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Insecticidal Potential of Essential Oils from *Ammi visnaga* L. and *Trachyspermum ammi* L. against *Sitophilus oryzae* (L.) and In Silico Study of Their Major Constituents

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Abstract: There is a high interest in utilizing natural bioactive products derived from plants as a substitute for synthetic chemicals in the industry. This research focuses on the phytochemical composition of essential oils (EOs) of *Ammi visnaga* L. and *Trachyspermum ammi* L and their insecticidal activity against *Sitophilus oryzae* (L.), a common pest found in stored cereals. The EOs were extracted through steam distillation and analyzed using gas chromatography coupled with mass spectrometry (GC-MS). The EOs of *A. visnaga* consisted of twenty-four components, with Abietadiene (41.23%) being the most abundant, followed by linalool (25.54%) and limonene (19.04%). On the other hand, the EOs of *T. ammi* consisted of twenty-eight main components, with isothymol being the most abundant (51.88%). The results revealed that the EOs of *T. Ammi* (DL_{50} = 0.1 μL EOs/L of air) were more toxic than *A. visnaga* (0.38 μL EOs/L of air), with the toxicity varying based on doses and exposure periods. To further understand the molecular mechanisms underlying this activity, molecular docking and dynamic simulations were performed using the major chemical constituents of the oils. The simulation results indicated that the major compounds, Abietadiene and isothymol, interact with the catalytic sites of the target proteins, inhibiting acetylcholinesterase and chitin synthase. These interactions form energetically favorable systems that remain stable throughout the molecular dynamic period. This research provides valuable insights into the potential of these EOs as natural insecticides and highlights the importance of molecular modeling in understanding the biological activities of plant-derived compounds.

Keywords: *Apiaceae*; essential oils; chemical composition; insecticidal activity; rice weevils; molecular docking

1. Introduction

The rice weevil (*Sitophilus oryzae*) is a significant pest that causes substantial damage to stored grains, particularly rice, wheat, barley, and occasionally peas. As a primary pest, the rice weevil is capable of infesting whole grains and causing extensive losses during storage [1,2]. The adult rice weevils feed directly on the whole grains, while the larvae develop within the kernels, consuming the internal contents. This feeding activity not only

reduces the overall grain quality and quantity but also contributes to heating and increased moisture levels in the stored grains, further exacerbating the damage [3,4]. In Western Europe and North America, where advanced pest control techniques are implemented, insects still destroy agricultural or forestry products worth over 3 billion dollars annually [5]. In Canada, for example, losses suffered by cereal productions range from 35 to 45% [6]. Postharvest losses in India exceed 12–16 million metric tons/year of food grain [7]. *S. oryzae* is widespread and highly damaging, causing about 10–65% of damage under moderate storage conditions and up to 80% under prolonged storage conditions [8]. In developing countries, especially those in the Mediterranean climate zone, estimates range from 30 to 50% of crops [8]. In other words, one-third to half of what is produced never reaches consumers, and the labor and money invested are irretrievably lost [8]. Therefore, the rice weevil causes severe quantitative and qualitative losses to stored rice and other cereal grains through its feeding, breeding, and the secondary effects it causes in the storage environment [9].

To combat this pervasive issue, chemical pesticides, such as organophosphates and synthetic pyrethroids, are commonly used for protecting food stocks and seeds, leading to environmental and health risks [10]. Despite the availability of alternative methods, such as biological control, improper use and abuse of pesticides persist in many developing countries, driven by factors such as illiteracy and a lack of effective control. This misuse not only contributes to resistance problems in pest insects but also contaminates the environment, posing threats to human health. Additionally, the improper application and handling of pesticides exacerbate these risks, leading to widespread contamination of soil, air, and water sources, further highlighting the urgent need for sustainable pest management practices [11].

The utilization of EOs from plants, such as *Ammi visnaga* L. and *Trachyspermum ammi* L. (Apiaceae), has shown promise in combating pests, such as the rice weevil (*S. oryzae*), in stored grains due to their repellent and fumigant activities, as well as their high concentration of monoterpene enantiomers, which exhibit both toxicity and repellent effects against insects [12]. EOs are known to possess bioactive compounds that exhibit insecticidal properties against various pests [13]. Studies have highlighted the effectiveness of EOs in repelling, fumigating, and affecting the enzyme activity of pests, such as the rice weevil [13]. *A. visnaga* (Khella) and *T. ammi* (Ajwain) are renowned for their medicinal benefits. *A. visnaga* is traditionally employed in the treatment of respiratory ailments, such as asthma and bronchitis, whereas *T. ammi* is celebrated for its potent antimicrobial and antifungal properties. EOs derived from the Apiaceae family, including those from Khella and Ajwain, are recognized for their insecticidal properties [13]. These oils have been proven effective against pests, such as *S. oryzae*, in stored grains, operating through mechanisms such as repulsion, fumigation, and enzyme activity disruption in various pests. This makes them promising natural alternatives to synthetic insecticides [14]. Moreover, EOs from *A. visnaga* and *T. ammi* have demonstrated insecticidal properties against different insects, highlighting their potential as natural substitutes for synthetic pesticides [15]. These oils exhibit repellent, fumigant, and anti-oviposition activities against pests, indicating their comprehensive impact on pest control [16,17]. The insecticidal toxicity of *T. ammi* EOs has been linked to constituents such as thymol, underscoring the necessity of understanding the chemical composition of these oils for effective pest management [16,17].

In this context, the aim of our study is to identify the chemical composition of the EOs extracted from two Moroccan plants, *A. visnaga* and *T. ammi*, and to assess their insecticidal potential against *S. oryzae*, a common pest of stored cereals. The mechanisms of interaction of the major chemical constituents present in the EOs are studied using doping techniques and molecular dynamics. Molecular docking studies were conducted to predict the binding affinities and interaction modes of these major compounds with target proteins in *S. oryzae*. The primary targets selected for this study are acetylcholinesterase (AChE) and chitin synthase, crucial enzymes in the nervous system of insects.

