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Research article

Biological Potential of *Myrtus communis* (L.) Essential Oils: Antimicrobial, Insecticidal Activities and Ecological Implications

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Abstract

This study aims to investigate the bioactive molecules in *Myrtus communis* essential oil (MCEO) using the GC-MS method, and to assess their antibacterial, antifungal, and insecticidal activities. The insecticidal evaluation focuses on toxicity via inhalation and contact, examining adulticidal (adult mortality), ovicidal (impact on egg laying), and larvicidal (reduction in offspring emergence) effects. Biochemical analysis identified 24 compounds, accounting for 99.3% of the total oil content, with major components being 1,8-cineole (32.5%), α-pinene (18.6%), myrtenyl acetate (18%), limonene (10.9%), and α-terpenyl acetate (7.3%). Antibacterial and antifungal activities were evaluated against pathogenic strains using MIC and MBC assays. Results indicate significant inhibition of fungal growth, with a notably low MIC of 1.03% recorded against the *Aspergillus niger* HO32. Conversely, MIC values for bacterial strains remained relatively high, suggesting some resistance to MCEO. Regarding insecticidal activity, the inhalation test showed higher efficacy (LC_{50} = 1.38 ± 0.15 µL/L air) compared to the contact test (LC_{50} = 2.48 ± 0.39 µL/100 g) after 24 hours of exposure. Furthermore, the contact test showed a significant reduction in fertility and insect emergence at a dose of 20 µL/mL.

Keywords: Bioinsecticide; Antibacterial; Antifungal; Adulticidal; Ovicidal; Larvicidal; inhalation; Contact.

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1. Introduction

Essential oils have attracted increasing attention as natural alternatives to synthetic chemicals, particularly for their varied biological properties, such as antimicrobial and insecticidal activity (Raveau et al., 2020). Among these oils, *Myrtus communis* (common myrtle), an aromatic Mediterranean plant, is particularly promising because of the richness and diversity of its bioactive compounds (Bouzabata et al., 2015). The antimicrobial and insecticidal effects of this essential oil justify in-depth studies to understand its potential in various fields, including medicine, agriculture and environmental protection.

Bacterial and fungal infections are a constant threat to human and animal health, leading to increasing resistance to antibiotics (Salam et al., 2023). Faced with this crisis, natural compounds, including essential oils, are seen as viable therapeutic alternatives. *Myrtus communis*, with its composition rich in monoterpenes and phenols, has proved effective against a wide range of pathogens, offering an interesting avenue for the development of new antimicrobial agents (Moura et al., 2023). At the same time, the impact of insect pests on crops and food stocks is a major challenge for world agriculture (Allali et al., 2020). Although chemical pesticides are effective, they pose significant environmental and health risks (Allali et al., 2020). The essential oil of *Myrtus communis* has

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insecticidal properties that can be exploited for sustainable insect pest management, particularly against species resistant to traditional pesticides (Benddine et al., 2023a).

The chemical composition of *Myrtus communis* essential oils (MCEO) varies according to various environmental factors, but compounds such as 1,8-cineole, α-pinene and myrtenol are often identified as being responsible for its biological effects (Caputo et al., 2022). These compounds exhibit not only antimicrobial actions, but also antibiofilm, cytotoxic, and anti-acetylcholinesterase and repellent effects on certain insects, making them attractive for use in crop protection and the preservation of agricultural produce (Caputo et al., 2022; Tavassoli et al., 2011). Such a multifaceted bioactivity profile underscores the potential of MCEO for integrated pest management (IPM) and the preservation of agricultural products. Indeed, recent research has increasingly emphasized the ecological benefits of plant-derived pesticides, given their biodegradability and lower impact on non-target organisms compared to conventional chemicals (Souto et al., 2021).

This study aims to explore the antimicrobial and insecticidal activity of MCEO, while discussing their potential mechanisms of action. The aim is to highlight the ecological applications of this plant, paving the way for greater use of natural resources to meet current challenges in public health and sustainable agriculture.

2. Materials and Methods

2.1. Plant Material

In this study, *Myrtus communis* leaves were used as the plant material. They were collected in May 2022 in the Taounate region of Morocco. The samples were identified by the laboratory botanists, based on botanical references and plant catalogues. The leaves were then cleaned and left to dry in the shade and open air for 15 days before starting the extraction process.

2.2. Essential Oil Extraction

Myrtus communis essential oil (MCEO) was extracted by hydrodistillation following the method described by (HADDOU et al., 2024). A modified Clevenger apparatus, comprising a 2 L flask, a water-cooled condenser, and a graduated separator, was employed. Briefly, 100 g of pre-dried and ground leaves were placed in the flask, and 1000 mL of distilled water was added. The mixture was heated on a magnetic stirrer at 100 °C for 3 hours. During this period, the steam entrained the essential oil, which then condensed in the water-cooled condenser and separated from the water in the graduated separator. The collected essential oil was transferred to a storage flask, and the extraction yield was calculated as a percentage relative to the initial mass of the plant material.

2.3. Chemical Composition Analysis of Essential Oils

The essential oils were analyzed using gas chromatography coupled with mass spectrometry (GC-MS). The analysis was performed on an Agilent-Technologies 6890N Network GC system equipped with a flame ionization detector and an HP-5MS capillary column (30m x 0.25mm, film thickness 0.25µm). The temperature was programmed from 35°C to 250°C, with a gradient of 5°C/min. Gas chromatography with two fused silica capillary columns (30m x 0.25mm) was used to determine retention indices, with the lower and upper temperatures maintained for 3 and 10 minutes, respectively. The flow rate of the carrier gas (helium) was set at 1 mL/min. A 1 µL sample was injected in splitless mode. The essential oil components were identified by comparing their mass spectra with those in the NIST-MS database.

2.4. Antimicrobial activity

2.4.1. Microbial strains

The antimicrobial activity of *Myrtus communis* essential oil (MCEO) was evaluated against both bacterial and fungal strains. The tested microorganisms included four bacterial strains: *Escherichia coli* (ATCC 10536), *Staphylococcus aureus* (ATCC 6538), *Listeria innocua* (ATCC 33090), and *Pseudomonas aeruginosa* (ATCC 15442), as well as three fungal strains: *Aspergillus niger* HO32, *Candida glabrata*, and *Penicillium digitatum*. All microbial strains were obtained from the Laboratory of bioresources, biotechnology, ethnopharmacology and health of the Faculty of Sciences, Oujda, Morocco.

