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Instar-specific susceptibility of *Culex quinquefasciatus* (Diptera: Culicidae) to aqueous botanical extracts: a comparative evaluation

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Abstract

Mosquito-borne diseases continue to pose significant public health challenges worldwide, necessitating the development of environmentally sustainable alternatives to synthetic insecticides. The present study evaluated the larvicidal efficacy of aqueous leaf extracts of *Mentha arvensis*, *Azadirachta indica*, *Eucalyptus globulus*, *Cymbopogon citratus*, and *Ocimum sanctum* against different larval instars of *Culex quinquefasciatus* under laboratory conditions. Larval bioassays were conducted using three concentrations (0.3, 0.6, and 0.9%) and mortality was recorded after 24 and 48 h of exposure. All botanical extracts exhibited larvicidal activity, with mortality generally increasing with concentration and exposure duration. Among the tested botanicals, *M. arvensis* showed the highest efficacy, causing 96.66% mortality in third instar larvae at 0.9% concentration after 48 h exposure, followed by *A. indica* (93.33%), *E. globulus* (90.00%), *C. citratus* (86.66%), and *O. sanctum* (80.00%). Larval susceptibility varied among developmental stages, with third instar larvae generally exhibiting greater mortality than other instars, whereas fourth instar larvae were comparatively less susceptible. The findings demonstrate the potential of aqueous botanical extracts, particularly *M. arvensis* and *A. indica*, as eco-friendly larvicidal agents against *C. quinquefasciatus*. Further investigations on active constituents, formulation development, and field validation are required to facilitate their incorporation into sustainable mosquito management programmes.

Keywords *Culex quinquefasciatus*, Botanical larvicides, *Mentha arvensis*, Mosquito management, Larval mortality, Plant extracts, Vector control

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Introduction

Mosquitoes (Diptera: Culicidae) are among the most medically important arthropods worldwide, serving as vectors of numerous pathogens that affect human and animal health. Species belonging to the genera *Aedes*, *Anopheles*, and *Culex* are responsible for the transmission of a wide range of diseases, including dengue, chikungunya, Zika, malaria, lymphatic filariasis, Japanese encephalitis, and several arboviral infections. Among these, *Culex quinquefasciatus* Say is recognized as an important vector of lymphatic filariasis and other mosquito-borne diseases, particularly in tropical and subtropical regions. The species is highly adaptable and commonly breeds in organically enriched aquatic habitats such as sewage drains, stagnant water bodies, and urban waste-water collections, thereby increasing its epidemiological significance (Meier et al., 2022).

Mosquito management programmes have traditionally relied on the extensive use of synthetic insecticides, including organophosphates, pyrethroids, and insect growth regulators. Although these compounds have contributed substantially to vector suppression, their indiscriminate and prolonged use has resulted in several challenges, including environmental contamination, adverse effects on non-target organisms, and the rapid development of insecticide resistance in mosquito populations (Barbosa et al., 2014; Sá et al., 2022; Benelli et al., 2017; Meier et al., 2022). These limitations have intensified the search for environmentally sustainable alternatives that can be integrated into modern vector management programmes. In this regard, plant-derived insecticides have received considerable attention because of their biodegradability, reduced environmental persistence, and the presence of multiple bioactive compounds that often act through diverse physiological pathways, thereby reducing the likelihood of resistance development (Benelli et al., 2017; Corzo-Gómez et al., 2024).

Recent studies have demonstrated the potential of botanical extracts and essential oils against a variety of medically important mosquito vectors, including *Aedes aegypti*, *Anopheles stephensi*, and *Culex* species. Plant-derived products rich in terpenoids, alkaloids, flavonoids, phenolics, and other secondary metabolites have been reported to interfere with larval feeding, respiration, development, and nervous system function, resulting in significant larval mortality (Goselle et al., 2017; Corzo-Gómez et al., 2024). The effectiveness of botanical insecticides, however, varies considerably among plant species because of differences in phytochemical composition, extraction methods, and target insect species. Consequently, comparative evaluations involving botanicals with distinct phytochemical profiles are necessary to identify promising candidates for mosquito larval management.

