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Comparative larvicidal efficacy of plant-derived essential oils and their active molecules against *Culiseta longiareolata* (Macquart, 1838)

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Introduction

I. INTRODUCTION

Pests represent a critical threat to ecosystem integrity, agricultural sustainability, and global public health. Effective pest management is essential not only for advancing entomological and parasitological research, but also for safeguarding food systems and curbing the transmission of vector-borne diseases (Li *et al.*, 2023). Over successive generations, insecticide development has evolved from basic chemical compounds to complex formulations. Nonetheless, reconciling high insecticidal potency with environmental safety remains a persistent challenge. The widespread and prolonged use of insecticides in agriculture, livestock production, and urban pest control has accelerated the evolution of multifaceted resistance mechanisms in arthropods—spanning physiological, biochemical, and behavioral adaptations. This resistance compromises the efficacy of vector control interventions, resulting in control failure, pest resurgence, and elevated disease transmission risks (Wang *et al.*, 2024).

Vector-borne diseases (VBDs) continue to impose a substantial burden on global health systems, with profound socio-economic implications in both urban and rural contexts (Swei *et al.*, 2020; Wilson *et al.*, 2020). They are responsible for an estimated 700,000 deaths annually—approximately 17% of all infectious disease cases— with the heaviest toll recorded in low-resource settings (WHO 2024; Dey *et al.*, 2023). Among diseases vectors, mosquitoes (Culicidae) are particularly notorious due to their role in transmitting protozoan, filarial, and arboviral pathogens, including *Plasmodium* spp. (malaria), *Wuchereria bancrofti* (lymphatic filariasis), and several arboviruses causing yellow fever, dengue, chikungunya, and Zika (Alemayehu, 2023; Jabeen *et al*., 2024; Piedrahita and Correa, 2024)

In Algeria's Tébessa Province, *Culiseta longiareolata* demonstrated notable ecological prominence. Recent entomological surveys by Benkhedim *et al*. (2023) identified *Culex pipiens* (57.7%) and *C. longiareolata* (25.1%) as the two most dominant species in the region. These findings are further supported by Bouabida *et al*. (2012), who reported high ecological indices for *C. longiareolata* across multiple study sites. This cold-tolerant, multivoltine species is capable of overwintering as larvae and as diapausing adult females. It is distributed across Europe, Africa, and Asia and has been implicated in the transmission of *Plasmodium relictum* (avian malaria), arboviruses, *Brucella* spp., highly pathogenic avian influenza (HPAI), and neuroinvasive strains of WNV (Khaligh *et al.*, 2020; Hazratian *et al.*, 2019). Therefore, early disruption of its developmental cycle is crucial, with larval control

representing the most strategic intervention point since it precedes pathogen acquisition and disease transmission, characterized by low mobility and confined to specific aquatic habitats (Pavela, 2015).

While synthetic chemicals such as organophosphates and pyrethroids have historically been the mainstay of vector control (Panda et al., 2025), their extensive use has precipitated unintended consequences. As a result, there is increasing interest in bio-insecticides derived from botanical sources—particularly essential oils and their bioactive compounds—which emerge as potent molecules with proven efficacy against a wide array of insect pests, displaying diverse modes of action (Abdelgaleil *et al*., 2021; Gupta *et al*., 2023).

Essential oils (EOs) are complex secondary metabolites produced by aromatic and medicinal plants. They exhibit high volatility, optical activity, and solubility in organic solvents. Their bioactivity is attributed to a diverse array of volatile constituents, primarily: monoterpenoids, sesquiterpenoids, and phenylpropanoids, which are biosynthesized via two major biochemical pathways: the mevalonic acid (MVA) and methylerythritol phosphate (MEP) pathways (Noriega, 2021). The MVA pathway, which occurs in the cytoplasm, leads to the formation of sesquiterpenes (C15) and triterpenes (C30), while the MEP pathway, located in plastids, produces hemiterpenes (C5), monoterpenes (C10), sesquiterpenes (C15) and diterpenes (C20). Aromatic compounds, including phenylpropanoids are less common and are derived mainly from the shikimate pathway, but a few phenols, such as carvacrol and cuminaldehyde, are derived from terpene biosynthesis by desaturation. EOs components are accumulated primarily in flowers, leaves, seeds, and fruits—often within glandular trichomes and secretory cavities (Zeni *et al.*, 2021).

Their qualitative and quantitative profiles are influenced by several factors, such as the plant part used, developmental stage, genetic differences, extraction method, seasonal variations, soil composition, and climatic conditions (Chaaban *et al*., 2019). Depending on their concentration and chemical composition, EOs can act as toxicants, repellents, or attractants, thereby modulating insect behavior or inducing direct mortality. In addition to their insecticidal properties, EOs exhibit a broad range of pharmacological activities, including antioxidant, antimicrobial, antifungal, antiviral, and antiparasitic effects (Souiy, 2024).

In this context, the main objective of the present study was to compare the larvicidal activity of *Eucalyptus globulus*, *Mentha spicata*, and *Citrus aurantium* EOs and their major

constituents against *C. longiareolata* larvae, to explore whether to focus on the entire EO, or simply isolate the specific molecule accountable for its effectiveness.

Materials and Methods

II. MATERIALS AND METHODS

2.1. Presentation of animal material

2.1.1. Presentation of Culicidae family

The family Culicidae, commonly known as mosquitoes, encompasses approximately 113 genera and around 3,570 species, distributed across a wide range of ecological regions worldwide. Since their recognition in 1878 as arthropod vectors capable of transmitting parasites to vertebrates, mosquitoes have been considered among the most significant arthropods affecting global public health (Khaligh *et al.*, 2020).

Mosquitoes are holometabolous insects, undergoing complete metamorphosis characterized by distinct developmental stages: egg, larva, pupa, and adult. Their life cycle encompasses two ecologically distinct phases. The immature stages—egg, larva, and pupa—are entirely aquatic (Bellver-Arnau et al., 2024), while adults emerge to occupy terrestrial and aerial habitats. This separation between aquatic and terrestrial phases represents an adaptive strategy that minimizes intraspecific competition and enhances survival by subjecting each stage to different environmental conditions and predator communities (Kaleka et al., 2019). Additionally, the pronounced morphological differentiation across life stages—particularly in the larva, pupa, and adult—provides valuable taxonomic markers, allowing precise identification at the subfamily, genus, and species levels (Ghorbani et al., 2025).

2.1.2. Presentation of *Culiseta longiareolata*

Among Culicidae species, *Cs. longiareolata* (Macquart, 1838) olds particular medical and veterinary importance. It is recognized as a vector of multiple pathogens, including viruses, bacteria, and parasites. Although it rarely bites humans, *Cs. longiareolata* contributes to the transmission of several zoonotic diseases, such as brucellosis, avian influenza, and West Nile encephalitis (Soltan-beiglu *et al*., 2020). Notably, it serves as an efficient vector of *Plasmodium* species responsible for avian malaria (Hazratian et al. 2019). Characterized by its thermophilic, bird-preferring, and multivoltine nature, *Cs. longiareolata* is widely distributed across the mediterranean basin and extends into parts of Africa, Europe, and Asia (Toubal *et al.*, 2022). The larva of this species is characterized by a dark, heavily pigmented head, a short antenna with a smooth cuticle, and a triangular mentum. The siphon is short and conical, bearing two tufts of basal setae. The siphonal comb extends along almost the entire length of the siphon, with irregularly arranged teeth. The adult of *Cs. longiareolata* is characterized by three white

longitudinal stripes on the thorax (Benkhedim *et al*., 2023). The systematic position of this species is as follows:

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Hexapoda

Class: Insecta

Order: Diptera

Family: Culicidae

Genus: Culiseta

Species: *Culiseta longiareolata*



Figure 1. Larval stage and adult female of *C. longiareolata*

2.2. Collection and breeding

From February to May 2025, mosquito larvae were systematically collected from untreated artificial accumulation sites in two regions of Tébessa Province: Bir El Ater and Lemridj. Upon collection, the larvae were promptly transported to Water and Environment Laboratory at Larbi Tebessi University, Tébessa. In the laboratory, a precisely tailored rearing protocol was implemented. The larvae were reared in plastic trays containing dechlorinated water and maintained at 25±2°C, with a 14:10 light and dark photoperiod cycle. They were fed with a finely powdered mixture of biscuits and dried yeast, at a ratio of 4:1 and water was renewed every two days.