2. Materials and Methods

2.1. Plants and EOs' Extraction

The aerial parts of *A. visnaga* and *T. ammi* were gathered from Moulay Idriss Zarhoun, located in the Meknes region of Morocco (34.120279, −5.539773). The region typically has fertile, well-drained soil. During the collection period, the air temperature typically ranges between 20 °C and 30 °C. The plants were dried in a shaded location, within an adequately aerated space. The process of steam distillation was employed to extract EOs using a Clevenger-type apparatus (Glassco®, Haryana, India) with a volume of 5 L. The process of hydro-distillation was conducted using 300 g for each plant in 3 L of distillated water for a duration of 3 h at a temperature of 100 degrees Celsius. Three repetitions were performed for each sample. The essential oils (EOs) were then collected, dried using anhydrous sodium sulphate, and stored in the dark at 4 °C until they were ready to be used.

2.2. Pest: Sitophilus oryzae (L.)

The adults of the rice weevil species, *Sitophioryzae* (*Coleoptera*: *Curculionidae*), were gathered from a grain market, and the taxonomic identification was confirmed by the Department of Animal Biology and Environment at the Faculty of Sciences in Kenitra, Morocco. The subjects were thereafter nourished with a diet consisting solely of rice grains, which were stored in glass jars. The temperature was maintained at a constant 24 °C, with a tolerance of ±1 °C, and the relative humidity was kept at 76%, with a tolerance of ±5%. These conditions were implemented in order to achieve a uniform and consistent adult population.

2.3. Gas Chromatography–Mass Spectrometry

The primary constituents of each essential oil (EO) were determined and measured using mass spectra acquired from a Hewlett–Packard GCD system gas chromatograph–mass spectrometer (GC-MS) (Thermo-Fischer Scientific, Waltham, MA, USA). The chromatographic separation was conducted using an HP Innowax capillary column with dimensions of 60 m × 0.25 mm × 0.25 µm (Agilent, Santa Clara, CA, USA). The oven temperature was programmed to climb at a rate of 4 °C per minute, starting from 50 °C and reaching 200 °C. Helium was employed as the carrier gas, maintaining a consistent flow rate of 1 mL/min. The injection mode was divided. The identification of the EO constituents was achieved by comparing their retention indices, which were determined using the retention times of a range of linear alkanes (C7–C40), with the values documented in existing literature. The obtained mass spectra were subsequently compared to the mass spectra in the NIST library/EPA/NIH mass spectral library, version 2.0 (2002) [18,19].

2.4. Insecticidal Activity

Fumigation with EOs derived from *A. visnaga* and *T. ammi* was conducted in airtight, transparent plastic boxes with a capacity of 1 L, used as exposure chambers to test the toxicity of the EOs against adult *S. oryzae*, as described [10,11,13]. Three Petri dishes were placed in each box, ensuring three repetitions. Each repetition consisted of ten adult *S. oryzae*. The tests were performed under rearing conditions. The EOs were spread on Whatman filter paper, which was placed inside the exposure chamber. Five doses were applied: 0.25, 0.5, 1, 5, and 10 microliters, with an untreated batch serving as a control. Mortality was monitored by counting dead insects from the first day of treatment until all individuals had died [11].

2.5. Molecular Docking Study

Molecular docking investigations were performed using the Maestro 11.5 software from Schrodinger suite. This analysis focused on the modes of interaction of the majority plant compounds and the active sites of the target proteins of acetylcholinesterase and chitin synthase in order to evaluate the insecticidal activity of the plants.

2.5.1. Protein Preparation

The crystal structures of acetylcholinesterase and chitin synthase were sourced from the RCSB data bank with the flowing IDs: 6ARY and 7STM, respectively. Protein preparation was carried out using the Maestro 11.5 Protein Preparation Wizard, focusing on preprocessing, refinement, and reduction. Hydrogen atoms were added, and hydroxyl groups, water molecules, and amino acids were reoriented to correct flaws, such as overlapping or missing atoms. The prepared protein was then subjected to limited minimization to enhance its structure [19].

2.5.2. Ligand Preparation

Ligands were prepared using the Ligprep wizard in Maestro 11.5 from Schrodinger suite. This process converted 2D ligand structures to 3D, introduced hydrogen atoms, resolved bond length and angle conflicts, and performed minimization using the OPLS3 force field. Ionization states were maintained, and chirality was preserved during this preparation phase [18].

2.5.3. Receptor Grid Generation

The receptor grid was generated using Glide molecular docking (Maestro 11.5 from Schrodinger suite), identifying the active site based on the coordinates of the original ligand. Ligands were positioned within the protein's X-ray crystal structure, facilitating potential interaction conformations. Sites, boundaries, rotatable groups, and excluded volumes were also defined [20].

2.5.4. Performing Molecular Docking

Docking was performed using the SP (Standard Precision) mode after completing ligand and protein preparation and grid construction. This mode was selected to evaluate the binding interactions between molecules. The binding energies were measured in kcal/mol, and other factors, such as internal energy, RMSD, desolvation energy, H-bonding, lipophilic interactions, and π-π stacking interactions, were assessed. This methodology ensures both speed and precision in molecular docking studies, providing a detailed analysis of ligand–protein binding interactions [21].

2.6. Statistical Analysis

In order to evaluate the toxic fumigant impact of the essential oils (EOs) being tested, a statistical analysis was conducted utilizing the square root of the percentage mortality (percentage mortality) in Microsoft Excel 2020. The survival probabilities were computed using the Kaplan–Meier analysis, and the impact of each concentration was assessed using the log-rank test [21]. The Probits method, as reported by Finey, was used to determine the lethal concentrations at 50% (LC_{50}) and 99% (LC_{99}) for fumigated insects [22].

