

# Insecticidal Properties and Chemical Characterization of Laurus nobilis L. Essential Oils from Two Regions of Morocco against Callosobruchus maculatus (Coleoptera: Bruchinae)

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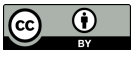
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# Insecticidal Properties and Chemical Characterization of *Laurus nobilis* L. Essential Oils from Two Regions of Morocco against *Callosobruchus maculatus* (Coleoptera: Bruchinae)

**Rachid El Baghazaoui [1,2], Saadia Belmalha [2], Abdellatif Boutagayout [1,2], Laila Nassiri [1], Salma El Alami [1,2], Jean-Michel Savoie [3,*] and El Houssine Bouiamrine [1]**

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**Abstract:** Morocco is a significant botanical reservoir that boasts a wealth of raw materials with promising applications across various industrial sectors, notably in pharmaceuticals and food. The objective of this study was to assess the effectiveness of essential oils (EOs) derived from *Laurus nobilis* L. leaves originating from the Tanger (EOT) and Meknes (EOM) regions in combating *Callosobruchus maculatus* infection. The chemical compositions of these oils were examined using Fourier transform infrared (FTIR) spectroscopy and gas chromatography–mass spectrometry (GC-MS). The biological activity of the EOs was evaluated via repulsion and fumigation tests against *C. maculatus* at varying concentrations. FTIR analysis revealed distinct vibrational bands indicative of various chemical compounds. GC-MS analysis was used to delineate the major chemical constituents of the EOs. The three predominant compounds in the EOT were 1,8-cineole (37.64%), linalool (16.40%), and adamantane (12.00%), whereas 1,8-cineole (47.84%), toluene (17.60%), and α-phellandrene (8.44%) were the most abundant in the EOM. Notably, the EOs exhibited significant repellent activity against *C. maculatus*, with repulsion percentages ranging from 51.11 to 90.00% in Tanger and 67.78 to 93.33% in Meknes. Mortality rates varied from 0 to 100% depending on the treatment. However, the mean concentrations showed mortality rates ranging from 29.44 to 65.56% for the EOT and from 21.11 to 67.78% for the EOM, with LD50 values of 11.96 µL/L and 5.22 µL/L. Docking studies revealed that 1,8-cineole had the highest binding affinity for the active site of acetylcholinesterase, thus confirming its toxic activity against *C. maculatus*. The findings of this study highlight the ability of EOs extracted from *L. nobilis* in the Moroccan regions of Tanger and Meknes to act as effective insecticides and repellents against *C. maculatus*, thereby highlighting avenues for further exploration of pest management and agricultural practices.

**Keywords:** *Callosobruchus maculatus*; fumigation; *Laurus nobilis* L.; repulsion; biocontrol

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## 1. Introduction

In Morocco, chickpea (*Cicer arietinum* L.) is the second largest cropped area among food legume crops, followed by faba bean (*Vicia faba* L.). It is grown in various regions and climates, including irrigated and not-irrigated areas [1]. In 2022, chickpeas were cultivated in over 60,985 ha, yielding 30,954 tons [2]. It is an important legume in the human diet because of its high protein, fiber, and micronutrient contents. Chickpeas are crucial for the sustainability of agricultural systems, especially in crop rotation, owing to their capacity

to fix atmospheric nitrogen in the soil [3,4]. Unfortunately, in Africa, insect infestations in stored grains lead to substantial quantitative and qualitative damage [5].

*Callosobruchus maculatus* (Fab.), belonging to the Chrysomelidae family, is an important pest of chickpeas and other pulses in South America and Africa in the field and in stored grains [6]. Larvae feeding inside the grain lead to significant weight loss, reduced germination potential, and lower nutritional value [7,8]. Currently, the control of *C. maculatus* relies on synthetic insecticides, which have many side-effects, including the development of resistance and the risk of intoxication for consumers and non-target organisms [9]. Therefore, it is essential to develop alternative control methods that are more environmentally friendly, cost-effective, and easier to apply. Botanical extracts, and especially essential oils, are considered an effective means of protecting stored grains against insect infestation [10,11]. Essential oils are particularly notable for their multifaceted insecticidal properties, including ovicidal, antifeedant, repellent, sterilizing, and toxic effects on various insects, but they have fewer non-targeted effects on natural enemies than synthetic chemicals [12–14].

*Laurus nobilis* L. (Lauraceae) is commonly known as laurel. Originally from the southern Mediterranean, this angiosperm is also grown in the United States and Europe, mainly for its decorative purposes [15], and is used as a valuable flavoring agent in the culinary and food industries. In Morocco, it grows spontaneously in the forests of the Eastern and Western Rif and Middle Atlas, and is one of the plants commonly used for the production of essential oils and aromatic extracts [16]. On average, Morocco exports 20 tons of laurel leaves annually [17].

The biopesticidal effectiveness of *L. nobilis* essential oils has been confirmed by various researchers. Notable among these are the repellent activities against pests such as *Acanthoscelides obtectus*, *Tribolium castaneum*, *Sitophilus zeamais*, *Cryptolestes ferrugineus*, *Tenebrio molitor*, *Sitophilus oryzae*, and *Rhyzopertha dominica* [18–20]. Additionally, Jemâa et al. [21] assessed the deterrent and lethal effects of laurel essential oils from Tunisia, Algeria, and Morocco on *R. dominica* and *T. castaneum*, with promising results. In an evaluation of the insecticidal activity of plant powders by contact effect, *L. nobilis* powder had a weak effect on egg-laying by females and the mortality of *C. maculatus* adults [22]. Investigating the insecticidal effects of essential oils from Labiatae and Lauraceae families against *C. maculatus*, it was observed that the essential oil laurel caused significant mortality in adults [23]. However, to the best of our knowledge, no research has been conducted on the effects of *L. nobilis* essential oil on *C. maculatus*.

Building on this foundation, our current study focused on analyzing the chemical components and evaluating the biopesticidal effectiveness of *L. nobilis* essential oils derived from Tanger and Meknes with their distinct differences in pedoclimatic conditions. We aimed to provide a detailed comparison of the composition and biopesticidal activities of these oils against *C. maculatus*, thus providing valuable insights into their potential application in pest control.

## 2. Materials and Methods

### *2.1. Insect Rearings*

Adults of *C. maculatus* came from the dry seeds of infested chickpea (*Cicer arietinum*). These seeds originated from the Meknes Grain Market. *C. maculatus* used for testing the insecticidal activity of essential oil were raised under laboratory conditions with a photoperiod of 14 h of light and 10 h of dark, and a temperature of 25 ± 1 °C [7]. All experiments were conducted under the same controlled conditions.

### *2.2. Plant Materials*

*Laurus nobilis* L. (Lauraceae) samples utilized in this research were gathered from Tanger (35°47′37.4″ N 5°51′57.3″ W) and Meknes (33°50′28.0″ N 5°28′37.8″ W), Morocco, during May and June 2023 (Figure 1). The Tanger region is characterized by a sub-humid Mediterranean climate (mild, wet winters and hot, dry summers), with an average rainfall of approximately 700 mm [24], and mild temperatures with an annual average of 17 °C,

varying from 0 °C to 37 °C depending on the season [25]. The soil in the study area belongs to the Arenosol class [26]. However, the Meknes region is characterized by a Mediterranean to continental climate (cold winters and hot summers) [27,28], with average annual rainfall in Meknes of between 500 and 600 mm [29]. The temperature is characterized by high interannual variability, with mean annual temperatures ranging from 11 °C to 24 °C [30].