2.4.2. Determination of MIC, MBC, and MFC

The minimum inhibitory concentration (MIC) of the essential oil was determined using the resazurin microtitration assay. In 96-well microplates, serial dilutions of the oil (ranging from 16% to 0.0015% v/v) were prepared in a suitable broth medium. A standardized inoculum of each microbial strain was added to the wells. For bacterial strains, the plates were incubated at 37°C for 24 hours, while for fungal strains, they were incubated at 25°C for 48 hours. After this initial incubation, resazurin was added to each well, and the plates were further incubated for 2 hours until a color change (from blue to pink) indicated microbial growth. Wells that remained blue were recorded as showing no growth, and the lowest concentration at which no color change occurred was noted as the MIC (Taibi, et al., 2024).

To determine the minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC), a 3 µL aliquot from each well that showed no visible growth (emained blue) was subcultured into agar plates. These plates were then incubated under the same conditions used during the MIC assay; 37°C for 24 hours for bacterial strains and 25°C for 48 hours for fungal strains. The MBC or MFC was established as the lowest concentration of the oil at which no colony growth appeared on the agar surface (Taibi et al., 2023).

2.5. Toxicity of Essential Oils against C. maculatus

2.5.1. Animal Material and Insect Rearing

The studied *Callosobruchus maculatus* population was initially collected from chickpea seeds (*Cicer arietinum*) obtained from a local stock in Fez, Morocco. For continuous rearing, insects were placed in 1 L glass jars containing chickpea seeds, serving both as a food source and an oviposition medium. These jars were kept in a climate-controlled rearing chamber under the following conditions: temperature of 27 ± 1°C, relative humidity of 65 ± 5%, and a photo-period of 10 hours of light and 14 hours of darkness. This environment was maintained consistently to promote multiple successive generations. The non-winged adult form, known for its higher reproductive capacity, was used for the experiments.

2.5.2. Contact Toxicity test

The insecticidal potential of the essential oil was evaluated against *C. maculatus* using a contact toxicity test. 100 g of chickpea seeds were placed in 1-liter glass containers with perforated covers. Each container was then infested with five pairs (male and female) of *C. maculatus* adults (aged 0–48 hours). The essential oil was applied directly to the chickpea grains at the designated concentrations (1, 5, 10, and 20 µL/100 g) using a micropipette, followed by manual shaking for approximately 2 minutes to ensure uniform distribution of the oil on the seeds. An untreated control group without the essential oil was maintained under the same conditions.

After 24 hours of exposure, the adult mortality was recorded, and any dead insects were removed. On day 12, the eggs laid on the seeds were counted by direct observation (using a magnifying lens or stereomicroscope) to ensure accurate quantification. Finally, the test continued until day 28, at which point the number of newly emerged adults was recorded to assess the oil's effect on the pest's life cycle. This protocol ensured a comprehensive evaluation of both adulticidal and reproductive inhibitory effects of the essential oil.

The observed mortality rate, corrected using Abbott's formula, is calculated as follows:

$$Pc = 100 \times \frac{P0 - Pt}{100 - Pt} \qquad (1)$$

Where:

Pc = corrected mortality percentage (%); $P0$ = observed mortality in the trial; Pt = observed mortality in the control.

The percentage reduction in the number of eggs and emerged adults for each concentration of essential oil was calculated relative to the control using the following formula:

$$PR = \frac{NC - NT}{CN} \times 100 \qquad (2)$$

Where:

PR = percentage reduction in egg-laying or emerged insects (%); NC = number of eggs or insects hatched in the control; NT = number of eggs or insects hatched in the treatment.

2.5.3. Inhalation Toxicity test

In this assay, ten adults *C. maculatus* (5 males and 5 females) aged 0–48 hours were introduced into 1-liter glass jars with airtight lids. A small cotton ball was suspended from the underside of each lid using a thread. The essential oil was then applied to the cotton ball using a micropipette at four concentrations; 1, 5, 10, and 20 µL per liter of air, ensuring each concentration was tested in triplicate. A control group (cotton ball without essential oil) was maintained under identical conditions. Mortality rates were recorded at regular intervals, and Abbott's formula was employed to correct for control mortality. This setup allowed a precise evaluation of the insecticidal effects of the essential oil via inhalation in a controlled environment.

$$Pc = 100 \times \frac{P0 - Pt}{100 - Pt} \qquad (3)$$

Where:

Pc = corrected mortality percentage (%); P0 = observed mortality in the trial; Pt = observed mortality in the control.

2.5.4. Data Analysis

Statistical analysis was performed using SPSS for Windows® (version 21.0). The data were subjected to one-way analysis of variance (ANOVA) to assess differences in the means of the groups. Fisher's Least Significant Difference (LSD) test was used to identify which means differed significantly at $\alpha = 0.05$. The lethal concentrations, LC_{50} and LC_{95}, along with their confidence intervals, were calculated using the probit method implemented in SPSS.

2.6. Molecular Docking

26.1. Ligand Preparation

The major compounds identified in our study from *Myrtus communis* essential oil were retrieved from the PubChem database in SDF format. Molecular preparation was performed using the LigPrep module in Schrödinger software (version 11.5), employing the OPLS3 force field. The ionization states of the molecules were optimized at a pH of 7.0 ± 2.0, resulting in the generation of up to 32 potential stereoisomers for each compound (Chebaibi et al., 2024).

2.6.2. Protein Preparation

The proteins utilized for docking were sourced from the Protein Data Bank, which included *Escherichia coli* beta-ketoacyl-[acyl carrier protein] synthase (PDB ID: 1FJ4), *Staphylococcus aureus* nucleoside diphosphate kinase (PDB ID: 3Q8U), *Aspergillus niger* beta-1,4-endoglucanase (PDB ID: 5I77), acetylcholinesterase (PDB ID: 6ARY), and Chitin Synthase 2 (PDB ID: 7STM). Protein preparation involved enhancing the structures by incorporating hydrogen atoms, adjusting bond orders, eliminating water molecules, assigning hydrogen bonds, optimizing the charges of receptor atoms, and reducing energy levels using the OPLS3 force field (Amrati et al., 2023; Tourabi et al., 2023).

