and chemical composition of the essential oil

The essential oil extraction yield from the aerial parts of *P. graveolens* by hydrodistillation was over 0.43 % per 100 g of dry plant matter. The obtained EO was a yellowish liquid with a strong lemony aromatic fragrance, compliant with AFNOR standards. Fifty-four components representing 96.40 % of the total content were identified (Table 1). The monoterpenes fraction dominates the chemical composition of the EO; Moreover, β -citronellol (28.67 %), geraniol (14.57 %), and linalool (6.09 %) are the main components.

Table 1. Chemical composition of the essential oil of *P. graveolens* identified by GC-MS.

Component	KI exp	KI Lit	Area %	Rt	Similarity (%)
α -pinene	929	932	0.47	5.468	97
β -myrcene	991	990	0.19	8.823	91
<i>P</i> -cymene	1023	1024	0.33	10.754	90
Limonene	1027	1029	0.48	10.960	76
<i>Cis</i> - β -ocimene	1044	1038	1.53	12.069	91
<i>trans</i> - β -ocimene	1054	1048	0.14	12.743	95
<i>trans</i> -linalool oxide	1087	1088	0.24	14.201	64
Linalool	1109	1100	6.09	17.053	95
Menthone	1150	1148	0.99	19.733	96
Iso-menthone	1163	1162	4.04	20.682	96
α -terpineol	1187	1185	0.88	22.551	91
β -citronellol	1235	1232	28.67	26.443	96
Geraniol	1266	1250	14.57	29.175	96
Citronellyl formate	1287	1286	3.29	29.421	86
Geranyl formate	1312	1320	4.22	31.062	83
α -cubebene	1341	1345	0.65	32.913	90
Phenethyl isobutyrate	1353	1401	0.16	33.662	78
α -copaene	1363	1365	0.81	34.354	96
β -bourbonene	1370	1373	0.94	34.811	94
β -cubebene	1377	1392	0.07	35.337	93
β -elemene	1380	1382	0.18	35.548	93
Geranyl acetate	1387	1390	1.12	36.011	91
β -caryophyllene	1401	1419	1.05	36.811	98
(<i>E,Z</i>)- α -farnesene	1427	1487	0.19	38.269	53
α -caryophyllene	1437	1422	0.79	38.840	95
Aromadendrene	1444	1441	0.69	39.258	91
Germacrene-D	1475	1479	3.59	40.698	98

Component	KI exp	KI Lit	Area %	Rt	Similarity (%)
γ-Gurjunene	1478	1473	0.54	41.025	95
(-)-Nerolidol	1480	1564	1.10	41.418	70
Ledene	1482	1491	0.99	41.567	96
α-Muurolene	1489	1484	0.43	42.001	93
α-Amorphene	1492	1496	0.92	42.378	95
Zingibrene	1495	1494	0.10	42.641	83
γ-Cadinene	1508	1509	0.72	42.871	96
δ-Cadinene	1514	1516	2.74	43.413	81
Neryl propionate	1516	-	0.45	43.567	86
Citronellyl propionate	1530	1445	0.47	44.327	87
Isoaromadendrene epoxide	1537	1580	0.47	44.670	54
β-neoclovene	1609	1556	2.62	48.791	45
α-elemene	1616	-	0.89	49.374	50
β-gurjunene	1624	1439	0.91	49.562	70
Valencene	1632	1628	0.92	49.980	83
β-eudesmol	1636	1651	0.83	50.225	98
Guaiol	1641	1600	1.39	50.488	90
E, E-.α-Farnesene	1654		0.16	51.174	50
Cycloisolongifolene, 8,9-dihydro-	1662	-	0.60	51.597	49
<i>trans</i>-carane	1667	-	0.44	51.866	60
<i>trans</i>-caryophyllene	1696	1600	0.13	53.500	27
Geranyl tiglate	1706	1701	3.09	53.998	90
Alloaromadendrene oxide-(1)	1729	1749	0.04	55.129	41
α-Caryophyllene	1757	-	0.15	56.524	91
Geranyl isovalerate	1912	1887	0.05	64.228	64
Farnesyl acetone c	1914	1921	0.08	64.360	38

KIexp = Kovats index in experiment; KILit = Kovats index in literature.

Insecticidal activity

The effectiveness of the essential oil was evidenced by the induction of mortality in target individuals. The toxic effects of *P. graveolens* essential oil are based on percent mortality and the determination of lethal doses (LD50 and LD90). Results show the percent mortality of *E. kuehniella* pupae scored at 72 h after exposure to different EO concentrations. The death of *E. kuehniella* pupae is mainly characterized by a complete absence of movement and a color change, shifting from brown to darker shades, or even black. As shown in Fig. 1, EO induced a dose-dependent increase in the mortality of *E. kuehniella*. Interestingly, the EO of *P. graveolens* at the lowest dose (10 $\mu\text{L mL}^{-1}$) caused mortality in *E. kuehniella* pupae ($41.11 \pm 8.39\%$). In contrast, at the highest dose (20 $\mu\text{L/mL}$), the EO induced a higher mortality rate of $62.26 \pm 3.84\%$ compared to the control series. According to the Probit analysis, the LD50 and LD90 values were 13.18 $\mu\text{L mL}^{-1}$ and 85.11 $\mu\text{L mL}^{-1}$, respectively. The insecticidal activity of *P. graveolens* EO against *E. kuehniella* is shown in Table 2.

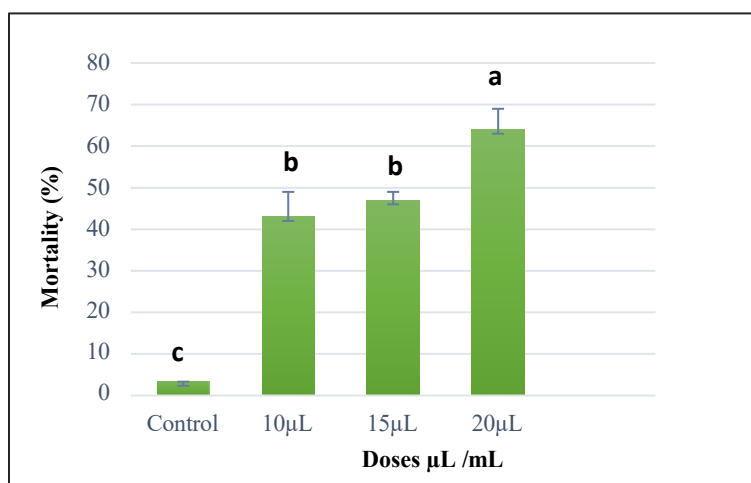


Fig. 1. Percentage of observed mortality of *E. kuehniella* pupae females treated by topical application with *P. graveolens* essential oil (mean $\pm$ SD); values followed by different letters are significantly different at $P < 0.05$.

Table 2. Lethal doses ($\mu\text{L mL}^{-1}$, FL= 95 %) of *P. graveolens* essential oil against pupae females of *E. kuehniella* after 72 h following treatments.

Treatment	Regression equation	Slope	LD50	LD90
<i>P. graveolens</i>	$Y=1.693 X+3.01$	3.01	(95% FL)	(95% FL)
			13.18 (10.54 – 16.60)	85.11 (68.08- 106.38)

Effect of *P. graveolens* EO on the duration of pupal development

The period duration of pupal development, identified by the number of days separating nymphal exuviation from adult exuviation, was determined. Thus, EO led to a highly significant reduction ($P=0.001$) in the duration of pupal development as compared to the control series. The values recorded were 15 ± 1.0 days for the treated series with LD50 against 10 ± 0.6 days for the control series.

Effect of EO on preoviposition and oviposition durations

The statistical test revealed a highly significant difference ($P=0.003$) between the values recorded in the treated and control groups. As shown in Table 3, the pre-oviposition duration in treated females was prolonged by an average of 2 days compared with the control group. In contrast, the oviposition duration was reduced by an average of 4 days.

Table 3. Effect of *P. graveolens* essential oil applied topically on newly molted pupae on preoviposition and oviposition duration of *E. kuehniella*.

Treatment/Duration	Preoviposition	Oviposition
Control	1.60 ± 0.54	6.00 ± 0.70
DL50	3.20 ± 0.83 **	2.60 ± 0.50 **

**: highly significant difference ($P \leq 0.01$).

Effect of EO on the fecundity

Female fecundity, determined by the number of eggs laid by females throughout their oviposition period, showed a highly significant difference ($P = 0.004$) between the control and treated series. The number of eggs counted for both series was 128.00 ± 7.38 for control females and 40.25 ± 4.35 eggs laid by the treated females.