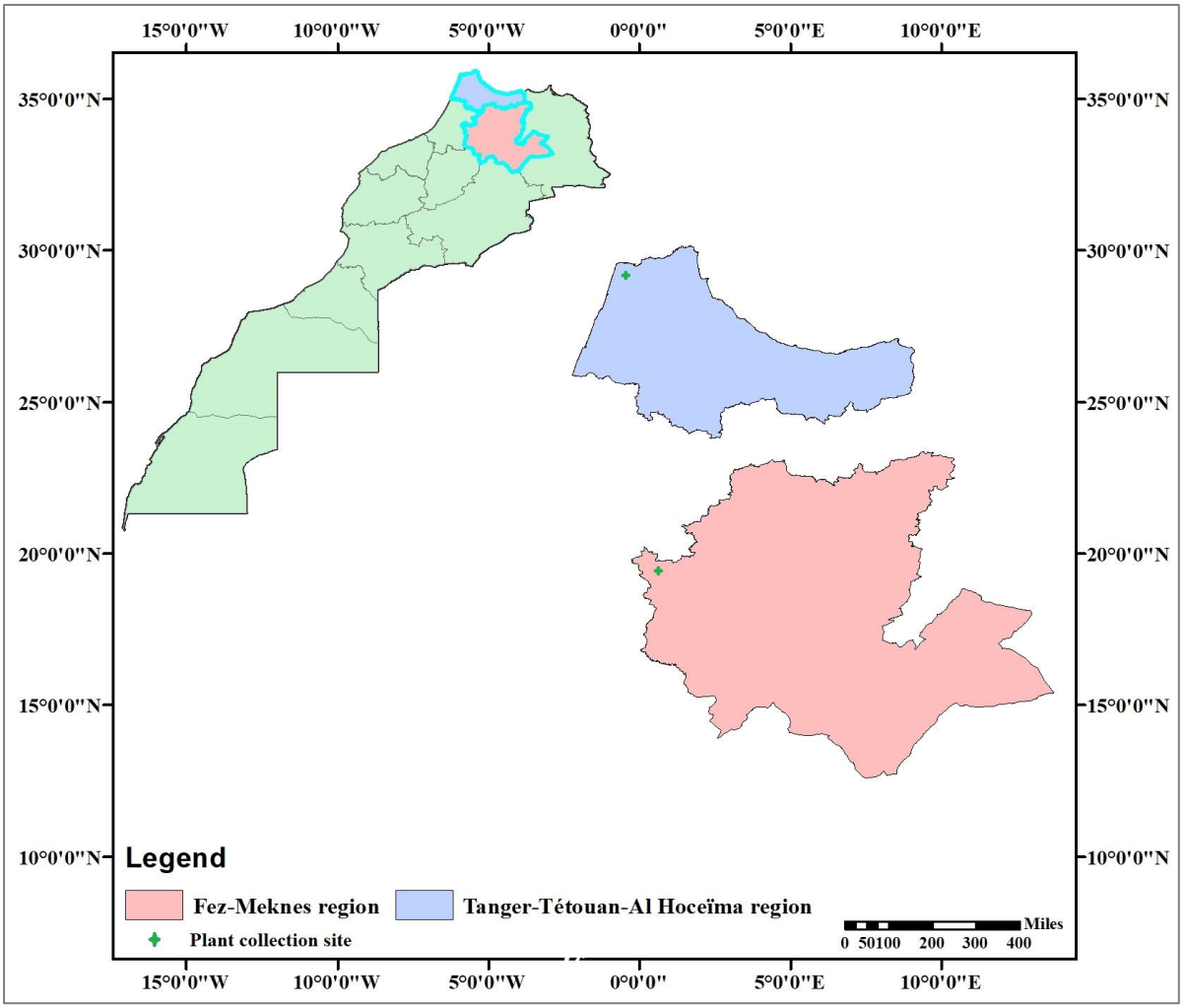



**Figure 1.** Map of *Laurus nobilis* L. collection sites from Tanger and Meknes, Morocco.

### *2.3. Essential Oil Extraction*

Noble laurel leaves were air-dried in the laboratory under controlled conditions and protected from light and moisture. The essential oil (EO) extraction process involved subjecting 100 g of dried leaves to hydrodistillation (4 h) using a Clevenger-type apparatus. The resulting EO was dehydrated using anhydrous sodium sulfate and stored in the dark at 4 °C [31].

### *2.4. FTIR Analyses*

Fourier transform infrared (FTIR) analyses were conducted using a Perkin Elmer Spectrum Two FTIR spectrometer (PerkinElmer, Waltham, MA, USA), performed on 20 µL of the samples. The FTIR spectroscopy of the essential oils was performed in the spectral interval of 400–4000 $cm^{-1}$. The samples were directly applied to the surface of a diamond ATR crystal. The measurements were performed thrice for each sample. Subsequently, baseline correction and smoothing were performed using Spectrum 10 software (Perkin Elmer, Waltham, MA, USA) and Origin Pro 9.1 (OriginLab Corporation, Northampton, MA, USA) [32].

### *2.5. GC–MS Analysis*

The GC-MS chemical analysis of the EO was carried out using a gas chromatograph (Trace GC ULTRA) coupled to a mass spectrometer (MS) (Polaris Q) equipped with a VB5 apolar column (30 m × 0.25 mm; film thickness 0.25 µm) and ion trap mass detector. The initial temperature of the column was adjusted to 40 °C for 2 min and then raised to 180 °C at 5 °C/min. The temperature was subsequently raised to 300 °C at a rate of 20 °C/min and held at this final temperature for 2 min. Helium served as the carrier gas at 1.5 mL/min. The injector temperature was maintained at 200 °C, and the transfer-line temperature was set to 250 °C. The ionization source temperature was set to 230 °C. One microliter of essential oils, diluted to 1:10 in hexane, was injected manually in the split mode. The components present in the essential oils were determined through analysis with the NIST MS Search database, and their identification was cross-referenced with the work of Adams [33].

### *2.6. Bioassay Tests*

#### 2.6.1. Repellency Test

The repellent activity of the essential oils against adult *C. maculatus* insects was assessed using the preferential area method on filter paper following the procedure outlined by McDonald et al. [34]. In brief, filter paper discs measuring 9 cm in diameter were each divided into two halves, each with an area equal to 31.80 $cm^2$, for the intended purpose. Experimental solutions were formulated by dissolving varying volumes of *L. nobilis* essential oils (1, 2, 4, 5, and 6 µL) in 1 mL of acetone. The solutions were evenly distributed over half of a filter paper disc using a micropipette, ensuring consistent coverage, resulting in dosages of 0.031, 0.063, 0.126, 0.157, and 0.189 µL/$cm^2$ per disc. The other half of the filter paper was treated with acetone as the control. The treated and control half discs were air-dried until the solvent had completely evaporated. Treated and untreated halves were taped on opposite sides and positioned in Petri dishes. Ten adult beetles, aged between 7 and 14 days and of both sexes, were introduced at the center of each filter paper disc. The Petri dishes were then securely sealed using Parafilm. The bruchid numbers on the parts of the discs that had been treated with essential oil were recorded against the numbers on the untreated section after 1, 2, 4, 6, 24, and 48 h. Three replicate experiments were performed. The percentage of repulsion (PR) was calculated using the following formula below [7]:

$$PR = \frac{No - Nt}{No + Nt} \times 100$$

*PR*: percentage of repulsion (%);
*No*: number of bruchids in the control zone;
*Nt*: number of bruchids in the treated zone.

The mean repellency percentage was determined for each EO classified into one of the different repellency classes, ranging from 0% to 100% [35]. The following classes were used to group the percentage repellency obtained: class 0 (0 to 0.1%), class I (0.2 to 10%), class II (20.1 to 40%), class III (40.1 to 60%), class IV (60.1 to 80%), and class V (80.1 to 100%) [35].

#### 2.6.2. Fumigant Activity Test

To assess the toxicity of the *L. nobilis* essential oil, small cotton balls were attached from strings to the lids of 380 mL glass jars containing 10 asexual adults each (7 to 14 days). Using a micropipette, doses of 2, 4, 5, and 6 µL of EO were deposited in the aforementioned cotton balls corresponding to fumigant concentrations of 5.3, 10.5, 13.2, and 15.8 µL/L of air, respectively. The caps were screwed firmly onto the vials. Three replicates were performed for each dose. When no movement of the legs or antennae was detected, the insect was considered dead. The observed mortality was corrected using Abbott's formula, as follows:

$$Pc = \frac{Po - Pt}{100 - Pt} \times 100$$

*Pc*: corrected mortality percentage (%);
*Po*: observed mortality in the test;
*Pt*: observed mortality in the control group.