2.6.3. Glide Standard Precision (SP) Ligand Docking

Ligand docking was conducted using the Standard Precision (SP) mode in Glide from Schrödinger-Maestro version 11.5. Penalties were applied to non-cis/trans amide bonds throughout the docking process. Ligand atoms' van der Waals interactions were scaled with a factor of 0.80, and the partial charge cutoff was set at 0.15. The resulting docking poses were assessed based on the Glide score, which was calculated from the energy-minimized conformations of the ligands obtained during the ligand preparation phase using the LigPrep module in Schrödinger. For each ligand, the pose exhibiting the lowest Glide score was chosen as the optimal docking result. (Beniaich et al., 2022; Bouslamti et al., 2023).

3. Results and discussion

3.1. Chemical composition

The chemical composition of MCEO was determined using GC/MS techniques. The percentages and retention indices (RI) of the identified components are compiled in Table 1, in order of their elution on the HP 5MS capillary column. Twenty-four compounds were identified, representing 99.30% of the total oil content. These compounds were classified into four categories: monoterpenes, sesquiterpenes, esters, and phenols (Table 1, and Figure 1). This oil is characterized by a very high percentage of monoterpenes (70.6%), with a significant presence of oxygenated compounds, among which 1,8-cineole (32.5%) is the most abundant oxygenated monoterpene. The main monoterpene hydrocarbons identified were α-pinene

(18.6%) and limonene (10.9%). Other major components detected included myrtenyl acetate (18%) and α-terpenyl acetate (7.3%).

According to several authors, the chemical composition can be influenced by various factors such as geographical conditions, plant parts, extraction method, drying process of plant material, and the phenological stage of the plant (Hennia et al., 2019). Despite the differences observed in essential oil yield and chemical composition across different studies on myrtle essential oils, the compounds 1,8-cineole, α-pinene, limonene, myrtenyl acetate, α-terpenyl acetate, and linalool consistently remain the most dominant (Asllani, 2000; Ben Hsouna et al., 2014; Bouzabata et al., 2015; Brada et al., 2012; Koutsaviti et al., 2015, 2018; Křůmal et al., 2015).

These molecules are highly significant due to their diverse biological activities, including antiparasitic, antifungal, and antibacterial properties. For instance, 1,8-cineole exhibits antiparasitic properties against the protoscoleces of *Echinococcus granulosus* (Almohammed et al., 2022), antifungal activity against *Alternaria tenuissima* (Singh et al., 2024), and antibacterial effects against *Escherichia coli* and *Staphylococcus aureus* (Sharma et al., 2024). Additionally, α-pinene shows anti-Leishmania activity against the amastigote forms of *Leishmania amazonensis* (Rodrigues et al., 2015) and insecticidal activity against *Myzus persicae* (Ali Chohan et al., 2023). Limonene is commonly used as a flavoring agent in the perfume, food, and pharmaceutical industries, and exhibits antibacterial, antioxidant, and antiproliferative properties (Aprotosoaie et al., 2019). Other compounds, such as α-terpinyl acetate, α-terpineol, linalool, and citral, also demonstrate antibacterial activity against *Escherichia coli* (Noui Mehidi et al., 2024).

Table 1. Chemical Composition of MCEO

Peak	Compounds	RI	Area%	Formula	Chemical class
1	α-Pinene	937	18.6	$C_{10}H_{16}$	Monoterpene
2	Camphene	954	0.1	$C_{10}H_{16}$	Monoterpene
3	Sabinene	972	0.2	$C_{10}H_{16}$	Monoterpene
4	β-Pinene	974	0.1	$C_{10}H_{16}$	Monoterpene
5	Myrcene	992	0.2	$C_{10}H_{16}$	Monoterpene
6	α-Phelladrene	1005	0.1	$C_{10}H_{16}$	Monoterpene
15	Perillene	1010	0.1	$C_{10}H_{14}$	Monoterpene
12	γ-Terpinene	1015	0.5	$C_{10}H_{16}$	Monoterpene
7	α-Terpinene	1017	0.3	$C_{10}H_{16}$	Monoterpene
8	ρ-Cymene	1025	0.9	$C_{10}H_{16}$	Monoterpene
9	Limonene	1028	10.9	$C_{10}H_{16}$	Monoterpene
10	1,8-Cineole	1030	32.5	$C_{10}H_{18}O$	Monoterpene
14	Terpinolene	1033	0.6	$C_{10}H_{16}$	Monoterpene
11	(Z)-p-ocimene	1060	0.3	$C_{10}H_{16}$	Monoterpene
13	(E)-p-ocirnene	1080	0.1	$C_{10}H_{16}$	Monoterpene
17	Linalool	1086	4.9	$C_{10}H_{18}O_2$	Monoterpene alcohol
16	Cis-linalool oxide (furanoid)	1190	0.1	$C_{10}H_{18}O_2$	oxidized monoterpene
18	Myrtenol	1196	0.1	$C_{10}H_{16}O$	Monoterpene alcohol
19	Myrtenyl acetate	1235	18	$C_{12}H_{18}O_2$	Terpene ester
20	α-Terpenyl acetate	1350	7.3	$C_{12}H_{20}O_2$	Terpene ester
21	Methyl eugenol	1360	0.6	$C_{11}H_{14}O_2$	Phenol
22	y-Patchoulene	1380	1.4	$C_{15}H_{24}$	Sesquiterpene
23	β-Caryophyllène	1419	1.1	$C_{15}H_{24}$	Sesquiterpene
24	Caryophyllene oxide	1579	0.3	$C_{15}H_{24}O$	Sesquiterpene
	Total identified (%)		99.3		