Figure 2. Artificial larval deposits

2.3. Presentation of *Mentha spicata*

Spearmint, a member of the Lamiaceae family, which comprises approximately 7,285 species distributed across 250 genera, primarily concentrated in the Mediterranean region (Prakash *et al*., 2016). It is a widely recognized aromatic and medicinal plant. The genus Mentha includes 18 species and 31 subspecies or varieties, including about 12 naturally occurring hybrids (Mansoori *et al*., 2022)**.** *M. spicata* is a rhizomatous perennial herb, typically growing to a height of 30 to 100 cm. It is characterized by erect, quadrangular, glabrous, and highly branched stems. The leaves are ovate to lanceolate in shape, ranging from 2 to 7 cm in length, and possess serrated margins. Its inflorescences are terminal, compact spikes measuring 3 to 12 cm in length and 5 to 10 mm in width, bearing pedicellate flowers that are pale pink to white and arranged in slender, interrupted spikes (Mahendran *et al*., 2021). This species is extensively cultivated for its essential oil, which holds significant commercial value in pharmaceutical, cosmetic, and food industries due to its rich composition of bioactive compounds. The oil is primarily composed of carvone, a monoterpenoid responsible for the plant's distinctive aroma and a variety of pharmacological activities (Mansoori *et al*., 2022). Furthermore, rosmarinic acid, the predominant phenolic compound in spearmint, contributes substantially to its therapeutic efficacy owing to its strong antioxidant properties (Sierra *et al*., 2022). The systematic position of this species is as follows:

Kingdom: Plantae
Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Order: Lamiales

Family: Lamiaceae

Genus: Mentha (Mint)

Species: *Mentha spicata* (spearmint)



Figure 3. *Mentha spicata*

2.4. Presentation of *Citrus aurantium*

C. aurantium, commonly known as bitter orange, sour orange, or Seville orange, is a small fruit-bearing tree from the Rutaceae family that typically reaches a height of about five meters and is characterized by its fragrant white flowers (Guimarães *et al*., 2024). It is extensively cultivated across various regions of the world, with notable prevalence in the Mediterranean basin and Southeast Asia (Ahranjani *et al*., 2025). *C. aurantium* has attracted considerable scientific attention due to its richness in bioactive compounds, including flavonoids, alkaloids, phenolic compounds, and essential oils (EOs), particularly rich in limonene, linalool, and β-pinene (Ellouze *et al*., 2024). These constituents contribute to the plant's wide range of pharmacological activities, such as antioxidant, antibacterial, anticancer, antidiabetic, immunomodulatory, and neuroprotective effects. The aromatic white flowers of *C. aurantium*, arranged singly or in clusters along the shoot axis, have long been valued in both culinary and therapeutic contexts. Traditionally used as flavoring agents in beverages and pastries, these flowers also exhibit medicinal properties, including antidepressant, antimicrobial, and sedative effects. Furthermore, their integration into skincare products highlights their versatility across

the food, pharmaceutical, and cosmetic industries (Seyyedi-Mansour *et al*., 2025). The systematic position of this species is as follows:

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosids

Order: Sapindales

Family: Rutaceae

Genus: *Citrus*

Species: *Citrus aurantium* L



Figure 4. *Citrus aurantium*

2.5. Presentation of *Eucalyptus globulus*

E. globulus, an evergreen broadleaf species native to Australia, belongs to the Myrtaceae family and is commonly known as “blue gum” or “Tasmanian blue gum.” It is one of more than 700 species in the Eucalyptus genus and exhibits significant morphological plasticity, ranging from multi-stemmed shrubs in harsh environments to medium-sized woodland trees up to 20 meters, or towering forest trees reaching 70–80 meters with broad crowns under optimal conditions (Praciak, 2015). Its global distribution has expanded widely due to its adaptability

to diverse environmental conditions, ease of cultivation, rapid growth, and high woody biomass production. Remarkably, *E. globulus* can grow in contaminated soils, reflecting its resilience. This adaptability is linked to its capacity to withstand biotic and abiotic stresses by selectively releasing defensive secondary metabolites such as monoterpenes, sesquiterpenes, hydrocarbons, and quinones (Shala and Gururani, 2021). The essential oil, concentrated mainly in the leaves, contains bioactive compounds with antioxidant, antibacterial, and anti-inflammatory properties. It is extensively used in healthcare, flavoring, perfumery, cosmetics, pharmaceuticals, and as a natural additive and preservative in the food industry. The leaves are particularly rich in 1,8-cineole (eucalyptol) (Soltani *et al*., 2025). The systematic position of this species is as follows:

Kingdom: Plantae
Phylum: Spermatophyta
Subphylum: Angiospermae
Class: Dicotyledonae
Order: Myrtales
Family: Myrtaceae
Genus: Eucalyptus
Species: *Eucalyptus globulus*



Figure 5. *Eucalyptus globulus*

2.6. Essential oils and chemicals

Leaves of *M. spicata*, *C. aurantium*, and *E. globulus* were collected from the Tebessa region. Essential oils were previously extracted by hydrodistillation using a Clevenger-type

apparatus, then chemically characterized by gas chromatography–mass spectrometry (GC–MS) to identify their constituents. Table 1 lists the five major compounds identified in each essential oil, along with the reference articles on which this study is based. The main compounds—limonene, linalool, carvone, eucalyptol, and β-pinene—were subsequently purchased from Sigma-Aldrich for experimental use.

Table 1. Major constituents of *M. spicata*, *C.aurantium* and *E. globulus* EOs

Plant	Major constituents	Relative percent (%)	Literature
M. spicata	Carvone	58.3	(Soltani et al., 2025)
	Limonene	24.6	
	Dihydrocarveol	2.1	
	cis-sabinene hydrate	1.9	
	terpinen-4-ol	1.6	
E.globulus	ρ-cymene	27.31	
	1,8-cineole	25.19	
	spathulenol	8.13	
	β-phellandrene	7.95	
	cryptone	6.21	
C. aurantium	Linalool	39.1	
	linalool acetate	36.6	
	α-Terpineol	5.9	
	geranyl acetate	3.1	
	sabinene	2.9	

2.7. Carvone

Carvone is a monoterpene ketone (2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one) with the molecular formula $C_{10}H_{14}O$ and a boiling point of 230 °C. It contains a stereogenic center, giving rise to two enantiomeric forms that share identical physicochemical properties but differ in their optical activity due to opposite specific rotations (Wojtunik-Kulesza *et al.*, 2024). Carvone occurs naturally in the essential oils of numerous aromatic plant species, particularly within the genera *Mentha*, *Origanum*, *Rosmarinus*, and *Thymus*. The (−)-carvone enantiomer is the dominant component in spearmint (*Mentha spicata*), whereas (+)-carvone is mainly found in the seed oils of caraway (*Carum carvi*) and dill (*Anethum graveolens*), where their concentrations can exceed 60% (Faliagkas *et al*., 2015). The concentration of this volatile compound varies depending on the plant species and is influenced by several factors, including geographical origin, developmental (phenological) stage, and the plant part (Koleva *et al.*, 2024). It is biosynthesized and secreted as a secondary metabolite within essential oils, although its primary physiological role in plants remains not fully elucidated (Pina *et al.*, 2022). It is

characterized by a density ranging from 0.9603 to 0.9659 at 20°C, a refractive index between 1.4988 and 1.5003, and a boiling point between 230 and 231°C. It exhibits polarization between +56° and +60° and -57° to -62°. Carvone is highly soluble in polar liquids, with solubility in 50% ethanol at 1:17, 60% ethanol at 1:4-5, and 70% ethanol at 1:1.5-2. It is insoluble in non-polar liquids and has a purity exceeding 98% (Koleva *et al.,* 2024). Beyond its physical properties, carvone has been shown to possess a range of promising pharmacological activities. Notably, it has demonstrated neuroprotective effects, suggesting its potential as a therapeutic agent for neurological disorders such as depression, anxiety, and epilepsy. Furthermore, carvone exhibits antidiabetic properties by preventing obesity and metabolic disturbances associated with high-fat diets. This effect is attributed to its ability to correct glycoprotein abnormalities and regulate glucose metabolism (Wojtunik-Kulesza *et al.,* 2024)**.** Carvone also shows potent antifungal activity against several fungal strains, including *Candida* spp., mycotoxigenic fungi (*Fusarium*, *Aspergillus*, and *Penicillium* spp.), and dermatophytes such as *Trichophyton*, *Epidermophyton floccosum*, and *Microsporum* spp.. It is effective in inhibiting fungal biofilm formation and germ tube development. The antibacterial properties of carvone are well-documented, including its efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA) .Its antibacterial mechanism involves disruption of bacterial membranes, leading to increased permeability and decreased membrane integrity. Additionally, carvone has demonstrated anticancer activity against various cancer cell lines, such as those of myeloma, melanoma, and breast cancer, with mechanisms including apoptosis induction and cell cycle arrest (Wei *et al.*, 2024). In addition to these activities, carvone also exhibits anti-inflammatory effects, with several studies exploring the molecular pathways involved (Cátia Sousa *et al*., 2021). Its antioxidant properties neuraminidase inhibitory effects against influenza virus (Jusoh *et al.*, 2018), and antiparasitic activity against *Culex quinquefasciatus*, *Cx. pipiens*, *Aedes aegypti*, and *Haemonchus contortus* (Pavela, 2015), further contribute to its broad-spectrum biological effects. These diverse bioactivities have expanded the application of carvone in multiple fields, including the disinfection of food packaging and medical devices (Bouyahya *et al.*, 2021).

2.8. Eucalyptol

Also called 1,8-cineole, an important natural compound that falls under the category of monocyclic monoterpene ether. This clear, colorless, and transparent liquid is characterized by a spicy, aromatic, and camphor-like odor (Hoch *et al*., 2023). Eucalyptol can also be synthetically produced through the isomerization of α-terpineol. It is widely distributed across

numerous plant species, including *Artemisia herbo-alba*, cardamom (*Elettaria cardamomum*), garland flower, eucalyptus (*Eucalyptus globulus*), peppermint (*Mentha* × *piperita*), Roman mugwort (*Artemisia pontica*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), Spanish sage (*Salvia lavandulifolia*), spearmint (*Mentha spicata*), sweet basil (*Ocimum basilicum*), and wormwood (*Artemisia absinthium*) (Deveci, 2022). Due to its pleasant spicy aroma and flavor, eucalyptol is widely utilized as a flavoring agent, as well as in perfumes and cosmetic formulations (Bhowal and Gopal, 2016). Eucalyptol exhibits a range of therapeutic properties, including antimicrobial, anti-inflammatory, antioxidant, analgesic, and spasmolytic effects, making it effective in the management of conditions such as colds, influenza, respiratory infections, rhinitis, and sinusitis. Research has shown that eucalyptol significantly inhibits proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β, while also exerting an analgesic effect in inflammatory models. Moreover, eucalyptol enhances the beat frequency of nasal cilia, improving mucus clearance, and possesses bronchodilatory effects. It has been reported to alleviate exacerbations in asthma, sinusitis, and chronic obstructive pulmonary disease by reducing cytokine-induced airway mucus hypersecretion. Eucalyptol also exhibits potent antioxidant properties through free radical scavenging and reduces intracellular calcium influx via calcium channels in cardiac muscle (Seol and Kim, 2016). In addition, eucalyptol demonstrates broad-spectrum insecticidal activity against a variety of pests, including stored grain beetles, human lice, German cockroaches, house flies, blow flies, sandflies, mosquitoes, and other species (Moretti *et al*., 2015).

