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Larvicidal Potential of Essential Oil of *Microtoena patchoulii* and Terpinolene against *Armigeres subalbatus*

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ABSTRACT: This study evaluated the larvicidal efficacy of *Microtoena patchoulii* essential oil (EOMP) and its principal constituent, terpinolene (Te), against *Armigeres subalbatus* larvae, with the objective of identifying a safer, plant-derived alternative to conventional chemical insecticides. EOMP was obtained through hydrodistillation using a Clevenger-type apparatus. Larvicidal bioassays were performed in accordance with World Health Organization (WHO) guidelines and demonstrated notable larvicidal effectiveness. The median lethal concentration (LC_{50}) values recorded at 24 h post-treatment were 1.573 µL/mL for EOMP and 0.592 µL/mL for terpinolene. The total phenolic content (TPC) of EOMP was measured as 410.47 ± 1.94 µg GAE/mL. The findings clearly indicate that both EOMP and terpinolene possess strong larvicidal activity against *A. subalbatus*, with terpinolene exhibiting comparatively higher potency. These results suggest that EOMP, particularly when applied through indoor air diffuser systems, may function as an effective and environmentally friendly natural insecticidal agent, potentially reducing dependence on synthetic mosquito repellents and their associated health risks.

Keywords: Antibacterial; Essential oil; GC-MS; *Microtoena patchoulii*; Terpinolene; Toxicity.

INTRODUCTION

Mosquito-borne diseases continue to pose a serious threat to public health worldwide, with several mosquito species acting as efficient vectors of pathogens. Among them, *Armigeres subalbatus*, a member of the subfamily Culicinae, is of particular concern due to its large body size, aggressive nature, and strong preference for blood feeding on both humans and animals (Harbach, 2007). This species is well recognized as a vector of filarial nematodes such as *Brugia pahangi* and *Wuchereria bancrofti*, the causative agents of lymphatic filariasis. In addition, *A. subalbatus* has been associated with the transmission of viral diseases including Japanese encephalitis and has also been suggested as a potential vector of Zika virus (Chen *et al*., 2008). Its role in spreading Getah virus among horses and pigs further highlights its epidemiological importance (Li *et al.*, 2017). The ecological adaptability of *A. subalbatus* allows it to thrive in human habitations, animal shelters, and surrounding grasslands, thereby increasing the frequency of human–vector contact and the risk of disease transmission (Dong *et al*., 2009).

The control of mosquito populations, particularly at the adult stage, largely depends on the extensive use of synthetic insecticides and mosquito coils. However, these methods are increasingly associated with serious health and environmental concerns. Continuous exposure to mosquito coil smoke has been reported to cause respiratory irritation and has been linked to an elevated risk of lung cancer (Chen *et al.*, 2000). Mosquito coils are widely used indoors in India and many other Asian countries, and to a lesser extent in Western regions (World Health Organization, 1998). According to the World Health Organization (World Health Organization, 1998), global consumption of mosquito coils reached nearly 29 billion units as early as 1998. Although pyrethrins are the main active ingredients, the burning of coils releases a variety of harmful gaseous pollutants and fine particulate matter (Lukwa & Chandiwana 1998), including polycyclic aromatic hydrocarbons, which can reach the lower respiratory tract. Such exposure is known to aggravate asthma and chronic wheezing, especially in children (Azizi & Henry 1991). Moreover, prolonged exposure to compounds like octachlorodipropyl ether can result in the formation of toxic substances such as hydrogen sulphide and formaldehyde, which may further react to produce bis(chloromethyl) ether, a highly potent lung carcinogen (Tou & Kallos 1974; ATSDR & EPA 1987; Gowers *et al*., 1993). Regular overnight use of mosquito coils in poorly ventilated spaces has also been linked to a higher incidence of nasopharyngeal carcinoma (West *et al*., 1993).

In view of these health and environmental risks, plant-derived essential oils have gained attention as safer and environmentally friendly alternatives for mosquito control. Since ancient times, plants have played an indispensable role in human life, serving as sources of food, medicine, dyes, cosmetics, and essential oils. Despite remarkable progress in synthetic chemistry, natural products continue to be central to modern drug discovery owing to their structural diversity, strong biological activities, and wide availability (Singh & Agrawal 2024). Essential oils, in particular, are biodegradable, generally safe to non-target organisms, and often exhibit potent insecticidal and repellent properties. The essential oil of *Microtoena patchoulii* (EOMP) has been reported to possess strong insecticidal activity against several mosquito species while remaining minimally toxic to mammals.