### *2.7. Molecular Docking*

To determine the interactions between the major components of the essential oils from Tangiers and Meknes, 1,8-cineole, linalool, toluene, and AChE were used. Initially, the crystal structure of AChE was retrieved from the Protein Data Bank (https://www.rcsb.org/; accessed on 25 June 2024) (PDB ID: 4EY7). The structure was carefully inspected and prepared using AutoDock MGL tools to ensure its suitability for docking studies. The ligands 1,8-cineole, linalool, and toluene were downloaded from PubChem and prepared using the Open Babel tool. Molecular docking simulations were performed using AutoDock Vina v1.1.2 software [36]. The best-docked poses of each ligand were analyzed to understand the molecular interactions using Discovery Studio Analyzer (Dassault Systèmes, San Diego, CA, USA) and PyMOL (Schrödinger, New York, NY, USA). In the Discovery Studio Analyzer [37], 2D interaction diagrams were generated to visualize the key interactions between the ligands and active site residues of the protein. This analysis included the identification of hydrogen bonds, hydrophobic interactions, π–π stacking, and van der Waals interactions, all of which are critical for ligand binding. Furthermore, 3D interaction maps were generated using PyMOL to provide a spatial representation of how each ligand fit within the active site. The grid box was centered at coordinates (7.279, −63.643, −15.945) with dimensions of 54, 56, and 62 Å along the x-, y-, and z-axes, respectively. Additionally, Solvent Accessibility Surface (SAS) analysis was conducted to evaluate the exposure of the ligands to the solvent, indicating how well the ligands were embedded within the binding pocket.

### *2.8. Statistical Analysis*

A completely randomized design was employed to assess the main effects of the dose and time, as well as their interactions, with three replications. Descriptive statistics were initially conducted to summarize the data on repellency and mortality across different treatments. The mean values and standard deviations were calculated for each treatment. Normality checks were performed on the collected data before conducting an analysis of variance (two-way ANOVA) to evaluate the effects of these factors on the measured parameters. Post hoc tests (Duncan's test) were subsequently applied to identify significant differences between individual means, where appropriate [21]. All statistical analyses were performed using the SPSS Statistics 21. A heatmap analysis of the insect repellency was performed using a plot correlation matrix in Pandas Python [38].

## 3. Results

### *3.1. Chemical Characterization of L. nobilis Essential Oils*

#### 3.1.1. FTIR Analysis

Typical FTIR spectra of *L. nobilis* essential oil are depicted in Figure 2. In the FTIR spectra of *L. nobilis* essential oil obtained from Tanger, significant vibrational bands were observed at 3607, 2900, 1998, and 1269 $cm^{-1}$. Conversely, the essential oil collected from Meknes exhibited notable spectral bands at 3607, 2900, 1998, 1732, 1465, 1374, 1269, 1213, 1169, 1132, 1080, 1053, 986, 920, and 842 $cm^{-1}$, as detailed in Table 1. Both essential oils shared common spectral bands at 3607, 2900, 1998, and 1269 $cm^{-1}$.

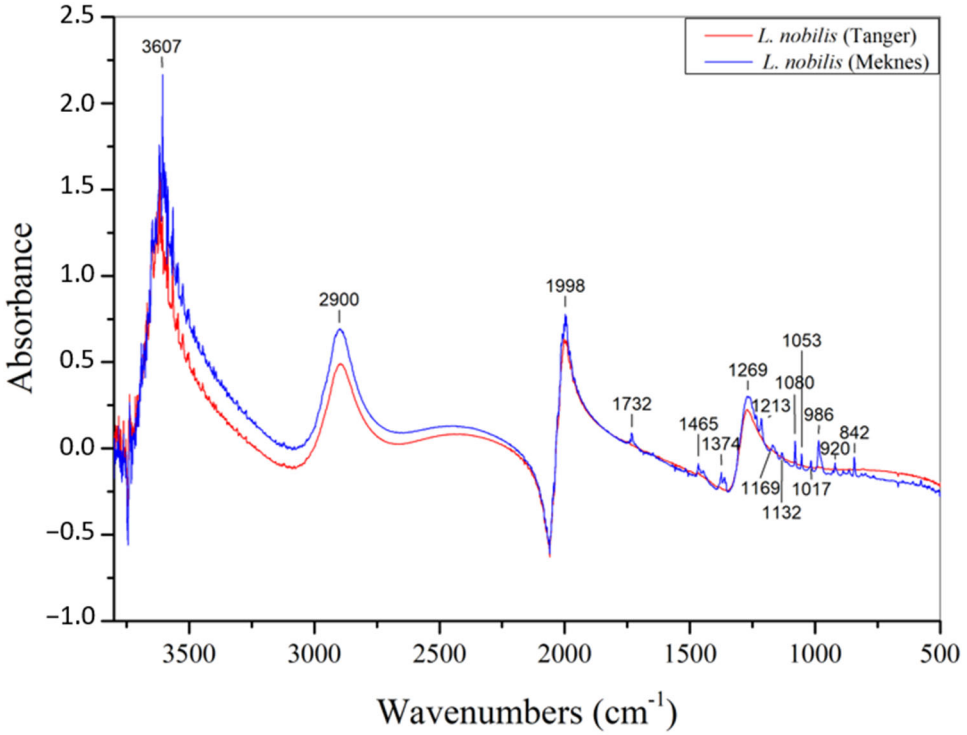



**Figure 2.** Fourier transform infrared spectroscopy (FTIR) bands related to the essential oils (EOs) of *Laurus nobilis* from Tanger and Meknes, Morocco.

**Table 1.** Assignment of the major FTIR assignments in the essential oils (EOs) of *Laurus nobilis* from Tanger (EOT) and Meknes (EOM), Morocco.

| Wavenumber ($cm^{-1}$) | EOT | EOM | Assignment | Relevant Constituent(s) |
|---|---|---|---|---|
| 3607 | * | * | $\upsilon_s$(OH) | linalool, terpinene-4-ol, α-terpineol |
| 2900 | * | * | $\upsilon_s(CH_2)$ | sabinene, linalool, β-pinene, 1,8-cineole |
| 1998 | * | * | –CHO | unidentified |
| 1732 | - | * | υ(C=O) | α-terpinyl, bornyl, linalyl acetates |
| 1465 | - | * | υ(C=C–C) (ring) or $\delta(CH_2)$ | methyleugenol, eugenol p-cymene |
| 1374 | - | * | $\upsilon_s$(CH3–C=O) $\delta_s(CH_3)$ gem | 1,8-cineole, α-terpinyl acetate |
| 1269 | * | * | υas(C–O–C) aromatic<br>υs(C–O–C) aromatic<br>υ (O=C–O) | methyleugenol, eugenol acetate esters |
| 1213 | - | * | υas(C–O–C) aromatic<br>υs(C–O–C) aromatic<br>υ (O=C–O) | methyleugenol, eugenol acetate esters |
| 1169 | - | * | υas(C–O–C) aromatic<br>υs(C–O–C) aromatic<br>υ (O=C–O) | methyleugenol, eugenol acetate esters |
| 1132 | - | * | υas(C–O–C) aromatic<br>υs(C–O–C) aromatic<br>υ(O=C–O) | methyleugenol, eugenol acetate esters |
| 1080 | - | * | υ(C–O–C) | 1,8-cineole |

**Table 1.** *Cont.*

| Wavenumber ($cm^{-1}$) | EOT | EOM | Assignment | Relevant Constituent(s) |
|---|---|---|---|---|
| 1053 | - | * | $\upsilon_{as}(CH_2–O–C=O)$ | acetates of primary alcohols |
| 986 | - | * | δ(C–H) | 1,8-cineole |
| 920 | - | * | $(CH_3)_3$–C–O or 5-membered cyclic ethers | |

*, present; -, absent; υ, stretching vibration; δ, in-plane deformation vibration.