2.9. Limonene

Limonene is a well-known cyclic monoterpene hydrocarbon ($C_{10}H_{16}$) characterized by its olefinic structure (Jongedijk *et al.*, 2016), and chemically designated as 1-methyl-4-(1-methylphenyl) cyclohexene (Satira *et al.*, 2021). It is naturally abundant in citrus fruits, with the highest concentrations found in grapefruit (95%), tangerine (94%), orange (91%), mandarin (72%), lemon (65%), and elemi resin (50**%)** (Anandakumar *et al*., 2021). Limonene is a colorless liquid that occurs in two optical isomeric forms—d-limonene and l-limonene—as well as in a racemic mixture. Its characteristic lemon-like aroma makes it a widely used additive for flavor and fragrance in various food products, including fruit juices, candies, chewing gums, soft drinks, and ice creams. Additionally, limonene ranks among the most commonly used and cost-effective fragrance ingredients in cosmetic formulations (Vieira *et al.*, 2018). Limonene exhibits repellent and toxic effects against a broad range of arthropods and is incorporated into more than 15 commercial insecticidal and repellent products (Showler *et al*., 2019). This

compound also possesses a variety of biologically active properties such as antioxidant, anti-inflammatory, anticancer, analgesic, and gastroprotective effects (Eddin *et al.*, 2021). In agriculture, limonene demonstrates effective insecticidal, antibacterial (Han *et al*., 2019), and herbicidal (Gettys *et al*., 2021) activities. In China, it has been officially registered as a pesticide, fungicide, and pesticide adjuvant. Due to its abundant natural sources, ease of extraction, and wide range of practical uses, limonene has become a growing focus of scientific research, with its applications steadily expanding across the food, pharmaceutical, and agricultural sectors (Luo *et al.*, 2023).

2.10. Linalool

Linalool ($C_{10}H_{18}O$), chemically known as 3,7-dimethyl-1,6-octadien-3-ol, is an acyclic monoterpenoid compound characterized by a tertiary alcohol functional group (Ferraz *et al.*, 2021). Linalool is a monoterpenoid alcohol notable for its characteristic floral fragrance (Rodríguez-López *et al*., 2020). It is present in the essential oils of more than 200 plant species, representing over half of all plant families (Quarshie *et al.*, 2024). Linalool exists in two enantiomeric forms: (R)-linalool and (S)-linalool. The (R)-enantiomer is characterized by a woody, lavender-like scent, whereas the (S)-enantiomer exhibits a sweet, floral, and herbaceous aroma. Variations in the ratio of these enantiomers influence the overall aroma and flavor profile of linalool-containing products (Poonphatanapricha *et al.*, 2021). It exhibits a broad range of biological activities making it valuable for applications in the pharmaceutical, cosmetic, and food industries. Linalool has demonstrated significant antibacterial activity against both Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. The antibacterial mechanism of linalool involves disrupting the integrity of bacterial membranes, increasing their permeability, and causing the leakage of intracellular components, ultimately leading to bacterial cell death (Krapež *et al.*, 2024). In addition to its antimicrobial properties, linalool exerts insecticidal effects by targeting the insect nervous system. It modulates neurotransmitter receptors, including gamma-aminobutyric acid (GABA) gated chloride channels and nicotinic acetylcholine receptors. These interactions disrupt neural transmission, causing paralysis and death in insects such as *Tribolium castaneum* (Pajaro-Castro *et al*., 2017). Beyond its antibacterial and insecticidal effects, linalool also possesses anti-inflammatory, antioxidant, and anticancer properties (Varia *et al.*, 2020).

2.11. β-Pinene

Pinene (C10H16) is a bicyclic monoterpenoid hydrocarbon characterized by the presence of a double bond (Winnacker, 2018). It exists in two principal structural isomers, α-pinene and β-pinene, which are commonly found in nature, particularly in EOs of coniferous trees such as pines. These compounds are prominent representatives of the monoterpene class. Each isomer exhibits chirality, with (+)- and (−)-enantiomers that differ in their optical rotation and cannot be superimposed, resulting in a total of four stereoisomers (Vespermann *et al.*, 2017). α-Pinene is a colorless organic liquid, insoluble in water but soluble in oils and ethanol, with a boiling point of approximately 155 °C. It is found in more than 40 essential oils. β-Pinene, also colorless and soluble in oil and ethanol, its boiling point ranges from 163 to 166 °C. It is obtained either through direct distillation or by isomerization of α-pinene. β-Pinene serves as a key intermediate in the synthesis of aromatic compounds such as menthol, ionones, linalool, geraniol, citronellal, citral, and citronellol, and is especially used in bakery products (Salehi *et al.*, 2019). It exhibits a broad spectrum of pharmacological activities, including modulation of antibiotic resistance, as well as anticoagulant, antimicrobial, antimalarial, antitumor, and anti-inflammatory effects (Zhang *et al.*, 2021).

2.12. Toxicity Bioassay

The larvicidal potential of three EOs—*E. globulus*, *C. aurantium*, and *M. spicata*—was assessed, along with their major constituents: limonene, carvone, eucalyptol, linalool, and β-pinene. Two stock solutions were applied: 900 ppm for eucalyptol and linalool, with concentrations ranging from 200 to 900 ppm, and 50 ppm for *E. globulus*, *C. aurantium*, *M. spicata*, limonene, carvone, and β-pinene, with concentrations ranging from 6.25 to 200 ppm. Each treatment was applied in containers filled with 150 mL of dechlorinated tap water, each containing 20 newly emerged fourth-instar larvae of *C. longiareolata*, with ethanol serving as solvent. In parallel, a separate set of cups received 1 mL of ethanol and served as positive controls while that of negative controls contained only water. After 24 h of exposure, larvae were removed, washed with untreated water and placed in clean dechlorinated tap water. The test was carried out with three replicates. Larval mortality was monitored at 24, 48, and 72 hours post-treatment. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region and unable to reach the water surface.

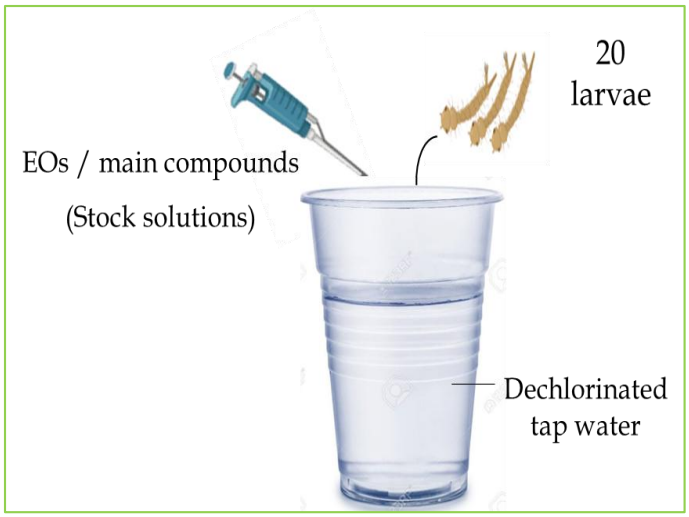


Figure 6. Larvicidal bioassay

2.13 Statistical Analysis

Statistical analysis was performed using GraphPad Prism 7. The results were expressed as mean±standard error of the mean (SEM). One-way analysis of variance (ANOVA), followed by Tukey's HSD multiple comparison test, was used to assess significant differences between groups.

Results

III. RESULTS:

3.1. Insecticidal activity:

After a screening test, different concentrations from *M. spicata*, *C. aurantium* and *E. globulus* EOs, and their main compounds Carvone, Limonene, Linalool, Eucalyptol and β-Pinene, were applied against newly emerged fourth-instar larvae of *C. longiareolata*. Parallel control groups were also set up. No mortality was observed in the control series.

3.1.1. Larvicidal efficacy of *M. spicata* EO and its major compounds:

The corrected mortality rates observed following treatment with Mentha spicata essential oil ranged from 1.66% at 24 h to 50% at 72 h at the lowest concentration (12.5 ppm), and from 71.66% at 24 h to 100% at 72 h at the highest concentration (100 ppm). In the case of carvone, corrected mortality ranged from 1.66% at 24 h to 23.33% at 72 h for the lowest concentration (25 ppm), and from 83.33% to 100% for the highest concentration (150 ppm). For limonene, mortality rates ranged from 1.66% at 24 h to 13.33% at 72 h at the lowest concentration (6.25 ppm), and from 55% to 100% at the highest concentration (50 ppm).

A significant increase in corrected mortality rates was noted depending on the applied doses and the time after treatment. For *M. spicata* EO, at 24 h ($F_{3,8}$= 56 ; $p<0.0001$), 48 h ($F_{3,8}$= 37.40 ; $p<0.0001$) and at 72 h ($F_{3,8}$= 38.80 ; $p<0.0001$). For Carvone, at 24 h ($F_{3,8}$= 48.91 ; $p<0.0001$), 48h ($F_{3,8}$= 186.2 ; $p<0.0001$) and at 72 h ($F_{3,8}$= 169.9 ; $p<0.0001$). For Limonene, at 24 h ($F_{3,8}$= 54.56; $p<0.0001$), 48 h ($F_{3,8}$= 30.31 ; $p = 0.0001$) and at 72 h ($F_{3,8}$= 146.9 ; $p<0.0001$).

Tukey's HSD test revealed three distinct groups across all three-time points for *M. spicata*, carvone, and limonene (Figure 7).