3. Results

3.1. Essential Oils' Chemical Composition

EO yields were 0.81 ± 0.12 and 1.75 ± 0.23% of dry weight for *A. visnaga* and *T. ammi*, respectively.
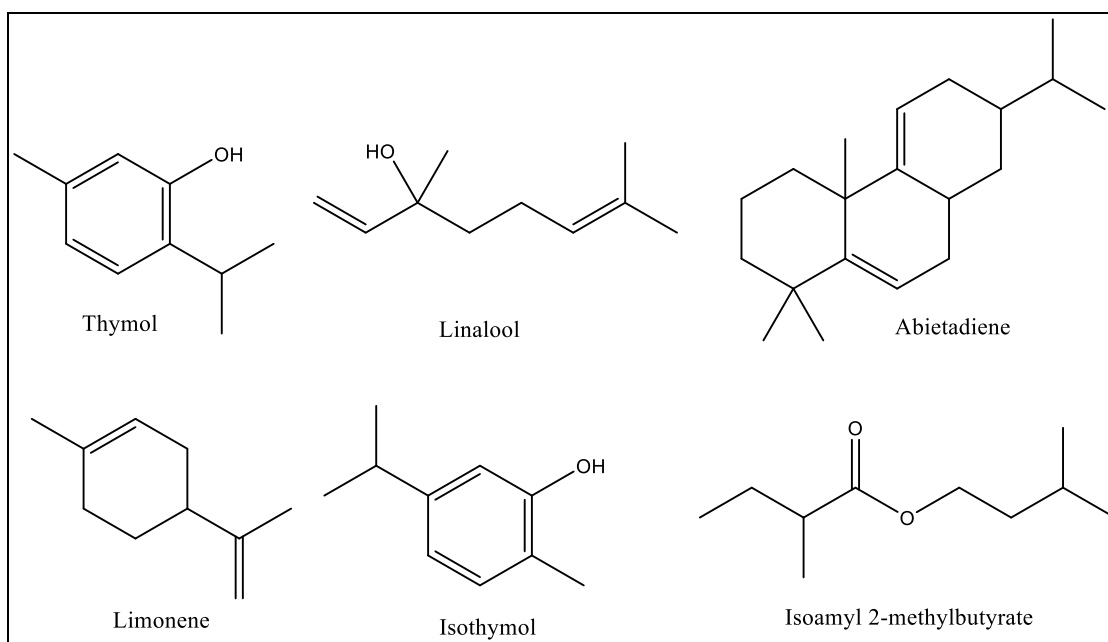
The chemical analysis results of the EOs extracted from *A. visnaga* and *T. ammi* are grouped in Table 1. For the two EOs, different compounds were identified. These compounds were divided into oxygenated and hydro-carbonated monoterpenes and oxygenated and hydro-carbonated sesquiterpenes. In total, 24 compounds were identified in the EOs of *A. visnaga* from the Meknes region (Morocco). The major compounds were Abietadiene (41.23%), linalool (25.54%), limonene (19.04%), isoamyl 2-methylbutyrate (4.78%), and terpinen-4-ol (1.34%; Figure 1).

Table 1. Chemical compositions of essential oils extracted from *Ammi visnaga* and *Trachyspermum ammi*.

No.	Constituent	RT	Area	
			A. visnaga	*T. ammi*
1	α-Hujene	4.64	0.12	0.08
2	α-Pinene	4.95	0.09	1.54
3	Sabinene	5.55	0.95	0.67
4	β-Pinene	5.57	0.04	0.87
5	Myrcene	5.64	-	1.08
6	Isobutyl isobutyrate	5.71	1.31	0.02
7	α-Phellandrene	5.73	-	0.01
8	Propyl-2-methylbutanoate	6.08	0.78	-
9	p-Cymene	6.12	0.23	1.12
10	Limonene	6.17	19.04	13.56
11	δ-3-Carene	6.25	-	0.03
12	Pulegone	6.41	0.52	0.76
13	γ-Terpinene	6.42	-	2.07
14	Cis-linalool oxide	6.47	0.03	0.05
15	Terpindene	6.55	1.06	-
16	Linalool	6.7	25.54	0.9
17	Isoamyl 2-Methylbutyrate	6.72	4.78	0.02
18	Isopentyl isovalerate	6.76	0.12	-
19	Terpinen-4-o1	6.85	1.34	1.08
20	α-Terpineol	7.01	0.78	1.45
21	β-Terpinol	7.03	0.06	-
22	Abietadiene	7.63	41.23	-
23	Methyl thymol	8.38	0.08	1.98
24	Abietal	8.48	0.05	-
25	Abietol	9.88	0.14	-
26	Methyl carvacrol	10.37	0.01	0.05
27	Isothymol	11.91	-	51.88
28	Thymol	12.94	-	10.9
29	Carvacrol	13.59	-	1.06
30	Trans-bergamoptene	14.17	0.04	-
31	Linalyl valerate	15.79	0.03	-
32	β-Sesquiphellandrene	16.43	0.01	0.03
33	Spathulenol	17.59	-	0.7
	Oxygenated monoterpenes		52.21	81.74
	Hydrocarbon monoterpenes		1.31	7.48
	Oxygenated sesquiterpenes		0.78	0.70
	Hydrocarbon sesquiterpenes		42.42	0.11
	Others		1.62	0.02
	Total		98.34	90.05
	Yield		0.81 ± 0.12	1.75 ± 0.23

Upon analysis of EOs from the aerial parts of *T. ammi*, 28 compounds were detected and identified, with the major ones being isothymol (51.88%), limonene (13.56%), thymol (10.9%), γ-terpinene (2.07%), and α-terpineol (1.45%; Table 1).

The variation in EO yields between *A. visnaga* and *T. ammi* can be attributed to differences in the chemical composition of the plant species and the extraction methods used.

Figure 1. Structures of principal compounds from the EOs of *Ammi visnaga* and *Trachyspermum ammi*.