The plant species selected for the present investigation—*Mentha arvensis*, *Azadirachta indica*, *Eucalyptus globulus*, *Cymbopogon citratus*, and *Ocimum sanctum*—were chosen based on their documented insecticidal properties, local availability, and diverse phytochemical composition. *Azadirachta indica* is well known for limonoids such as azadirachtin that function as insect growth regulators, whereas *Mentha arvensis* contains monoterpenoids including menthol and menthone with reported insecticidal activity. Similarly, *Eucalyptus globulus* is rich in eucalyptol (1,8-cineole), *Cymbopogon citratus* contains citral and citronellal, and *Ocimum sanctum* possesses phenolic compounds and essential oil constituents such as eugenol (Manzoor et al., 2013; Benelli et al., 2017; Goselle et al., 2017). These botanicals therefore represent distinct classes of bioactive compounds with potential larvicidal activity against mosquitoes.

Despite increasing interest in botanical larvicides, most studies have focused on individual plant species, solvent-based extracts, or specific larval stages. Comparative evaluations of multiple aqueous botanical extracts across different larval instars remain limited, particularly under conditions relevant to the Indian subcontinent. Furthermore, larval susceptibility may vary considerably among developmental stages because of differences in physiology, cuticle development, feeding behaviour, and detoxification capacity, which can influence the effectiveness of control measures (Ebe et al., 2014; Roba et al., 2015). Therefore, the present study was undertaken to evaluate the comparative larvicidal efficacy of aqueous leaf extracts of selected botanicals against different larval instars of *C. quinquefasciatus* under laboratory conditions, with particular emphasis on concentration-dependent responses, exposure duration, and instar-specific susceptibility patterns.

Materials and methods

Study area and collection of mosquito larvae

The study was conducted in the Department of Entomology, Guru Kashi University, Talwandi Sabo, Punjab, India (29°57' N, 75°07' E; 213 m above mean sea level) during August–October 2024. The region is characterized by a semi-arid climate, with summer temperatures ranging from 35 to 45 °C. Mosquito larvae were collected from various stagnant water habitats within and around the university campus, including sewage drains, artificial ponds, discarded tyres, storm-water catch basins, and water-filled containers. Sampling was performed using standard dipping methods with plastic containers. Collected larvae were pooled and subsequently reared under laboratory conditions to obtain sufficient numbers of uniform developmental stages for bioassays.

Rearing and identification of larvae

Collected larvae were transported to the laboratory in habitat water and reared under semi-field simulated conditions. Rearing units consisted of plastic trays lined with polythene sheets and filled with a 1:1 mixture of fresh water and sewage water to mimic natural breeding environments. Larvae were fed finely powdered fish meal throughout development. Environmental conditions were maintained at 26 ± 2 °C, $70 \pm 5\%$ relative humidity, and a 12:12 h (light) photoperiod. Larvae were monitored daily until pupation. Pupae were transferred to screened emergence cages for adult emergence. Emerged adults were maintained in mosquito cages and provided with a 10% sucrose solution as a carbohydrate source. Oviposition was facilitated by placing small containers containing organically enriched water inside the cages. Egg rafts produced by gravid females were collected and maintained under laboratory conditions to obtain uniform larval cohorts for observations and bioassays. Larval instars (I–IV) were separated and identified based on morphological characteristics such as siphon length, head capsule width, and body size using standard taxonomic keys (Gerberg et al., 1994). Only healthy and actively moving larvae were used for experimentation.

Collection of plant material and preparation of extracts

Fresh, mature leaves of *Mentha arvensis*, *Azadirachta indica*, *Eucalyptus globulus*, *Cymbopogon citratus*, and *Ocimum sanctum* were collected locally from cultivated plants. The selected species were chosen based on their reported insecticidal properties, local availability, and representation of diverse phytochemical groups. Leaves were washed thoroughly with tap water followed by distilled water and shade-dried at room temperature (26 ± 3 °C) for seven days. The dried plant material was ground into fine powder using an electric grinder. Aqueous extracts were prepared by cold maceration, wherein 10 g of leaf powder was soaked in 100 mL of distilled water (10% w/v stock extract) and maintained at room temperature for 72 h with intermittent stirring. The extracts were filtered through Whatman No. 1 filter paper and stored in amber-coloured bottles at 4 °C until use. Working concentrations of 0.3%, 0.6%, and 0.9% (w/v) were prepared by diluting the stock extract with distilled water immediately before bioassay. The concentrations were selected based on preliminary screening trials and published reports demonstrating larvicidal activity of botanical extracts against mosquito larvae.

