Vol. 1 / Issue 2 / 2024

FEZ MULTIDISCIPLINARY JOURNALS

Journal of Biology and Biomedical Research

ISSN: 3009-5522

Journal homepage: https://fmjpublishing.com/index.php/J2BR

Satureja calamintha essential oil: Chemical composition and assessing insecticidal efficacy through activity against acetylcholinesterase, chitin, juvenile hormone, and molting hormone

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Abstract

Plant-derived insecticides based on essential oils (EOs) are currently considered as an excellent option to reduce the adverse effects associated with synthetic pesticides. In this study, we attempted to analyze the chemical composition, insecticidal properties and inhibition of biological parameters, as well as to evaluate the mode of action of the bioactive components of *Satureja calamintha* EO in relation to molecular targets in three life stages of the insect *Callosobruchus maculatus* (*C. maculatus*). Gas chromatography-mass spectrometry (GC-MS) was used to investigate the phytochemical profile of the EO. Inhalation and contact tests with the insect *Callosobruchus maculatus* were carried out to evaluate the insecticidal activity, and *in silico* molecular docking techniques were used to evaluate the mode of action. The extraction of the EO yielded 1.72%, and chromatographic analysis identified 19 potentially active compounds, with pulegone (52.4%) and neo-dihydrocarveol (16.1%) as the predominant constituents. Toxicity tests conducted via contact and inhalation demonstrated significant insecticidal activity against *C. maculatus* after 24 hours of exposure, achieving 87% and 100% mortality, respectively. The LC_{50} values were determined to be 9.34 µL/L for contact toxicity and 4.43 µL/L for inhalation toxicity, while the LC_{95} values were 22.26 µL/L and 12.92 µL/L, respectively. Additionally, exposure to EO inhibited oviposition and emergence across all insect life stages tested. This inhibition was dose-dependent and reached complete suppression at a concentration of 20 µL/L. In addition, exposure to EO showed inhibition of oviposition and emergence at all insect life stages tested. This inhibition increased with increasing dose, reaching complete inhibition at a concentration of 20 µL/L.

Keywords: Biopesticides, *Satureja calamintha*, toxicity, *in vivo*, *in silico*, biological parameters

Citation: Taibi, M.; Rezouki, S.; Moubchir, T.; El Abdali, Y.; Mssillou, B.; El Barnossi, A.; Elbouzidi, A.; Haddou, M.; Baraich, A.; Berraaouan, D.; Mellouk, F.; Idrissi Yahyaoui, M.; Bellaouchi, R.; Saalaoui, E.; khoulati, A.; Asehraou, A.; Addi, M.; El Guerrouj, B.; and Chaabane, K. *Satureja calamintha* essential oil: Chemical composition and assessing insecticidal efficacy through activity against acetylcholinesterase, chitin, juvenile hormone, and molting hormone. *Journal of Biology and Biomedical Research*. 2024, 1(2), 79-91. https://doi.org/10.69998/j2br6

Edited by: Allali Aimad

Received: 18 May 2024; **Revised:** 10 December 2024; **Accepted:** 27 December 2024; **Published:** 05 January 2025

1. Introduction

Food legumes play an important role in a variety of areas, including food security, nutrition, agriculture, the environment and socio-economics. In addition to their contribution to a balanced diet for humans and animals, pulses play a crucial role in revitalizing soil fertility and maintaining the sustainability of production systems. From an economic and social point of view, small-scale farmers grow food legumes, providing essential workdays and income for rural families (Sauer et *al.*, 2018).

Chickpea (*Cicer arietinum*) is recognized as one of the most nutritionally beneficial grain legumes worldwide, due to its high protein, mineral and vitamin content (Allali et *al*., 2020) (Allali et *al.*, 2020). Insects, fungi and rodents pose a threat to stored foodstuffs, with insect damage being the most notable concern. Although this problem is widespread, it is particularly serious in developed nations, and particularly prevalent on the African continent due to climatic conditions that favor their proliferation (Ndomo et *al.*, 2009). Yield loss in leguminous crops during storage, mainly due to bruchid attacks, poses a major challenge for farmers and traders (Matos et *al*., 2020). Among these pests, *Callosobruchus maculatus*, commonly known as chickpea weevil, is one of the main destroyers of chickpeas. The negative economic impacts caused by this insect are linked to the penetration of larvae to feed inside the seeds, resulting in significant weight loss and a reduction in nutritional values and germination potential (Anderson et *al*., 1990). The conventional approach to managing this pest relies on synthetic insecticides like phosphine, but this method results in adverse effects on germination, along with residues that diminish seed quality (Niu et *al*., 2012).

Most insect control methods in agriculture depend heavily on chemical pesticides, resulting in growing resistance and issues like environmental pollution. These methods adversely affect public and animal health globally (Ammar et *al.*, 2020; El Jilali et *al.*, 2023). In response to this trend, numerous researchers have concentrated on developing plant-based biopesticides, like essential oils (EOs), which are efficient and eco-friendly for agricultural purposes. Plant essential oils, recognized for their bioactive elements, provide a dual benefit of cost-effectiveness, eco-friendliness, biodegradability, and safety for non-target organisms (Aimad et *al.*, 2021a).

Satureja calamintha (L.), known locally as “minta”, is a perennial aromatic species widely harvested for various biological applications (Abbad et *al.*, 2014; Abbad et *al.*, 2023a). In this study, we assess the bio-insecticidal activity of *Satureja calamintha* essential oils on *Callosobruchus maculatus* adults, female fecundity, emergence of new individuals, repellency, and conduct an *in silico* molecular docking study.