3.2. Insecticidal Activity of Ammi visnaga and Trachyspermum ammi EOs against Sitophilus oryzae

In this study, we assessed the insecticidal properties of *A. visnaga* and *T. ammi* EOs against the weevil *Sitophilus oryzae*. The tested concentrations demonstrated significant fumigant activity, with the extent of this activity depending on both the concentration applied and the duration of exposure. As the concentration and exposure period increased, the survival rate of the insects decreased. Individual responses from our *S. oryzae* adult subjects to different concentrations of EOs were synthesized using survival curves (Figure 2). The mortalities induced by all tested EOs were significantly higher compared to those observed in the controls.
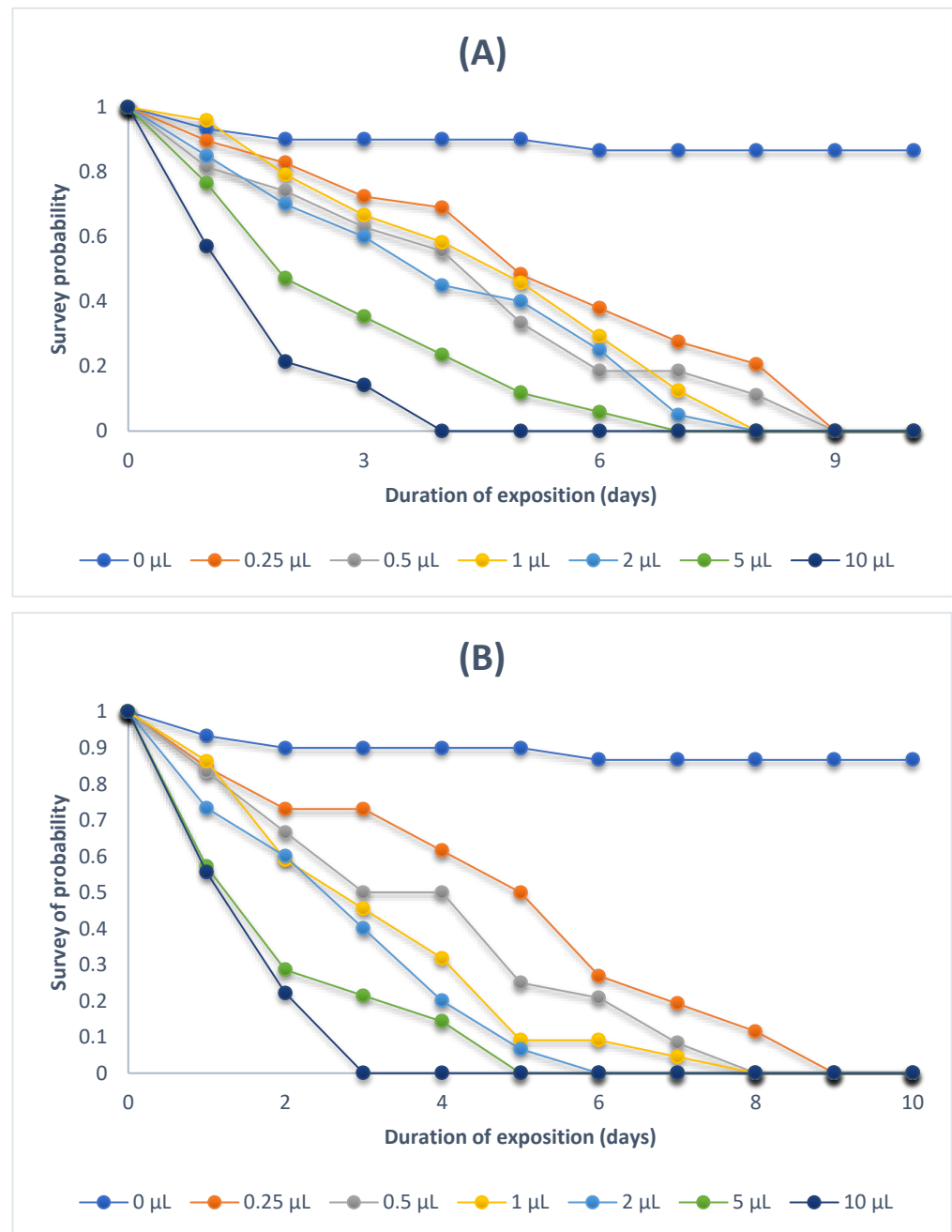
The survival probability of the controls was always higher than 70% on the days of exposure (Figure 2). In both tested plant species, the application of 10 μL of EOs/L of air resulted in high toxicity, leading to the mortality of all treated individuals within the first four days for *A. visnaga* and three days for *T. ammi* EOs. Conversely, the use of 5 μL of EOs/L of air showed lower toxicity. Complete mortality of the treated weevils occurred on the 7th and 5th days after the initiation of treatment with *A. visnaga* and *T. ammi* EOs, respectively.

In the case of *A. visnaga*, concentrations of 0.25, 0.5, and 1 μL of EOs/L of air yielded significantly different results from those of 2.5 or 10 μL of EOs/L of air. However, mortalities of weevils caused by 2, 5, or 10 μL of EOs/L of air were statistically comparable (Figure 2).

The interaction between the weevils and the EOs persisted from 6 to 10 days, with mortality observed within the weevil population as early as the first day, even at the lowest concentration. By the fourth day, insects exposed to 10 μL of EOs/L of air were completely decimated. At lower concentrations, insect mortality progressively affected the entire population by the tenth day of fumigation.

The 50% (LT_{50}) and 99% (LT_{99}) mortality times of adults of *S. oryzae* exposed to various concentrations of the two EOs ranged from 10.64 h to 3.33 days and approximately 4 to 8 days, respectively, depending on the concentration. In contrast, adults in the control group survived an average of 35 and 73 days, respectively (Table 2). Additionally, LT_{50} and LT_{99} showed a negative correlation with the tested concentrations.

Figure 2. Survival curves of *Sitophilus oryzae* (adults) treated with EOs. (**A**) EOs of *A. visnaga* and (**B**) EOs of *T. ammi*.

In assessing the toxicity levels of *A. visnaga* and *T. ammi* EOs on *S. oryzae* adults, lethal concentrations were determined. Comparison of the LC_{50} values revealed that the EOs from *T. ammi* (0.1 µL/L) exhibited greater toxicity than *A. visnaga* (0.38 µL/L) when applied 24 h post-treatment. Over time, both EOs led to a decrease in LC_{50} and LC_{99} values as the exposure duration of weevils to the oils increased (Figure 2). Notably, all concentrations of *A. visnaga* and *T. ammi* EOs resulted in significantly higher mortality rates compared to the control group, indicating acute toxicity against the targeted pest. This observed toxicity can be attributed to the volatile compounds released by the respective EOs.

Table 2. LT$_{50}$ and LT$_{99}$ of the *Sitophilus oryzae* cohort treated with *Ammi visnaga* or *Trachyspermum ammi* essential oils.