Rationale for selection of larval stages

Larval stages were selected because larval control represents one of the most effective approaches for mosquito management. Mosquito larvae are confined to aquatic habitats, making them easier to target than mobile adults.

Furthermore, larval stages are generally more susceptible to botanical compounds than pupae, which are non-feeding and have reduced exposure to toxicants. Therefore, evaluation of larval susceptibility provides a practical basis for developing botanical-based mosquito management strategies.

Larval bioassay

Larvicidal activity was evaluated following standard World Health Organization guidelines (WHO, 1997) with minor modifications. Larvae used in the bioassays were obtained from the laboratory-maintained colony, and separate bioassays were conducted using first (L1), second (L2), third (L3), and fourth (L4) instar larvae of *Culex quinquefasciatus*. For each treatment, 25 larvae of a given instar were introduced into Petri dishes containing 100 mL of test solution at concentrations of 0.3%, 0.6%, and 0.9% (w/v). Each treatment was replicated three times. A control containing distilled water was maintained simultaneously. The bioassay was conducted under controlled laboratory conditions (26 ± 2 °C and $70 \pm 5\%$ RH). Larval mortality was recorded at 24 and 48 h post-exposure. Larvae were considered dead if they showed no movement when gently probed with a fine brush.

Statistical analysis

Percentage mortality data were subjected to arcsine transformation prior to analysis to stabilize variance. The effects of botanical extract, concentration, exposure duration, and larval instar on larval mortality were analyzed using analysis of variance (ANOVA) in OPSTAT software (CCS Haryana Agricultural University, Hisar, India). Treatment means were separated using least significant difference (LSD) at the 5% level of significance ($p < 0.05$).

Results

Larvicidal activity of botanical extracts

All five aqueous botanical extracts exhibited larvicidal activity against different larval instars of *Culex quinquefasciatus*, with mortality generally increasing with concentration and exposure duration (Tables 1, 2, 3, 4 and 5). No mortality was observed in the control treatment throughout the experimental period. Among the tested botanicals, *Mentha arvensis* exhibited the highest larvicidal activity. At 0.9% concentration and 48 h exposure, mortality reached 96.66% in third instar larvae, followed by 80.00%, 76.66%, and 73.33% mortality in second, first, and fourth instar larvae, respectively (Table 1). Similarly, *Azadirachta indica* caused a maximum mortality of 93.33% in third instar larvae at 0.9% concentration after 48 h exposure (Table 2). High larvicidal activity was also recorded for *Eucalyptus globulus*, which produced 90.00% mortality in third instar larvae under the

Table 1 Larval mortality (%) of *Culex quinquefasciatus* exposed to *Mentha arvensis* leaf extract

Larval instar	Exposure (h)	0.3%	0.6%	0.9%	Control
First instar	24	33.33 ± 5.77	40.00 ± 10.00	50.00 ± 10.00	0.00
	48	43.33 ± 5.77	56.66 ± 5.77	66.66 ± 5.77	0.00
Second instar	24	40.00 ± 10.00	53.33 ± 5.77	63.33 ± 5.77	0.00
	48	53.33 ± 5.77	70.00 ± 10.00	80.00 ± 10.00	0.00
Third instar	24	50.00 ± 10.00	66.66 ± 5.77	76.66 ± 11.54	0.00
	48	66.66 ± 5.77	76.66 ± 11.54	96.66 ± 5.77	0.00
Fourth instar	24	43.33 ± 5.77	56.66 ± 5.77	66.66 ± 5.77	0.00
	48	56.66 ± 5.77	66.66 ± 5.77	73.33 ± 5.77	0.00

Values are mean ± SD

Table 2 Larval mortality (%) of *Culex quinquefasciatus* exposed to *Azadirachta indica* leaf extract

Larval instar	Exposure (h)	0.3%	0.6%	0.9%	Control
First instar	24	26.66 ± 5.77	33.33 ± 5.77	43.33 ± 5.77	0.00
	48	40.00 ± 10.00	53.33 ± 5.77	63.33 ± 5.77	0.00
Second instar	24	33.33 ± 5.77	50.00 ± 10.00	60.00 ± 10.00	0.00
	48	50.00 ± 10.00	63.33 ± 5.77	76.66 ± 5.77	0.00
Third instar	24	43.33 ± 5.77	60.00 ± 10.00	70.00 ± 10.00	0.00
	48	56.66 ± 5.77	70.00 ± 10.00	93.33 ± 11.54	0.00
Fourth instar	24	40.00 ± 10.00	53.33 ± 5.77	63.33 ± 5.77	0.00
	48	50.00 ± 10.00	66.66 ± 5.77	80.00 ± 10.00	0.00