2. Materials and Methods

2.1. Plant material collection and Essential Oil Extraction

Aerial parts of *Satureja calamintha* were collected in April 2022 from the Ghafsai region, Taounate province in North Morocco. Specimens have been preserved in the Herbarium of the Laboratory of Plant, Animal, and Agro-Industrial Production at the Department of Biology, Faculty of Science, Ibn Tofail University. The identification of specimens was conducted at the National Agency for Aromatic and Medicinal Plants (ANPAM). The harvested plant samples underwent washing with water followed by drying in the shade at a well-ventilated, dry location with a temperature of 25°C for 7 days. Subsequently, 200g of plant material was combined with 1.25L of distilled water and subjected to hydrodistillation using a Clevenger apparatus. The extracted oils were dehydrated with anhydrous sodium sulfate and stored in opaque bottles at 4°C until chemical analysis.

2.2. Phyto-chemical analysis of EO

Essential oils were analyzed using gas chromatography-mass spectrometry (GC-MS) on an Agilent-Technologies 6890 N Network GC system. The system was equipped with a flame ionization detector and an HP-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm). Temperature settings ranged from 35°C to 250°C, with a gradient of 5°C/min. Retention indices were determined through gas chromatography on two fused silica capillary columns (30 m × 0.25 mm) from Agilent-Technologies, Little Falls, CA, USA. Temperature programming spanned from 35 to 250°C at a rate of 5°C/min, with the lower and upper temperatures maintained for 3 and 10 minutes respectively. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. A 1.0 µl sample was injected in fractionated mode (split ratio, 1:100). Essential oil constituents were identified by comparing their mass spectra with those in a specified database (NIST-MS).

2.3. Insecticide bioassay

2.3.1. Adult mortality

The insect bioassay followed the protocol outlined in Aimad et *al.*, (2022). Screening involved testing initial concentrations of 1, 2, 5, 8, 10, 15, 20, 25, and 30 µL/L of air. Doses leading to 10-95% mortality were selected for further experiments. Four doses were excluded: 2, 8, 15, 25, and 30 µL/L of air. Ten newly emerged adults (24 hours old) of the *Callosobruchus maculatus* species underwent two toxicity tests with varying concentrations of essential oils (1, 5, 10, and 20 µL/L of air).

For the inhalation toxicity test, insects were placed in 1-liter glass jars. Various doses of essential oils were applied using a micropipette on cotton masses inside the lid. Distilled water was used for the control. Each concentration had three replicates. Adult mortality was monitored daily post 24-hour exposure.

For the contact toxicity test: insects were placed in 1-liter glass jars with 100g of healthy chickpea seeds, and different doses of essential oils were applied to the seeds using a pipette. Distilled water was used in the control group. Dead insects were counted daily until the experiment ended. Three replicates were conducted to evaluate the effectiveness of the insecticide, shown as a percentage of the average mortality of *C. maculatus* adults

The mortality percentage adjusted for both tests was calculated using Abbott's method:

$$Pc=100\times((P0-Pt)/(100-Pt))$$

2.3.2. Impact of essential oils on female fecundity

The effect of essential oils on female fecundity was evaluated by comparing the number of eggs laid by *C. maculatus* females on chickpea seeds treated with essential oils (contact EO toxicity test) with that of the control. The egg reduction rate was calculated using the formula:

$$PR=(NC-NT)/NC\times100$$

In this formulation, PR represents the rate of egg-laying reduction (%), while NC represents the number of eggs observed in the control, and NT designates the number of eggs during treatment.

2.3.3. Effect of essential oils on Callosobruchus maculatus eggs

The number of eggs laid in each jar was counted and then subjected to the influence of essential oils until new individuals emerged. Daily monitoring was conducted, and the reduction rate in emergence was calculated using the formula below:

$$PR=(NC-NT)/NC\times100$$

Where PR is the percentage reduction in emerged insects (%), NC is the number of insects hatched during control and NT is the number of insects hatched during treatment.

2.4. In silico evaluation of mechanism of action

2.4.1. Protein Preparation

The crystal structures of acetylcholinesterase (PDB ID: 6ARY) (Rants'o et *al.*, 2022), Chitin Synthase 2 crystal structure (PDB ID: 7STM) (Ren et *al.*, 2022), juvenile hormone (PDB ID: 5V13) (Ramos et *al.*, 2020), and molting hormone (PDB ID: 1R1K)(Feng et *al.*, 2023) were obtained from the Protein Data Bank. Before docking ligands into the protein's active site, the protein was prepared using the protein preparation wizard in Maestro 11.5 from Schrodinger's software. The preparation process involved assigning bond orders, removing water molecules and heteroatoms, adding hydrogen atoms to determine ionization and tautomeric states, and optimizing hydrogen bonds using the PROPKA tool. This preparation included optimization and minimization steps, resulting in the final form of the protein for grid positioning (El Abdali et *al.*, 2023).

A grid box was created with the following dimensions:

	Acetylcholinesterase (PDB ID: 6ARY)	**Chitin Synthase 2 (PDB ID: 7STM)**	**Juvenile hormone (PDB ID: 5V13)**	**Molting hormone (PDB ID: 1R1K)**
X	-59.984	113.982	235.248	6.575
Y	57.429	139.656	-4.962	25.737
Z	17.731	134.805	362.364	257.632

The grid box had a volumetric spacing of 20 × 20 × 20. The ligand was docked into the protein-generated grid box using 'standard Precision' (SP), and the SP GScore was used to assess the results.