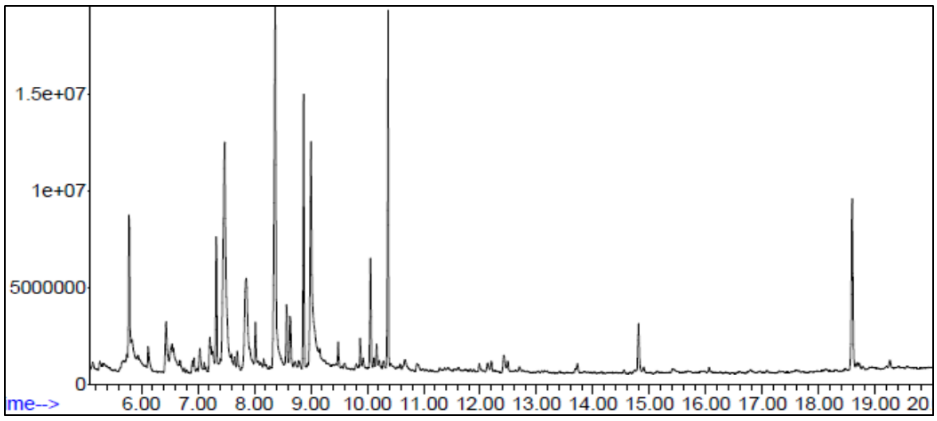

Figure 1. Chromatogram of GC MS analysis of MCEO

3.2. Antimicobial properties of MCEO

The antibacterial activity of MCEO (*Myrtus communis* essential oil) was assessed against Gram-positive bacteria (*S. aureus, L. innocua*) and Gram-negative bacteria (*P. aeruginosa, E. coli*). The antibacterial activity was determined by measuring the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values. The results showed that MCEO exhibits antibacterial activity, with higher effectiveness against Gram-negative bacteria compared to Gram-positive ones, and variable MIC values (Table 2). The MIC values obtained were relatively high compared to other plants, indicating that the tested strains were either less sensitive or resistant. The MIC values were found to be 16% v/v for *E. coli* and *P. aeruginosa*, while *S. aureus* and *L. innocua* exhibited no inhibition even at the highest tested concentration (16% v/v), suggesting that these strains are less susceptible to MCEO under the conditions tested. Similarly, for the MBC, no bactericidal activity was observed for any of the tested strains at concentrations up to 16% v/v. This indicates that, within the tested concentration range, MCEO does not possess bactericidal properties. It is possible that higher concentrations may yield inhibitory or bactericidal effects; however, such concentrations were not evaluated in this study. In contrast to its moderate antibacterial activity, MCEO demonstrated potent antifungal activity, with significantly lower MIC values: 1% against *A. niger*, 4% against *C. glabrata*, and 8% against *P. digitatum* (Table 3).

Table 2. Antibacterial Efficacy Evaluation

Microorganisms	*E. coli*	*S. aureus*	*L. innocua*	*P. aeruginosa*
MIC (% v/v)	16	-	-	16
MBC (% v/v)	-	-	-	-

(-): No inhibition or bactericidal effect was observed for the tested strains at the highest concentration evaluated (16% v/v). This indicates that, within the range of concentrations tested (up to 16% v/v), the respective microorganisms did not exhibit susceptibility to MCEO. Testing at higher concentrations may be necessary to determine if inhibitory or bactericidal effects could be achieved.

Furthermore, MCEO exhibited fungicidal activity against *A. niger* with an MFC of 16%, making it particularly effective against this fungal strain. This suggests that MCEO could be more effective as an antifungal agent than as an antibacterial, with notable fungicidal potential against *A. niger*. These findings align with previous studies, such as Yadegarinia et al. (2006), who tested *Myrtus communis* oils against *E. coli*, *S. aureus*, and *C. albicans*, finding that *C. albicans* was the most sensitive microorganism, with the lowest MIC value (4 µL/mL). Owlia et al., (2009) reported significant activity of MCEO against *P. aeruginosa*, with a MIC of 64 µg/mL.

Other studies have attributed the antimicrobial effects of MCEO primarily to the presence of 1,8-cineole, the major compound in these oils. Research has shown that 1,8-cineole possesses relatively strong antimicrobial properties, targeting several pathogens, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Bacillus subtilis* (Merghni et al., 2018; Papadopoulos et al., 2008). However, its antibacterial activity is generally lower than its antifungal activity (Honório et al., 2015; Morcia et al., 2011). These results support the traditional use of myrtle essential oil as a disinfectant and antiseptic.

Table 3. Assessment of Antifungal Activity

Microorganisms	*Aspergillus niger*	*Candida glabrata*	*Penicillium digitatum*
MIC (% v/v)	1	4	8
MFC (% v/v)	16	-	-

(-): No fungicidal effect was observed for the tested strains at the highest concentration evaluated (16% v/v). This indicates that, within the range of concentrations tested (up to 16% v/v). Testing at higher concentrations may be necessary to determine if fungicidal effects could be achieved.

3.3. Insecticidal activity

The insecticidal activity of myrtle essential oil was studied in this work using two methods: inhalation toxicity and contact toxicity. For the latter, the toxicity of the oils was evaluated on adults, eggs, and the emergence of new individuals after a complete life cycle.

3.4. Adulticidal activity

In this experiment, various concentrations of MCEO essential oil (0, 1, 5, 10, and 20 µL/L of air) were tested to assess their inhalation toxicity on adult *C. maculatus*. Adult mortality was recorded every 24 hours over a period of 4 days, and the results are shown in Figure 2.

MCEO demonstrated a notable insecticidal effect on the longevity of treated adults. A positive correlation was observed between the increase in applied doses and the duration of exposure to the essential oil and adult insect mortality. Specifically, higher doses of myrtle essential oil led to a progressive increase in mortality, with particularly marked results during prolonged exposures. At a dose of 10 µL/L of essential oil, after 96 hours of exposure, a significant mortality rate of 90% was recorded in chickpea bruchid adults (*C. maculatus*). This high mortality rate illustrates the strong insecticidal effect of the oil, suggesting that even at moderate doses, myrtle essential oil can be an effective insecticidal agent, especially in the context of agricultural pest control

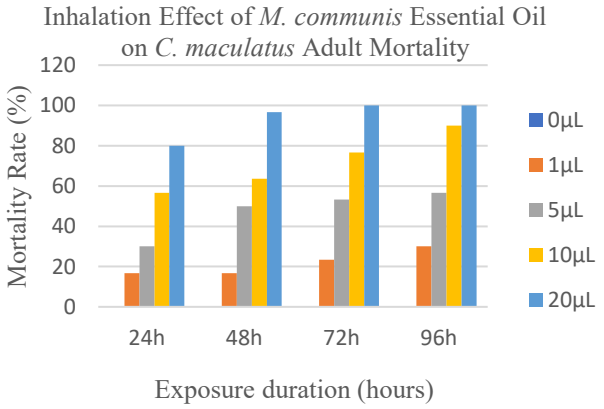

Figure 2. Inhalation Toxicity of *Myrtus communis* Essential Oil on *C. maculatus.* Adult mortality rate (%) of *C. maculatus* following exposure to various concentrations of *Myrtus communis* essential oil (0, 1, 5, 10, and 20 µL per L air) via inhalation at four time points (24, 48, 72, and 96 hours).