Microtoena patchoulii [(C.B. Clarke ex J.D. Hooker) C.Y. Wu et Hsuan], commonly known as "Sangbrei," is a perennial aromatic herb belonging to the family Lamiaceae and holds considerable ethnobotanical and pharmacological significance in Northeast India. The plant grows profusely in shaded, moist, humus-rich forest areas of Manipur and is also distributed across northeastern India, Myanmar, southern China, and other parts of Southeast Asia (Li & Hedge 1994; Wang & Hong 2011; Toms *et al*., 2025). Among the Meitei community, fresh leaves of *M. patchoulii* are traditionally used as insect repellents, particularly against silverfish, mites, and cockroaches (Devi *et al*., 2014). Leaf decoctions are commonly employed to treat cough, allergic asthma, abdominal pain, menstrual disorders, and enteritis (Das & Khatoon 2015). Externally, the plant is applied to reduce swelling, heal bruises, and treat bone injuries, while root extracts are valued for their hemostatic properties, especially in the management of bleeding piles (Khomdram *et al*., 2011). The plant also serves as an important ingredient in "Ching-hee," a traditional hair lotion (Khomdram & Singh 2012). These diverse medicinal and domestic uses are largely attributed to the presence of aromatic volatile organic compounds in the plant.

Previous phytochemical investigations of *M. patchoulii* essential oil have identified terpinolene, 1-octen-3-ol, and patchouli alcohol as major constituents. Terpinolene is known for its sedative properties and central nervous system depressant activity (Ito & Ito 2011), whereas patchouli alcohol exhibits pronounced antibacterial and anti-inflammatory effects (Pavlovic, 2011; Nonomura, 2002). Terpinolene is also widely used in the cosmetic and food industries and represents one of the dominant volatile components of EOMP. It is well established that the chemical composition of essential oils can vary considerably with geographical location, climatic conditions, and extraction techniques. Therefore, region-specific chemical profiling is essential for accurately evaluating their biological efficacy and therapeutic potential.

In the present investigation, fresh leaves and young shoots of *M. patchoulii* were collected during early November 2021 from four different locations in Manipur, namely Awangkhunou (Lat.24.9073240˚; Long.93.8599630˚), Nongchup Kameng (Lat.24.852010˚; Long.93.895028˚), Sagolband Heinoubok (Lat.24.7898490˚; Long.93.842330˚, and Bishnupur (Lat.24.6318760˚; Long.93.7602450˚). These regions differ in climatic conditions, with Imphal West areas receiving annual rainfall of 108.5–143.4 cm and temperatures ranging from 8-25°C, whereas Bishnupur experiences approximately 205 mm of rainfall with temperatures between 7–25°C. The plant specimens were authenticated by the Botanical Survey of India (BSI) under the voucher number BSI/ERC/Tech/2018-19/178 and the herbarium (Accession number 001538) was deposited at Manipur University.

Although earlier studies have reported certain biological properties of *M. patchoulii*, detailed investigations on its phenolic content and the larvicidal potential of its essential oil and major constituent, terpinolene, remain limited. Notably, no comprehensive study has yet documented the larvicidal efficacy of *Microtoena patchoulii* against mosquito vectors. Owing to its long-standing traditional use as an insect repellent, this gap in knowledge is particularly significant. Therefore, the present study was designed to analyze the chemical composition of *M. patchoulii* essential oil using GC-MS, determine its total phenolic content, and evaluate the larvicidal activity of EOMP and terpinolene against *Armigeres subalbatus* following WHO guidelines (World Health Organization, 2005), with the aim of exploring their potential as novel and sustainable mosquito control agents.

MATERIALS AND METHODS

A. Isolation and extraction of essential oil

Young shoots, leaves, and flowers of *M. patchoulii* (approximately 3 kg fresh weight) were thoroughly washed with tap water followed by distilled water to remove adhering debris. The material was then air-dried at room temperature and coarsely chopped. Hydro-distillation was performed for 3 h using a 3 L Clevenger-type apparatus. The distilled essential oil of *M. patchoulii* (EOMP) was collected in clean microfuge tubes, dried over anhydrous sodium sulfate to remove residual moisture, and stored at 4 °C until further analysis. The average yield of essential oil was 0.2 ± 0.1% (w/w) per 500 g of fresh plant material. The extracted oil was yellowish in color and possessed a strong characteristic aroma.