Effect of EO on ovarian morphometric measurements

The treatment applied to newly exuviated female *E. kuehniella* pupae significantly reduced ovary weight ($P=0.019$), basal oocyte length ($P=0.002$), and basal oocyte volume ($P=0.011$) compared with controls. On the other hand, it induced a highly significant reduction in basal oocyte width ($P=0.001$), and in the number of oocytes per pair of ovaries ($P=0.004$), as the number of oocytes in controls was almost double that in the ovaries of treated females (Table 4).

Table 4. Effect of *P. graveolens* essential oil applied on pupae on ovarian weight (mg), number of oocytes, basal oocyte size (μm), and volume (mm^3) in newly emerged adult females of *E. kuehniella* (mean ± SD, $N=30$).

Treatment	Ovarian weight (mg)	Oocyte number	Basal oocyte Length (μm)	Basal oocyte Width (μm)	Basal oocyte Volume (mm^3)
Control	4.70 ± 0.05	108.66 ± 2.08	525.00 ± 25.00	260.67 ± 12.80	0.01 ± 0.00
DL50	3.00 ± 0.04*	64.00 ± 4.35**	308.32 ± 14.40*	183.30 ± 14.40**	0.00 ± 0.00 *

*: Significant difference ($p \leq 0.05$); **: highly significant difference ($p \leq 0.01$).

Effect of EO on vitellin amounts

Quantitative assessment of vitellin content recorded in newly emerged adult female ovaries reveals a significant difference ($P= 0.046$) between vitellin content determined in the ovaries of treated (73.40 ± 1.90) and control (174.31 ± 19.97) samples.

Molecular docking

Prior to docking the EO constituents, the protocol was validated by re-docking the co-crystallized ligand JH III into the active site of mJHBP. The obtained RMSD value of 0.22 Å between the crystallographic and predicted poses confirmed the reliability and accuracy of the docking protocol, as RMSD values below 2.0 Å are generally considered acceptable for docking validation (Fig. 2).

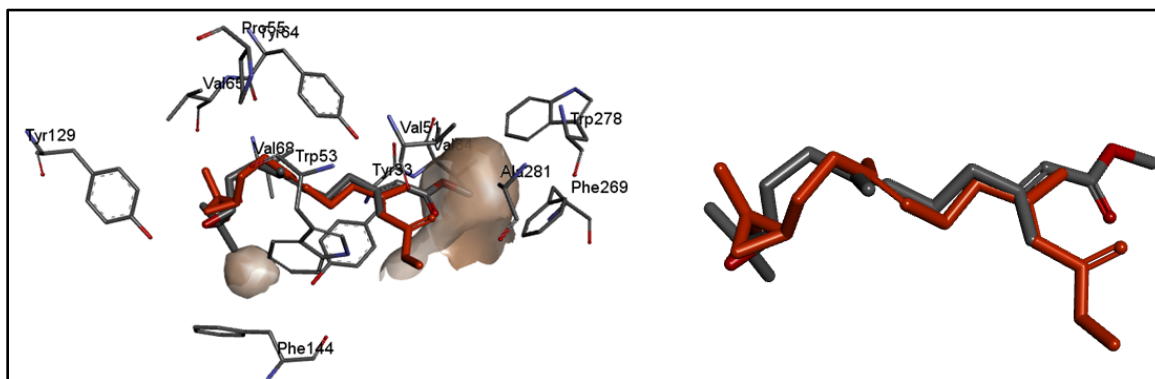


Fig. 2. Re-docking the co-crystallized ligand of the juvenile hormone-binding protein (JHBP) with the ligand JH III (PDB ID: 5V13).

Analysis of the binding pocket revealed a predominantly hydrophobic cavity formed by residues Tyr-33, Trp-53, Tyr-64, Val-65, Val-68, Leu-72, Leu-74, Tyr-129, Phe-144, and Val-51. These residues contribute significantly to the stabilization of the natural ligand JH III through hydrophobic contacts and hydrogen-bond interactions (Fig. 3) [52-54].

Fig. 3 illustrates the crystal structure of the juvenile hormone-binding protein (JHBP) with the ligand JH III (PDB ID: 5V13) at a resolution of 1.84 Å.

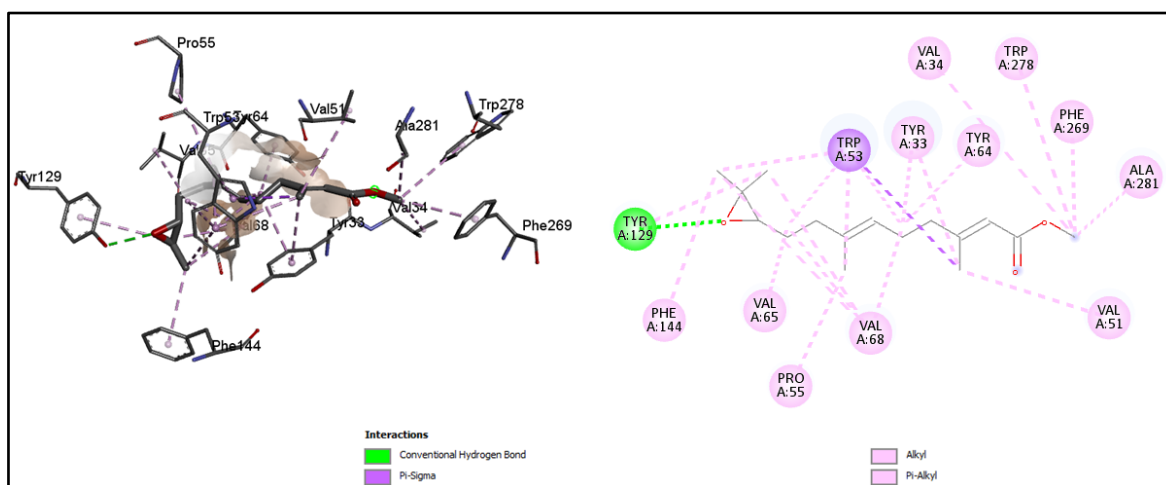


Fig. 3. The crystal structure of the juvenile hormone-binding protein (JHBP) with the ligand JH III (PDB ID: 5V13).

The binding free energy values of the main components studied (β -citronellol, geraniol, linalool, geranyl formate, citronellyl formate, geranyl tiglate, and citronellyl propionate) of the EO extracted from *P. graveolens* and the ligand (JH III) are mentioned in Table 5.

Table 5. Summary of docking results in the active site of the juvenile hormone-binding protein (JHBP) (PDB ID: 5V13).

N	Protein	Compound	RMSD	Free Energy of Binding	Inhibition Constant, Ki	Amino acids involved in interactions	
1	JHBP	JH III	0.22	-9.13	201.77 nM	Hydrogen interactions	Tyr-129
						Hydrophobic interactions	Phe-144, Tyr-64, Trp-53, Val-65, Val-68, Leu-72, Leu-74, Val-51, Trp-278, Phe-269, Pro-55 and Tyr-33
2	JHBP	Linalool	-	-5.84	52.7 μ M	Hydrogen interactions	Tyr-133
						Hydrophobic interactions	Tyr-64, Trp-53, Val-65, Val-68, Val-51 and Tyr-33
3	JHBP	Citronellyl formate	-	-6.2	28.55 μ M	Hydrophobic interactions	Tyr-133, Tyr-64, Trp-53, Val-68, Leu-37, Val-51, Pro-55 and Tyr-33
4	JHBP	Citronellyl propionate	-	-7.09	6.38 μ M	Hydrogen interactions	Trp-53
						Hydrophobic interactions	Tyr-133, Tyr-64, Trp-53, Val-51, Pro-55 and Tyr-33
5	JHBP	β -citronellol	-	-5.48	95.84 μ M	Hydrogen interactions	Val-51
						hydrophobic interactions	Tyr-133, Tyr-64, Trp-53, Val-51, Pro-55 and Tyr-33
6	JHBP	Geraniol	-	-5.68	69.05 μ M	Hydrogen interactions	Gly-146
						Hydrophobic interactions	Tyr-64, Trp-53, Val-51, Val-68, Ala-281 and Tyr-33
7	JHBP	Geranyl formate	-	-6.45	18.72 μ M	Hydrogen interactions	
						Hydrophobic interactions	Pro-55, Tyr-64, Trp-53, Val-65, Val-68, Leu-37 and Tyr-33
8	JHBP	Geranyl tiglate	-	-8.24	917.81 nM	Hydrogen interactions	Tyr-129
						Hydrophobic interactions	Phe-144, Tyr-64, Trp-53, Val-65, Val-68, Leu-72, Leu-74, Val-51 and Tyr-33

Among the tested compounds, geranyl tiglate exhibited the strongest binding affinity toward mJHBP, with a binding free energy of -8.24 kcal/mol and an estimated inhibition constant (K_i) of 917.81 nM. The compound established a hydrogen bond with Tyr-129, the same residue involved in the interaction of the native ligand JH III, together with several hydrophobic interactions involving Phe-144, Tyr-64, Trp-53, Val-65, Val-68, Leu-72, Leu-74, Val-51, and Tyr-33. The similarity of these interactions to those observed for JH III suggests that geranyl tiglate may effectively occupy the hormone-binding pocket and interfere with the biological function of mJHBP (Fig. 4) [52-54].