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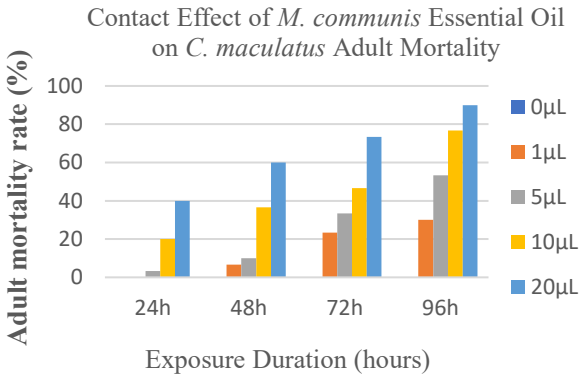


Figure 3. Contact Toxicity of *Myrtus communis* Essential Oil on *C. maculatus*. Adult mortality rate (%) of *C. maculatus* following exposure to various concentrations of *Myrtus communis* essential oil (0, 1, 5, 10, and 20 µL per 100 g seeds) via contact at four time points (24, 48, 72, and 96 hours).

Figure 3 illustrates the effect of contact application of MCEO on adult mortality of *C. maculatus*. The results indicate a gradual increase in mortality based on the dose applied and the duration of contact with the oil. Each treatment was replicated three times, and the mortality percentages reported represent the mean values obtained from these replicates. When higher doses of MCEO were used, or when the contact duration approached 96 hours, adult mortality of *C. maculatus* became significantly higher. At the lowest concentration tested (1 µL/100 g), direct contact with MCEO resulted in an average mortality rate of 40% in adults after 96 hours of exposure. Although modest, this result underscores the efficacy of the essential oil even at low doses. However, when tested by inhalation at the same concentration, the average mortality exceeded 80% after 96 hours, suggesting that inhalation exposure is more effective than direct contact at lower concentrations, likely due to the improved dispersion of volatile oil components in the insects' immediate environment. At the highest concentration (20 µL/100 g), MCEO achieved 100% mortality in both modes of application, regardless of whether the exposure was by inhalation or direct contact. These findings highlight the powerful insecticidal action of myrtle essential oils when applied at high doses, with both contact and inhalation methods resulting in complete adult mortality, far surpassing that observed in the control samples. These data confirm the insecticidal potential of myrtle essential oils and underscore a dose-dependent and time-dependent relationship, where prolonged exposure or higher doses significantly enhance efficacy. This dual effectiveness, through contact and inhalation, suggests that myrtle essential oils could be used flexibly to combat insect infestations, particularly in food storage systems where protection against pests like *C. maculatus* is crucial.

(LC_{50}) and the 95% lethal concentration (LC_{95}) values obtained for the two application methods of the essential oil, namely the contact and inhalation tests (Table 4). LC_{50} and LC_{95} represent the concentrations required to kill 50% and 95% of the tested insects, respectively, and were calculated using probit analysis implemented in SPSS. Statistical significance was determined using the chi-square goodness-of-fit test. In the contact test, the LC_{50} was determined to be 24.26 µL/L of air, indicating that a

relatively high concentration of essential oil was required to achieve 50% mortality of the exposed insect population. In contrast, the inhalation test demonstrated significantly higher effectiveness, with an LC_{50} value of 7.15 µL/L of air. This result suggests that a much lower concentration of essential oil (expressed as µL of oil per liter of air) is needed to achieve the same mortality level through inhalation. The LC_{95} values further confirm that inhalation of the essential oil is substantially more toxic to the insects, requiring considerably lower doses to reach 95% mortality. Control samples, in which insects were exposed only to air (without MCEO), were included in each experimental run to ensure that the observed effects were attributable solely to the essential oil.

3.5. Ovicidal and larvicidal activity

In this study, the ovicidal and larvicidal activities of *Myrtus communis* essential oil (MCEO) were evaluated using two distinct concentration metrics: µL/100 g: Denotes the volume of essential oil applied per 100 g of seeds in direct contact tests, and µL/L : Denotes the volume of essential oil per liter of air in inhalation tests. Each treatment was performed in triplicate, and the values are reported as mean ± standard deviation. Statistical significance was determined using one-way ANOVA followed by Fisher's LSD test at $\alpha = 0.05$.

Table 4. LC_{50} and LC_{95} values (µL/L air) calculated based on adult mortality of *C. maculatus* after 24 hours for both tests.

Test	df	Slope+SD	LC_{50}	LC_{95}	X^2
Contact	2	2.48 + 0,39	24.26	111.75	1.09
Inhalation	2	1.38±0,15	7.15	111.1	10.09

Exposure of *C. maculatus* females to MCEO resulted in a marked, dose-dependent reduction in egg-laying (Figure 4). For untreated females, the average number of eggs laid was 184.67. At the lowest dose of 1 µL/100 g, this average dropped to 72 eggs per female, indicating a significant reduction in reproductive activity ($p < 0.05$). At the highest tested concentration (20 µL/100 g), egg-laying was further reduced to an average of 7 eggs per female.

A similar trend was observed in larval emergence. At 1 µL/100 g, the average number of emerging individuals was 54.66, compared to 111.66 ± 6.50 emergences in the control group. When the MCEO concentration was increased to 20 µL/100 g, larval emergence was significantly inhibited, demonstrating that higher doses of MCEO effectively limit both egg-laying and embryonic development