2.4.2. *Ligand Preparation*

All molecules identified in *Satureja Calamintha* essential oil were downloaded from the PUBCHEM platform in .SDF format. Subsequently, the ligand structures were processed using Schrodinger Suite's ligprep tool, which involved refining the ligands, optimizing their geometry using the OPLS3e force field, and ionizing the forms using Epic (Lafraxo et *al.*, 2022).

2.5. Data analysis

We performed a comprehensive statistical analysis using the SPSS software for Windows® (version 21) to guarantee the validity of our findings. In this case, the homogeneity and normality of the data were assessed using the t-test and the Shapiro-Wilks test, respectively. Subsequently, in order to identify significant differences between the extreme values of the groups, we performed a one-way analysis of variance (ANOVA). To distinguish between significant and nonsignificant means, the Fisher's Least Significant Difference (LSD) test was applied at a significance level of $\alpha = 0.05$. Concurrently, we employed the probit technique to determine the lethal concentrations LC_{50} and LC95, along with the associated confidence intervals. These rigorous methodological approaches have ensured the reliability and robustness of our results.

3. Results and discussion

3.1. Chemical Composition of Essential Oils

Table 1 displays the findings of the physicochemical analysis of *S. calamintha* essential oils, showing the quantity produced and the chemical composition of the oils tested. According to GC/MS data, pulegone was the most abundant compound in *S. calamintha* EO at 52.4%, followed by neo-Dihydrocarveol at 16.1% and Dihydrocarveol at 9.6%.

The chemical composition of the oils analyzed displayed similarities with previous studies, especially concerning the existence of the compound pulegone (Abbad et *al.*, 2023b). Pulegone has also been detected in *M. pulegium* essential oils from Mediterranean regions, albeit in different quantities

(Aimad et *al*., 2021b). Pulegone is a ketone monoterpene found in the essential oils of various aromatic plants, and it exhibits numerous bioactivities in cells and animals (Božović & Ragno, 2017). de Sousa et *al*., (2011) reported that pulegone possesses analgesic properties. Roy et al. (Roy et *al*., 2018) demonstrated that pulegone can reduce lipopolysaccharide (LPS)-induced inflammation by mitigating the effects of NF-κB, indicating its potential for treating and preventing various inflammatory diseases. Additionally, pulegone-rich *Mentha pulegium* oils exhibited high efficacy (100% mortality) against *Callosobruchus maculatus* adults, with a lethal concentration (LC_{50}) of 1.41±0.48 µ L/L of air after 24 hours of exposure, suggesting pulegone's effectiveness as an insecticide against these cereal seed pests (Aimad et *al*., 2021c).

Table 1: Chemical composition of essential oils from *Satureja calamintha*.

Peak	Compounds	Ri	Area (%)	Chemical class
1	α-Pinene	937	0.6	Monoterpene
2	Sabinene	972	2.3	Monoterpene
3	β-Pinene	977	0.7	Monoterpene
4	1.8-Cineole	1028	8.9	Monoterpene oxygenated
5	Borneol	1164	0.3	Monoterpene alcohol
6	Terpinen-4-ol	1173	5.2	Monoterpene alcohol
7	Dihydrocarveol	1191	9.6	Monoterpene alcohol
8	neo-Dihydrocarveol	1192	**16.1**	Monoterpene alcohol
9	Pulegone	1233	**52.4**	Ketonic monoterpene
10	Carvone	1241	0.2	Ketonic monoterpene
11	Dihydroedulan II	1247	0.1	Monoterpene alcohol
12	Dihydro carveol acetate	1302	0.1	Monoterpene acetate
13	Cis-Carvyl acetate	1337	0.3	Monoterpene acetate
14	β-Bourbonene	1380	0.2	Sesquiterpene
15	(E)-Caryophyllene	1401	0.1	Sesquiterpene
16	Trans-calamenene	1516	1.6	Sesquiterpene
17	Spathulenol	1559	0,1	Sesquiterpene alcohol
18	Epi-a-Cadinol	1639	0,1	Sesquiterpene alcohol
19	α-Cadinol	1643	0,1	Sesquiterpene alcohol
		Identified compounds		**Percentage**
Monoterpenes		13		81.9%
Sesquiterpene		6		17,9%
Total identified (%)		**19**		**99.8**

3.2. Essential oil' insecticidal activity:

3.2.1. Essential oil' toxicity against C. maculatus:

Experiments on the insecticidal activity of *S. calamintha* essential oils applied by direct contact to *C. maculatus* have yielded promising results. The study found that essential oils extracted from *S. calamintha*, containing a significant amount of pulegone (52.4%), were notably effective against C. maculatus, a common pest of stored seeds. Observation of direct contact effects showed a significant reduction in the survival of *C. maculatus* individuals exposed to different doses of the essential oils. This reduction reached a maximum value of 100% at a dose of 20µL/L of air after 3 days of exposure, while for the other doses (1, 5, and 10µL/L of air), total mortality of all insects was achieved only after 4 days of exposure to *S. calamintha* essential oils by direct contact with the insects (Figure 1).