According to previous research, the spectral bands at 3607 $cm^{-1}$ were identified as indicative of the stretching vibrations of the OH functional group of alcohols, potentially corresponding to compounds such as linalool, terpinene-4-ol, and α-terpineol. The spectral band observed at 2900 $cm^{-1}$ was likely linked to the $CH_2$ ring vibrations of volatile compounds, including sabinene, linalool, β-pinene, and 1,8-cineole. The presence of spectral bands at 1269 $cm^{-1}$ indicated C–O–C stretching vibrations, potentially corresponding to methyl eugenol and eugenol acetate esters.

The essential oil from Meknes was distinguished by additional spectral bands at 1732, 1465, 1374, 1213, 1169, 1132, 1080, 1053, 986, 920, and 842 $cm^{-1}$, which may correspond to compounds such as α-terpinyl, bornyl, linalyl acetates, methyleugenol, p-cymene, eugenol, and 1,8-cineole, with the latter being the most abundant.

### 3.1.2. GC-MS Analysis

The chemical compositions of the essential oils, along with their retention times and percentage peak areas analyzed by GC-MS, are summarized in Tables 2 and 3. Both essential oils were found to contain several compounds, including α-pinene, β-pinene, 1,8-cineole, α-phellandrene, cyclofenchene, α-terpineol, eugenol, methyleugenol, adamantane, and terpinolene, albeit in varying quantities.

**Table 2.** Components detected in the essential oils (EOs) of *Laurus nobilis* from Tanger (EOT) using GC-MS analysis.

| N | Compounds | Chemical Formula | RT | Peak Area (%) | MW |
|---|---|---|---|---|---|
| 1 | α-Pinene | $C_{10}H_{16}$ | 8.68 | 2.62 | 136 |
| 2 | β-Pinene | $C_{10}H_{16}$ | 10.16 | 7.57 | 136 |
| 3 | 1,8-cineole | $C_{10}H_{18}O$ | 12.26 | 37.64 | 154 |
| 4 | α-phellandrene | $C_{10}H_{16}$ | 13.26 | 1.06 | 136 |
| 5 | Linalool | $C_{10}H_{18}O$ | 14.94 | 16.40 | 154 |
| 6 | Cyclofenchene | $C_{10}H_{16}$ | 16.90 | 0.83 | 136 |
| 7 | Isobornyl acetate | $C_{12}H_{20}O_2$ | 17.60 | 1.23 | 196 |
| 8 | α-terpineol | $C_{10}H_{18}O$ | 18.12 | 2.01 | 154 |
| 9 | Phenol, p-tert-butyl- | $C_{10}H_{14}O$ | 21.90 | 0.51 | 150 |
| 10 | Eugenol | $C_{10}H_{12}O_2$ | 23.78 | 1.02 | 164 |
| 11 | Methyleugenol | $C_{11}H_{14}O_2$ | 25.30 | 8.03 | 178 |
| 12 | Nopol | $C_{11}H_{18}O$ | 25.71 | 0.98 | 166 |
| 13 | Benzene | $C_{13}H_{20}$ | 25.79 | 5.21 | 176 |
| 14 | Adamantane | $C_{12}H_{20}$ | 28.06 | 12.00 | 136 |
| 15 | Terpinolene | $C_{10}H_{16}$ | 30.67 | 1.46 | 136 |
| 16 | Butylated Hydroxytoluene | $C_{15}H_{24}O$ | 32.73 | 0.49 | 220 |

**Table 2.** *Cont.*

| N | Compounds | Chemical Formula | RT | Peak Area (%) | MW |
|---|---|---|---|---|---|
| 17 | Unknown | | | 0.52 | |
| 18 | Octanal, 2-(phenylmethylene)- | $C_{15}H_{20}O$ | 33.44 | 0.42 | 216 |
| | Total (%) | | | 100 | |
| | Yield (%) | | | 0.84 | |

RT—retention time (min); MW—molecular weight (g/mol).

**Table 3.** Components detected in the essential oils (EOs) of *Laurus nobilis* from Meknes (EOM) using GC-MS analysis.

| N | Compounds | Chemical Formula | RT | Peak Area (%) | MW |
|---|---|---|---|---|---|
| 1 | α-Pinene | $C_{10}H_{16}$ | 8.69 | 3.71 | 136 |
| 2 | Camphene | $C_{10}H_{16}$ | 9.19 | 0.40 | 136 |
| 3 | α-Phellandrene | $C_{10}H_{16}$ | 9.95 | 8.44 | 136 |
| 4 | β-Pinene | $C_{10}H_{16}$ | 10.21 | 2.47 | 136 |
| 5 | α -Myrcene | $C_{10}H_{16}$ | 10.80 | 0.48 | 136 |
| 6 | Tricyclene | $C_{10}H_{16}$ | 11.44 | 0.59 | 136 |
| 7 | α-Terpinene | $C_{10}H_{16}$ | 11.69 | 0.47 | 136 |
| 8 | o-Cymene | $C_{10}H_{14}$ | 12.08 | 0.41 | 134 |
| 9 | 1,8-Cineole | $C_{10}H_{18}O$ | 12.30 | 47.84 | 154 |
| 10 | Cyclofenchene | $C_{10}H_{16}$ | 13.80 | 0.99 | 136 |
| 11 | Terpinolene | $C_{10}H_{16}$ | 14.90 | 6.53 | 136 |
| 12 | Isopulegol | $C_{10}H_{18}O$ | 17.60 | 1.60 | 154 |
| 13 | α -Terpineol | $C_{10}H_{18}O$ | 18.13 | 1.71 | 154 |
| 14 | Adamantane | $C_{12}H_{20}$ | 22.37 | 0.59 | 136 |
| 15 | Toluene | $C_7H_7NO_2$ | 23.52 | 17.60 | 137 |
| 16 | Eugenol | $C_{10}H_{12}O_2$ | 23.79 | 2.28 | 164 |
| 17 | Methyleugenol | $C_{11}H_{14}O_2$ | 25.28 | 1.55 | 178 |
| 18 | Benzene, 1,3,5-trimethyl-2-nitro- | $C_9H_{11}NO_2$ | 25.71 | 0.44 | 165 |
| 19 | Benzoic acid, 3,4-dimethyl- | $C_9H_{10}O_2$ | 29.89 | 0.64 | 150 |
| 20 | Benzene, hexamethyl- | $C_{12}H_{18}$ | 30.55 | 0.39 | 162 |
| 21 | Terpinolene | $C_{10}H_{16}$ | 30.66 | 0.60 | 136 |
| 22 | Thymoquinone | $C_{10}H_{12}O_2$ | 32.73 | 0.29 | 164 |
| | Total | | | 100 | |
| | Yield (%) | | | 0.55 | |

RT—retention time (min); MW—molecular Weight (g/mol).

In the essential oil from Tanger (EOT), the most prominent components are 1,8-cineole (37.64%), linalool (16.40%), adamantane (12.00%), methyleugenol (8.03%), β-pinene (7.57%), and benzene (5.21%). Conversely, the essential oil from Meknes (EOM) is characterized by the presence of 1,8-cineole (47.84%), toluene (17.60%), α-phellandrene (8.44%), terpinolene (6.53%), α-pinene (3.71%), β-pinene (2.47%), and eugenol (2.28%).

### 3.2. Repellent Activity

The repellent test conducted using the *L. nobilis* EOT demonstrated significant repellent activity toward *C. maculatus* adults (Figure 3). The heatmap results for insect repellence by essential oils varied considerably with dose and time. In the EOT, higher doses generally produce a stronger and more consistent repellency. For example, at a dose of 0.189 µL/cm$^2$, the repellency remained high at 93.33% after 1 h, 100% after 2 h, and 93.33% after 4 and 6 h. Even after 24 h, the repulsion remained significant at 73.33%, increasing to 86.67% after 48 h. In contrast, at the lowest dose of 0.031 µL/cm$^2$, the initial repulsion was moderate at 60%, decreased sharply to 0% after 24 h, and then increased to 53.33% after 48 h. These results indicate that higher doses of essential oils are more effective in maintaining high repellency over an extended period, although fluctuations may occur. For the EOM, insect repellency also showed strong dose and time dependency. At a dose of 0.189 µL/cm$^2$, the repellency remained high (93.33%) after 1 h, reached 100% after 2 h, and was maintained at 86.67% after 24 and 48 h. At the lowest dose of 0.031 µL/cm$^2$, repulsion was initially elevated to 93.33%, maintained at 80% after 6 h, and remained elevated to 100% after 24 h and 86.67% after 48 h. These results suggest that, even at lower doses, the repellency of *C. maculatus* could be maintained at high levels over time by the EOM.