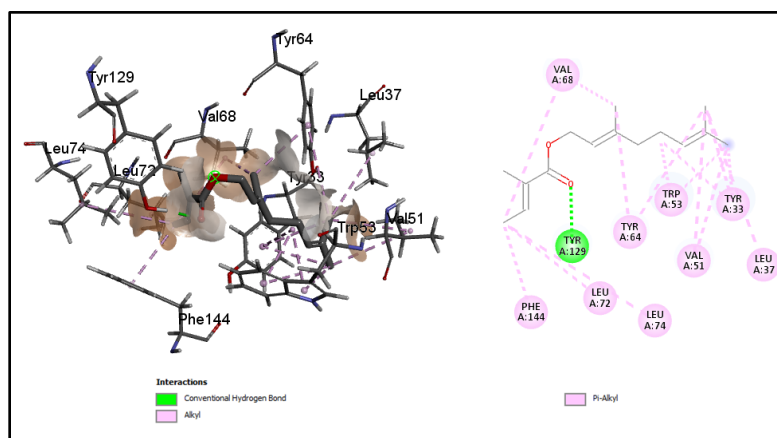


Fig. 4. Binding site structure of geranyl tiglate-juvenile hormone-binding protein (JHBP).

Citronellyl propionate also displayed a relatively favorable binding affinity (-7.09 kcal/mol), forming a hydrogen bond with Trp-53 and several hydrophobic contacts within the active site (Fig. 7). In contrast, linalool, β -citronellol, geraniol, citronellyl formate, and geranyl formate showed weaker binding energies, ranging from -5.48 to -6.45 kcal/mol, indicating lower affinity toward the receptor (Figures 5, 6, 8, 9, 10).

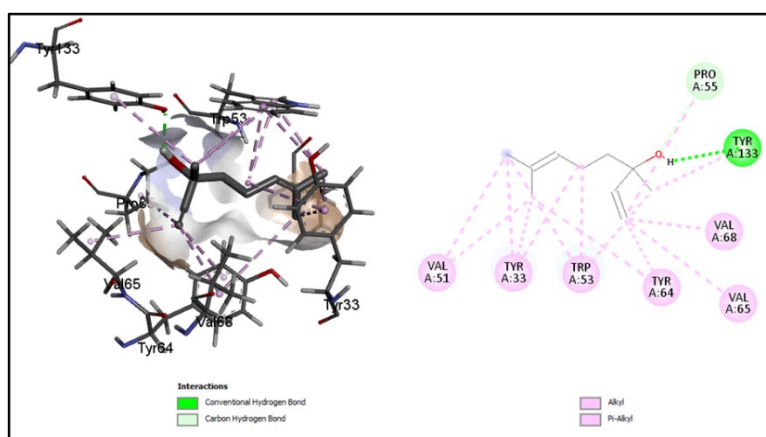


Fig. 5. Binding site structure of linalool-juvenile hormone-binding protein (JHBP).

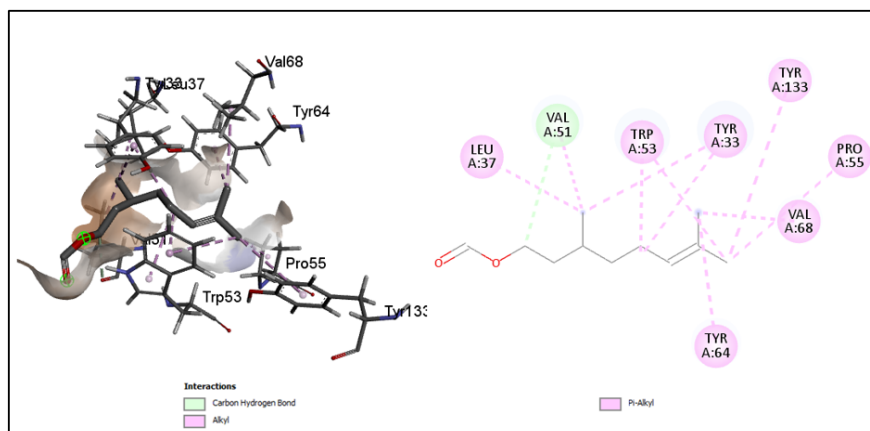


Fig. 6. Binding site structure of citronellyl formate-juvenile hormone-binding protein (JHBP).

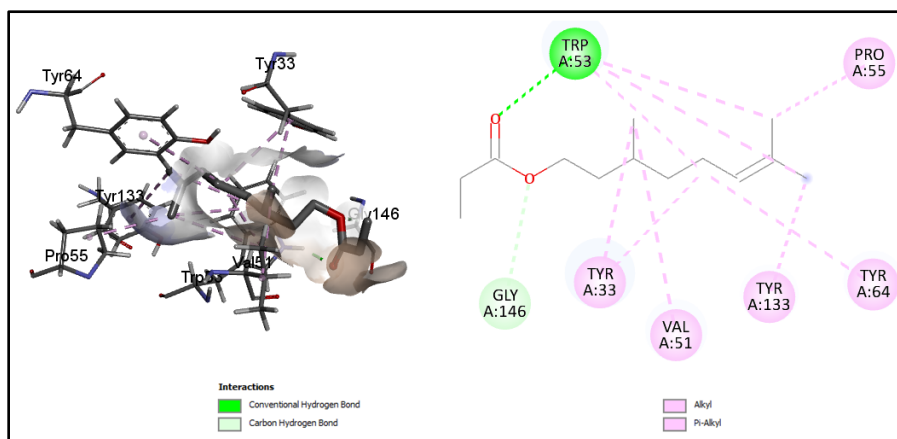


Fig. 7. Binding site structure of citronellyl propionate-juvenile hormone-binding protein (JHBP).

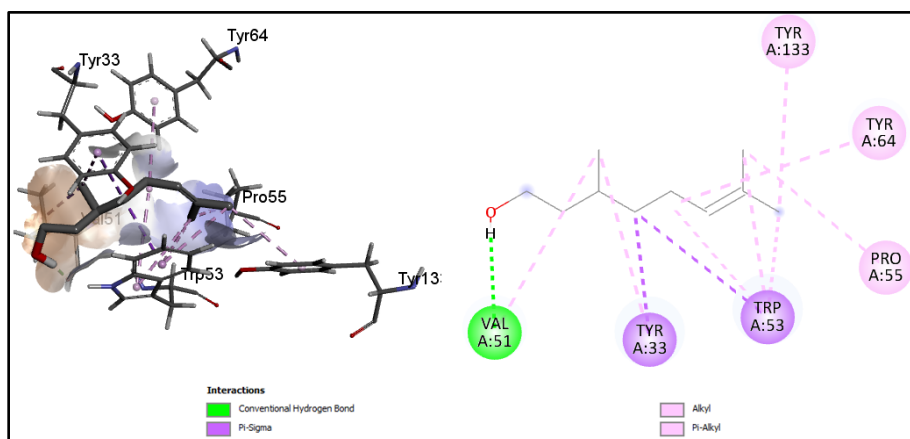


Fig. 8. Binding site structure of β -citronellol-juvenile hormone-binding protein (JHBP).

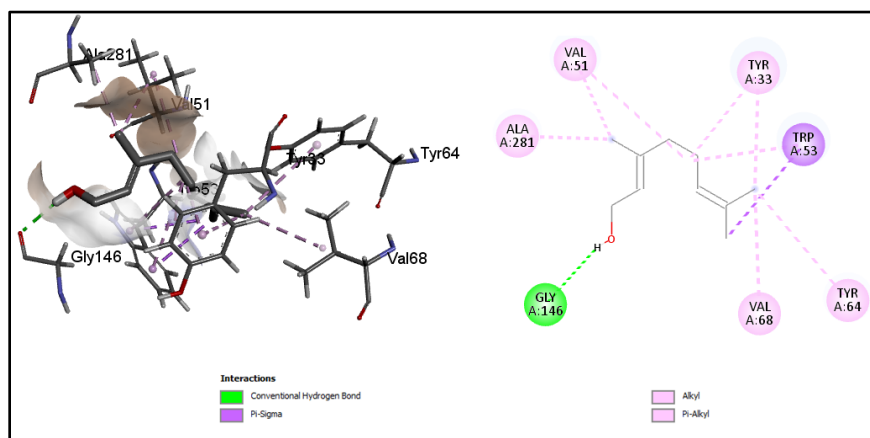


Fig. 9. Binding site structure of geraniol-juvenile hormone-binding protein (JHBP).