In the inhalation toxicity test, adult insect mortality is higher than in the contact toxicity test, reaching 100% after only one day of exposure to essential oils (Figure 2). The presence of pulegone, an active compound in *Satureja* oils, contributes to their insecticidal properties. This biological activity, either alone or in synergy with other compounds, is crucial for pest control in grain storage environments. Inhalation insecticidal activity of essential oils provides advantages over direct contact application. It ensures uniform coverage, reaching insects in inaccessible areas, and allows volatile compounds

to penetrate insects' respiratory systems effectively, leading to higher mortality rates. Natural plant extracts may serve as viable substitutes for traditional crop pesticides due to their low toxicity to mammals, rapid degradation, and local availability (El Jilali et *al.*, 2023). Research indicates that inhalation of essential oils derived from aromatic plants significantly impacts the lifespan of *C. maculatus* adults. Typically, mortality rates rise with higher doses and longer exposure periods. These findings align with previous studies demonstrating the insecticidal properties of essential oils from various aromatic plants (Abdelli et *al.*, 2016; Dutra et *al.*, 2016; Pourya et *al.*, 2018). This is mainly because of the high content of monoterpenes in our essential oil, which are lipophilic and can quickly escape, enter the respiratory tract, and cause physiological harm to insects (Lee et *al.*, 2004).

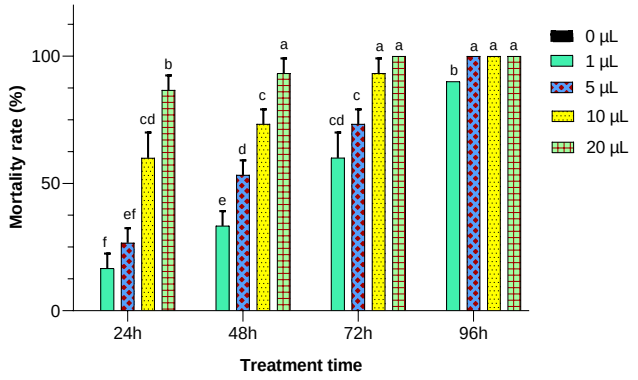


Figure 1: Mortality rate of different concentrations of *Satureja calamintha* essential oil against *C. maculatus* adults in contact toxicity tests after different exposure times.

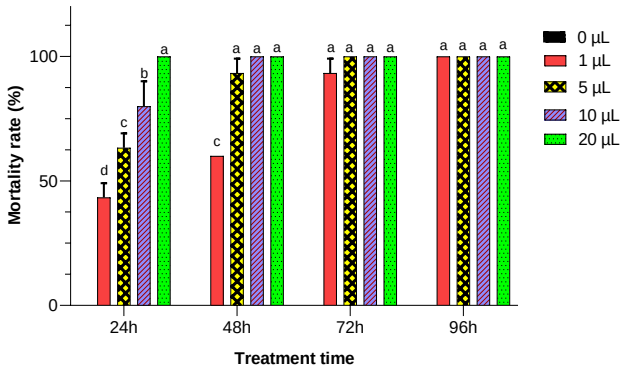


Figure 2: Mortality rate of different concentrations of *Satureja calamintha* essential oil against *C. maculatus* adults in inhalation toxicity tests *after* different exposure times.

LC_{50} and LC_{95} values, indicating the doses needed to induce 50% and 95% mortality in *C. maculatus* adults following exposure to various essential oil doses, were determined utilizing the Probit method. The results are detailed in Table 2. The LC_{50} and LC_{95} values for contact toxicity were 9.34 and 22.26 µg/L, while for inhalation toxicity they were 4.43 and 12.92 µg/L, after 24 hours of exposure to essential oils by the insects. These results validate the potent insecticidal properties of our essential oil against *C. maculatus*, aligning with prior studies on insecticidal impacts of essential oils from other Lamiaceae family species like *Ocimum basilicum* (LC_{50} = 1548.15 ppm (72 h)), *Satureja hortensis* (LC_{50} = 68.728 µl/L air (24 h)), *Salvia officinalis* (LC_{50} = 8.79 µg/mL air (24 h)), *Mentha longifolia* (LC_{50} = 2.05 µL/L air (24 h)), and *Mentha piperita* (LC_{50} = 1.76 µL/mL (24 h)) (Ebadollahi et *al.*, 2012; Islam Adel et *al.*, 2015; Jayaram et *al.*, 2022; Kaya et *al.*, 2018; Khani & Asghari, 2012).

Table 2: LC_{50} and LC_{95} (in µL/L of air) of essential oils tested against *Callosobruchus maculatus* insects after each 24-hour interval for 4 days.

Test	Exposure time (h)	df	Slope+SD	LC_{50}	LC_{95}	Intercept+SD	pValue	X^2
Contact	24	3	0.13±0.01	9.34	22.26	-1.33±0.1	0.00	18.71
	48	3	0.14±0.012	6.37	18.27	-0.88±0.09	0.00	39.83
	72	3	0.25±0.02	2.78	9.46	-0.88±0.09	0.00	68.23
	96	3	3.73±0.46	0.64	1.08	-2.39±0.42	0.81	0.98
Inhalation	24	3	0.19±0.02	4.43	12.92	-0.86±0.01	0.00	47.81
	48	3	0.55±0.06	1.66	4.64	-0.92±0.12	0.00	51.35
	72	3	4.68±1.07	0.67	1.02	-3.14±1.05	0.99	0.13
	96	*	*	*	*	*	*	*

The chi-square test ($\chi 2$) is used to determine the slope of a line. Slope is determined by probit (p) = constant + Bx (covariates x transformed using log base 10); df: degrees of freedom; SD: standard deviation; LC_{50} and LC_{95} lethal concentrations (50% and 95% mortality of *C. maculatus* adults).

* Mortality rate has been observed for this dosage.