B. Chemicals

Chloropyriphos was used as a standard positive control in larvicidal assays. Ethanol (EtOH) served as the solvent for preparing stock and working solutions of the essential oil and terpinolene. Folin-Ciocalteu reagent and sodium carbonate (Na_2CO_3) were used for the estimation of total phenolic content. Gallic acid was employed as the reference standard for constructing the calibration curve.

GC-MS analysis of essential oil

The chemical composition of EOMP was analyzed using a Shimadzu GC-MS QP-2010 Plus system. Identification of individual constituents was achieved by comparing their mass spectra with those available in the Wiley 8, NIST 14, and FFNSC 2 spectral libraries. A total of 53 compounds were identified, with monoterpene and sesquiterpene hydrocarbons and alcohols constituting the major fraction of the essential oil.

C. Determination of Total Phenolic Content

The total phenolic content (TPC) of EOMP was determined using the Folin-Ciocalteu colorimetric method with slight modifications based on the procedure described (Slinkard & Singleton 1977). Briefly, 1 mL of the essential oil extract was mixed with 1 mL of Folin-Ciocalteu reagent. This was followed by the addition of 2 mL of 8% (w/v) sodium carbonate solution and 2 mL of distilled water. The reaction mixture was incubated in the dark at room temperature (25 ± 2 °C) for 30 min. Absorbance was recorded at 700 nm using a UV-Visible spectrophotometer. A calibration curve was prepared using gallic acid (200-1000 µL, 0.1 mg/mL), and results were expressed as milligrams of gallic acid equivalents per gram of essential oil (mg GAE/g). The TPC of EOMP was calculated to be 410.47 ± 1.94 µg GAE/mL.

D. Mosquito collection and laboratory rearing

Healthy larvae of *Armigeres subalbatus* were collected from stagnant water bodies within the Manipur University campus, Imphal, India (24.753526°N, 93.932218°E). Larvae were reared in 24-well cell culture plates under controlled laboratory conditions. The rearing environment was maintained at a temperature of 25 ± 2 °C, relative humidity of 65–75%, and a photoperiod of 12 h light and 12 h dark. Larvae were fed daily with commercially available fish food (Microbits or Tetrabits), and dechlorinated water was used throughout the rearing period. All containers were covered with fine mosquito nets to prevent contamination and escape of emerging adults.

E. Larvicidal bioassay

The larvicidal activity of EOMP and terpinolene was evaluated following the standard World Health Organization protocol (World Health Organization, 2005), with minor modifications. Stock solutions of the essential oil and terpinolene (20 µL/mL) were prepared in 2% ethanol. From these stock solutions, a series of working concentrations was prepared. For EOMP, test concentrations ranged from 31 to 49 µL/mL, whereas terpinolene concentrations ranged from 1 to 10 µL/mL.

Ten healthy late third or early fourth instar larvae were introduced into each well of a 24-well culture plate containing the respective test solutions. Control groups consisted of larvae exposed to 2% ethanol and dechlorinated water, while chloropyriphos (30 µL/mL) served as the positive control. Larval mortality was assessed after 24 h of exposure. Larvae were considered dead if they failed to show any movement when gently probed with a glass rod. All experiments were performed in triplicate at 25 ± 2 °C, and percentage mortality was calculated according to WHO (World Health Organization, 2005) guidelines.

F. Statistical analysis

EC_{50} and LC_{50} values were obtained via nonlinear regression (SPSS, Microsoft Excel). One-way ANOVA with Tukey's test (GraphPad Prism 8.0) identified significant differences ($p < 0.05$). All assays were conducted in triplicate.

RESULTS

The chemical characterization of EOMP by GC-MS analysis revealed a complex mixture dominated by monoterpenes and sesquiterpenes, with terpinolene identified as the major constituent (Table 1).

Table 1: GCMS Analysis of the Chemical composition of the EOMP.