The dose-response curve expressing the percentage of mortality as a function of the logarithm of the applied doses allowed for the estimation of lethal concentrations (LC) along with their confidence intervals and Hill Slope (Table 2).

Interestingly, limonene alone exhibited a significantly higher larvicidal effect than both the whole EO and carvone.

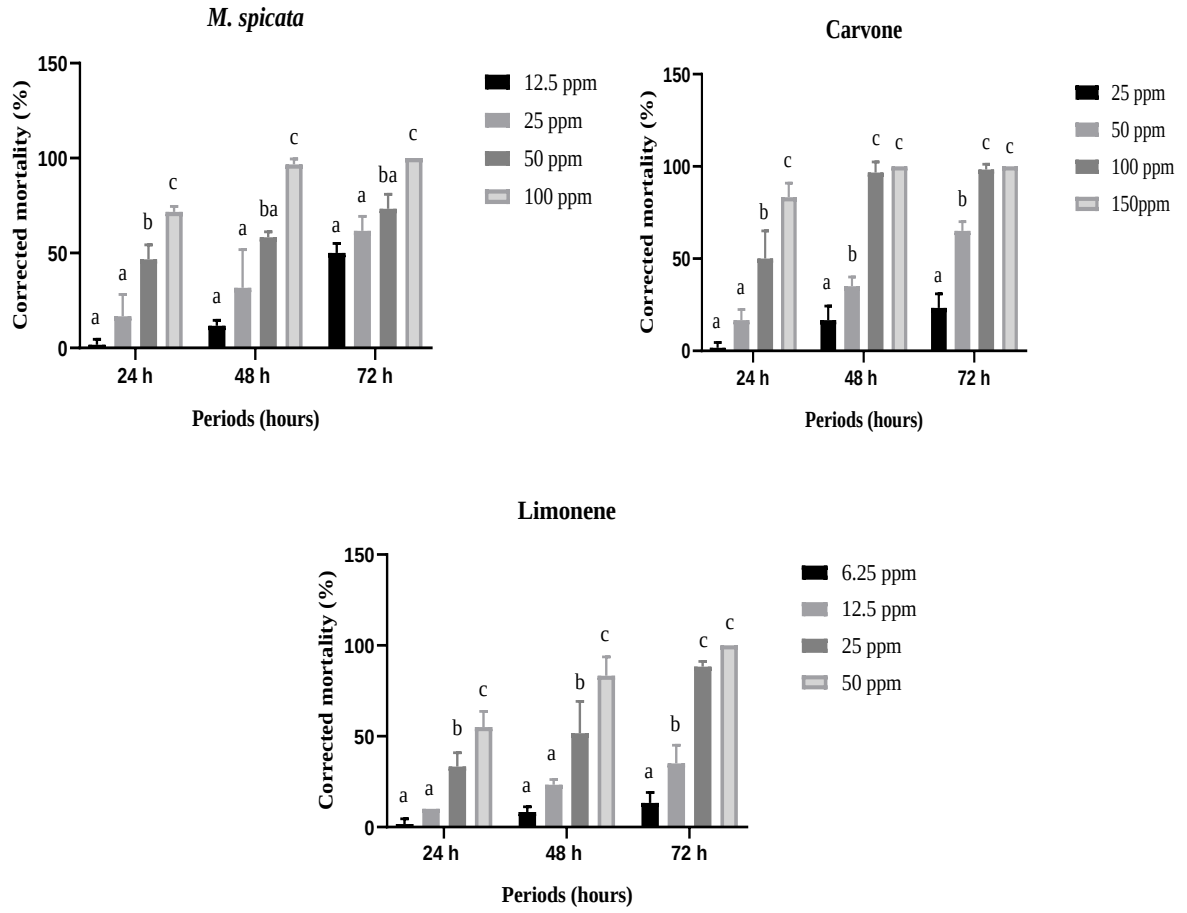


Figure 7. Toxicity of *M. spicata* EO and its major compounds Carvone and Limonene against *C. longiareolata* larvae at different periods: Corrected mortality (%) (mean ± SEM, n = 3 repetitions of 20 individuals each): Tukey's HSD test.

Table 2. Efficacy of *M. spicata* EO and its major compounds Carvone and Limonene against *C. longiareolata* larvae: determination of lethal concentrations and their confidence intervals (95%).

Treatments	Periods	R^2	Hill slope	LC_{25} CI	LC_{50} CI	LC_{90} CI
Mentha spicata	24 h	0.99	1.88	31.99 21.78 ± 43.63	57.27 44.61 ± 76.21	183.60 107.40 ± 444.20
	48 h	0.97	2.09	22.26 8.07 ± 34.05	37.60 22.65 ± 60.28	104.20 48.65 ± 486.40
	72 h	0.87	1.10	10.63 6.11 ± 21.52	14.21 11.38 ± 34.76	107.30 20.76 ± 560.35

Carvone	24 h	0.98	2.93	65.04 43.06 ± 91.11	94.59 74.49 ±116.30	200.10 130.90 ± 393.30
	48 h	0.96	4.08	42.74 10.31 ± 76.89	55.94 28.23 ± 140.22	95.80 44.89 ± 532.80
	72 h	0.99	2.92	26.69 18.36 ± 35.93	38.87 28.23 ± 91.37	82.45 57.98 ± 132.50
Limonene	24 h	0.97	1.66	21.82 13.50 ± 31.90	42.25 30.55 ± 76.96	158.40 76.30 ± 1002
	48 h	0.99	1.95	13.29 10.95 ± 15.82	23.32 20.57 ± 26.45	71.75 54.67 ± 100.70
	72 h	0.98	3.24	10.31 5.67 ± 15.00	14.47 10.41 ± 19.51	28.50 17.45 ± 66.80

3.1.2. Larvicidal efficacy of *C. aurantium* EO and its major compound:

The corrected mortalities recorded after treatment by *C. aurantium* EO ranged from 1.66 % at 24h to 26.66 % at 72 h for the lowest dose (50 ppm), and from 98.33 % at 24 h to 100 % at 72 h for the highest dose (200 ppm). For Linalool, the corrected mortalities ranged from 11.66 % at 24 h to 48.33 % at 72 h for the lowest dose (500 ppm), and from 81.66 % at 24 h to 100 % at 72 h for the highest dose (800 ppm).

A significant increase in corrected mortality rates was noted depending on the applied doses and the time after treatment. For *C. aurantium* EO, at 24 h ($F_{3,8}$= 144.2 ; $p<0.0001$), 48 h ($F_{3,8}$= 107.6; $p<0.0001$) and at 72 h ($F_{3,8}$= 83.81 ; $p<0.0001$). For Linalool, at 24 h ($F_{3,8}$= 20.86 ; $p = 0.0004$), 48 h ($F_{3,8}$= 29.36; $p = 0.0001$) and at 72 h ($F_{3,8}$= 26.17 ; $p = 0.0002$).

Tukey's HSD test revealed four distinct groups at 24 h and three groups at both 48 h and 72 h for *C. aurantium* EO. For linalool, two groups were identified at 24 h and three groups at 48 h and 72h (Figure 8).

The dose-response curve expressing the percentage of mortality as a function of the logarithm of the applied doses allowed for the estimation of lethal concentrations (LC) along with their confidence intervals and Hill Slope (Table 3).

C.aurantium EO, dominated by linalool, demonstrated greater larvicidal efficacy than linalool alone.

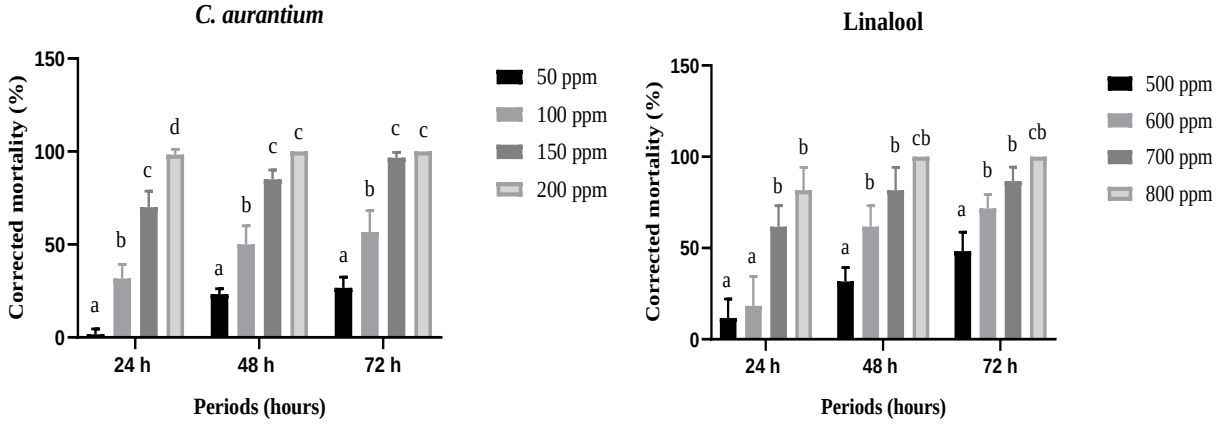


Figure 8. Toxicity of *C. aurantium* EO and its major compound Linalool against *C. longiareolata* larvae at different periods: Corrected mortality (%) (mean ± SEM, n = 3 repetitions of 20 individuals each): Tukey's HSD test.

Table 3. Efficacy of *C. aurantium* EO and its major compound Linalool against *C. longiareolata* larvae: determination of lethal concentrations and their confidence intervals (95%).