3.2.2. Essential oil's impact on egg-laying :

The study shows a significant decrease in oviposition of *C. maculatus* females when exposed to *S. calamintha* essential oils. At low concentrations of 1µL/l of air, the average number of eggs laid per female was 18±2, indicating a 90.25% reduction in oviposition compared to the control group which had 184.67±17.78 eggs laid (Figures 3 and 4). The decrease in female fertility is in line with the insecticidal effects of the plant's essential oils. As the doses of essential oils increased, we noticed a corresponding decline in the egg count, with numbers dropping to 8±1.33 eggs, indicating a 90% reduction at higher doses. The dose-response relationship indicates a dose-dependent efficacy of *S. calamintha* essential oils in inhibiting the reproduction of *C. maculatus* females. These findings underscore the potential of *S. calamintha* essential oils as reproductive inhibitors in C. maculatus. The notable decrease in the number of eggs laid by females could have a significant impact on insect population dynamics in cereal crops. This reproductive inhibition may help reduce insect population density and consequent damage to cereal crops by interfering with key hormonal processes involved in egg maturation, oocyte development, and reproductive organ formation in females.

In the literature, few studies have explored the impact of essential oils on insect eggs and the mechanism of action of these oils on potential molecular targets. Ketoh et al (Ketoh et *al.*, 2006) found that *Cymbopogon schoenanthus* oils hindered egg-laying by *C. maculatus* at low doses. Moreover, (Mssillou, et *al.*, (2022) observed that applying 20 µL of *Dittrichia viscosa* L. essential oil to *Callosobruchus maculatus* in one liter of air led to a 91% decrease in the number of eggs laid by this insect. In a different study, the insecticidal efficacy of Atalantia monophylla's essential oil, containing high levels of eugenol (19.76%) and sabinene (19.57%), against *C. maculatus* was evaluated through a fumigation test, demonstrating notable ovicidal effects (Nattudurai et *al.*, 2017). Furthermore, essential oils derived from Moroccan *Mentha pulegium* rich in pulegone, a monoterpene, led to a reduction of more than 70% in egg-laying (Aimad et *al.*, 2021c). This active component exhibited significant insecticidal potency against various pests, including *C. maculatus* (Abdelgaleil et *al.*, 2009; Franzios et *al.*, 1997).

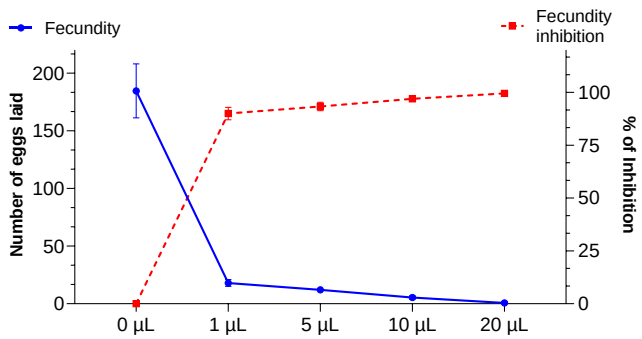


Figure 3: Rate of reduction in the number of eggs laid by *Callosobruchus maculatus* females subjected to different concentrations of *Satureja calamintha* essential oil.

3.2.3. Essential oil's impact on emergence:

The impact of *S. calamintha* essential oils on the developmental stages (larval and pupal stages) of *C. maculatus* is remarkably demonstrated by a significant reduction in emergence. Our study results show that exposure to these essential oils results in a significant decrease in the number of emerging individuals, indicating an inhibitory effect on the larval and pupal stages of insect development and survival. This decrease escalates with higher doses, from 111.67± 4.44 individuals in the control group to 12± 2 individuals for the 1µL/L air dose, 10.33± 2.89 for the 5µL/L air dose, 8.33± 0.89 for the 10µL/L air dose, and 6± 0.67 for the 20µL/L air dose, resulting in percentage reductions of 90%, 96%, 100%, and 100%, respectively. This notable reduction in emergence may be due to the various mechanisms of action of the active compounds found in essential oils, including hormonal disruption, altered embryonic development, and direct toxicity to pre-imaginal stages. These results show a significant larvicidal effect of the compounds in our essential oil, preventing several larvae from completing their life cycle, and consequently halting development and totally reducing emergence. In this respect, several authors have contributed to the study of the impact of plant bioactive molecules on insect larvae. Dris et *al.* (Dris et *al.*, 2017) found that *Ocimum basilicum* oil with linalyl acetate and linalool as main compounds showed strong larvicidal activity against *Culex pipiens* house mosquito larvae (Diptera: Culicidae). Carvone, on the other hand, was observed to deter feeding, inhibit growth, cause contact, fumigant toxicity, and impair egg hatching and reproduction in the caterpillar *Pseudaletia unipuncta* (Lepidoptera: Noctuidae) (de Sousa et *al.*, 2013, 2015). These findings indicate that carvone has various toxic effects and mechanisms of action (Govindarajan et *al.*, 2012). Within 24 hours of treatment, the essential oil of *A. nilotica* seeds demonstrated notable toxic effects on the larvae and adults of several insect pests, including *Spodoptera litura*, *Tenebrio molitor*, *Oncopeltus hyalinipennis*, and *Aphis fabae* (Perumal et *al.*, 2023). Similarly, essential oils from the seeds of *A. nilotica* and the crude extract of its pods have demonstrated significant toxicity in the laboratory against the larvae of the pathogenic mosquitoes *Aedes aegypti*, *Anopheles stephensi*, and *Culex quinquefasciatus* (Vivekanandhan et *al.*, 2018).