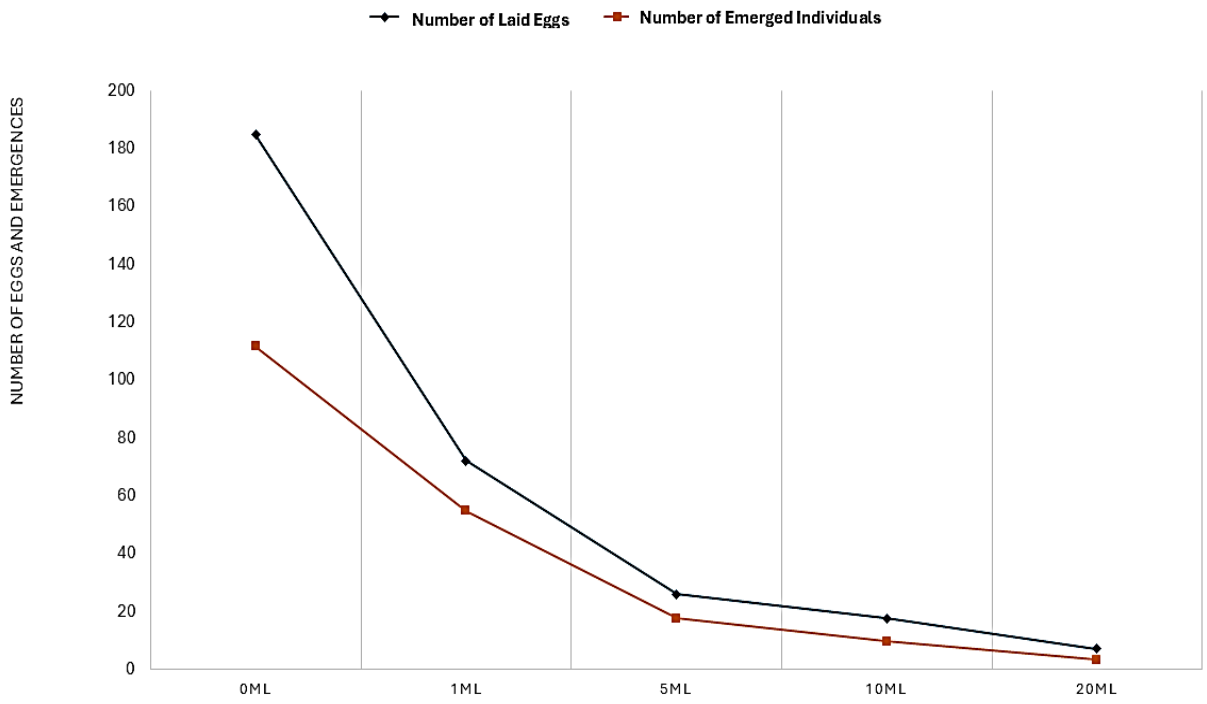


Figure 4. Inhibition of egg-laying and adult emergence of *C. maculatus* following direct contact toxicity testing with various concentrations of MCEO.

The results of this study are consistent with previous findings on essential oils, particularly those from the Lamiaceae family, which have also demonstrated significant insecticidal activity against stored-product pests. For example, Houzi et al., (2024) reported that *Rosmarinus officinalis* essential oil, containing the same major

compound (1,8-cineole) as in MCEO was highly effective against *C. maculatus*, with a notable reduction in adult survival observed at a concentration of 1 µL/L air (LC_{50} = 0.62 µL/L air in inhalation tests). Similarly, (Ainane et al., 2019) documented strong toxic effects of *R. officinalis* essential oil against *Tribolium confusum*, achieving complete mortality at a dose of 12×10^{-2} µL/cm³ after one day. Furthermore, Benddine et al., (2023) demonstrated acute field toxicity of *Myrtus communis* essential oil against heterogeneous populations of *Rhopalosiphum maidis*, with corrected mortality rates of 88.13% and 81.47% after five days of exposure. Additional studies on essential oils from *Origanum compactum*, *Lavandula dentata*, and *Mentha pulegium* have reported LC_{50} values of 5.3, 4.01, and 1.41 ± 0.48 µL/L air, respectively.

These findings suggest that the major bioactive compounds in these essential oils, such as 1,8-cineole, are primarily responsible for the observed insecticidal effects. To further investigate this hypothesis, we conducted an *in-silico* study on the major compounds present in MCEO to identify potential molecular targets in insects, as discussed in the following section.

3.6. Molecular Docking

3.6.1. Antimicrobial Activity

In antimicrobial activity against *E. coli*, myrtenyl acetate, 1,8-cineole, and α-pinene were the most active molecules, with glide scores of -6.122, -5.703, and -5.661 kcal/mol, respectively. For *S. aureus*, α-terpinyl acetate, myrtenyl acetate, and limonene demonstrated the highest activity, with glide scores of -4.698, -4.541, and -4.390 kcal/mol (Table 5). Figure 5A and 6A illustrate that myrtenyl acetate formed a single hydrogen bond with THR 300 residue in the active site of *E. coli* β-ketoacyl-[acyl carrier protein] synthase. This interaction with THR 300 may be critical for inhibitory activity, as β-ketoacyl-ACP synthase is essential for bacterial fatty acid biosynthesis. Inhibition of this enzyme would disrupt cell membrane formation, potentially leading to bacterial cell death. The strong binding affinity of myrtenyl acetate suggests it could serve as a lead compound for developing novel antibiotics, particularly against gram-negative bacteria.

For *S. aureus*, despite the strong binding affinity of α-terpinyl acetate to nucleoside diphosphate kinase (glide score -4.698 kcal/mol), no hydrogen bonding was observed. This suggests that hydrophobic interactions or van der Waals forces may be the predominant binding mechanisms. Inhibition of nucleoside diphosphate kinase could impair DNA replication and bacterial growth, offering another mechanism for antimicrobial action.

3.6.2. Antifungal Activity

Regarding antifungal properties, 1,8-cineole, α-pinene, and myrtenyl acetate exhibited remarkable inhibitory activity against *A. niger*, with glide scores of -4.543, -4.301, and -4.083 kcal/mol, respectively (Table 5). Figure 5C and 6C display the interaction between 1,8-cineole and the active site of *A. niger* β-1,4-endoglucanase, where no bond formation was observed despite showing the lowest glide score (indicating strongest binding) among the tested compounds. The strong binding of 1,8-cineole to β-1,4-endoglucanase suggests a potential mechanism for antifungal activity, as this enzyme is essential for fungal cell wall synthesis and remodeling, particularly during hyphal growth. Inhibition of β-1,4-endoglucanase would significantly impair fungal cell wall integrity, potentially explaining the observed antifungal activity. The absence of hydrogen bonding suggests that hydrophobic interactions may be the primary binding mechanism, similar to α-terpinyl acetate in *S. aureus*.

3.6.3. Insecticidal Activity

Regarding antifungal properties, 1,8-cineole, α-pinene, and myrtenyl acetate exhibited remarkable inhibitory activity against *A. niger*, with glide scores of -4.543, -4.301, and -4.083 kcal/mol, respectively (Table 5). Figure 5C and 6C display the interaction between 1,8-cineole and the active site of *A. niger* β-1,4-endoglucanase, where no bond formation was observed despite showing the lowest glide score (indicating strongest binding) among the tested compounds.