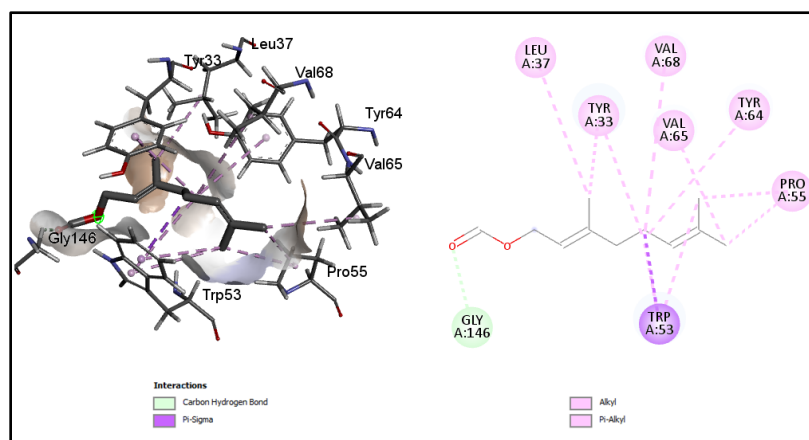


Fig. 10. Binding site structure of geranyl formate-juvenile hormone-binding protein (JHBP).

Overall, the docking results suggest that esterified monoterpenes, particularly geranyl tiglate, possess a greater capacity to interact with the active site of mJHBP than alcohol-containing monoterpenes. These findings support the hypothesis that certain major constituents of *P. graveolens* essential oil may contribute to the observed insecticidal activity by interfering with the juvenile hormone signaling pathway. While this static docking provides robust predictions of binding modes, future Molecular Dynamics (MD) simulations are planned to further validate the dynamic stability and residence time of these protein-ligand complexes over extended periods [54].

Discussion

Essential oil yield

The procedure for extracting EO from the aerial parts (leaf, stem, and flower) of *P. graveolens* harvested during the flowering period in northeastern Algeria gave a satisfactory yield of 0.43 % (w/w), which is higher than that indicated in AFNOR standards (0.2 %), as well as those reported in several other studies. From a nearby sub-humid geographical area, the yield was estimated at 0.15 % (w/w) (Blida, North Algeria) [57], and from Tlemcen (West Algeria) was 0.25 % (w/w) based on the mass of the fresh plant [58]. From Tunisia, Boukhris *et al.* [59]

studied yield during all vegetative stages of the plant; the highest was estimated at 0.16 % (w/w). Benelhadj-Ali *et al.* [60] recorded a yield varying between leaves (0.15 %) (w/w) and flowers (0.18 %) (w/w).

Several intrinsic and extrinsic factors influence the yield of essential oils. These include climatic, geological, genetic, and technical conditions [61-63]. As far as climatic factors are concerned, temperature and rainfall play a very important role, with extreme temperatures (drought/humidity) and water deficit reducing yields by up to 50 % [64-67]. Abu-El-Wafa *et al.* [68] studied the yield of *P. graveolens* during several periods of the year and recorded the highest percentage in autumn, when the climate is moderate. In addition, the harvesting technique and the phenological cycle at harvest are involved. Hazrati *et al.* [69] showed the influence of this period on essential oil yield. The results showed a 50 % reduction over a few hours on the same day.

Chemical composition

Out of a total of 96.40 % spread over 54 compounds, chemical analysis of *P. graveolens* essential oil reveals a dominance of β -citronellol (28.67 %), geraniol (14.57 %), and linalool (6.09 %). These molecules belong to the oxygenated monoterpene class, which accounts for 88.69 % of all monoterpenes. This is in line with that obtained by Fayed. [70] and Boukhris *et al.* [59] who reported that *P. graveolens* essential oil was rich in oxygenated components. In line with our results, Rana *et al.* [71] from North India recorded the same components in dominance with a percentage of (33.60 %) for β -citronellol, (26.80 %) for geraniol, and (10.50 %) for linalool. Boukhatem *et al.* [57] and Boukhris *et al.* [72,59], on the other hand, highlight the predominance of citronellol, citronellyl formate, and geraniol. Furthermore, other studies from different geographical areas (Serbia, India, Egypt, and Tunisia) reported similar results, but linalool came fourth, preceded by citronellyl formate (10.66 %; 12.14 %) [73,74] and menthol (6.70 %) [75]. From Egypt, Abouelatta *et al.* [7] reported that the EO of *P. graveolens* grown in Cairo, a subtropical region, has citronellol (29.76 %), geraniol (12.53 %), δ -selenine (9.28 %), and citronellyl formate (7.10 %) as major components. Likewise, in Tajikistan (Central Asia), the chemical composition of *P. graveolens* EO shows dominance of citronellol, geraniol, caryophyllene oxide, and menthone [76]. In contrast, from South Africa, isomenthone and decanoid acid were the main compounds of the EO, with percentages of (65.8 %) and (12.9 %), respectively; while citronellol, citronellol, and citronellyl formate were present in trace amounts [77].

The chemical composition of the plant's essential oil varies due to a complex interplay of factors affecting the biosynthesis of its molecules. In addition to plant genetic factors [67,37], external factors such as those mentioned above that influence essential oil yield (climatic, geological, and technical factors) are responsible for the variation in chemical composition from one species to another and even within the same species. However, the majority of compounds for a given species are often the same, with percentage differences.

Insecticidal activity

Essential oils (EOs) have recently demonstrated powerful insecticidal efficacy, making them promising new products for pest control strategies with minimal impact on the environment and human health [78-81]. They have shown significant toxicological activity against a wide variety of insects, primarily acting via contact [82,83]. Contact toxicity occurs after contact across the entire surface of the insect's cuticle [84] or through direct application of droplets onto the insect's body surface [85], as well as via indirect contact with a surface treated with the insecticide [86]. In the present study, the toxicity of the essential oil extracted from *P. graveolens* collected at Annaba, a sub-humid area, was evaluated against *E. kuehniella* by topical application for the first time. Furthermore, few studies have reported the contact-mode effect of *P. graveolens* EO on pest insects. The toxicological trials of *P. graveolens* EO showed lethality, evidenced by increased pupal mortality and inhibition of adult emergence in *E. kuehniella*, particularly at the highest concentrations. At 20 $\mu\text{L mL}^{-1}$, the EO induced a mortality rate of 62.26 % compared to the control, with an LD50 of 13.18 $\mu\text{L/mL}$ and an LD90 of 85.11 $\mu\text{L mL}^{-1}$. According to Kim *et al.* [87], the toxic effects of essential oils vary based on several factors, including the insect species, the oil source, the mode of application, and the duration of exposure. Previous studies on the insecticidal activity of the EO from *P. graveolens* leaves have reported potent insecticidal activity against *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrichidae) [88] and *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) [89]. Furthermore, the toxicity of the vapors of geraniol, linalool, and citronellol, the main chemical components of this EO, was found to be comparable to that of the whole oil [90-92]. These findings suggest that the EO of *P. graveolens* and its associated monoterpenes could be used to develop effective natural-product-based pest management compounds.

Reproduction is a fundamental parameter that ensures the survival of living beings, whatever their division. It also plays a crucial role in maintaining the ecological balance and diversity of ecosystems. Developing new strategies to prevent and control their infestation is therefore of crucial interest.

In the normal state, sexual development, oogenesis, and vitellogenesis occur during the pupal stage in female *E. kuehniella*. In addition, mating takes place a few hours after adult exuviation, and oviposition is no longer delayed. Similarly, the oviposition period averages 4 days [38,93]. In this respect, the treatment used was administered to newly ecdysed female pupae during the first few hours of their nymphal.