The average repulsion increased with the dose, with *C. maculatus* adults showing an average repulsion of over 50% for all doses. For the EOT, the dose averages showed repellency ranging from 51.11% to 90%, with classifications ranging from class III to V. The time averages showed more varied values, with an initial repulsion of 77.33% at 1 h, decreasing to 66.67% after 2 h, rising to 74.67% after 4 h, reaching a minimum of 49.33% after 24 h, before rising to 65.33% after 48 h. Dose averages for the EOM showed higher repellencies, ranging from 67.78% to 93.33%, with most doses classified as class V, indicating a high efficacy. The time averages initially showed high repellency, decreasing slightly with time, with *p*-values indicating the statistically significant effects of the dose ($p < 0.0001$) and time ($p = 0.03$) (Table 4).

**Table 4.** Repellency (%) of *C. maculatus* with *L. nobilis* essential oil from Tangier (EOT) and Meknes (EOM) as a function of mean dose and time.

| | **Repellency (%)** | | | |
|---|---|---|---|---|
| | **Essential Oil from Tangier** | | **Essential Oil from Meknes** | |
| **Mean of Dose (µL/cm$^2$)** | **Mean Repellency (%)** | **Class** | **Mean Repellency (%)** | **Class** |
| 0.031 | 51.11 ± 7.95 c | Class III | 87.78 ± 12.15 a | Class V |
| 0.063 | 52.22 ± 5.15 c | Class III | 67.78 ± 19.57 b | Class IV |
| 0.126 | 78.89 ± 4.98 ab | Class IV | 90.00 ± 17.15 a | Class V |
| 0.157 | 71.11 ± 4.35 b | Class IV | 93.33 ± 9.70 a | Class V |
| 0.189 | 90 ± 2.91 a | Class V | 92.22 ± 10.03 a | Class V |
| **Mean of Time (h)** | **EOT** | | **EOM** | |
| 1 | 77.33 ± 5.47 a | | 93.33 ± 9.76 a | |
| 2 | 66.67 ± 6.67 ab | | 92.00 ± 10.14 ab | |
| 4 | 74.67 ± 7.16 a | | 86.67 ± 17.99 ab | |
| 6 | 78.67 ± 5.33 a | | 82.67 ± 18.31 ab | |
| 24 | 49.33 ± 8.92 b | | 84.00 ± 17.24 ab | |
| 48 | 65.33 ± 5.33 ab | | 78.67 ± 22.00 b | |
| *p*-value dose | <0.0001 | | <0.0001 | |
| *p*-value time | 0.024 | | 0.03 | |

According to Duncan's test, values with different letters in the same column indicate significant differences ($p < 0.05$).

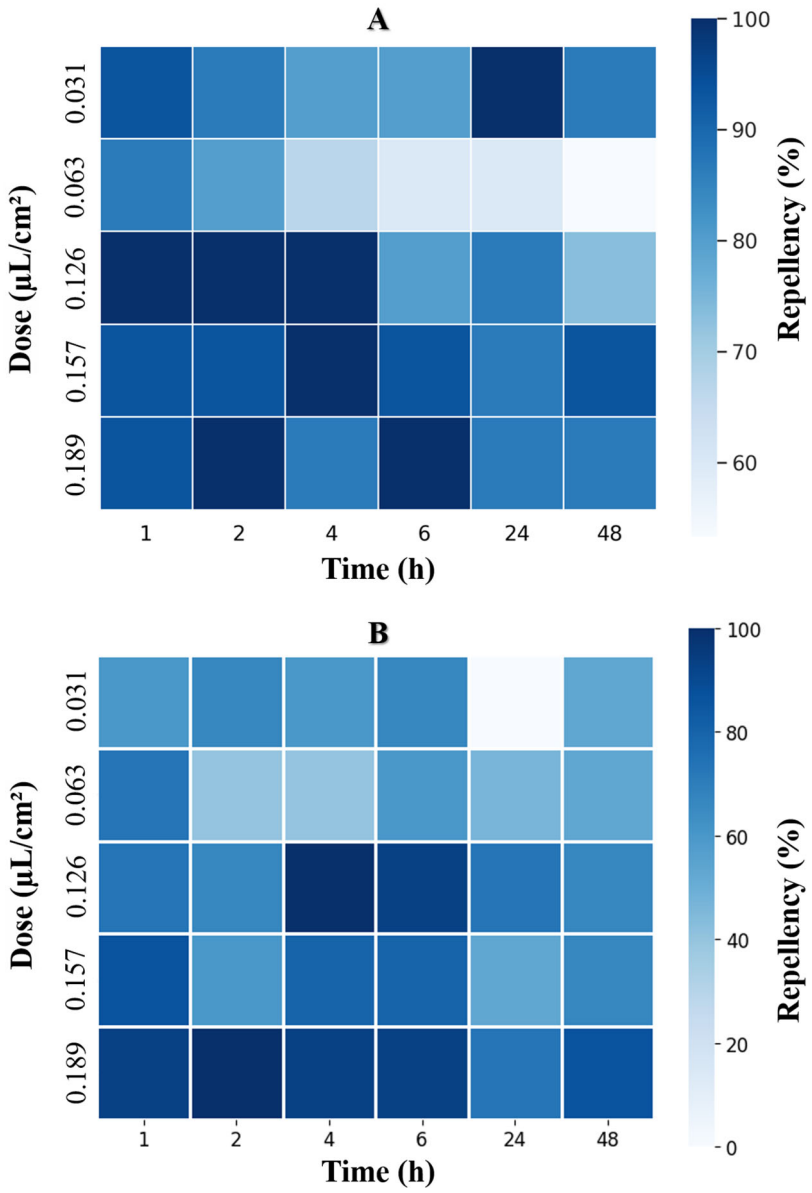



**Figure 3.** Heatmaps showing the effect of *L. nobilis* essential oil from Meknes (**A**) and Tanger (**B**) on *C. maculatus* repellency (%).

### *3.3. Fumigant Toxicity*

The insecticidal activity of the EOs extracted from *L. nobilis* against cowpea bruchids was evaluated (Figure 4). The results from the EOT showed that insect mortality varied with time and treatment with increasing doses of essential oil. The data showed a progressive increase in mortality over time for all of the treatments. For example, for treatment T0 (without essential oil), the mortality remained at zero for up to 3 h, began to increase at 24 h (16.7%), and reached 70% after 96 h. At higher doses, such as T4 (15.8 µL/L air), mortality reached 100% as early as 96 h. The results for the EOM also showed an increase in insect mortality with time and the essential oil dose. However, mortality in this region was generally higher for the same treatments than in the Tangier region. For example, for treatment T1 (5.3 µL/L air), mortality was 100% after 96 h, whereas, in the Tangier region, mortality was 70% for the same treatment and duration.