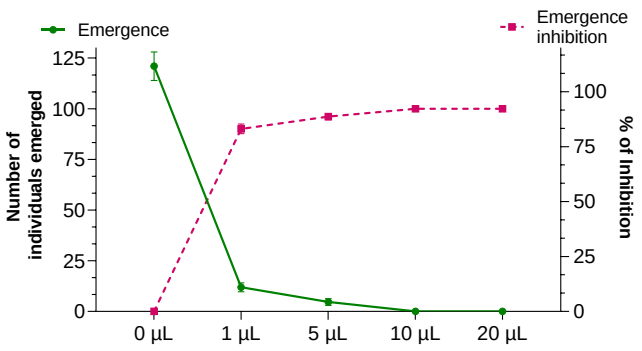


Figure 4: Rate of reduction in emergence caused by different concentrations of *Satureja calamintha* essential oil.

3.3. In silico evaluation of mechanism of action

A thorough molecular analysis was conducted to discover and elucidate the potential action mechanisms of the *S. calamintha* EO in its insecticidal efficacy against various parameters of *C. maculatus*. This involved employing computer-aided drug design techniques to examine and predict interactions between diverse ligands and molecular targets associated with various biological functions. The key EO compounds and insect protein targets, namely acetylcholinesterase (PDB ID: 6ARY), chitin synthase 2 (PDB ID: 7STM), juvenile hormone (PDB ID: 5V13), and molt hormone (PDB ID: 1R1K), were utilized in molecular docking simulations (Table 3).

Inhibition of acetylcholinesterase (AChE) is a common mechanism employed by many insecticides to disrupt the nervous system of insects, leading to paralysis and eventually death. Acetylcholinesterase is an enzyme responsible for breaking down the neurotransmitter acetylcholine (ACh) into choline and acetate, thereby terminating the nerve signal transmission at cholinergic synapses (Araújo et *al.*, 2023; Mssillou, et *al.*, 2022).

In our *in silico* study, Dihydroedulan II, Borneol, and Terpinen-4-ol were the most active molecules against the active site of acetylcholinesterase with glide gscore of -7.589, -7.254, and -6.476 kcal/mol (Table 3).

Moreover, inhibition of chitin biosynthesis is another common mechanism employed by insecticides to control pests. Chitin is a structural polysaccharide found in the exoskeletons of insects and other arthropods. It provides rigidity and support to the exoskeleton, making it an essential component for insect survival and growth (Cohen, 2010). Insecticides that target chitin biosynthesis typically disrupt the process of chitin formation, leading to defects in the insect's exoskeleton and ultimately causing mortality (Yang et *al.*, 2021).

Among the chemical compounds docked in the active site of chitin synthase 2, trans-calamenene, alpha-Cadinol, and terpinen-4-ol presented the highest inhibitory activity with glide g score of -6.540. -5.782, and -5.481 kcal/mol (Table 3).

Inhibition of juvenile hormone is another strategy employed by insecticides to disrupt the development and reproduction of insects. Juvenile hormone is a key regulator of insect growth and development, playing crucial roles in processes such as metamorphosis, reproduction, and behavior (Zhang et *al.*, 2019).

In insects, juvenile hormone is produced by the corpora allata gland and acts as a signaling molecule that regulates various physiological processes, particularly during the immature stages (larvae and pupae). It prevents the premature development of larvae into adults and maintains them in a juvenile state until they are ready to undergo metamorphosis (Shinoda, 2016). Juvenile hormone inhibition in our *in silico* study showed strong activity of Borneol, Terpinen-4-ol, and Dihydroedulan II with glide gscore of -7,490, -7,219, and -6,843 kcal/mol (Table 1). The effect of essential oil EO compounds on insect juvenile hormone has been experimentally validated, and these results are consistent with those of other similar studies (Herrera-Calderon et *al.*, 2022; Shah et *al.*, 2021).

The molting hormone in insects, known as 20-hydroxyecdysone (20E), is produced as ecdysone by a pair of endocrine glands situated either in the prothorax of Lepidoptera and other insects (prothoracic glands) or in the ventroposterior region of the head (ventral glands). Ecdysone undergoes conversion into 20E, which exerts its effects by inducing molting through interactions with specific amino acid residues within the ligand-binding domain of the ecdysone receptor (EcR) protein. Insects undergo molting from the embryonic stage onwards, with the molting process continuing through larval, pupal, and adult stages in holometabolous insects, and nymphal instars and adults in hemimetabolous insects. Molting is essential for insects to accommodate growth under the influence of 20E, with regulation occurring through timing and levels of Juvenile Hormone (JH). These hormones play dual roles, transitioning to regulate reproductive processes during the adult stages of insects. In our study, inhibition of 20-hydroxyecdysone showed strong inhibitory activity of Spathulenol, trans-calamenene, and alpha-Cadinol with glide gscore of -7.952, -7.850, and -7.773 kcal/mol (Table 3).

Previous research has revealed the significant influence of compounds found in essential oils on the molting hormone. These compounds can act in various ways on the insect molting process, including interfering with the synthesis, release, or action of the molting hormone itself. For example, certain compounds may function as hormone mimetics, activating or inhibiting hormone receptors and thereby

disrupting normal insect development. Others may modulate the production or degradation of the molting hormone, affecting its level within the insect's body. By understanding how essential oil compounds interact with the molting hormone, it is possible to develop effective pest control strategies based on these specific mechanisms of action (Kilani-Morakchi et *al*., 2021; Osman et *al*., 2016).

In the active site of acetylcholinesterase, Dihydroedulan II established a single hydrogen bond with TYR residue 282 (Figure 5A and 6A). While in the active site of chitin synthase, trans-calamenene established a Pi-Pi stacking type bond with the TRP residue 647 (Figure 5B and 6B).