Our results are consistent with a few studies that have investigated the reproduction of *E. kuehniella*. Delimi *et al.* [94,35] studied the insecticidal effect of *Artemisia herba alba* EO administered by inhalation and topical application with different doses. The results revealed a dose-response disturbance in the reproductive potential of *E. kuehniella*. Pupal development and the pre-oviposition period have been prolonged, and the oviposition period and the number of eggs laid have been reduced, affecting fertility. Even after the eggs hatch, the emerging larvae cannot live for more than 24 hours. Taibi *et al.* [6] reported that Oregano (*Origanum vulgare*) EO administered by inhalation in female pupae disrupts the reproductive potential of the moth. The pupal development period was elongated, with a highly significant difference between the controls and the treated, and oviposition duration was reduced after treatment, thereby affecting female fertility. Lahcene *et al.* [95] tested four different *Olea europaea* leaf and stem extracts, and it was shown that the oviposition period is significantly reduced after exposure of females to the tested extracts. Shehu *et al.* [96] observed disruption in the development and reproduction of *Ephestia* sp. following treatment with neem EO. In addition, Bachrouche *et al.* [97] demonstrate that exposure of *E. kuehniella* females to bio-insecticides represented by EO from *Pistacia lentiscus* L. significantly reduced the fecundity, hatching rate, and even reduced copulation rate. Hami *et al.* [36] studied the bio-insecticidal effect of two aromatic plants EOs against *P. interpunctella*; the results showed a disturbance in reproduction with a reduction in fecundity, with 166.6 eggs/female for the control compared with 106 and 40.8 eggs female⁻¹ for the treated ones by *Urtica dioica* and *Lantana Camara* EOs, respectively. Furthermore, Abouelatta *et al.* [7] showed that *P. graveolens* EO had a deterrent effect on the oviposition of adult *Callosobruchus maculatus* females with all doses used; however, the highest dose completely deterred the females' ability to lay eggs.

Two main hormones, juvenile hormone (JH) and ecdysone, secreted by corpora allata, the prothoracic gland, and reproductive organs, regulate insect reproduction [98-101]. Hormone secretion is indirectly influenced by several external and internal factors, such as temperature, photoperiod, nutrition, and hormonal factors [102-106]. Furthermore, both hormones act in coordination and play a significant role in metamorphosis [107]. It has been shown that they are also involved in oogenesis, ovulation, control of vitellogenesis, and ovarian development [108-111]. The application of some ecdysteroid agonists in *E. kuehniella* disrupted ovarian weight, oocyte size, and number [18, 111]. Moreover, the application of certain HJ analogs, such as methoprene and pyriproxyfen, has adversely affected reproductive potential in several species [112,113]. In addition, it has been shown that the above-mentioned hormones regulate reproductive diapause in lepidopteran species, such as *E. kuehniella* [114] and *P. caureum* [115]. Accordingly, the reduction in ovarian morphometry, as reflected in the number, weight, length, width, and volume of basal oocytes after treatment with *P. graveolens* EO, could be explained by the effect of *P. graveolens* EO on hormonal secretion, thereby disrupting oogenesis and vitellogenesis.

Molecular docking

The Juvenile Hormone-Binding Protein (JHBP) plays a vital role in regulating Juvenile hormones (JHs) [51,52], which govern insect growth, development, and reproduction by stabilizing and transporting JH II and JH III. Targeting JHBP provides a precise way to disrupt these processes, leading to developmental and reproductive failures in pests. By mimicking or blocking JHBP activity, inhibitors can serve as eco-friendly and species-specific insecticides, offering a promising strategy to combat insect resistance while minimizing environmental impact [53,54]. This study highlights JHBP's potential as a key biological target for innovative pest control solutions

Firstly, to determine the blocking mechanism of the JHBP receptor, the JH III binding site in the receptor's crystal structure has been well studied. The N-terminal binding pocket of JHBP comprises amino acid residues that play a crucial role in interacting with the JH III molecule. This pocket features a hydrophobic environment formed by residues such as Phe-144, Tyr-64, Trp-53, Val-65, Val-68, Leu-72, Leu-74, Val-51, and Tyr-33, which stabilize the isoprenoid chain of JH III. Additionally, several specific interactions contribute to this affinity: the phenolic hydroxyl group of Tyr-129 forms a hydrogen bond with the epoxy group of JH III.

At the same time, an ordered water molecule establishes a hydrogen bond with the methyl ester group of the ligand. Finally, the epoxy end of JH III is deeply embedded in the pocket, while the methyl ester group is oriented toward the surface, suggesting a directional interaction [52-54,116,117]. This arrangement allows the JHBP binding pocket to show a strong affinity and high selectivity for the natural form of JH III.

In addition, various interactions were recorded between the EO compounds and the biological target. Hydrophobic interactions and hydrogen bonding effectively stabilize the compounds and limit access to the active site, highlighting the potential insecticidal properties of the compounds studied. Consequently, the *in silico* study confirms the hypothesis and provides evidence of EO molecules interacting with the biological target.

Conclusions

The current study, conducted to address the various threats to food safety posed by harmful insect pests, demonstrated *in vitro* and *in silico* the efficacy of the essential oil from the aromatic plant *Pelargonium graveolens* against the Mediterranean flour moth, *Ephestia kuehniella*. Topical application of the EO exhibits significant toxicity and disturbs the moth's development and reproduction. In addition, molecular docking explains the results obtained and demonstrates the different interactions between the main components of the EO, as determined by GC-MS, and the biological target. Our findings indicate that the EO exhibits significant insecticidal activity against the targeted insect pest, highlighting its potential as an effective and sustainable alternative to conventional chemical insecticides in stored-product pest control strategies.

Acknowledgements

The National Fund for Scientific Research, the Laboratory of Applied Animal Biology, Badji Mokhtar University, Annaba, and the Ministry of Higher Education and Scientific Research of Algeria supported this research.

References

1. Aisha, K.; Visakh, N. U.; Pathrose, B.; Mori, N.; Baeshen, R. S. and Shawer, R. *Molecules*. **2024**, *29*, 344. DOI: <https://doi.org/10.3390/molecules29020344>
2. Kim, K. H.; Kabir, E.; Jahan, S.A. *Sci. Total Environ.* **2017**, *575*, 525-535. DOI: <https://doi.org/10.1016/j.scitotenv.2016.09.009>
3. Domingues, P.M.; Santos, L. *Ind. Crops Prod.* **2019**, *139*, 111534. DOI: <https://doi.org/10.1016/j.indcrop.2019.111534>
4. Gupta, S.; Gupta, K., in: *Pesticides in Crop Production: Physiological and Biochemical Action*; John Wiley & Sons Ltd., **2020**; 55–67. DOI: <https://doi.org/10.1002/9781119432241.ch4>
5. Novato, T.P.L.; Araújo, L.X.; de Monteiro, C. M. O., Maturano, R.; Senra, T.D.O.S.; da Silva Matos, R.; Gomes de Carvalho, M.G.; Daemon, E. *Vet. Parasitol.* **2015**, *212*, 331-335. DOI: <https://doi.org/10.1016/j.vetpar.2015.08.021>
6. Taibi, F.; Boumendjel, M.; Zaafour, M.; Sekiou, O.; Khaldi, T.; Delimi, A.; Abdssamad, S.; Rebani, H.; Chnouga, H.; Siakhène, N.; Boumendjel, A.; Mahfoud, M. *Cell Mol. Biol.* **2018**, *64*, 5-11. DOI: <https://doi.org/10.14715/cmb/2018.64.10.2>
7. Abouelatta, A.M.; Keratum, A.Y.; Ahmed, S.I.; El-Zun, H.M. *Int. J. Trop. Insect Sci.* **2020**, *40*, 1021-1030. DOI: <https://doi.org/10.1007/s42690-020-00161-4>