EOs	Concentration (µL L^{-1} Air)	LT$_{50}$ (Days)	Correlation Coefficient	LT$_{99}$ (Days)	Correlation Coefficient
A. visnaga	0.25	5.72	−0.9	10.64	0.75
	0.5	4.64		9.46	
	1	4.46		9.29	
	2	3.36		8.75	
	5	1.94		7.82	
	10	0.46		7.25	
T. ammi	0.25	4.85	−0.87	9.85	0.75
	0.5	3.86		8.85	
	1	3.15		7.66	
	2	1.96		5.98	
	5	1.52		4.98	
	10	1.13		3.33	

3.3. Molecular Docking Study

Insecticides often target critical biological processes in insects, with two prominent mechanisms being the inhibition of acetylcholinesterase (AChE) and chitin synthesis. AChE inhibitors, such as organophosphates and carbamates, prevent the breakdown of acetylcholine, leading to continuous nerve signal transmission, overstimulation, paralysis, and rapid insect death. In contrast, chitin synthesis inhibitors, including benzoylureas, disrupt the production of chitin, an essential component of the insect exoskeleton and gut lining, resulting in developmental issues and mortality over time. These mechanisms provide effective pest control, with AChE inhibitors offering quick action and chitin inhibitors providing longer-term population management, while also reducing non-target toxicity and resistance development.

In our in silico study, all molecules exhibited remarkable inhibitory activity against acetylcholinesterase and chitin synthase.

In the inhibition of acetylcholinesterase, isothymol, Abietadiene, and limonene were the most active, with glide g-scores of −6.747, −6.656, and −5.002 kcal/mol, respectively (Table 3).
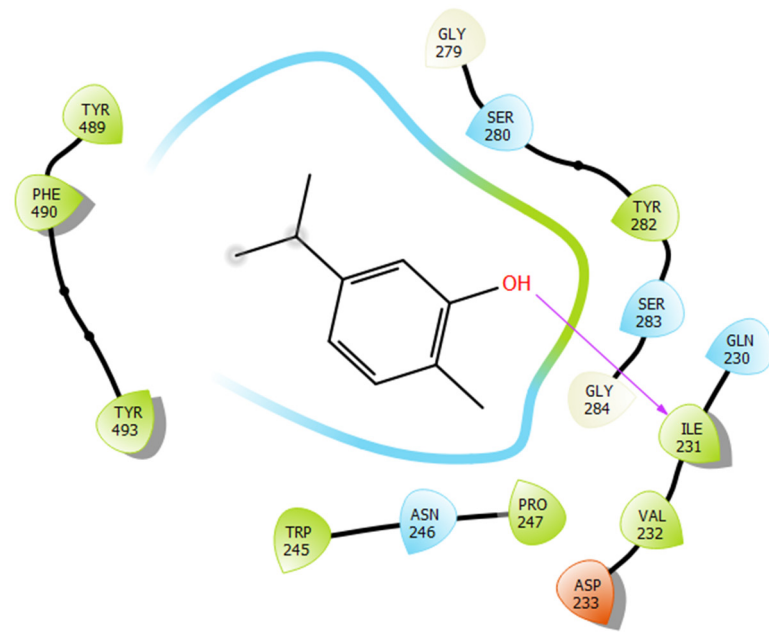
Table 3. Docking results with ligands in different receptors.

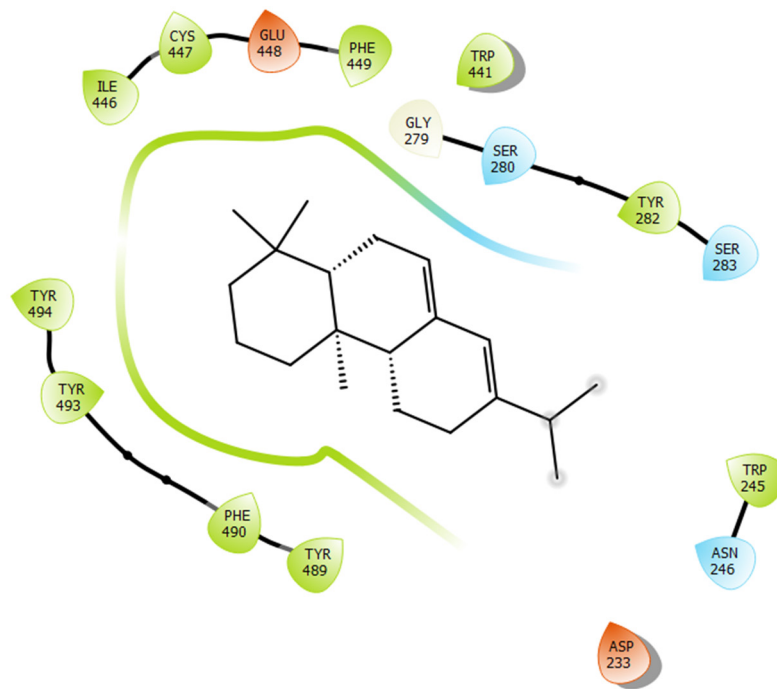|	6ARY			7STM—Minimized		
	Glide G-Score	**Glide Emodel**	**Glide Energy**	**Glide G-Score**	**Glide Emodel**	**Glide Energy**
Abietadiene	−6.656	−31.186	−22.774	−5.564	−30.728	−23.602
Isoamyl 2-methylbutyrate	−4.629	−31.752	−27.192	−3.505	−28.241	−24.543
Isothymol	−6.747	−35.464	−25.354	−5.442	−36.678	−25.386
Limonene	−5.002	−21.261	−16.375	−2.96	−18.591	−15.629
Linalool	−3.991	−27.208	−23.225	−3.568	−36.123	−28.3

In the active site of acetylcholinesterase, isothymol established a single bond with the ILE residue 231 (Figures 3 and 4).