Borneol established a single hydrogen bond with the residue GLN C 164 in the active site of juvenile hormone (Figure 5C and 6C). Furethermore, Spathulenol also made a single hydrogen bond with the THR residue 346 in the active site of molting hormone (Figure 5D and 6D).

Table 1: Docking results with ligands in active sites.

	Glide gscore (kcal/mol)			
	acetylcholinesterase (PDB ID: 6ARY)	**Chitin Synthase 2 (PDB ID: 7STM)**	**juvenile hormone (PDB ID: 5V13)**	**molting hormone (PDB ID: 1R1K)**
(E)-Caryophyllene	-5.662	-3.65	-6.153	-6.488
1,8-Cineole	-4.665	-4.18	-6.32	-6.224
alpha-Cadinol	-6.36	-5.782	-4.457	-7.773
alpha-Pinene	-5.731	-3.611	-6.493	-5.981
beta-Bourbonene	-6.473	-3.785	-6.568	-7.552
beta-Pinene	-5.972	-4.001	-6.653	-6.084
Borneol	-7.254	-5.441	-7.49	-7.46
Carvone	-6.06	-4.372	-5.844	-6.148
cis-Carvyl acetate	-4.83	-4.665	-6.095	-7.118
Dihydro carveol acetate	-5.291	-3.93	-5.493	-6.612
Dihydrocarveol	-6.34	-4.886	-6.411	-6.496
Dihydroedulan II	-7.589	-4.741	-6.843	-7.447
epi-alpha-Cadinol	-6.227	-5.187	-4.86	-6.935
neo-Dihydrocarveol	-4.11	-4.595	-6.403	-6.496
Pulegone	-6.145	-4.568	-5.881	-6.454
Sabinene	-5.56	-3.582	-6.234	-6.268
Spathulenol	-5.978	-5.034	-7.219	-7.952
Terpinen-4-ol	-6.476	-5.481	-7.106	-6.855
trans-calamenene	-6.23	-6.54	-	-7.85

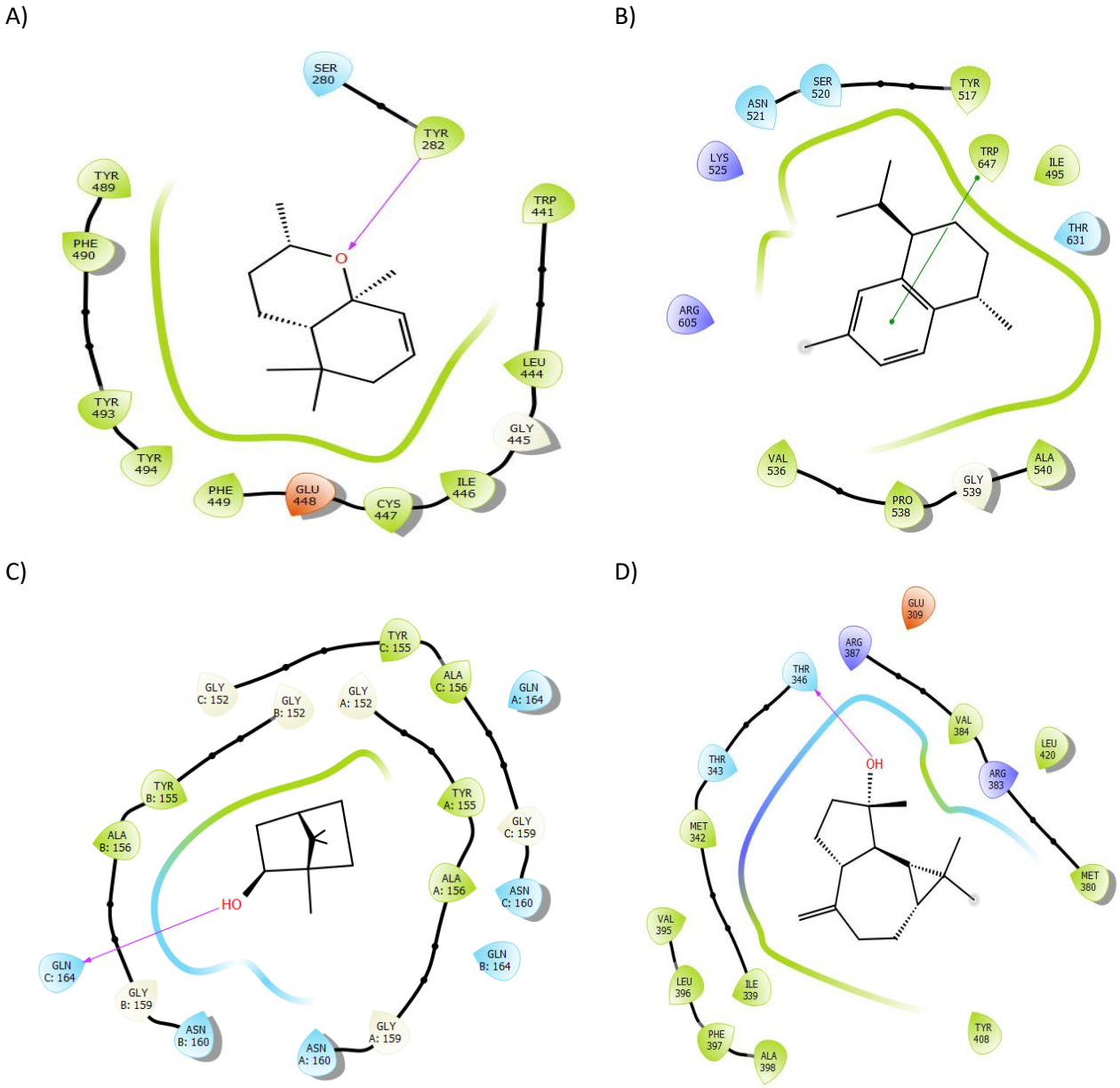
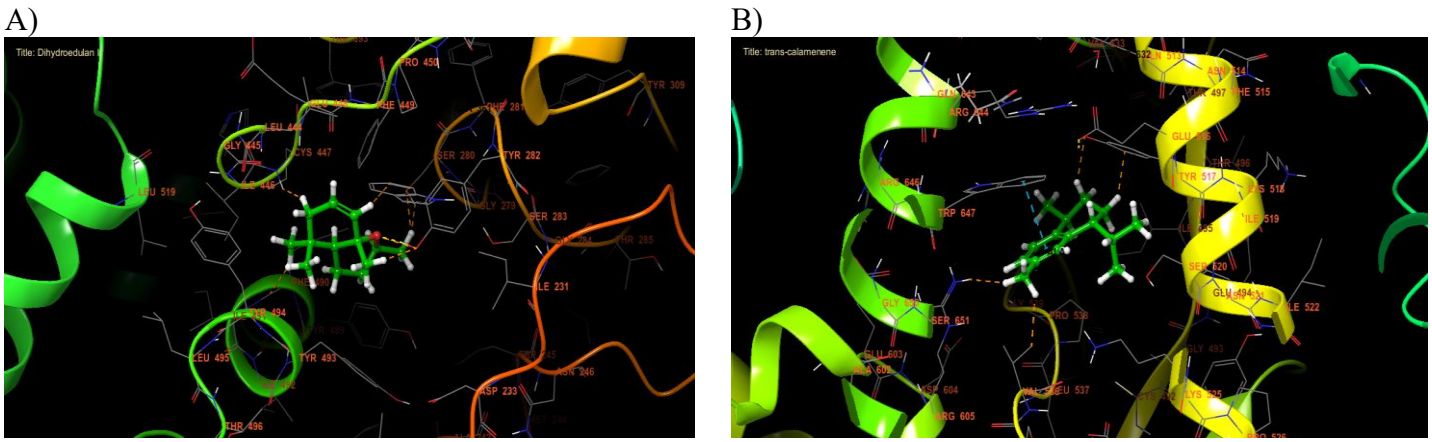