Sr. No.	Compound	RT (min)	Peak area (%)	ID
1	Terpinolene	12.64	10.15	MS
2	α-Guaiene	27.87	8.81	MS
3	Patchouli alcohol (Patchoulol)	37.02	7.52	MS
4	Pogostol	36.77	6.49	MS
5	β-Sesquiphellandrene	28.15	4.95	MS
6	Oct-1-en-3-ol	8.44	4.86	MS
7	β-Caryophyllene	27.03	4.14	MS
8	trans-β-Bergamotene	28.78	4.06	MS
9	Azulene	30.61	3.01	MS
10	Aciphyllene (Guaia-4,11-diene)	30.19	2.76	MS
11	(+)-Sesquithujene	26.88	2.49	MS
12	cis-β-Elemene	25.80	2.36	MS
13	β-Selinene	29.92	2.14	MS
14	δ-Germacrene	29.61	2.06	MS
15	α-Pinene	6.48	2.38	MS
16	Neointermedeol	39.24	1.24	MS
17	Limonene	10.04	1.24	MS
18	α-Farnesene	30.71	1.17	MS
19	α-Thujene	6.23	1.15	MS
20	Palustrol	35.40	1.08	MS

RT = retention time; ID = identification based on comparison of mass spectra with NIST library. *Top 20 major components of EOMP is only presented in the table.

The total phenolic content of EOMP was determined to be 410.47 ± 1.94 µg GAE/mL, reflecting a high level of phenolic compounds.

Microtoena patchoulii (C.B. Clarke ex Hook. f.) C.Y. Wu & S.J. Hsuan

Larvicidal bioassays conducted against *Armigeres subalbatus* larvae demonstrated a clear concentration-dependent increase in mortality following treatment with *Microtoena patchoulii* essential oil (EOMP) and its major constituent, terpinolene (Te). EOMP exhibited larvicidal activity at concentrations ranging from 31 to 45 µL/mL, while terpinolene was effective at much lower doses, between 1 and 10 µL/mL (Fig. 1 A-B).

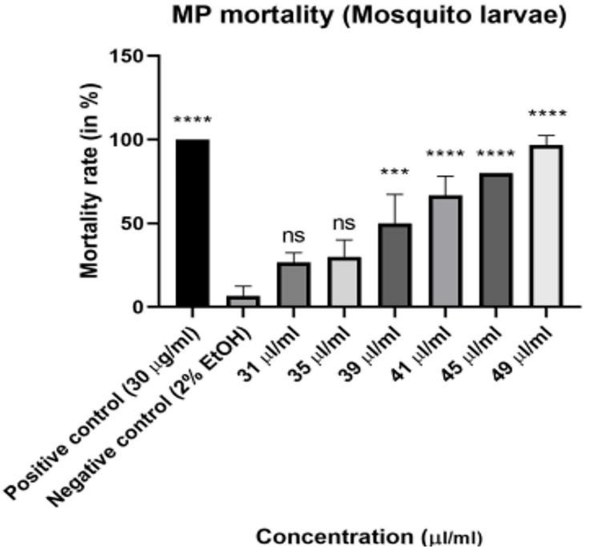

Fig. 1(A). Larvicidal activity of different concentrations of EOMP against *Armigeres subalbatus* larvae. Chloropyriphos (30 %) and ethanol 2% are used as positive and negative control respectively.

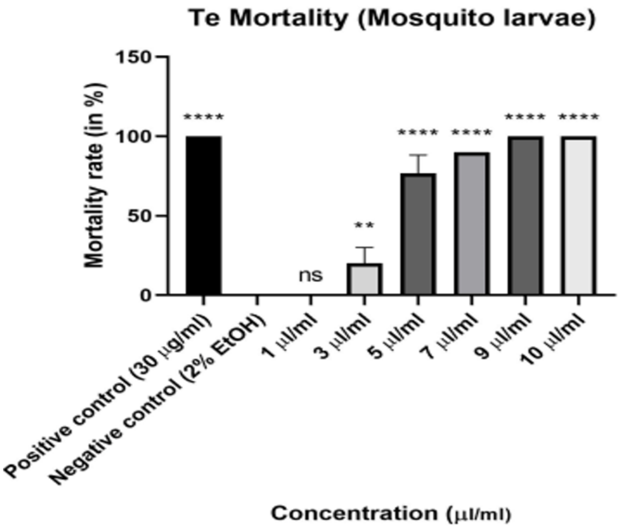

Fig. 1(B). Larvicidal activity of different concentrations of terpinolene (Te) against *Armigeres subalbatus* larvae. Chloropyriphos (30 %) and ethanol 2% are used as positive and negative control respectively.