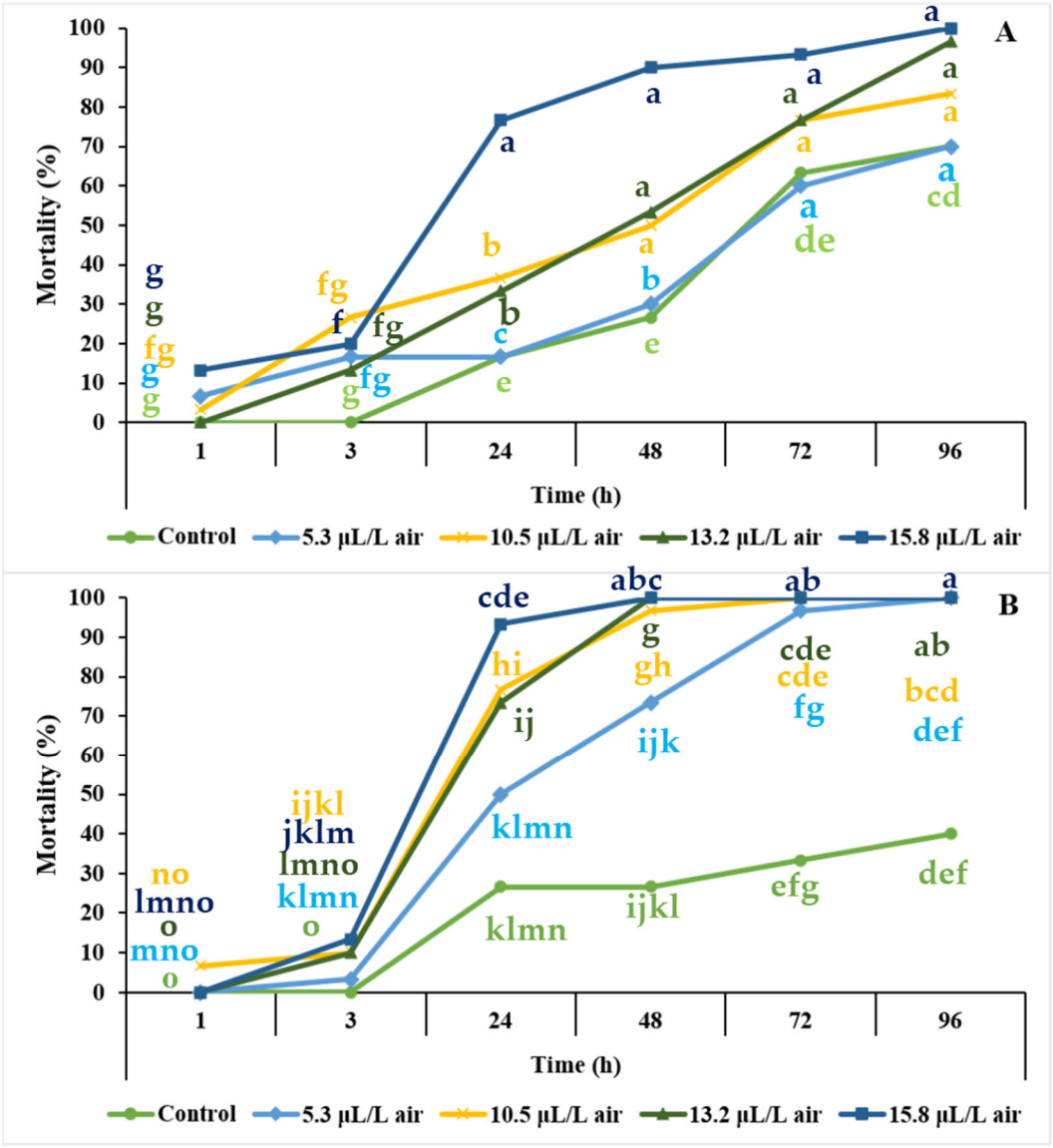



**Figure 4.** Mortality (%) of *L. nobilis* essential oil from Meknes (**A**) and Tanger (**B**) on *C. maculatus*. According to Duncan's test, the means of mortality in a curve followed by a similar letter with the same color were not significantly different ($p < 0.05$).

Table 5 shows the increase in the mortality rates as a function of the dose and duration of exposure. For essential oil from the Tangier region (EOT), mean concentrations showed a progressive increase in mortality, rising from 29.44% for the control to 65.56% for the highest dose. Similarly, for essential oil from the Meknes region (EOM), the concentration averages also showed an increase in mortality, ranging from 21.11% for the control to 67.78% for the highest dose. This trend of increasing mortality was also observed over time for both essential oils, with rates ranging from 4.67% to 84.00% for the EOT and from 1.33% to 88.00% for the EOM.

Comparing the two regions, it was clear that the Meknes region showed higher insect mortality at the same doses and durations. For example, at 48 h for treatment T3 (13.2 µL/L air), the mortality in the Tangier region was 53.3%, whereas it was 100% in the Meknes region. Furthermore, mortality exceeded 50% when exposed to a dose of 5.3 µL/L of air, whereas the EOT only induced mortality above 50% at a higher dose of 15.8 µL/L of air. This suggests that the EOM is more effective in killing insects. The LD50 values (dose lethal to 50% of insects) confirm this difference, with an LD50 of 11.96 µL/L of air ($y = 1.0692x + 4.2323$, $R^2 = 0.9805$) for the Tangier region and 5.22 µL/L of air ($y = 1.0692x + 4.2323$,

$R^2$ = 0.9805) for the Meknes region, indicating that larger doses are needed in the EOT to achieve the same mortality of the EOM.

**Table 5.** Mortality (%) of *C. maculatus* with *L. nobilis* essential oil from Tangier (EOT) and Meknes (EOM) as a function of mean dose and time.

| | | Mortality (%) | |
|---|---|---|---|
| | | **EOT** | **EOM** |
| Mean of concentration | Control | 29.44 ± 7.12 b | 21.11 ± 4.11 b |
| | 5.3 µL/L air | 33.33 ± 6.00 b | 53.89 ± 9.87 a |
| | 10.5 µL/L air | 46.11 ± 6.87 ab | 65.00 ± 10.01 a |
| | 13.2 µL/L air | 45.56 ± 8.37 ab | 63.89 ± 10.45 a |
| | 15.8 µL/L air | 65.56 ± 8.72 a | 67.78 ± 10.56 a |
| Mean of time | 1 h | 4.67 ± 1.65 d | 1.33 ± 0.91 c |
| | 3 h | 15.33 ± 2.91 d | 7.33 ± 2.06 c |
| | 24 h | 36.00 ± 6.39 d | 64.00 ± 6.53 b |
| | 48 h | 50.00 ± 6.47 b | 79.33 ± 7.59 ab |
| | 72 h | 74.00 ± 3.63 a | 86.00 ± 7.22 b |
| | 96 h | 84.00 ± 3.75 a | 88.00 ± 6.49 a |
| $LD_{50}$ | | 11.96 | 5.22 |
| *p*-value dose | | 0.01 | 0.003 |
| *p*-value time | | <0.0001 | <0.0001 |

The data are presented as the mean ± standard deviation of three replicates. According to Duncan's test, values with different letters in the same column indicate significant differences ($p < 0.05$). LD50 was defined as the lowest concentration that caused 50.0% mortality.

### *3.4. Molecular Docking*

Molecular docking showed that 1,8-cineole was the most active compound against acetylcholinesterase, followed by toluene and linalool, with a binding energy of −6.7 Kcal/mol, −5.7 kcal/mol, and −4.8 kcal/mol, respectively. Figures 5 and 6 show the 2D and 3D structures of the 1,8-cineole, toluene, and linalool interactions with the active site of AChE (PDB: 4EY7). The 1,8-cineole formed Pi-Alkyl interactions with TRP residue 86 and hydrophobic interactions between the amino acids' aromatic rings and the ligand's alkyl groups. The same ligand formed a Pi-Sigma-type interaction with TYR 337 and PHE 338, and van der Waals-type interactions with THR 83, TYR 341, TYR 124, ASP 74, GLY 121, and HIS 447, which are weak, non-covalent interactions between molecules or parts of molecules close to each other (Figure 5A). Toluene, in turn, formed Pi-Pi stacked interactions with the residues TYR 341 and TRP 286, and its residues formed π-π stacked interactions with the aromatic ring of toluene. The same ligand exhibited a Pi-alkyl interaction with VAL 294 and van der Waals interactions with PHE 297, PHE 338, PHE 295, and SER 293 (Figure 5B). Linalool formed two bonds, the first of the van der Waals type with the residues GLN 413, CYS 409, ASN 533, PRO 410, GLY 234, ASN 233, and PRO 235, while the second was of the Pi-alkyl type with the residues HIS 405, TRP 532, LEU 536, PRO 537, and LEU 540 (Figure 5C). Thus, all of the compounds tested formed hydrophobic bonds, as well as charged and polar interactions with the target protein. The SAS analysis showed that 1,8-cineole was deeply embedded within the binding pocket, suggesting a strong and stable interaction, whereas toluene and linalool were less embedded, correlating with their lower binding affinities.