Values are mean ± SD

Table 3 Larval mortality (%) of *Culex quinquefasciatus* exposed to *Eucalyptus globulus* leaf extract

Larval instar	Exposure (h)	0.3%	0.6%	0.9%	Control
First instar	24	23.33 ± 5.77	26.66 ± 5.77	36.66 ± 5.77	0.00
	48	36.66 ± 5.77	46.66 ± 5.77	56.66 ± 5.77	0.00
Second instar	24	26.66 ± 5.77	40.00 ± 10.00	56.66 ± 5.77	0.00
	48	43.33 ± 5.77	56.66 ± 5.77	73.33 ± 5.77	0.00
Third instar	24	36.66 ± 5.77	53.33 ± 5.77	60.00 ± 10.00	0.00
	48	46.66 ± 5.77	66.66 ± 5.77	90.00 ± 10.00	0.00
Fourth instar	24	33.33 ± 5.77	46.66 ± 5.77	53.33 ± 5.77	0.00
	48	43.33 ± 5.77	63.33 ± 5.77	76.66 ± 5.77	0.00

Values are mean ± SD

Table 4 Larval mortality (%) of *Culex quinquefasciatus* exposed to *Cymbopogon citratus* leaf extract

Larval instar	Exposure (h)	0.3%	0.6%	0.9%	Control
First instar	24	20.00 ± 10.00	26.66 ± 5.77	33.33 ± 5.77	0.00
	48	33.33 ± 5.77	43.33 ± 5.77	53.33 ± 5.77	0.00
Second instar	24	26.66 ± 5.77	36.66 ± 5.77	50.00 ± 10.00	0.00
	48	40.00 ± 10.00	53.33 ± 5.77	66.66 ± 5.77	0.00
Third instar	24	40.00 ± 10.00	53.33 ± 5.77	60.00 ± 10.00	0.00
	48	60.00 ± 10.00	70.00 ± 10.00	86.66 ± 5.77	0.00
Fourth instar	24	30.00 ± 10.00	43.33 ± 5.77	50.00 ± 10.00	0.00
	48	40.00 ± 10.00	60.00 ± 10.00	70.00 ± 10.00	0.00

Values are mean ± SD

same treatment conditions (Table 3). *Cymbopogon citratus* and *Ocimum sanctum* were comparatively less effective than *M. arvensis*, *A. indica*, and *E. globulus*, although substantial larval mortality was still observed at higher concentrations and longer exposure periods. The highest mortality recorded for *C. citratus* was 86.66% in third instar larvae at 0.9% concentration after 48 h exposure,

whereas *O. sanctum* produced a maximum mortality of 80.00% under similar conditions (Tables 4 and 5).

Effect of concentration and exposure duration

A concentration-dependent increase in larval mortality was observed for all botanical extracts and larval instars. Across all treatments, mortality was generally lowest at

Table 5 Larval mortality (%) of *Culex quinquefasciatus* exposed to *Ocimum sanctum* leaf extract

Larval instar	Exposure (h)	0.3%	0.6%	0.9%	Control
First instar	24	16.66 ± 5.77	23.33 ± 5.77	30.00 ± 10.00	0.00
	48	30.00 ± 10.00	40.00 ± 10.00	46.66 ± 5.77	0.00
Second instar	24	23.33 ± 5.77	33.33 ± 5.77	43.33 ± 5.77	0.00
	48	36.66 ± 5.77	50.00 ± 10.00	60.00 ± 10.00	0.00
Third instar	24	36.66 ± 5.77	50.00 ± 10.00	56.66 ± 5.77	0.00
	48	53.33 ± 5.77	70.00 ± 10.00	80.00 ± 10.00	0.00
Fourth instar	24	26.66 ± 5.77	40.00 ± 10.00	46.66 ± 5.77	0.00
	48	36.66 ± 5.77	53.33 ± 5.77	66.66 ± 5.77	0.00

Values are mean ± SD

Table 6 Comparative larvicidal efficacy of botanical extracts against third instar larvae (48 h exposure)