Treatments	Periods	R^2	Hill slope	LC_{25} CI	LC_{50} CI	LC_{90} CI
C. aurantium	24 h	0.98	4.71	94.77 65.72 ± 120.90	119.70 97.27 ± 142.20	190.80 136.80 ± 304.80
	48 h	0.94	2.87	60.34 5.22 ± 113.40	88.47 30.44 ± 136.30	190.20 89.77 ± 238.30
	72 h	0.92	2.95	54.04 0.55 ± 109.50	78.37 9.52 ± 129.20	164.90 20.76 ± 201.60
Linalool	24 h	0.97	9.78	605.10 494.70 ± 675.90	677.10 615.10 ± 745.60	790.12 675.33 ± 980.02
	48 h	0.97	7.49	482.70 382.30 ± 541.90	558.90 498.00 ± 607.10	749.20 643.20 ± 960.90
	72 h	0.97	6.32	428.00 292.70 ± 488.80	493.45 395.35 ± 547.95	720.70 610.60 ± 950.20

3.1.3. Larvicidal efficacy of *E. globulus* EO and its major compound:

The corrected mortalities recorded after treatment by *E. globulus* EO ranged from 13.33 % at 24h to 51.66 % at 72 h for the lowest dose (30 ppm), and from 75 % at 24 h to 100 % at 72 h for

the highest dose (45 ppm). For Eucalyptol, the corrected mortalities ranged from 1.66 % at 24h to 68.33 % at 72 h for the lowest dose (200 ppm), and from 68.33 % at 24 h to 100 % at 72 h for the highest dose (800 ppm).

A significant increase in corrected mortality rates was noted depending on the applied doses and the time after treatment. For *E. globulus* EO, at 24 h ($F_{3,8}$= 60.63 ; p<0.0001), 48 h ($F_{3,8}$= 18.30; p= 0.0006) and at 72 h ($F_{3,8}$= 88.11 ; p<0.0001). For Eucalyptol, at 24 h ($F_{3,8}$= 132.6 ; p<0.0001), 48 h ($F_{3,8}$= 42.18 ; p<0.0001) and at 72 h ($F_{3,8}$= 12.49 ; p = 0.0022).

Treatment with *E. globulus* EO resulted in four distinct dose groups at 24 h and three groups at both 48 h and 72 h. In contrast, treatment with its major compound, eucalyptol, revealed three groups at 24 h and 48 h, and two groups at 72 h (Figure 9).

The dose-response curve expressing the percentage of mortality as a function of the logarithm of the applied doses allowed for the estimation of lethal concentrations (LC) along with their confidence intervals and Hill Slope (Table 4).

Considering the lethal doses and the concentration of the treatments in the stock solutions *E.globulus* EO, with eucalyptol as a major compound, also outperformed its isolated constituent.

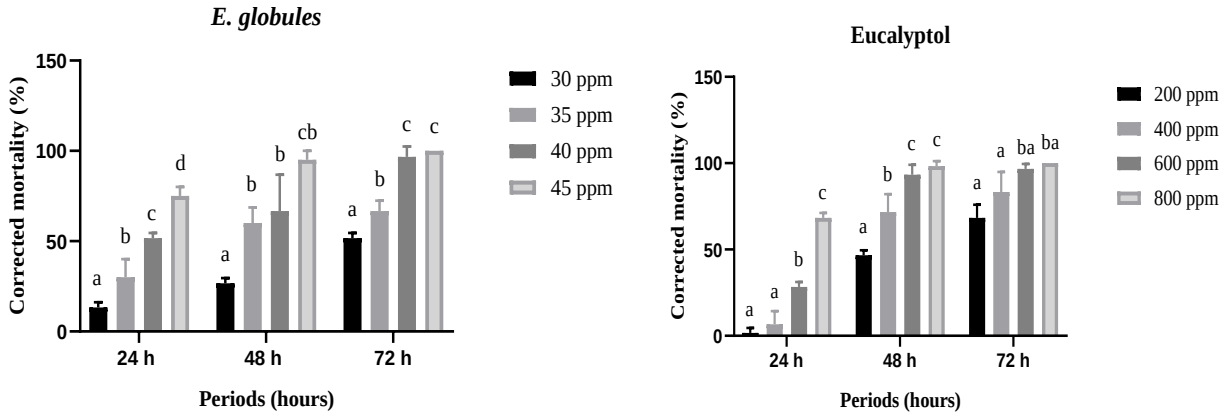


Figure 9. Toxicity of *E. globulus* EO and its major compound Eucalyptol against *C. longiareolata* larvae at different periods: Corrected mortality (%) (mean ± SEM, n = 3 repetitions of 20 individuals each): Tukey's HSD test.

Table 4. Efficacy of *E. globulus* EO and its major compound Eucalyptol against *C. longiareolata* larvae: determination of lethal concentrations and their confidence intervals (95%).

Treatments	Periods	R^2	Hill slope	LC_{25} CI	LC_{50} CI	LC_{90} CI
E. globulus	24 h	0.99	7.39	33.83 32.15 ± 35.30	39.25 38.12 ± 40.44	52.08 43.19 ± 243.80
	48 h	0.92	7.25	29.32 8.24 ± 34.91	34.11 21.59 ± 39.42	46.18 35.80 ± 194.30
	72 h	0.90	7.79	26.28 7.15 ± 31.75	30.26 12.87 ± 34.80	40.12 35.06 ± 52.15
Eucalyptol	24 h	0.99	5.59	576.00 517.70 ± 625.70	701.00 661.20 ± 745.40	1038 908.10 ± 1290
	48 h	0.95	2.13	131.80 13.24 ± 223.90	220.70 66.02 ± 323.30	618.90 275.60 ± 2585
	72 h	0.92	1.84	74.31 0.02 ± 154.93	135.00 0.97 ± 207.90	445.30 20.34 ± 2341

3.1.4. Larvicidal efficacy of β-Pinene:

The corrected mortalities recorded after treatment by β-Pinene ranged from 1.66 % % at 24h to 50 % at 72 h for the lowest dose (12.5 ppm), and from 93.33 % at 24 h to 100 % at 72 h for the highest dose (100 ppm).

A significant increase in corrected mortality rates was noted depending on the applied doses and the time after treatment at 24 h ($F_{3,8}$= 139.4 ; p<0.0001), 48 h ($F_{3,8}$= 381.5 ; p<0.0001) and at 72h ($F_{3,8}$= 13.60 ; p = 0.0017).

Based on Tukey's HSD test, four statistically distinct groups were identified at 24 h and 48 h, and two groups at 72 h (Figure 10).

The dose-response curve expressing the percentage of mortality as a function of the logarithm of the applied doses allowed for the estimation of lethal concentrations (LC) along with their confidence intervals and Hill Slope (Table 5).

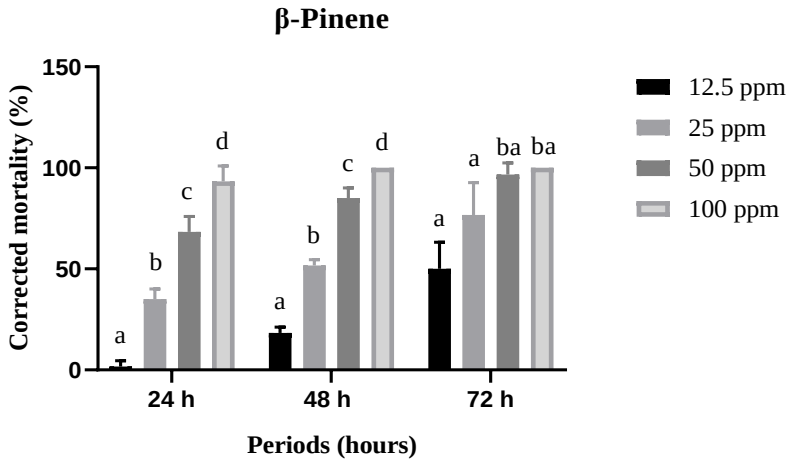


Figure 10. Toxicity of β-Pinene against *C. longiareolata* larvae at different periods: Corrected mortality (%) (mean ± SEM, n = 3 repetitions of 20 individuals each): Tukey's HSD test.

Table 5. Efficacy of β-Pinene against *C. longiareolata* larvae: determination of lethal concentrations and their confidence intervals (95%).

Treatments	Periods	R^2	Hill slope	LC_{25} CI	LC_{50} CI	LC_{90} CI
β-Pinene	24 h	0.98	2.47	22.45 12.45 ±34.87	34.97 24.44 ± 50.74	84.83 41.32 ± 240.70
	48 h	0.99	2.40	15.18 12.08 ± 18.36	23.99 20.82 ± 27.54	59.93 45.28 ± 83.45
	72 h	0.98	1.98	7.31 3.57 ± 10.26	12.73 8.78 ± 15.80	38.57 24.79 ± 69.22

In terms of overall larvicidal efficacy against *C. longiareolata*, the tested substances demonstrated varying levels of potency. β-Pinene exhibited the highest efficacy, followed by limonene, which outperformed the essential oil of *M. spicata*. This EO, in turn, was more effective than *E. globulus*. Carvone showed lower activity, followed by *C.aurantium* EO. The least effective were the individual compounds eucalyptol and linalool.

Discussion

IV. DISCUSSION

Essential oils (EOs) are complicated mixtures of natural chemicals that are mainly obtained from aromatic plants using a method called hydrodistillation. Renowned for their insecticidal, antimicrobial, and antioxidant properties, their chemical composition was characterized using gas chromatography (GC)—a powerful analytical technique that allows precise separation and identification of both major and minor constituents. In this context, a comparative analysis was conducted between the chemical profiles of Mentha, Citrus, and Eucalyptus essential oils, alongside the insecticidal activity of their principal compounds—limonene, linalool, carvone, β-pinene, and eucalyptol—providing a deeper understanding of their potential as botanical insecticides against *Culiseta longiareolata*.

4.1. Comparative analysis of essential oils composition

4.1.1. *Mentha spicata* EO

The chemical composition of the essential oils analyzed in this study aligns largely with findings from previously published works, albeit with some minor variations.