The highest larval mortality was observed at 45 µL/mL for EOMP and 10 µL/mL for terpinolene, indicating greater potency of terpinolene. No mortality was recorded in the negative control treated with 2% ethanol, confirming the absence of solvent-induced toxicity. In contrast, the positive control, chlorpyriphos, produced 100% larval mortality at 20 µL/mL, validating the experimental protocol. The median lethal concentration (LC_{50}) values calculated at 24 h post-treatment were observed to be 1.573 µL/mL for EOMP and 0.592 µL/mL for terpinolene, further confirming the higher larvicidal efficacy of terpinolene against *A. subalbatus*. In addition to mortality, treated larvae exhibited delayed development, morphological abnormalities, and failure to complete metamorphosis when compared with the untreated control group.

DISCUSSION

Armigeres subalbatus is a commonly found in domestic and peri-domestic environments, where its larvae develop in stagnant water bodies (Magalhães *et al.*, 2010). The present findings demonstrate that EOMP and terpinolene exert strong toxic effects on both larval and adult stages of this species. The observed dose-dependent mortality, along with impaired larval development and disrupted metamorphosis, suggests that these compounds interfere with essential physiological and developmental processes.

The lower LC_{50} value obtained for terpinolene compared to the crude essential oil highlights its role as a key bioactive constituent responsible for the mosquitocidal activity of EOMP. According to previously proposed classification systems for natural larvicides, compounds with LC_{50} values ≤ 50 mg/L are considered highly active (Komalamisra *et al.*, 2005), while LC_{50} values < 100 mg/L indicate strong larvicidal potential (Ravi Kiran *et al.*, 2006). The LC_{50} values reported in this study fall well within these thresholds, supporting the classification of EOMP and terpinolene as potent natural larvicides.

The GC-MS profile of EOMP revealed a predominance of monoterpenes and sesquiterpenes, which are widely reported to possess insecticidal and neurotoxic effects on mosquitoes. The dominance of terpinolene is consistent with earlier studies on related plant species (Ito & Ito 2011), although variations in chemical composition may occur due to environmental factors, plant age, or extraction methods. The high total phenolic content of EOMP further supports its biological activity, as phenolic compounds are known to contribute to oxidative stress and cellular damage in insects.

The adulticidal activity observed in this study is particularly relevant, as *A. subalbatus* adults are frequently encountered in poorly maintained septic tanks and domestic environments, increasing the risk of pathogen transmission, including Japanese encephalitis and Zika virus (Yang *et al*., 2022). The rapid knockdown and mortality induced by EOMP suggest its potential utility beyond larval control.

Due the increasing resistance of mosquito populations to synthetic insecticides and the ecological concerns associated with their widespread use, plant-derived essential oils offer a promising alternative. However, the use of chemical pesticides for the control of mosquitoes can be replaced by the use of EOMP (patchoulii oil), which is a naturally obtained mosquitocidal agents (Balkrishna *et al.*, 2021). The biodegradability, environmental safety, and broad-spectrum activity observed for EOMP and terpinolene underscore their suitability as eco-friendly agents for integrated mosquito management programs.

CONCLUSION

GC-MS analysis confirmed the predominance of monoterpenes and sesquiterpenes, while the high phenolic content supports the strong biological activity of EOMP. The present study demonstrated significant dose-dependent larvicidal activity of *Microtoena patchoulii* essential oil (EOMP) and its major constituent, terpinolene, against *Armigeres subalbatus* larvae, with terpinolene showing higher potency and a lower LC_{50} value. Overall, these findings highlight EOMP and terpinolene as effective, eco-friendly alternatives to chemical insecticides, with potential application in sustainable mosquito control strategies. Further field-based validation is required to confirm their practical efficacy.

FUTURE SCOPE

The promising larvicidal efficacy of *Microtoena patchoulii* essential oil and its major constituent, terpinolene, against *Armigeres subalbatus* highlights their potential as ecofriendly alternatives to synthetic larvicides. Future studies focus on field-based validation of *Microtoena patchoulii* essential oil and terpinolene to confirm their larvicidal efficacy under natural conditions. Development of stable and effective formulations, evaluation of safety on non-target organisms, and investigation of their mode of action will further support their use as eco-friendly agents in sustainable mosquito control programs.

Author Contributions. Birjit Singh Waikhom performed the experiments, Sangeeta assisted in performing the experiments. Reema Khangembam executed data evaluation, O. Kshetrimayum carry out revision, and Maibam Damayanti Devi supervised the study. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest. The authors declare no conflict of interest.

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