8. Benchaâbane, S.; Ayad, A.S.; Loucif-Ayad, W.; Soltani, N. *Comp. Biochem. Physiol C: Toxicol. Pharmacol.* **2022**, 257, 109334. DOI: <https://doi.org/10.1016/j.cbpc.2022.109334>
9. Serradj, N.; Bendali-Saoudi, F.; Soltani, N. *Pol. J. Entomol.* **2022**, 91, 174-183. DOI: <https://doi.org/10.5604/01.3001.0016.1930>
10. Li, Y.; Wu, W.; Jian, R.; Ren, X.; Chen, X.; Hong, W. D.; Wu, M.; Cai, J.; Lao, C.; Xu, X.; Sheng, Z. *J. Pest Sci.* **2023**, 96, 961-971. DOI: <https://doi.org/10.1007/s10340-022-01555-8>
11. Gacem, H.; Bendali-Saoudi, F.; Serradj, N.; Houmani, M.; Soltani, N. *Appl. Ecol. Environ. Res.* **2023**, 21. DOI: http://dx.doi.org/10.15666/aer/2102_11431155
12. Mulhair, P.O.; Holland, P.W. *Semin. Cell Dev. Biol.* **2024**, 152, 4-15. DOI: <https://doi.org/10.1016/j.semdb.2022.11.010>
13. Lopez-Vaamonde, C.; Agassiz, D.; Augustin, S.; De Prins, J.; De Prins, W.; Gomboc, S.; Ivinskis, P.; Karsholt, O.; Koutroumpas, A.; Koutroumpa, F.; Laštůvka, Z.; Marabuto, E.; Olivella, E.; Przybyłowicz, L.; Roques, A.; Ryrholm, N.; Sefrova, H.; Sima, P.; Sims, I.; Sinev, S.; Skulev, B.; Tomov, R.; Zilli, A.; Lees, D. *BioRisk*. **2010**, 4, 603–668. DOI: <https://doi.org/10.3897/biorisk.4.50>
14. Suckling, D. M.; Conlong, D. E.; Carpenter, J. E.; Bloem, K. A.; Rendon, P.; Vreysen, M. J. B. *Biol. Invasions*. **2017**, 19, 1107-1119. DOI: <https://doi.org/10.1007/s10530-016-1325-9>
15. Vreysen, M.J.; Klassen, W.; Carpenter, J.E. *Fla. Entomol.* **2016**, 99, 1-12. DOI: <https://doi.org/10.1653/024.099.sp102>
16. Trajković, A.; Žikić, V. *Sustainability*, **2023**, 15, 2881. DOI: <https://doi.org/10.3390/su15042881>
17. Karthi, S.; Vasantha-Srinivasan, P.; Senthil-Nathan, S.; Han, Y. S.; Shivakumar, M. S.; Murali-Baskaran, R. K.; Kalaivani, K.; Radhakrishnan, N.; Park, K.B.; Malafaia, G. *Biocatal. Agric. Biotechnol.* **2024**, 58, 103146. DOI: <https://doi.org/10.1016/j.bcab.2024.103146>
18. Hami, M.; BI, F.; Smagghe, G.; Soltani-Mazouni, N. *Comm. Appl. Biol. Sci.* **2005**, 70.
19. Abdi, A.; Naseri, B.; Fathi, S.A.A. *J. Entomol. Soc.* **2014**, 33, 29-41.
20. Sakka, M.K.; Gourgouta, M.; Athanassiou, C.G. *J. Agron.* **2024**, 14, 1307. DOI: <https://doi.org/10.3390/agronomy14061307>
21. Titouhi, F.; Amri, M.; Messaoud, C.; Haouel, S.; Youssfi, S.; Cherif, A.; Mediouni-BenJemâa, J. *J. Stored Prod. Res.* **2017**, 72, 11-20. DOI: <https://doi.org/10.1016/j.jspr.2017.02.007>
22. Bendjedid, H.; Yezli-Touiker, S.; Taffar, A.; Hamel, T.; Soltani, N. *J. Essent. Oil-Bear. Plants*. **2021**, 24, 792-807. DOI: <https://doi.org/10.1080/0972060X.2021.1984315>
23. Tine-Djebbar, F.; Trad, M.; Tine, A.O.; Tine, S.; Soltani, N. *J. Plant Dis. Prot.* **2023**, 130, 509–518. DOI: <https://doi.org/10.1007/s41348-023-00727-7>
24. Abes, I.F.Z.; Soltani, M.; Torchane, N.; Tine, S.; Tine-Djebbar, F.; Soltani, N. *J. Essent. Oil- Bear. Plants*. **2024**, 27, 1157-1174. DOI: <https://doi.org/10.1080/0972060X.2024.2383203>
25. Merzougui, M.; Tine, S.; Tine-Djebbar, F.; Soltani, N. *Int. J. Trop. Insect Sci.* **2024**, 1-13. DOI: <https://doi.org/10.1007/s42690-024-01204-w>
26. Chen, W.; Viljoen, A.M. Geraniol—a review update. *S. Afr. J. Bot.* **2022**, 150, 1205-1219. DOI: <https://doi.org/10.1016/j.sajb.2022.09.012>
27. Huang, D.; Liu, H.; Yin, X.; Yuan, F.; Song, B.; Teng, Y.; Ding, Y.; Chen, Q.; Liu, T. *J. Chem. Eng.* **2024**, 486, 150358. DOI: <https://doi.org/10.1016/j.cej.2024.150358>
28. Isman, M. B.; Grieneisen, M. L. *Trends Plant Sci.* **2014**, 19, 140-145.
29. Campos, E. V.; Proença, P. L.; Oliveira, J. L.; Bakshi, M.; Abhilash, P. C.; Fraceto, L. F. *Ecol. Indic.* **2019**, 105, 483-495. DOI: <https://doi.org/10.1016/j.ecolind.2018.04.038>

30. Bachrouh, O.; Zarroug, Y.; Bourgou, S.; Charradi, K.; Sriti, J.; Msaada, K.; Jallouli, S.; Chaibi, K.; Haouel-Hamdi, S.; Abderraba, M.; Ben Jemâa, J. M. *J. Mex. Chem. Soc.* **2023**, *67*, 152-162. DOI: <http://dx.doi.org/10.29356/jmcs.v67i2.1875>
31. Grine, S.; Taibi, F.; Berredjem, M.; Dekir, A.; Benaliouche, F.; Rachedi, K. O.; Soltani, N. *J. Mol. Struct.* **2023**, *1294*, 136546. DOI: <https://doi.org/10.1016/j.molstruc.2023.136546>
32. Nishimura, H. *Aroma. Res.* **2001**, *2*, 257-267.
33. Aouadi, G.; Haouel, S.; Soltani, A.; Ben Abada, M.; Boushih, E.; Elkahoui, S.; Taibi, F.; Mediouni-BenJemaa, J.; Bennadja, S. *J. Plant Dis. Prot.* **2020**, *127*, 471-482. DOI: <https://doi.org/10.1007/s41348-020-00340-y>
34. Rekioua, N.; Boumendjel, M.; Taibi, F.; Samar, M.F.; Jemaa, J.M.B.; Benaliouch, F.; Negro, C.; Nicoli, F.; De Bellis, L.; Boushih, E.; Haouel, S. *Cell Mol. Biol.* **2022**, *68*, 144-157. DOI: <https://doi.org/10.14715/cmb/2022.68.4.18>
35. Delimi, A.; Taibi, F.; Bouchelaghem, S.; Boumendjel, M.; Hennouni-Siakhène, N.; Chefrour, *Int. J. Biosci.* **2017**, *10*, 130-137. DOI: <https://doi.org/10.12692/ijb/10.2.130-8>
36. Hami, M.; Zidi, S.; Hamdiken, M.; Grara, N.; Atoki, A.V.; Messaoudi, M. *J. Biopest.* **2024**, *17*, 72-85. DOI: <https://doi.org/10.57182/jbiopestic.17.2.72-85>
37. Tine, S.; Tine-Djebbar, F.; Debab, A.; Mesloub, A.; Soltani, N. *J. Plant Dis. Prot.* **2023**, *130*, 769-780. DOI: <https://doi.org/10.1007/s41348-023-00766-0>
38. Soltani-Mazouni, N.; Hami, M.; Gramdi, H. *Invertebr. Reprod. Dev.* **2012**, *56*, 157-163. DOI: <https://doi.org/10.1080/07924259.582695>
39. Roy, S.; Prasad, A.K. *Phytoparasitica.* **2018**, *46*, 405-410. DOI: <https://doi.org/10.1007/s12600-018-0670-x>
40. Hamaidia, K.; Soltani, N. *J. Entomol. Res.* **2019**, *43*, 125-130. DOI: <https://doi.org/10.5958/0974-4576.2019.00024.0>
41. Bensafi-Gheraibia, H.; Kissoum, N.; Hamida, Z.C.; Farine, J.P.; Soltani, N. *Invertebr. Reprod. Dev.* **2021**, *65*, 35-47. DOI: <https://doi.org/10.1080/07924259.2020.1862315>
42. Merad, I.; Rabei, R.; Soltani, N. *Molluscan Res.* **2024**, *44*, 11-21. DOI: <https://doi.org/10.1080/13235818.2023.2277834>
43. Soltani, N.; Delbecque, J.P.; Delachambre, J. *Pestic. Sci.* **1983**, *14*, 615-622. DOI: <https://doi.org/10.1002/ps.2780140609>
44. Abbott W.S. *J. Econ. Entomol.* **1925**, *18*, 265-267.
45. Fisher, R. A.; Yates, F. *Statistical Tables for Biological, Agricultural and Medical Research*; Oliver & Boyd: London, **1957**; 138.
46. Finney, D. J. in: *Probit Analysis*, Cambridge University Press, Cambridge, UK, **1971**.
47. Swaroop, S.; Gilroy, A. B.; Uemura, K. *Statistical Methods in Malaria Eradication*; WHO Monograph Series No. 51; World Health Organization: Geneva, **1966**; 164.
48. Lumbreras, C. J.; Galante, E.; Mena, J. *Ann. Entomol. Soc. Am.* **1991**, *84*, 190-194.
49. Postlethwait, J.H.; Bownes, M.; Jowett, T. *Dev. Biol. J.* **1980**, *79*, 379-387.
50. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248-254.
51. Shinoda, T.; Itoyama, K. *Proc. Natl. Acad. Sci.* **2003**, *100*, 11986-11991. DOI: <https://doi.org/10.1073/pnas.2134232100>
52. Kim, I.H.; Pham, V.; Jablonka, W.; Goodman, W.G.; Ribeiro, J. M.; Andersen, J. F. *J. Biol. Chem.* **2017**, *292*, 15329-15339.
53. Ramos, R. S.; Macêdo, W. J.; Costa, J. S.; da Silva, C.H.D.P.; Rosa, J. M.; da Cruz, J. N.; de Oliveira, M.S.; Andrade, E.H.D.A.; Silva, R.B.L.E.; Souto, R.N.P.; Santos, C. B. *J. Biomol. Struct. Dyn.* **2020**, *38*, 4687-4709. DOI: <https://doi.org/10.1080/07391102.2019.1688192>