Furthermore, in the inhibition of chitin synthase, Abietadiene, isothymol, and linalool were the most active, with glide g-scores of −5.564, −5.442, and −3.568 kcal/mol, respectively (Table 3). Isothymol established a single bond with the GLU 321 residue in the active site of chitin synthase (Figures 3 and 4).
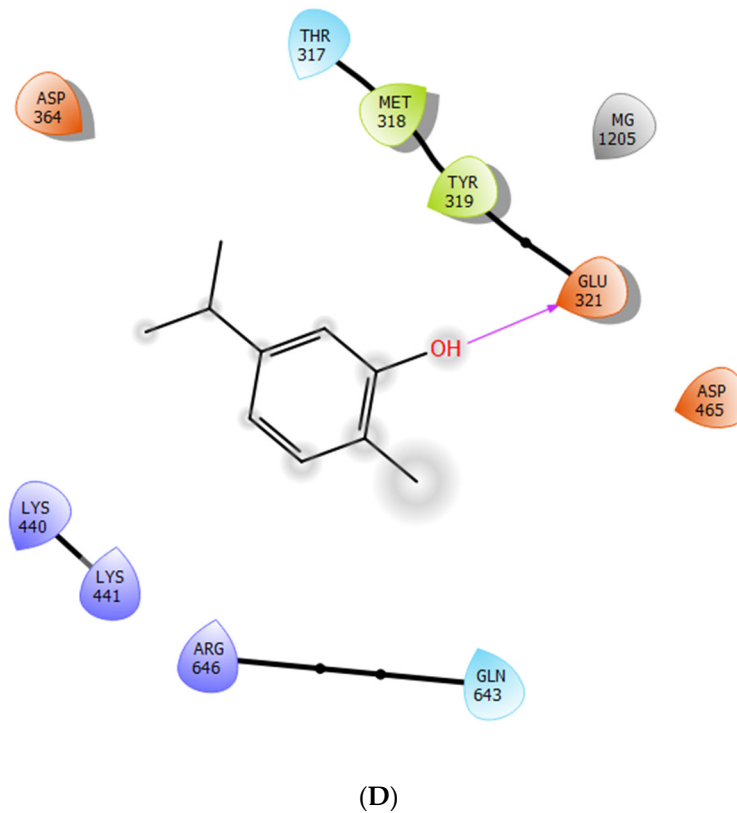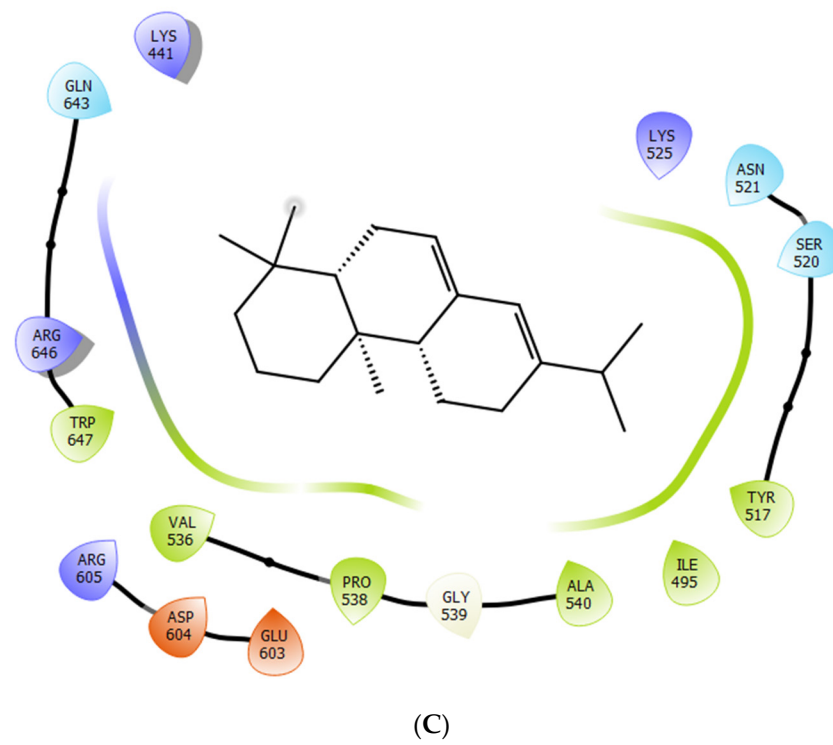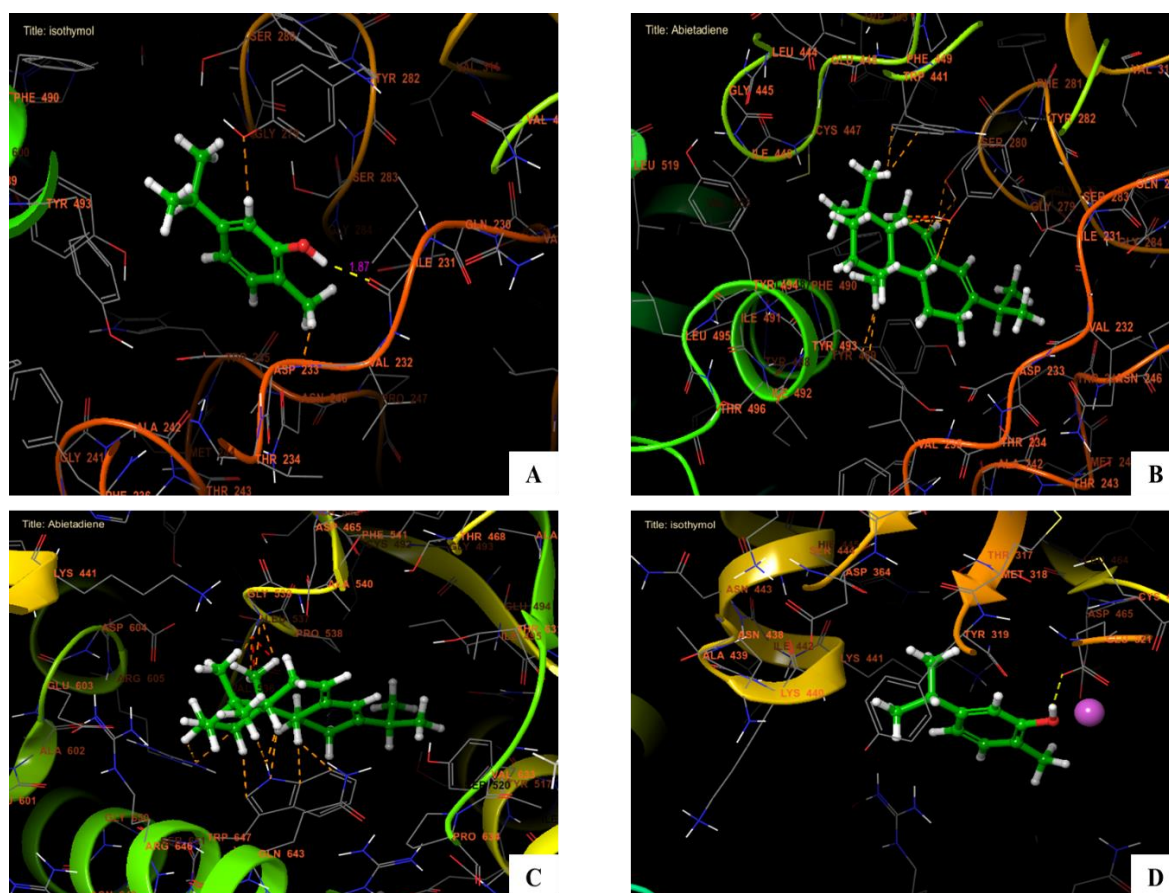
(**A**)