Figure 5: 2D diagrams of ligand interactions with the active sites: A: Dihydroedulan II interactions with the active site of acetylcholinesterase; B: trans-calamenene interactions with the active site of Chitin Synthase 2; C: Borneol interactions with the active



C) D)

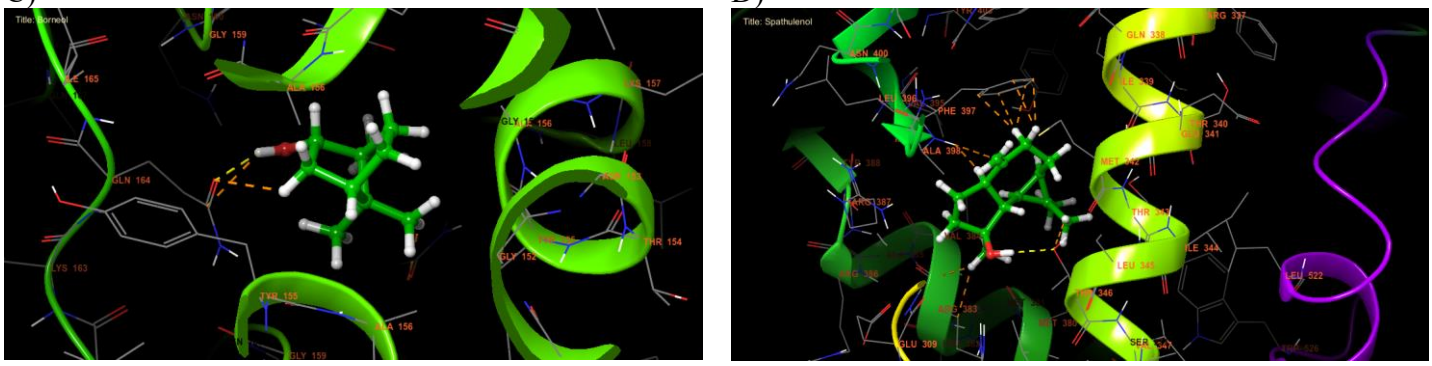


Figure 6: 3D diagrams of ligand interactions with the active sites: A: Dihydroedulan II interactions with the active site of acetylcholinesterase; B: trans-calamenene interactions with the active site of Chitin Synthase 2; C: Borneol interactions with the active

4. Conclusion

Our results show that the EO of Moroccan calament has promising insecticidal properties, particularly against the insect C. maculatus. These laboratory results are supported by *in silico* analyses, which confirm the inhibitory effect of EO compounds on insect acetylcholinesterase, juvenile hormone and chitin synthase 2. Consequently, these results highlight its potential utility as an effective bio-insecticidal agent for agricultural crops and legume storage. The biological activities observed can be attributed, at least in part, to the presence of monoterpene compounds in the essential oil. The various advantages of this essential oil, as elucidated in this study, provide a sound basis for further exploration and development of its practical applications in crop protection and storage management programs for chickpea pest control.

Funding

This research received no external funding

Conflicts of Interest

The authors declare no conflicts of interest.

Data availability statement

The data was not deposited in public repositories

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