54. Hiroyoshi, S.; Reddy, G.V.; Mitsuhashi, J. *J. Comp. Physiol.* **2017**, *203*, 635-647. DOI: <https://doi.org/10.1007/s00359-017-1179-3>
55. Djouad, S.E.; Berredjem, M.; Aoul, F.Z.H.; Bouchareb, F.; Guerfi, M.; Ben Hadda, T.; Aissaoui, M.; Belhani, B. *J. Indian Chem. Soc.* **2022**, *99*, 100650. DOI: <https://doi.org/10.1016/j.jics.2022.100650>
56. Madhavi Sastry, G.; Adzhigirey, M.; Day, T.; Annabhimoju, R.; Sherman, W. *Aided Mol. Des.* **2013**, *27*, 221-34.
57. Boukhatem, M.N.; Kameli, A.; Saidi, F. *Food Control.* **2013**, *34*, 208-213. DOI: <https://doi.org/10.1016/j.foodcont.2013.03.045>
58. Medjdoub, H.; Bouali, W.; Semaoui, M.; Benaissa, A.; Chaib, F.; Azzi, A. *Chem. Pap.* **2024**, 1-8. <https://doi.org/10.1007/s11696-024-03859-8>
59. Boukhris, M.; Hadrich, F.; Chtourou, H.; Dhoub, A.; Bouaziz, M.; Sayadi, S. *Ind. Crops Prod.* **2015**, *74*, 600-606. DOI: <https://doi.org/10.1016/j.indcrop.2015.05.051>
60. Ben Elhadj-Ali, I.; Tajini, F.; Boulila, A.; Jebri, M. A.; Boussaid, M.; Messaoud, C.; Sebaï, H. *Ind. Crops Prod.* **2020**, *158*, 112951. DOI: <https://doi.org/10.1016/j.indcrop.2020.112951>
61. Ben-Chaabane, S.; Haouel-Hamdi, S.; Bachrouch, O.; Mahjoubi, K.; Mediouni-BenJemâa, J. *Int. J. Environ. Health Res.* **2024**, *34*, 419-431. DOI: <https://doi.org/10.1080/09603123.2022.2152431>
62. Lončar, B.; Pezo, L.; Pezo, M.; Jovanović, A.; Šuput, D.; Radosavljević, M.; Acimović, M. *Horticult.* **2024**, *10*, 577. DOI: <https://doi.org/10.3390/horticulturae10060577>
63. Rakic, Z.; Johnson, C.B. in: *Breeding Research on Aromatic and Medicinal Plants*. CRC Press, **2024**, 157-162.
64. Bettaib, I.; Zakhama, N.; Wannes, W.A.; Kchouk, M.E.; Mabrouk, B. *Sci. Hortic.* **2009**, *120*, 271-275. DOI: <https://doi.org/10.1016/j.scienta.2008.10.016>
65. Correl, M.; Garcia, M. C.; Contreras, J. I.; Segura, M. L.; Cermeño, P. *Commun. Soil Sci. Plant Anal.* **2012**, *43*, 419-425. DOI: <https://doi.org/10.1080/00103624.2012.641811>
66. Ferrante, A.; Mariani, L. *Hortic.* **2018**, *4*, 21. DOI: <https://doi.org/10.3390/horticulturae4030021>
67. Valkovszki, N.J.; Szalóki, T.; Székely, Á.; Kun, Á.; Kolozsvári, I.; Szalókiné-Zima, I.; Tavaszi-Sárosi, S.; Jancsó, M. *Hortic.* **2023**, *9*, 1037. DOI: <https://doi.org/10.3390/horticulturae9091037>
68. Abu El Wafa, S. S.; El-Ashmawy, A.A.; Kassem, H.A.; Eissa, I.H.; Abu-Elghait, M.; Younis, N.A.; Younis, I.Y. *Sci. Rep.* **2023**, *13*, 19887. DOI: <https://doi.org/10.1038/s41598-023-47170-0>
69. Hazrati, S.; Beidaghi, P.; Beyraghdar Kashkooli, A.; Hosseini, S.J.; Nicola, S. *Horticult.* **2022**, *8*, 149. DOI: <https://doi.org/10.3390/horticulturae8020149>
70. Fayed, S. A. *Res. J. Agric. Biol. Sci.* **2009**, *5*, 740-747.
71. Rana, V. S.; Juyal, J. P.; Blazquez, M.A. *Int. J. Aromather.* **2002**, *12*, 216-218. DOI: [https://doi.org/10.1016/S0962-4562\(03\)00003-1](https://doi.org/10.1016/S0962-4562(03)00003-1)
72. Boukhris, M.; Simmonds, M.S.; Sayadi, S.; Bouaziz, M. *Phytother. Res.* **2013**, *27*, 1206-1213. DOI: <https://doi.org/10.1002/ptr.4853>
73. Dzamic, A. M.; Sokovic, M.D.; Ristic, M.S.; Grujic, S.M.; Mileski, K.S.; Marin, P.D. *J. Appl. Pharm. Sci.* **2014**, *4*, 001-005. DOI: <https://doi.org/10.7324/JAPS.2014.40301>
74. Gogoi, R.; Begum, T.; Sarma, N.; Tamang, R.; Chanda, S.K.; Lal, M.; Pervin, K.; Khan, F.; Marinković, J. *J. Essent. Oil-Bear. Plants.* **2024**, *27*, 135-151. DOI: <https://doi.org/10.1080/0972060X.2024.2311825>
75. Kačániová, M.; Vukic, M.; Vukovic, N.L.; Čmiková, N.; Verešová, A.; Schwaržová, M.; Babošová, M.; Porhajašová, J.I.; Kluz, M.; Waszkiewicz-Robak, B.; Ben Hsouna, A.; Ben Saad, R.; Garzoli, S. *Foods.* **2023**, *13*, 33. DOI: <https://doi.org/10.3390/foods13010033>
76. Sharopov, F.S.; Zhang, H.; Setzer, W.N. *Am. J. Essent. Oil.* **2014**, *2*, 13-16.
77. Lalli, J. Y.; Viljoen, A.M.; Başer, K.H.C.; Demirci, B.; Özek, T. *J. Essent. Oil Res.* **2006**, *18*, 89-105. DOI: <https://doi.org/10.1080/10412905.2006.12067128>