In the case of *Mentha spicata* EO, our analysis identified carvone (58.3%) and limonene (24.6%) as the predominant components (Soltani *et al*., 2025), consistent with the findings reported by several authors (Mahboubi, 2021). It is worth noting that the chemical profile of *M. spicata* exhibits significant variability between wild and cultivated forms, as evidenced by a wide range of chemotypes documented in the literature. These chemotypes are characterized by dominance of carvone, pulegone, or a combination of pulegone/menthone (İsfendiyaroğlu *et al.*, 2024) . A related study conducted in Ghardaïa, Algeria, identified cis-carvone oxide (44.06%), 1,8-cineole (15.32%), cis-dihydrocarvone (8.85%), and limonene (5.80%) as major constituents (Laggoune *et al.*, 2016). Similarly, a study on essential oils from the Algerian Saharan Atlas reported carvone (49.5%), limonene (16.1%), 1,8-cineole (8.7%), cis-dihydrocarvone (3.9%), β-caryophyllene (2.7%), germacrene D (2.1%), and β-pinene (1.1%). Regarding chemical classes, oxygenated monoterpenes were the most abundant (67.2%), followed by monoterpene hydrocarbons (20.8%), sesquiterpene hydrocarbons (7.5%), and oxygenated sesquiterpenes (1.2%) (Bardaweel *et al.*, 2018) .

In Tunisia, the EO of *M.spicata* was predominantly composed of oxygenated monoterpenes (50.6%) and monoterpene hydrocarbons (45.1%), with a smaller fraction of sesquiterpene hydrocarbons (2.8%). The major constituents were carvone (40.8% ± 1.23%) and limonene (20.8% ± 1.12%), followed by 1,8-cineole (17.0% ± 0.60%), β-pinene (2.2% ± 0.25%), cis-dihydrocarvone (1.9% ± 0.49%), and dihydrocarveol (1.7% ± 0.31%) (Snoussi *et al.*, 2015). In Palestine, *M. spicata* EO exhibited significant variation in constituent concentrations, with carvone (36.9–76.8%), limonene (6.23–9.79%), and dihydrocarveol (2.27–13.76%) as major components (Ali-Shtayeh *et al.*, 2019). In Egypt, the main constituents identified were menthone (32.43%), 1,8-cineole (18.79%), cis-iso-pulegone (16.65%), pulegone (10.01%), β-pinene (7.12%), α-cadinol (5.30%), and α-pinene (5.03%) (Omar et *al.*, 2009). A Romanian study identified carvone (41.215% ± 4.18%) as the major component, followed by menthol (12.774% ± 2.48%), menthone (7.225% ± 1.81%), and pulegone (3.763% ± 1.04%) (Mogosan *et al.*, 2017). In Argentina, the essential oils were characterized by carvone (60.72–68.09%), limonene (14.23–16.41%), α-pinene (0.12–3.14%), β-myrcene (0.35–2.33%), 1,8-cineole (1.69–2.73%) (Ariel Meloni *et al.*, 2019). In Turkey, carvone (67.8%) was identified as the predominant component, with limonene (10.6%) as the second major constituent (İsfendiyaroğlu *et al.*, 2024). In contrast, a study from Iran reported a distinct profile, dominated by iso-menthone (10.3%), piperitone oxide (19.3%), and α-terpinene (19.7%) (Rasooli *et al.*, 2007). While a Serbian essential oil exhibited a profile of limonene (5.8 %), menthone (21.9%), and carvone (69.5%) (Soković *et al.*, 2009). In samples collected from four different regions of Turkey with varying soil and climate conditions, *M. spicata* EO displayed considerable chemical diversity. The main monoterpene hydrocarbons were limonene (3.2–5.21%) and β-phellandrene (1.31–2.55%), whereas the principal sesquiterpenes were trans-caryophyllene (5.23–8.01%) and germacrene D (3.0–5.32%) (Telci *et al.*, 2010). Overall, carvone, limonene, and 1,8-cineole are typically the main constituents of spearmint essential oils, although their biosynthesis can be affected by factors such as genotype, agricultural practices (including irrigation and fertilization), and environmental conditions like temperature and photoperiod (Meloni *et al*., 2021) .

4.1.2. *Citrus aurantium* EO

In Tébessa, Algeria, 19 compounds were identified, representing 99.7% of the total composition. Oxygenated monoterpenes (49.4%) and esters (42.1%) were the predominant chemical classes, with linalool (39.1%) and linalool acetate (36.6%) as their major

constituents. Significant amounts of α-terpineol (5.9%) and geranyl acetate (3.1%) were also detected (Soltani *et al.*, 2025). In Annaba, Eastern Algeria, the EO analysis revealed 23 constituents, with β-linalool being the dominant compound (44.52%), while most components belonged to the monoterpene hydrocarbon class, including α-terpineol (8.34%) and a geraniol ester (3.48%) (Salıma *et al.*, 2019). In Constantine, Algeria, 38 compounds were identified in the leaf EO, representing 95% of the total oil. The major constituents were linalool (18.6%), γ-terpinene (6.9%), and α-terpineol (15.1%). In the peel EO, 32 compounds representing 96% of the total were identified, with linalool (12.0%), cis-linalool oxide (8.1%), trans-carveol (11.9%), endo-fenchyl acetate (5.5%), and carvone (5.8%) as main components (Abderrezak *et al.*, 2014). In Algeria, linalyl acetate, linalool, α-terpineol, geraniol, and geranyl acetate were identified as the major components. Other detected compounds included β-pinene, neryl acetate, trans-β-ocimene, β-myrcene, and nerol, each contributing at least 2% of the total essential oil content. These findings confirm that the essential oil from Algerian *C. aurantium* leaves corresponds to the linalool/linalyl acetate chemotype, the most common profile reported (Oulebsir *et al.*, 2022).

In Iran, the major constituents of the leaf EO was linalool (32.60%), whereas the peel EO was dominated by limonene (59.88%–81.6%) (Azhdarzadeh *et al.*, 2016). In Serbia, the EO of *C. aurantium* was rich in monoterpene hydrocarbons such as limonene (90.4%), hydrocarbons such as β-myrcene (2.6%) and β-pinene (1.5%) were found, alongside monoterpene alcohol α-terpineol (1.7%) and monoterpene ester linalool acetate (1.6%) (Vukić et al., 2023). In Tunisia, the EO was predominantly composed of monoterpene hydrocarbons (88.31%), with limonene (67.1%) as the major compound, followed by linalool (8.37%), β-pinene (4.02%), myrcene (3.17%), β-ocimene (2.36%), and α-pinene (1.18%) (Chaieb *et al.*, 2018). In Greece, the composition of *C. aurantium* EOs varied by plant part. Neroli oil (from flowers) was rich in linalool (29.14%), β-pinene (19.08%), and limonene (12.04%). Peel oil was dominated by limonene (94.67%) with minor amounts of myrcene and linalool. Petitgrain oil (from leaves) primarily contained linalool (36.03%–58.21%), α-terpineol, and esters like geranyl acetate. Monoterpene hydrocarbons dominated in the peel (98.30%), while oxygenated monoterpenes prevailed in leaf and flower oils, especially from older leaves (88.09%). Sesquiterpenes were found only in neroli oil (7.67%) (Sarrou *et al.*, 2013).

Leaves of the *Citrus* genus are notable for their bioactive components, including ascorbic acid, flavonoids, and various phenolics, and are rich in essential oils composed mainly of limonene, linalool, α-terpineol, and linalyl acetate (Maksoud *et al.*, 2021). In another study,

the volatile profile of *C. aurantium* EO was marked by the presence of eucalyptol, sabinene, linalool, and α-terpineol as dominant compounds. The chemical composition of *Citrus* species EOs varies significantly based on species, geographic origin, climate, season, ripening stage, and extraction and analysis methods used (Elshafie, 2022). Additionally, the EOs extracted from various parts of *C. aurantium* typically feature linalool as the predominant component (Bnina *et al.*, 2019). Generally, the chemical composition of EOs varies significantly due to a combination of intrinsic and extrinsic factors. Intrinsic factors include the plant's genetic makeup, chemotype, and developmental stage, which determine the biosynthesis pathways of key compounds. Extrinsic factors such as geographic location, climate conditions (temperature, humidity, sunlight), soil type, altitude, and agricultural practices (irrigation, fertilization, harvesting time) also play crucial roles in shaping the essential oil profile. These variables influence not only the quantity but also the quality and relative abundance of specific chemical constituents across different regions and even within the same species, resulting in notable variability in EOs activities and efficacy (Khan *et al.*, 2023).