The strong binding of 1,8-cineole to β-1,4-endoglucanase suggests a potential mechanism for antifungal activity, as this enzyme is essential for fungal cell wall synthesis and remodeling, particularly during hyphal growth. Inhibition of β-1,4-endoglucanase would significantly impair fungal cell wall integrity, potentially explaining the observed antifungal activity. The absence of hydrogen bonding suggests that hydrophobic interactions may be the primary binding mechanism, similar to α-terpinyl acetate in *S. aureus*.

3.6.4. Molecular Interactions

No bond formation was observed for α-terpinyl acetate in the active sites of *S. aureus* nucleoside diphosphate kinase and Chitin synthase 2, despite having the lowest glide scores (indicating strongest binding affinity) among the tested compounds (Figure 5B, 5E, and 6B, 6E). Similarly, 1,8-cineole, which showed the strongest binding affinity (lowest glide score) to *A. niger* β-1,4-endoglucanase, exhibited no bond formation in the active site (Figure 5C and 6C). These findings suggest that non-covalent interactions, such as hydrophobic effects, van der Waals forces, or π-π stacking, may play dominant roles in the binding mechanisms of these compounds.

Comparing the docking results across different compounds reveals important structure-activity relationships. Myrtenyl acetate consistently showed strong binding across multiple targets (antimicrobial, antifungal, and insecticidal), suggesting its bicyclic structure with an acetate group provides versatile binding properties. α-terpinyl acetate demonstrated particularly strong binding to insect targets, suggesting specificity that could be exploited for selective insecticidal applications. The multi-target activity of these essential oil compounds may explain their role in plant defense mechanisms, providing comprehensive protection against bacterial, fungal, and insect threats.

Table 5. Docking results of essential oil compounds in different receptor active sites, presented as glide scores (kcal/mol). Lower glide scores indicate stronger binding affinity between the ligand and the receptor. (*E. coli* β-ketoacyl-ACP synthase (1FJ4); *S. aureus* nucleoside diphosphate kinase (3Q8U); *A. niger* β-1,4-endoglucanase (5I77); Acetylcholinesterase (6ARY); Chitin synthase 2 (7STM)).

Compound	1FJ4	3Q8U	5I77	6ARY	7STM
1,8-Cineole	-5.703	-3.275	-4.543	-4.665	-4.18
alpha-Pinene	-5.661	-4.324	-4.301	-5.731	-3.611
alpha-Terpinyl acetate	-5.52	-4.698	-3.646	-5.834	-4.754
Limonene	-5.101	-4.39	-3.938	-5.002	-2.96
Linalool	-3.99	-3.158	-2.721	-3.991	-3.568
myrtenyl acetate	-6.122	-4.541	-4.083	-5.907	-4.133