(**B**)

Figure 3. *Cont.*

(**C**)

(**D**)

Figure 3. The 2D viewer of ligands' interactions with the active site. (**A**,**D**) Isothymol interactions with the active site of acetylcholinesterase and chitin synthase. (**B**,**C**) Abietadiene interactions with the active site of acetylcholinesterase and chitin synthase.

Figure 4. The 3D viewer of ligands' interactions with the active site. (**A**,**D**) Isothymol interactions with the active site of acetylcholinesterase and chitin synthase. (**B**,**C**) Abietadiene interactions with the active site of acetylcholinesterase and chitin synthase.

4. Discussion

For *A. visnaga*, the yield of EOs obtained from the flowering period was found to be 1.4% *v/w* [14] when compared to previously reported yields for similar species from Tunisia and Algeria by Khadhri et al. [16,23] and Khalfallah et al. [24], which ranged from 0.2% to 1.3%. Additionally, it surpassed yields obtained during the fruiting period for the same Moroccan species by Satrani et al. [25] and for a Turkish species by Günaydin et al. [26], which ranged between 0.1% and 0.27%. Discrepancies in the extract yields could be attributed to various factors, including geographical origin, species variation, extraction techniques, harvest timing, growth stages, and environmental conditions [24–27].

In contrast, the EO yield of *T. ammi* has been reported to range from 1.20% to 2.64% *v/w* [28], depending on the extraction method used. The highest yield was obtained using the supercritical carbon dioxide (SC-CO$_2$) extraction technique, which is considered a sustainable and green extraction method. This method yielded an EO content of 2.64% *v/w*, which is significantly higher than the other extraction methods [17,29].

For the chemical composition, the EOs of *A. visnaga* contained various compounds identified through GC-MS. Some of the major components included campholenal, longipinene, longifolene, estragole, and β-mentha 1-b-8-ol. Khalil et al. (2020) found that the EOs of *A. visnaga* contain primarily monoterpenes, with smaller amounts of diterpenes and sesquiterpenes. This distribution emphasizes the significance of monoterpenes in influencing the biological properties of the EOs [30]. Moreover, Sellami et al. (2011) identified butanoic acid 3-methyl-1-ethenyl-1,5-dimethylhexenyl ester (linalyl isovalerate) as a derivative present in the chemical composition of *A. visnaga* EOs, showcasing its antimicrobial properties against bacteria and molds [31]. Other components identified in different

studies include butanoic acid, 2-methyl-, pentyl ester, (Z)-β-ocimene, D-limonene, linalool, pulegone, and lavandulyl-butyrate [14,32].

The composition discussed above presents similarities to the profile of *A. visnaga* from Morocco, particularly in terms of linalool abundance: 19.27%, contrasting with 22.7% reported by Satrani et al. [25] and 23.5% by Zrira et al. [33]. Notable differences are seen, specifically regarding the most prevalent compound and identified esters [34]. Satrani et al. [25] pinpointed isoamyl 2-methylbutyrate (27.7%) as the primary compound, while Zrira et al. [33] highlighted butyl 2-methylbutyrate at 41.8%. In contrast, the EOs from the Middle Atlas Mountains of Morocco exhibit isoamyl 2-methylbutyrate (5.33%) in lower concentrations. The chemical profile of *A. visnaga* EOs from Morocco exhibits significant variability based on regions of origin, plant material, and extraction methods [35].

The EOs derived from the northern Tunisian species are characterized by high levels of linalool (23 to 32%), isoamyl 2-methylbutyrate (24 to 36%), and isopentyl isovalerate (10 to 14.8%) [23]. Conversely, the EOs of *A. visnaga* from Giza, Egypt, and Constantine, Algeria, contain significant amounts of 2,2-dimethylbutyric acid (28.9% and 30.1%, respectively) and thymol (13.2% and 6%, respectively). In Turkey, nerol (29.98%) and bisabolol (20.86%) dominate this EOs [24,36].

For *T. ammi*, studies have shown that its EOs are primarily composed of monoterpene compounds. The major components identified in the EOs are consistent with our study: γ-terpinene (266.28 mg/g oil), thymol (201.97 mg/g oil), p-cymene (194.91 mg/g oil), and β-pinene (38.49 mg/g oil) [37,38]. Gandomi et al. (2013) analyzed the chemical composition of *T. ammi* EOs and identified thymol (63.4%), p-cymene (19%), and γ-terpinene (16.9%) as the major components [39]. The study by Moein et al. (2014) further supported these findings, identifying γ-terpinene (48.07%), p-cymene (33.73%), and thymol (17.41%) as the major constituents of *T. ammi* EOs. These compounds contribute to the antimicrobial properties of the EOs, making them effective against various pathogens [38].