78. Papanikolaou, N.E.; Kavallieratos, N.G.; Iliopoulos, V.; Evergetis, E.; Skourti, A.; Nika, E.P.; Haroutounian, S.A. *J. Insects*. **2022**, *13*, 165. <https://doi.org/10.3390/insects13020165>
79. Song, C.; Zhao, J.; Zheng, R.; Hao, C.; Yan, X. *J. Asia Pac. Entomol.* **2022**, *25*, 101881. DOI: <https://doi.org/10.1016/j.aspen.2022.101881>
80. Acidi, A.; Siakhene, N.; Grine, S.; Bouasla, R.; Rizi, A.; Otmane-Rachedi, K.; Dekir, A.; Benaliouche, F.; Bahadi, R.; Taibi, F.; Ferkous, H.; Berredjem, M. *Chem. Afr.* **2025**, *8*, 1365-1376. DOI: <https://doi.org/10.1007/s42250-025-01225-z>
81. Bouzeraa, N.; Saoudi, B.; Grine, S.; Bouzeraa, H.; Samar, M. F.; Negro, C.; Luvisi, A.; De Bellis, L.; Djahoudi, A.; Benaliouche, F.; Houali, K.; Taibi, F.; Boumendjel, M. *Sustainability*. **2025**, *17*, 11380. DOI: <https://doi.org/10.3390/su172411380>
82. Pang, X.; Feng, Y.-X.; Qi, X.-J.; Xi, C.; Du, S.-S. *Int. J. Food Prop.* **2021**, *24*, 304-315. DOI: <https://doi.org/10.1080/10942912.2021.1876088>
83. Achimón, F.; Peschiutta, M.L.; Brito, V.D.; Beato, M.; Pizzolitto, R.P.; Zygadlo, J.A.; Zunino, M.P. *Plants*. **2022**, *11*, 3070. DOI: <https://doi.org/10.3390/plants11223070>
84. Upadhyay, N.; Dwivedy, A.K.; Kumar, M.; Prakash, B.; Dubey, N. K. *J. Essent. Oil Bear. PL.* **2018**, *21*, 282-297. <https://doi.org/10.1080/0972060X.2018.1459875>
85. Ishaaya, I.; Perry, R., in: *Insecticides in agriculture and environment*; Springer Berlin Heidelberg, **1998**; 12-13.
86. Lee, M.J.; Lee, S.E.; Kang, M.S.; Park, B.; Lee, S.G.; Lee, H.S. *Appl. Biol. Chem.* **2018**, *61*, 481-488. DOI: <https://doi.org/10.1007/s13765-018-0379-z>
87. Kim, E.H.; Kim, H.K.; Ahn, Y.J. *J. Agric. Food Chem.* **2003**, 885-889. DOI: <https://doi.org/10.1021/jf0208278>
88. Bouzenna, H.; Krichen, L. *Nat. Prod. Res.* **2013**, *27*, 841-846. DOI: <https://doi.org/10.1080/14786419.2012.711325>
89. Odeyemi, O.O.; Masika, P.; Afolayan, A.J. *Nat. Prod. Commun.* **2008**, *3*, 1097-1102.
90. Gallardo, A. Picollo, M. I.; González-Audino, P.; Mougabure-Cueto, G. *J. Mol. Entomol.* **2019**, *49*, 332-335. DOI: <https://doi.org/10.1603/ME11142>
91. Lokesh, M.; Sreekrishnakumar, A. K.; Sahu, U.; Vendan, S. E. *Environ. Sci. Pollut. Res.* **2024**, *31*, 35455-35469. DOI: <https://doi.org/10.1007/s11356-024-33483-8>
92. Santos, R. C.; Paes, J. S.; Ribeiro, A. V.; Santos, A. A.; Picanço, M. C. *Entomol. Commun.* **2020**, *2*, 2675-1305. DOI: <https://doi.org/10.37486/2675-1305.ec02913>
93. Yezli-Touiker, S.; Soltani-Mazouni, N.; Kirane-Amrani, L.; Soltani, N. *Fresenius Environ. Bull.* **2016**, *25*, 1190-1205.
94. Delimi, A.; Taibi, F.; Fissah, A.; Gherib, S.; Bouhkari, M.; Cheffrou A. *Afr. Sci.* **2013**, *9*, 82- 90.
95. Lahcene, S.; Taibi, F.; Mestar, N.; Ahmed, S.A.,; Boumendjel, M.; Ouafi, S.; Houali, K. *Cell. Mol. Biol.* **2018**, *64*, 6-12. DOI: <https://doi.org/10.14715/cmb/2018.64.11.2>
96. Shehu, A.; Obeng-Ofori, D.; Eziah, V.Y. *Int. J. Trop. Insect Sci.* **2010**, *30*, 207-213. DOI: <https://doi.org/10.1017/S1742758410000378>
97. Bachrouche, O.; Jemâa, J. M.B.; Wissem, A.W.; Talou, T.; Marzouk, B.; Abderraba, M. *J. stored prod. Res.* **2010**, *46*, 242-247. DOI: <https://doi.org/10.1016/j.jspr.2010.07.001>
98. Gäde, G.; Hoffmann, K.H. *Physiol. Entomol.* **2005**, *30*, 103-121. DOI: <https://doi.org/10.1111/j.1365-3032.2005.00442.x>
99. Raikhel, A.; Brown, M.; Belles, X. *Comprehens. Mol. Insect Sci.* **2005**, *3*, 433-91.
100. Hentze, J. L.; Moeller, M.E.; Jørgensen, A. x.; Bengtsson, M.S.; Bordoy, A.M.; Warren, J.T.; Gilbertm L.I.; Andersen, O.; Rewitz, K.F. *PLoS One*, **2013**, *8*, e55131. DOI: <https://doi.org/10.1371/journal.pone.0055131>

101. Guo, S.; Tian, Z.; Wu, Q. W.; King-Jones, K.; Liu, W.; Zhu, F.; Wang, X. P. *PLoS Genet.* **2021**, 17, e1009352. DOI: <https://doi.org/10.1371/journal.pgen.1009352>
102. Lafont, R.; Dauphin-Villemant, C.; Warren, J.T.; and Rees, H., in: *Comprehensive molecular insect science*, Vol. 6; Elsevier: London, **2005**, 125-195.
103. Garelli, A.; Gontijo, A.M.; Miguela, V.; Caparros, E.; Dominguez, M. *Science.* **2012**, 336, 579-582. DOI: <https://doi.org/10.1126/science.1216735>
104. Hiruma, K.; Kaneko, Y. *Curr. Top. Dev. Biol.* **2013**, 103, 73-100. DOI: <https://doi.org/10.1016/B978-0-12-385979-2.00003-4>
105. Ali, H.M.S.; Mirza, J.H.; Kamran, M.; Alatawi, F.J. *Agriculture.* **2024**, 14, 2295. DOI: <https://doi.org/10.3390/agriculture14122295>
106. Xue, J.B.; Huang, Y.; Che, J.T.; Wang, R.; Liu, M.K.; Wang, W.K. *Int. J. Fauna Biol. Stud.* **2024**, 11, 33-41. DOI: <https://doi.org/10.22271/23940522.2024.v11.i2a.1018>
107. Wu, X.; Chen, Z.; Gao, Y.; Wang, L.; Sun, X.; Jin, Y.; Liu, W. *FEBS J.* **2018**, 285, 3945-3958. DOI: <https://doi.org/10.1111/febs.14652>
108. Hoffmann, K.H.; Meyering-Vos.; Lorenz, W.M. *Eur. J. Entomol.* **1999**, 96, 255-266.
109. Ganter, G.K.; Walton, K.L.; Merriman, J.O.; Salmon, M.V.; Brooks, K.M.; Maddula, S.; Kravitz E.A. *Behav. Genet.* **2007**, 37, 507-512. DOI: <https://doi.org/10.1007/s10519-006-9140-1>
110. Tufail, M.; Nagaba, Y.; Elgendy, A.M.; Takeda, M. *J. Entomol. Sci.* **2014**, 17, 269-282. DOI: <https://doi.org/10.1111/ens.12086>
111. Bakli, D.; Kirane-Amrani, L.; Soltani-Mazouni, N. ; Soltani, N. *Afr. Entomol.* **2016**, 24, 453-459. DOI: <https://hdl.handle.net/10520/EJC195080>
112. Tong-Xian, L.; Stansly, A. *Sc. Biol. Control.* **2004**, 30, 298-305. DOI: <https://doi.org/10.1016/j.biocontrol.2004.01.007>
113. Aribi, N.; Smagghe, G.; Lakbar, S.; Soltani-Mazouni, N.; Soltani, N. *Pestic. Biochem. Physiol.* **2006**, 84, 55-62. DOI: <https://doi.org/10.1016/j.pestbp.2005.05.008>
114. Delhoum, H.; Aribi, N.; Kilani-Morakchi, S.; Soltani, N. *J. Anim. Plant Sci.* **2016**, 26, 823-832.
115. Hiroyoshi, S.; Reddy, G.V.; Mitsuhashi, J. *J. Comp. Physiol.* **2017**, 203, 635-647. DOI: <https://doi.org/10.1007/s00359-017-1179-3>
116. Verma, S.; Dubey, A.; Singh, R.; Tayade, R.; Mishra, V. K. *Chemistselect.* **2025**, 10, e202403269. DOI: <https://doi.org/10.1002/slct.202403269>
117. Ja'afaru, S. C.; Uzairu, A.; Mishra, V. K.; Sallau, M. S.; Ibrahim, M. T.; Dubey, A. *Mol. Simul.* **2024**, 50, 1274-1291. DOI: <https://doi.org/10.1080/08927022.2024.2399150>