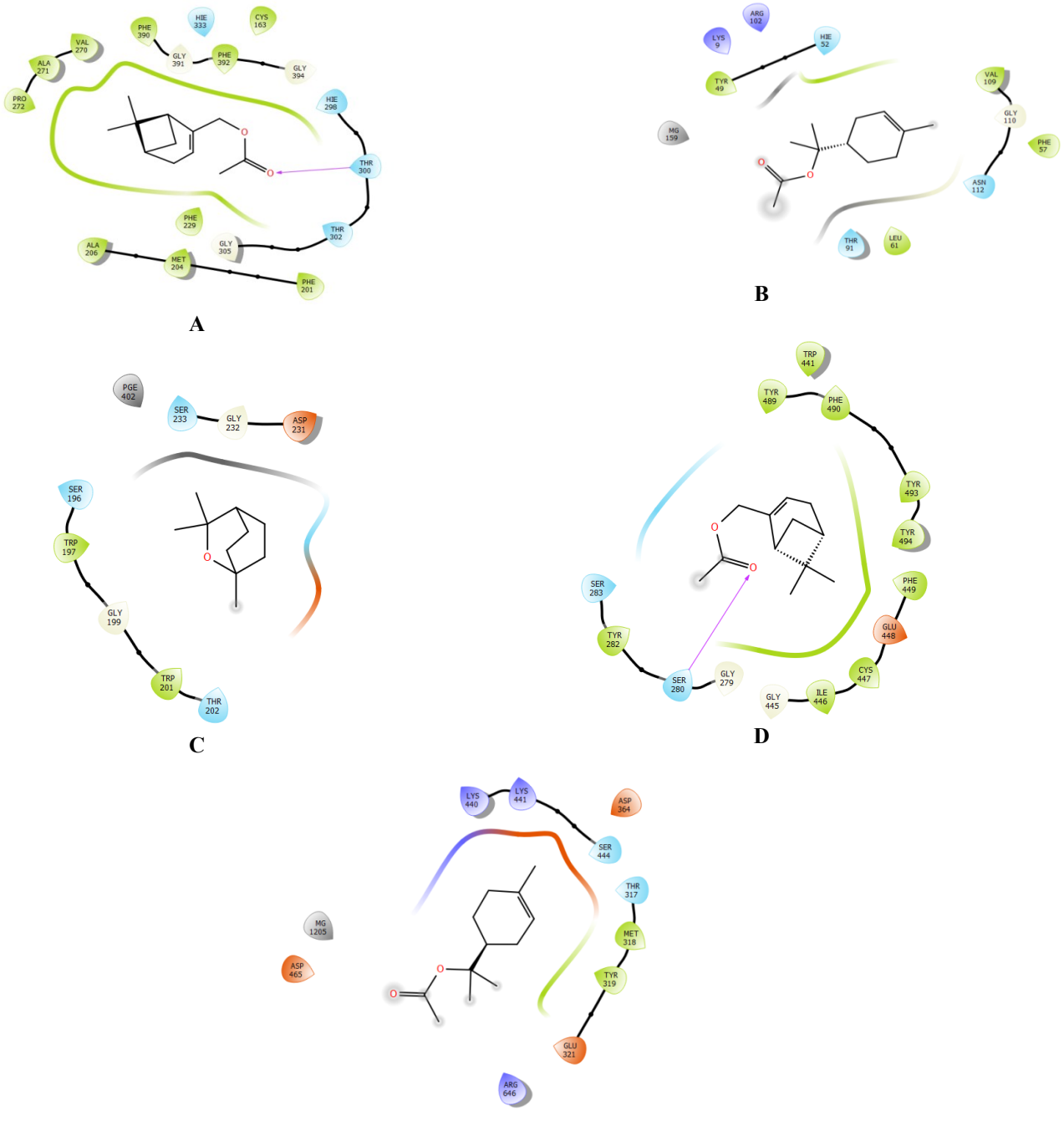
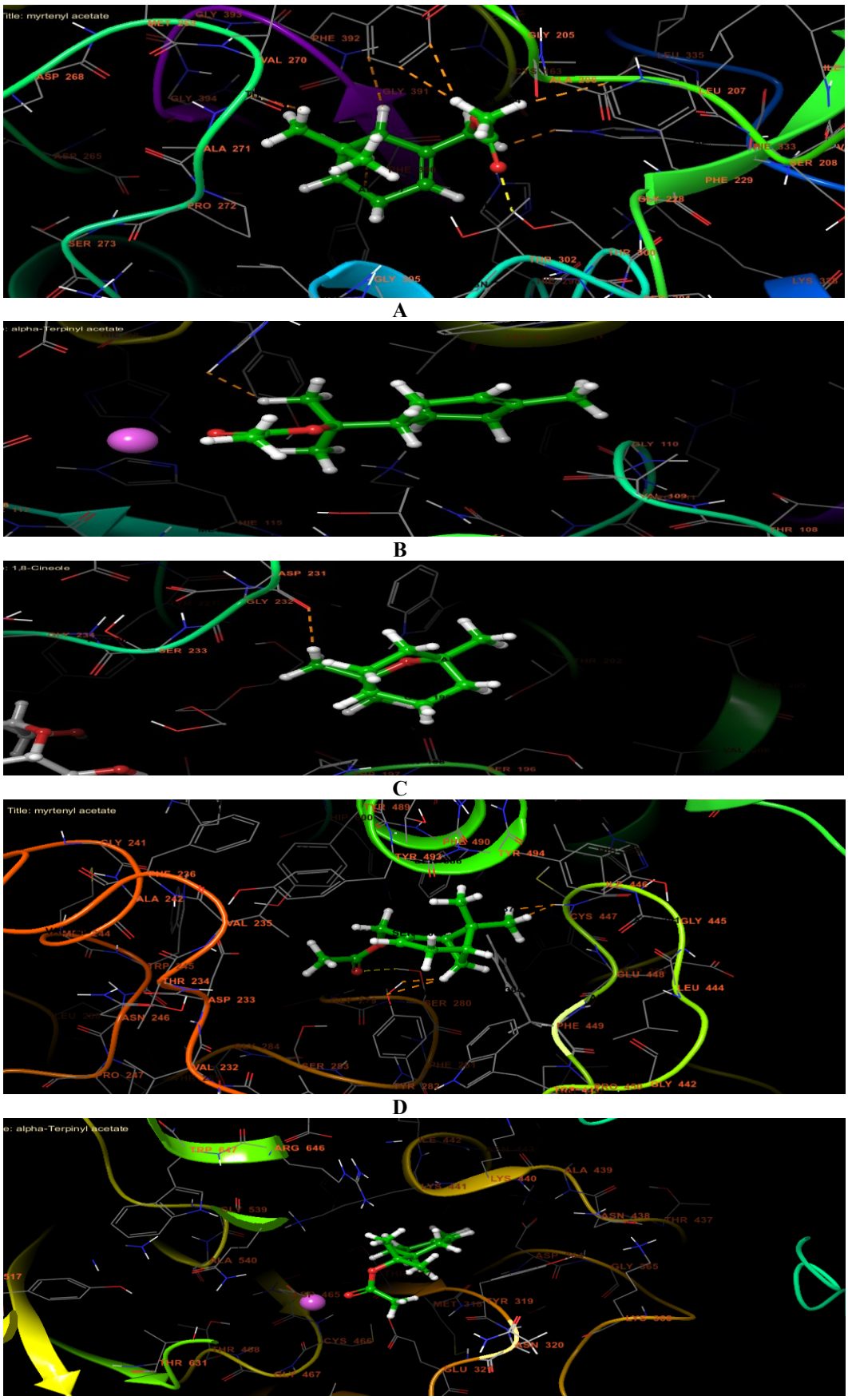


Figure 5. Two-dimensional (2D) representations of ligand interactions with active sites. (A) Myrtenyl acetate interaction showing hydrogen bond formation with THR 300 residue in *E. coli* β-ketoacyl-[acyl carrier protein] synthase. (B) α-Terpinyl acetate interaction with *S. aureus* nucleoside diphosphate kinase active site. (C) 1,8-Cineole interaction with β-1,4-endoglucanase active site from *A. niger*. (D) Myrtenyl acetate interaction showing hydrogen bond formation with SER 280 residue in Acetylcholinesterase. (E) α-Terpinyl acetate interaction with Chitin synthase 2 active site.



A

B

C

D

E

Figure 6. Three-dimensional (3D) representations of ligand interactions with active sites. (A) Myrtenyl acetate interaction showing hydrogen bond formation with THR 300 residue in *E. coli* β-ketoacyl-[acyl carrier protein] synthase. (B) α-Terpinyl acetate interaction with *S. aureus* nucleoside diphosphate kinase active site. (C) 1,8-Cineole interaction with β-1,4-endoglucanase active site from *A. niger*. (D) Myrtenyl acetate interaction showing hydrogen bond formation with SER 280 residue in Acetylcholinesterase. (E) α-Terpinyl acetate interaction with Chitin synthase 2 active site.

4. Conclusion

This study highlighted the impact of myrtle essential oil (MCEO) on various bacterial and fungal strains, as well as its adulticidal, ovicidal, and larvicidal activity against *Callosobruchus maculatus*. The results show that myrtle oil has a moderate effect on the tested bacterial strains but a strong effect on fungal strains, even at low concentrations, suggesting that MCEO could be more effective as an antifungal agent than as an antibacterial one. Regarding its insecticidal effect, myrtle oil acts on all stages of the insect's life cycle, causing cycle failure in over 98% of cases. These findings indicate that MCEO could serve as an effective and safe fungicide and insecticide, with potential applications in the medical and food sectors on a commercial scale. Further research is needed to test the bioactive molecules identified in the chemical composition of this essential oil to further optimize the results obtained.

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Conflicts of Interest
The authors declare no conflicts of interest.

Data availability statement
Data will be available upon request from the corresponding author.

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