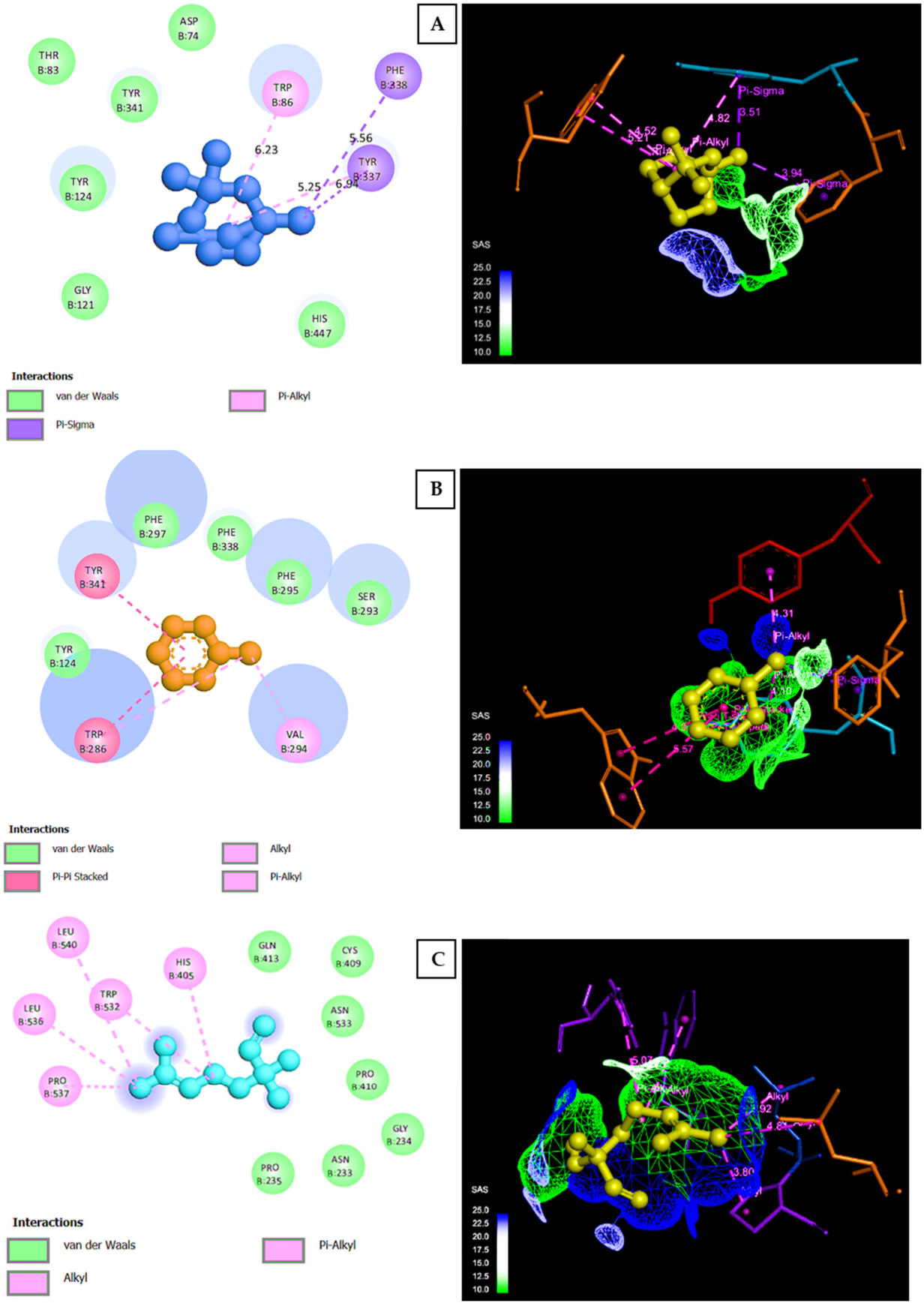



**Figure 5.** The 2D diagrams of ligand interactions with Ache (PDB: 4EY7) (left) and solvent accessibility (SAS) (Wright): (**A**) 1,8-cineole, (**B**) toluene, and (**C**) linalool.

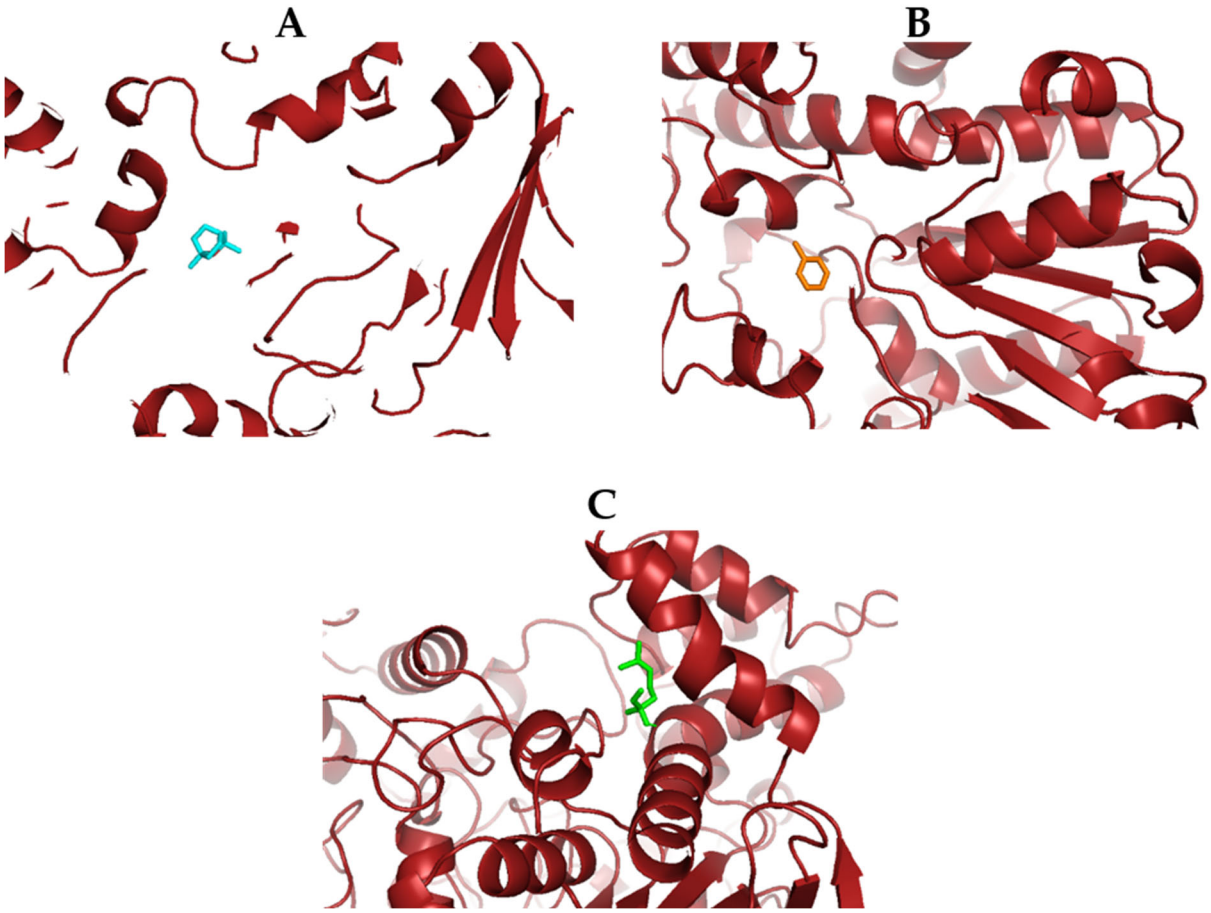



**Figure 6.** The 3D diagrams of ligand interactions with Ache (PDB: 4EY7): (**A**) 1,8-cineole, (**B**) toluene, and (**C**) linalool.