Moreover, Bisrat and Jung (2020) highlighted that the EOs of *T. ammi* are dominated by monoterpenoids, with γ-terpinene (32.72%), p-cymene (27.92%), and thymol (24.36%) being the major constituents. These compounds play a crucial role in the insecticidal properties of *T. ammi* EO, making it a potential natural insecticide [17].

The distribution of chemical compounds also correlates with the species' developmental stage and the selected organs for extraction, as noted by Sellami et al. [31]. According to the desired product during the exploitation of the species, the selection of organs, vegetative stage, and region prove very useful in obtaining very precise chemotypes [35].

Our two plants demonstrated insecticidal activity against various weevil species in several studies. According to the search results, *A. visnaga* EOs and extracts have shown efficacy in protecting stored grains against the granary weevil *S. granarius* and the rice weevil *S. oryzae* [39]. The ethanol extract of *A. visnaga* fruit has been found to inhibit lipid content in the hemolymph of nymphs and adults of these weevil species [39]. Khalil et al. (2020) [30] investigated the larvicidal and insecticidal properties of *A. visnaga*, demonstrating its efficacy against nymphs of *Oncopeltus fasciatus* and larvae of *Aedes aegypti*. This highlights the insecticidal potential of *A. visnaga*, indicating its ability to effectively combat insect pests [13]. Overall, while direct studies on the insecticidal activity of *A. visnaga* against weevils are limited, findings from related studies on *Apiaceae* plants and insect pests suggest that *A. visnaga* may indeed exhibit insecticidal properties that could be effective against weevils. Moreover, *T. ammi*, also known as ajwain, has shown promising insecticidal properties against various insect pests, including weevils. According to the search results, the EOs derived from *T. ammi* fruits have demonstrated strong contact and fumigant toxicities against the small hive beetle (*Aethina tumida Murray*), an invasive pest that threatens honeybee colonies [17]. The study by Chaubey (2012) [14] focused on the bioactivity of EOs from *T. ammi* and *N. sativa* against rice weevils, *S. oryzae*. Although this study specifically targeted *T. ammi*, it provided insights into the insecticidal properties of EOs from related plants, which could be indicative of the potential insecticidal activity of *A. visnaga* against weevils. Additionally, the research by Pavela et al. (2017) [40] highlighted

the larvicidal activity of EOs from various Apiaceae taxa, including *T. ammi*, against *Culex quinquefasciatus* [40]. This suggests that plants within the Apiaceae family, such as *A. visnaga*, may possess insecticidal properties that could be effective against weevils [41]. Thymol was found to be the most toxic component, exhibiting high contact ($LD_{50} = 41.79$ μg/adult) and fumigation ($LC_{50} = 52.66$ mg/L air) toxicities against the small hive beetle. The high insecticidal activity of thymol is attributed to its high vapor pressure and phenolic nature, which are generally toxic to insects [17]. The insecticidal properties of *T. ammi* have been further supported by studies on its fumigant activity against adult stages of other stored-product pests, such as *Oryzaephilus surinamensis*, *Rhyzopertha dominica*, and *Tribolium confusum* [13,17].

Research into the insecticidal activities of various active compounds extracted from *A. visnaga* and *T. ammi* EOs has revealed promising potential for several substances. Thymol, a phenolic monoterpene, showed significant insecticidal properties against pests such as *Plutella xylostella* and *Spodoptera litura*, with studies indicating high toxicity and inhibition of developmental stages [42]. Linalool, another monoterpene, exhibited insecticidal and synergistic effects, especially when combined with substances such as eugenol and pyrethroids, enhancing its efficacy against pests such as *Triatoma infestans* and *Spodoptera frugiperda* [43]. Abietadiene derivatives, particularly dehydroabietyl amides, have demonstrated high potency against *Leishmania donovani* and *Trypanosoma cruzi*, suggesting potential for broader insecticidal applications [44].

The inhibition of chitin synthesis in insects is a critical mechanism for pest control, as chitin is an essential component of the insect exoskeleton and gut lining. Chitin synthesis inhibitors, such as benzoylureas, disrupt the production of chitin, leading to developmental issues and mortality over time. In contrast, acetylcholinesterase (AChE) inhibitors, such as organophosphates and carbamates, prevent the breakdown of acetylcholine, leading to rapid insect death [45,46].

The exact mechanisms of chitin synthesis inhibition are still not fully understood, but several studies have shed light on the biochemical properties of chitin synthases and the inhibition of these enzymes by various compounds. For example, diflubenzuron and related benzoylphenylureas have been found to inhibit chitin synthesis in insect integuments and midguts, although the exact mode of action remains unknown [46,47].

Recent studies have also provided structural insights into the inhibition of chitin synthases. For instance, peptidyl nucleoside inhibitors have been shown to inhibit chitin synthases using a unique mechanism, where the nucleoside binds to the active site and prevents the synthesis of chitin [48]. Additionally, nikkomycin Z and polyoxin D have been found to inhibit chitin synthesis by competitively occupying the UDP-GlcNAc binding site and by inhibiting the enzyme through a second mechanism [48].

In summary, the inhibition of chitin synthesis in insects is a critical mechanism for pest control, and various compounds have been identified as effective inhibitors of this process. Further research is needed to fully understand the biochemical and structural mechanisms of chitin synthesis inhibition, which will help in the development of more effective and targeted insecticides.

These findings indicate that *A. visnaga* and *T. ammi* EOs and their active compounds could serve as potential alternatives to synthetic insecticides for the control of weevils and other stored-product insects.

5. Conclusions

This study demonstrated the significant potential of EOs from *A. visnaga* and *T. ammi* as natural insecticides. The chemical analysis identified Abietadiene and isothymol as the primary active compounds. Their effectiveness was confirmed through toxicity tests against *S. oryzae*, with *T. ammi* oil showing higher toxicity. Molecular docking and dynamics simulations further revealed that these compounds stably interacted with and inhibited acetylcholinesterase and chitin synthase, elucidating the molecular basis of their insecticidal properties. These findings underscore the promise of plant-derived compounds in

developing safer, natural alternatives to synthetic insecticides, and highlight the role of molecular modeling in advancing our understanding of their biological mechanisms.

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