4.1.3. *Eucalyptus globulus* EO

The leaves of *E. globulus* can contain up to 3.5% (w/w) essential oil. The major constituent is 1,8-cineole (also known as eucalyptol), which characterizes the typical chemotype of this species. However, other chemotypes have also been reported, featuring dominant compounds such as α-phellandrene, p-cymene, γ-terpinene, ethanone, and spathulenol (Akolade *et al*., 2012). In Tebessa region (semi-arid), located in Eastern Algeria, thirty compounds were identified in *E. globulus* EO, representing 98.1% of the total chemical composition. The predominant chemical class was monoterpene hydrocarbons, accounting for 72.3%, followed by oxygenated monoterpenes (10.6%) and oxygenated sesquiterpenes (8.8%). Sesquiterpene hydrocarbons were present in a negligible amount (0.1%). The most abundant compounds were ρ-cymene (27.3%), 1,8-cineole (5.2%), spathulenol (8.1%), and β-phellandrene (8%) (Soltani et al., 2025). However, in other sample, the major compounds were Sabinene (35.38 %) and α-Phellandrene (12.64 %) (Yahia *et al*., 2023). The chemical analysis of *E. globulus* EO from Saida, Algeria, revealed that the major constituents were p-cymene (20.24%), spathulenol (14.10%), and eucalyptol (11.30%) (Benabdesslem et al., 2020). Analyses of the essential oils from the leaves of *E. globulus* collected in Bejaia and Blida (northern Algeria) reported eucalyptol as the major constituent, accounting for 55.29% and 51.08%, respectively. Other significant compounds identified were spathulenol (7.44%) and α-terpineol (5.46%) in Bejaia, and α-pinene (24.6%) in Blida (Harkat-Madouri *et al*.,

2015; Boukhatem *et al*., 2014). The EO of *E. globulus* leaves from Seraïdi, wilaya of Annaba, northeastern Algeria was dominated by linalool (30.09%), followed by β-linalyl oxide (13.93%), camphor (12.09%), eucalyptol (10.95%), and bergamotene (10.03%). This indicates a linalool chemotype, unlike the typical eucalyptol-rich E. globulus oils (Moussa *et al*., 2024). In Mascara, the major constituents of this essential oil were: (-)-spathulenol (32.66%), o-cymene (28.30%) and (+-)-cryptone (12.13%). Other notable compounds identified were: terpinen-4-ol (4.60%), cuminaldehyde (3.05%) and caryophyllene oxide (2.10%) (Bourakna *et al*., 2022). GC/MS analysis of leaves collected from the herbarium of Draa Naga, Djbel El Ouahch, east of Constantine, Algeria, identified 39 compounds, mainly oxygenated monoterpenes (86.01%), monoterpenes (5.74%), monoterpene alcohols (4.05%), and sesquiterpene alcohols (2.74%). The major constituents were 1,8-cineole (78.45%), o-cymene (2.18%), isopinocarveol (1.74%), α-pinene (1.69%), pinocarvone (1.34%), and veridiflorol (1.31%) (Atmani-Merabet *et al*., 2018).

In Tunisia, the essential oil profile of *E. globulus* is characterized by α-pinene (13.61%) and 1,8-cineole (43.18%) as the major compounds (Slimane *et al*., 2014). However, that from Egypt contained 76.25% oxygenated monoterpenes, mainly eucalyptol (74.31%), 14.34% monoterpene hydrocarbons—primarily cymene (6.81%), d-limonene (3.67%), α-pinene (2.12%), and γ-terpinene (1.55%)—and 7.39% oxygenated sesquiterpenes, with globulol at 4.06% (Salman *et al*., 2024). In Tanzania, the EO was mainly composed of oxygenated and non-oxygenated monoterpenes, along with oxygenated sesquiterpenes. The primary oxygenated monoterpenes were eucalyptol (51.62%) and terpinen-4-ol (2.74%), while the main non-oxygenated monoterpenes included α-pinene (23.62%), p-cymene (10%), and β-myrcene (8.74%) (Almasa *et al*., 2021). *E. globulus* essential oil (EO), obtained from Nitra, Slovakia and cultivated in Spain, had 1,8-cineole—a monoterpene epoxide—as its major compound, accounting for 63.1% of the total composition, monoterpene hydrocarbons were the second most abundant class, with the main components including p-cymene (7.7%), α-pinene (7.3%), α-limonene (6.9%), γ-terpinene (3.6%), β-pinene (3.0%), and β-myrcene (1.7%) (Čmiková *et al*., 2023). In India, the main components of *E. globulus* EO include eucalyptol (54.79%), beta-pinene (18.54%), alpha-pinene (11.46%), beta-eudesmol (4.68%) and alpha-phellandrene (2.06%) (Joshi *et al*., 2016). In Iran, 1,8-cineole was the predominant compound in Eucalyptus EO, accounting for 51.25%, followed by camphor (9.58%), borneol (7.63%), and camphene (3.77%) (Abbasi *et al*., 2021). Finally, in Portugal, the major

components of *E. globulus* EO were 1,8-cineole (72.3%) and α-pinene (9.4%) (Moreira *et al*., 2022).

4.2.Comparative Larvicidal activity

Culiesta longiareolata is a widespread mosquito species commonly found in temporary water bodies, urban reservoirs, and artificial containers, particularly in arid and semi-arid regions (Boumaza *et al.*, 2022). Mosquitoes serve as vectors for numerous infectious diseases caused by parasitic and viral agents, including lymphatic filariasis, chikungunya, and Zika, all of which are responsible for significant illnesses in both humans and animals (Nebbak *et al.*, 2022)

Research has identified that the most promising botanical insect-control agents are found within the families Asteraceae, Annonaceae, Canellaceae, Rutaceae, Lamiaceae, and Meliaceae. Their highly volatile components grant them potent fumigant properties, enabling these compounds to penetrate insects via their respiratory systems (Rosa *et al.*, 2020). Their larvicidal effects against *C. longiareolata* larvae have been extensively documented (Bouabida and Dris, 2022). The toxic effects of these oils are mainly due to their primary components, which are largely composed of monoterpenes (El-Minshawy *et al.*, 2018). Several monoterpenoids have been investigated for their insecticidal activity, including β-Pinene, 1,8-Cineole, Linalool, Limonene, and Carvone (Yildirim *et al*., 2013).

Eucalyptus globulus oil, rich in 1,8-cineole and β-pinene, was shown to be effective against *Culex pipiens* and *Aedes aegypti* (Vivekanandhan *et al.*, 2020). The acute toxicity of Eucalyptus globulus essential oil may be primarily attributed to its major constituent, eucalyptol (1,8-cineole), which exhibits a high penetration capacity into insect tissues. This facilitates its interaction with critical molecular targets such as acetylcholinesterase (AChE), resulting in the disruption of neurotransmission and ultimately leading to insect mortality. (Park *et al.*, 2024). Morphometric analysis revealed that *E. globulus* EO inhibited the development of various life stages of *C. longiareolata*. Additionally, it significantly decreased carbohydrate and lipid reserves in larvae, pupae, and male and female adults, while inducing an increase in total protein content. These findings highlight its promising mosquitocidal potential (Yahia *et al.*, 2023). This aligns with previous studies highlighting the effectiveness of *E. globulus* EO as a natural insecticide, demonstrating significant toxicity against various insect pests. Notably, it has shown efficacy against stored-product pests such as *Oryzaephiluss urinamensis* (Abd El-Salam *el al*., 2019), *Tribolium confusum* (Tine *et al.*,

2023), *T. castaneum* (Ebadollahi and Taghinezhad, 2020), *Sitophilus granarius* (Demeter *et al.*, 2021), and *Ephestia kuehniella* (Rekioua *et al.*, 2022). Its toxicity is mainly attributed to its volatile constituents such as eucalyptol, α-pinene, linalool, and limonene (Kheloul *et al.*, 2023). Similarly, (Riat and Kocher, 2019) evaluated the larvicidal efficacy of *E. globulus* oil against *Anopheles stephensi*, a principal malaria vector. Testing concentrations ranging from 50 to 100 ppm on fourth-instar larvae over 48 hours, they observed LC_{50} and / LC_{90} values of 46.11 ppm and 69.33 ppm, respectively. Among the tested doses, 90 ppm was identified as the most effective concentration, highlighting the oil's strong larvicidal potential as a promising eco-friendly alternative for vector control programs. Extending these findings, (Vivekanandhan *et al.*, 2020) assessed the oil's toxicity against three major mosquito species: *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*. After 24 hours, the EO demonstrated potent activity with LC_{50} / LC_{90} values of 30.20/103.39 ppm (*An. stephensi*), 13.58/106.76 ppm (*Ae. aegypti*), and 7.47/32.45 ppm (*Cx. quinquefasciatus*). Toxicity increased after 48 hours, with LC_{50} / LC_{90} values decreasing to 12.58/49.38 ppm, 7.93/34.47 ppm, and 4.41/21.05 ppm, respectively. Chemical profiling via GC-MS and FT-IR identified 1,8-cineole (71.7%) and α-pinene (9.14%) as the predominant bioactive constituents, underlying the oil's larvicidal efficacy. These collective findings support the potential of *Eucalyptus globulus* essential oil as a sustainable and effective agent for mosquito larval control.

Numerous studies have investigated both the chemical composition and insecticidal potential of different Citrus species, including *C. aurantium*, *C. sinensis*, *C. medica*, *C. limonum*, and *C. grandis* (da Câmara and Mahmoud, 2015; Akono *et al.*, 2016; Mossa, 2016;, Guettal *et al.*, 2021). *C. aurantium* EO has demonstrated potent insecticidal and repellent activities against multiple mosquito species, including *Aedes aegypti* (Soares *et al.*, 2024) *Anophele sstephensi* (Sanei-Dehkordi *et al.*, 2016) and *Culex quinquefasciatus* (Sahayaraj *et al.*, 2022). Its efficacy is primarily attributed to major bioactive constituents such as limonene and linalool, which disrupt the mosquito nervous system, leading to mortality or behavioral deterrence (Jian *et al.*, 2022). These properties underscore the potential of *C. aurantium* EO as a natural alternative to synthetic insecticides in vector control strategies. Supporting this, Campolo *et al.* (2014) reported similar insecticidal effects for five Citrus essential oils against *Tribolium confusum*, while Patel *et al.* (2023) demonstrated the efficacy of aromatic compounds and volatile monoterpenes against *Sitophilus granarius* adults. Numerous studies reinforce the promising bio-insecticidal potential of *C. aurantium* EO. For example, the peel oil, rich in limonene,

exhibited strong larvicidal activity against *Anopheles stephensi* larvae with an LC_{50} of 31.20 ppm (Sanei-Dehkordi *et al.*, 2016). Additionally, bark-derived EO showed significant larvicidal effects against *Aedes aegypti* (LC_{50} = 22.64 mg/L), highlighting its applicability in controlling arbovirus vectors (El-Akhal *et al*., 2015). Furthermore, essential oils from *C.aurantium* and *C. paradisi*, both rich in Dl-limonene, displayed considerable larvicidal activity against *A. stephensi*, with LC_{50} values of 31.20 ppm and 35.71 ppm, respectively. A clear dose–response relationship was observed, with nearly 100% mortality at the highest concentration tested (80 ppm) (Sanei-Dehkordi *et al*., 2016). Collectively, these findings position *C. aurantium* EO, particularly due to its limonene content, as a promising eco-friendly alternative for mosquito vector management.