## 4. Discussion

### *4.1. Chemical Characterization and Yield*

The yields of *L. nobilis* essential oil, calculated based on the dry matter weight, ranged from 0.55% for the EOM to 0.84% for the EOT. Jemâa [21] reported a yield of 0.65% for Moroccan *L. nobilis* essential oil. Haouel-Hamdi et al. [31] recorded a yield of 0.55%, which is the same as the EOM yield. Our study demonstrated that the essential oils from both origins were predominantly composed of 1,8-cineole, as confirmed by the FT-IR analysis, which is consistent with previous studies that analyzed *L. nobilis* EO from plants freshly harvested from the Moroccan territory [21,39]. A comparison of the chemical compositions of the essential oils and essential oils from other regions showed significant similarities and differences based on the existing literature. The 1,8-cineole content was 37.64% in the EOT and 47.84% in the EOM, which closely aligned with the 1,8-cineole levels observed in laurels from Morocco (52.43%) [39], France (30.08%) [40], and Turkey (59.94%) [41]. Furthermore, essential oils from some Mediterranean countries had lower 1,8-cineole contents, such as Algeria (22.42%) [42], Tunisia (21.15%) [31], and Georgia (29.2%) [43]. Other constituents present after 1,8-cineole are linalool (16.40%), adamantane (12.00%), methyleugenol (8.03%), β-pinene (7.57%), and benzene (5.21%) for the EOT. On the other hand, they are toluene (17.60%), α-phellandrene (8.44%), terpinolene (6.53%), α-pinene (3.71%), β-pinene (2.47%), and eugenol (2.28%) for the EOM. Some elements have been reported in previous studies in Morocco [21,39]. Several factors may be responsible for the quantitative and qualitative differences in the chemical profiles of the oil, including the plant growth stage and harvesting time, environmental conditions, soil type, genetics, cultivar, plant parts, climate, season, and geographical location [44–48]. The difference in

the efficacy of the EOs in controlling stored food pests is largely attributed to variations in their chemical constituents, which greatly influence their biological activities [49].

### *4.2. Repellency Test*

Our findings indicate that the repellent activity of the two *L. nobilis* essential oils varied. Oil from the Meknes region (class V dominance) was more active against *C. maculatus* than oil from the Tanger region. Some studies have confirmed the repellent properties of bay laurel essential oils against other insects that affect stored food. Jemâa et al. [20] highlighted the repellent activity of *L. nobilis* essential oil collected from Morocco (Marrakesh). The essential oil of *L. nobilis* demonstrated its highest effectiveness with half-dose values (RD50) of 0.045 mL/cm$^2$ against *T. castaneum* and 0.013 mL/cm$^2$ against *R. dominica*. Studies carried out with six types of essential oils, including bay laurel, have shown that the essential oil derived from *L. nobilis* possesses strong repellent properties against *S. granarius* and *T. castaneum* [50]. Another study showed the remarkable repellent activity of *L. nobilis* essential oil against *Acanthoscelides obtectus* [18]. The main reason for the strong repellent activity of *L. nobilis* leaf oil against stored food insects may be the presence of phytochemicals such as 1,8-cineole and linalool [21,42,51].

The fluctuations in the repellent efficacy of essential oils extracted from plants in the Tangier and Meknes regions observed in this study can be attributed to several factors. The variable chemical composition of oils, influenced by region-specific growing conditions such as light, rainfall, soil, and the growing site, plays a major role [52]. Plant age can also influence the composition of essential oils [52,53]. The volatility and degradation of active compounds over time, insect adaptation to repellent doses, and insect behavior also contribute to these variations [54,55]. Understanding these dynamics is essential for optimizing the use of essential oils as natural repellents.

### *4.3. Fumigant Toxicity*

The essential oil derived from noble laurel exhibited significant fumigant activity against *C. maculatus* adults, with notably higher efficacy observed in the oil harvested from Meknes than that from Tanger. This disparity in effectiveness may be attributed to variations in the quantities of the active components present in oils sourced from different regions. Our findings align with several prior studies that have assessed the fumigant properties of bay laurel essential oil against other stored-product pests. For instance, Jemâa et al. [20] showed the fumigant properties of Moroccan noble laurel essential oil against *T. castaneum* and *R. dominica*, with LC50 values varying from 68 to 172 µL/L, respectively. Koutsaviti et al. [56] reported the significant fumigant toxicity of *L. nobilis* essential oil against adults of the rice weevil *S. oryzae*, with an LC50 value of 8 µL/L of air. Similarly, Papachristos and Stamopoulos [18] noted the toxic effects of rosemary essential oil against *A. obtectus* adults (LD50 of 2.1 to 3.3 µL/L of air) compared to laurel essential oil (LD50 of 5.7 to 10.3 µL/L of air).

In contrast, Teke and Mutlu [50] found no fumigant activity for laurel essential oil against *S. granarius* L. and *T. castaneum* (Herbst). The lethal effects of other essential oils on *C. maculatus* reported in the literature were lower than those observed in the present study. An LC50 of 109–148 µL/L was observed in adults treated with EO from *Citrus aurantium* peel [57]. We observed that the EO containing the highest percentage of 1,8-cineole and potentially linalool was the most effective, with a lower LC50. Selecting *L. nobilis* essential oils (EOs) with a specific composition could improve their potential to control *C. maculatus*. A dose of 13.2 µL of EO per liter of air resulted in a 100% insect mortality within 48 h. Considering the extraction yield, the EO obtained from one kilogram of dried leaves could be used to treat a volume of 420 L in storage facilities.

### *4.4. Docking Analysis*

Molecular docking simulations revealed that, among the three active ingredients, 1,8-cineole had the highest binding affinity with the active site of recombinant AChE, as

shown by the docking scores and detailed interaction analyses. Other studies have shown that compounds such as 1,8-cineole and linalool identified in *Laurus nobilis* EO have been reported to contribute significantly to fumigant properties [58–61]. The fumigant action of essential oils is often associated with their high monoterpenoid composition, which acts as an inhibitor of acetylcholinesterase (AChE), the enzyme responsible for the degradation of acetylcholine, a key neurotransmitter in nerve impulses [62–64]. Studies on the inhibition of AChE by oils from sage species, such as *Salvia officinalis* and *Salvia lavandulaefolia*, revealed that 1,8-cineole and α-pinene are particularly active compounds against AChE in bovine erythrocytes [62]. A study has arisen regarding the potential contribution of minor components, such as α-terpineol and isobornyl acetate, to the overall toxicity profile of essential oils [21], where α-terpineol caused a 100% mortality in adult *Sitophilus zeamais* after 96 h of exposure to a dose of 30 µL/µg under laboratory conditions [65]. However, based on docking scores, it seems unlikely that the main components of EOs are solely responsible for these biological activities. They may contribute actively or insignificantly, but the bioactivity of different essential oils may result from synergistic, additive, or antagonistic interactions between their major and minor components [66,67].

The significance of this study lies in its contribution to the advancement of environmentally sustainable pest management strategies. By demonstrating the effectiveness of bay laurel essential oils against *C. maculatus* adults, this study highlights the potential of natural substances as effective substitutes for synthetic insecticides, thus reducing the reliance on chemicals harmful to both human health and the environment.

## 5. Conclusions

The analysis of essential oils using FTIR and GC-MS revealed a diversity of active phytochemical compounds. Remarkably, both oils showed significant repellent and fumigant effects on adult *C. maculatus*. According to molecular docking tests, this effectiveness can be attributed to their chemical profile, in particular to the predominance of 1,8-cineole and linalool in the Tangier essential oils, and of 1,8-cineole and toluene in the Meknes essential oils, while recognizing the potential synergistic role of minor compounds. Based on our findings, we strongly advocate for the inclusion of bay laurel essential oil in integrated pest management programs that target bruchids and other stored-product pests.

The prospects for this research include an in-depth exploration of the synergies between the major and minor compounds of *Laurus nobilis* essential oils and their mechanisms of action to maximize their insecticidal efficacy. It would also be relevant to conduct studies on the impact of different environmental and growth conditions on the chemical composition of essential oils and insect behavior. Finally, the reformulation, large-scale application, and evaluation of these oils under actual storage conditions would validate their potential as alternatives to synthetic insecticides.

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