Mentha spicata (spearmint) EO has similarly been studied extensively for its insecticidal potential against mosquito species. Its major constituents, carvone and limonene, are credited with significant larvicidal and repellent activities (Govindarajan *et al.*, 2012). *M. spicata* exhibited the highest larvicidal activity against Culex larvae, achieving a mortality rate of 94% and the lowest LC_{50} value (1.91 mg/dL), underscoring its strong potential as a botanical larvicide (Phulpoto et al., 2021). Similarly, *M. spicata* EO and its major constituents—carvone, cis-carveol, and limonene—were assessed for their larvicidal efficacy against *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles stephensi*. GC-MS analysis identified 18 compounds in the oil, with carvone (48.60%) as the predominant component. The essential oil demonstrated notable larvicidal activity, with LC_{50} values of 62.62 ppm for *C. quinquefasciatus*, 56.08 ppm for *A. aegypti*, and 49.71 ppm for *A. stephensi*. When tested individually, the major constituents showed higher toxicity, particularly against *A. stephensi*, with LC_{50} values of 19.33 ppm for carvone, 28.50 ppm for cis-carveol, and 8.83 ppm for limonene (Govindarajan et al., 2012). In addition to its larvicidal properties, the methanolic leaf extract of *M. spicata* also exhibited adulticidal activity against *A. aegypti*, with LC_{50} and LC_{90} values of 230.00 ppm and 457.40 ppm, respectively. The presence of bioactive phytochemicals—such as alkaloids, flavonoids, and terpenoids—likely contributes to its insecticidal potential, despite slightly lower efficacy compared to *Coleus amboinicus* (Bandi et al., 2024). Further research by Bendifallah *et al.* (2020) extended the insecticidal efficacy of *M. spicata* EO to stored-product pests such as *Sitophilus granarius* and *Tribolium confusum*, reinforcing its broad-spectrum bioactivity. The larvicidal activity of *M. spicata* EO is mainly attributed to carvone and limonene, with carvone recognized for its insecticidal and repellent properties. The U.S. Environmental Protection Agency (EPA) acknowledges l-

carvone as a candidate for formulation into mosquito control products, underscoring its validated role in vector management. However, correlating the insecticidal activity of essential oils to single constituents is complex, as synergistic interactions among major and minor compounds significantly influence overall efficacy (Ebadollahi, 2013). The insecticidal effectiveness of EOs depends on factors such as chemical structure, membrane penetration, volatility, lipophilicity, and the presence of specific functional groups in their components (Ben Miri, 2025).

In addition to carvone and limonene, other compounds such as linalool, 1,8-cineole, and β-pinene—common in *Mentha*, *Citrus*, and *Eucalyptus* species—exhibit strong insecticidal and repellent effects. Linalool acts as a competitive inhibitor of acetylcholinesterase (AChE) and disrupts neurotransmission via GABAergic and glutamatergic pathways, showing fumigant and repellent properties against *Aedes aegypti* and *Culex quinquefasciatus* (Tong and Coats, 2010; de Sena Filho *et al.*, 2023, Ferrati *et al.*, 2023). Carvone's toxicity is linked to interference with insect nervous system function, while limonene affects both octopaminergic and AChE systems. Compounds like 1,8-cineole and β-pinene demonstrate toxicity through cuticular penetration and enzymatic disruption in mosquitoes (Ben Miri, 2025).

Nevertheless, the variation in mosquito species' responses and developmental stages, coupled with synergistic effects among EO constituents, complicates the identification of a single active agent (Ebadollahi, 2013). Therefore, understanding the whole essential oil's complex chemistry is crucial for developing effective bio-insecticides.

The larvicidal activity of various essential oils was evaluated against *C. longiareolata* larvae, revealing significant differences in potency among the tested oils. According to the 24-hour LC_{50} and LC_{90} values, *T. vulgaris* demonstrated the highest larvicidal potency (LC_{50} = 25.64 ppm; LC_{90} = 50.53 ppm), while *A. herba-alba* was the least effective. Probit regression analysis indicated that larval mortality increased significantly with concentration. Specifically, each unit increase in concentration resulted in mortality rates multiplied by 4.36 in *T. vulgaris*, 3.66 in *J. phoenicea*, 6.08 in *A. herba-alba*, 7.16 in *R. officinalis*, and 5.28 in *E. globulus* (Ismahane, 2020). Complementary studies have confirmed the efficacy of *Ruta* species essential oils. The larvicidal activity of *Ruta montana* and *Ruta graveolens* against *C. longiareolata* larvae revealed LC_{50} values of 30.24 ppm and 27.21 ppm, respectively, highlighting their strong toxic potential. In addition to their lethal effects, these oils caused significant biochemical disruptions in the larvae, notably reducing carbohydrate, lipid, and

protein contents (Bendjazia *et al.*, 2025). Furthermore, *R. graveolens* essential oil was shown to exhibit potent larvicidal activity in another study, with LC_{50} and LC_{90} values of 39.4 µL/mL and 89.57 µL/mL, respectively (Bouabida and Dris, 2022)

Conclusion and perspectives

V. CONCLUSION AND PERSPECTIVES

The present findings underscore that the larvicidal efficacy of essential oils is not exclusively attributable to their major bioactive constituents, but rather to the intricate interplay between these dominant compounds and a suite of minor constituents that may act synergistically. This highlights the necessity of a holistic approach in analyzing essential oil compositions, moving beyond the isolated effects of single molecules toward understanding their combined actions. A nuanced understanding of both the individual potency and the synergistic dynamics among EO components is thus indispensable for designing effective, sustainable mosquito control strategies.

In light of these findings, several promising perspectives emerge for future research and application:

- Enhancing the stability of active compounds.
- Utilize advanced tools to predict larvicidal potential and molecular interactions.
- Conduct transcriptomic and epigenetic analyses to identify potential adaptations or resistance mechanisms in *C. longiareolata*.
- Perform field trials under real environmental conditions to validate laboratory findings and assess practical efficacy.
- Collaborate with biotechnology start-ups to facilitate the transfer of our research findings to the green industry.

ABSTRACT

ABSTRACT

This study evaluates the comparative larvicidal efficacy of certain plant-derived essential oils and their main constituents against *Culiseta longiareolata* (Macquart, 1838), a mosquito species of medical importance. Three essential oils — *Mentha spicata*, *Citrus aurantium*, and *Eucalyptus globulus* — were tested, along with their dominant compounds: limonene and carvone (*M. spicata*), linalool (*C. aurantium*), and eucalyptol (*E. globulus*). Bioassays revealed that β-pinene exhibited the highest larvicidal activity, followed by limonene, which was more effective than the full essential oil of *M. spicata* and carvone. In contrast, the essential oils of *C. aurantium* and *E. globulus* demonstrated greater efficacy than their respective major compounds, suggesting the presence of synergistic interactions. These findings highlight the potential of both isolated terpenes and complex essential oil mixtures as eco-friendly larvicidal agents, and the importance of assessing both pure compounds and whole oils in the development of sustainable mosquito control strategies.

RESUME

Cette étude évalue l'efficacité larvicide comparative de certaines huiles essentielles d'origine végétale et de leurs principaux constituants contre *Culiseta longiareolata* (Macquart, 1838), une espèce de moustique d'importance médicale. Trois huiles essentielles — *Mentha spicata, Citrus aurantium* et *Eucalyptus globulus* — ont été testées, ainsi que leurs composés dominants: le limonène et le carvone (*M. spicata*), le linalool (*C. aurantium*) et l'eucalyptol (*E. globulus*). Les tests biologiques ont révélé que le β-pinène présentait l'activité larvicide la plus élevée, suivi du limonène, plus efficace que l'huile essentielle complète de *M. spicata* et que le carvone. À l'inverse, les huiles essentielles de *C. aurantium* et *E. globulus* ont montré une efficacité supérieure à celle de leurs composés majeurs respectifs, suggérant l'existence d'interactions synergiques. Ces résultats soulignent le potentiel des terpènes isolés ainsi que des mélanges complexes d'huiles essentielles en tant qu'agents larvicides écologiques, et l'importance d'évaluer à la fois les molécules pures et les huiles dans leur ensemble pour des stratégies de lutte anti-moustiques durables.

الملخص

تهدف هذه الدراسة إلى تقييم الفعالية القاتلة لليرقات لبعض الزيوت الأساسية النباتية ومركباتها الرئيسية ضد بعوض *Culiseta longiareolata*(ماكوار، 1838)، وهو نوع ذو أهمية طبية. تم اختبار ثلاث زيوت أساسية :النعناع *السنبلي* (*Mentha spicata*)، *النارنج* (*Citrus aurantium*)، *والكافور* (*Eucalyptus globulus*)، إلى جانب مركباتها الأساسية: الليمونين والكارفون (من *النعناع السنبلي*(، اللينالول) *النارنج*(، والأوكاليبتول) *الكافور* .(أظهرت نتائج الاختبارات الحيوية أن-β بينين كان الأكثر فعالية ضد اليرقات، يليه الليمونين، الذي تفوق على زيت *النعناع السنبلي* والكارفون. وعلى العكس، أظهرت زيوتا *النارنج والكافور* فعالية أكبر من مركباتها الأساسية، مما يشير إلى وجود تفاعلات تعاونية داخل الزيت الكامل. تؤكد هذه النتائج على أهمية كل من المركبات الفردية والزيوت الأساسية الكاملة كعوامل طبيعية فعالة لمكافحة يرقات البعوض، وتدعم اعتماد استراتيجيات صديقة للبيئة في برامج المكافحة.

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