Botanical extract	0.3%	0.6%	0.9%
<i>Mentha arvensis</i>	66.66 ± 5.77 c	76.66 ± 11.54 b	96.66 ± 5.77 a
<i>Azadirachta indica</i>	56.66 ± 5.77 c	70.00 ± 10.00 b	93.33 ± 11.54 a
<i>Eucalyptus globulus</i>	46.66 ± 5.77 c	66.66 ± 5.77 b	90.00 ± 10.00 a
<i>Cymbopogon citratus</i>	60.00 ± 10.00 c	70.00 ± 10.00 b	86.66 ± 5.77 a
<i>Ocimum sanctum</i>	53.33 ± 5.77 c	70.00 ± 10.00 b	80.00 ± 10.00 a

Values are mean ± SD. Means within a row followed by different letters differ significantly ($P \leq 0.05$)

0.3% concentration and increased progressively at 0.6% and 0.9% concentrations (Tables 1, 2, 3, 4 and 5). Exposure duration also influenced larvicidal activity. Mortality recorded after 48 h was consistently greater than that observed after 24 h exposure for all botanical treatments. For example, third instar larvae exposed to *M. arvensis* at 0.9% concentration exhibited mortality of 76.66% after 24 h, which increased to 96.66% after 48 h (Table 1). Similarly, mortality caused by *A. indica* increased from 70.00% to 93.33%, while that caused by *E. globulus* increased from 60.00% to 90.00% between 24 and 48 h exposure periods (Tables 2 and 3). These observations indicate that both concentration and exposure duration play important roles in determining the larvicidal efficacy of aqueous botanical extracts against *C. quinquefasciatus* larvae.

Instar-specific susceptibility

Larval susceptibility varied among developmental stages. In general, third instar larvae exhibited the highest mortality across most botanical treatments, particularly at higher concentrations and longer exposure periods (Tables 1, 2, 3, 4 and 5). At 0.9% concentration and 48 h exposure, third instar mortality ranged from 80.00% in *O. sanctum* to 96.66% in *M. arvensis*.

First and second instar larvae also showed considerable susceptibility, although mortality levels varied among botanical treatments. Notably, *C. citratus* produced the highest mortality in first instar larvae (80.00%) at 0.9% concentration after 48 h exposure (Table 4). Fourth instar larvae generally exhibited comparatively lower mortality than third instar larvae under similar treatment

conditions, indicating relatively reduced susceptibility in the later developmental stage.

Comparative efficacy of botanical extracts

A comparison of maximum mortality values recorded for the five botanical extracts revealed differences in larvicidal effectiveness (Table 6). *Mentha arvensis* was the most effective botanical, producing 96.66% mortality in third instar larvae at 0.9% concentration after 48 h exposure. This was followed by *Azadirachta indica* (93.33%), *Eucalyptus globulus* (90.00%), *Cymbopogon citratus* (86.66%), and *Ocimum sanctum* (80.00%). Overall, the results demonstrated that all tested botanicals possessed larvicidal activity against *C. quinquefasciatus* larvae, with efficacy increasing as concentration and exposure duration increased. Among the evaluated extracts, *M. arvensis* consistently exhibited the highest larvicidal potential under the experimental conditions.

Discussion

The present study demonstrated that aqueous leaf extracts of *Mentha arvensis*, *Azadirachta indica*, *Eucalyptus globulus*, *Cymbopogon citratus*, and *Ocimum sanctum* possess considerable larvicidal activity against different larval instars of *Culex quinquefasciatus*. Mortality increased progressively with increasing concentration and exposure duration, indicating a clear dose-dependent and time-dependent response. Similar concentration-dependent larvicidal effects of botanical extracts against mosquito vectors have been reported previously by Kamaraj et al. (2008), Ghosh et al. (2012), and Hillary et al. (2024), who emphasized that increasing concentrations generally enhance the availability of bioactive phytochemicals responsible for larval toxicity.

Among the tested botanicals, *M. arvensis* exhibited the highest larvicidal activity, causing 96.66% mortality in third instar larvae at 0.9% concentration after 48 h exposure. The superior efficacy of *Mentha* extracts may be associated with the presence of monoterpenoids such as menthol and menthone, which have been reported to interfere with respiratory activity, nervous system function, and membrane integrity in insects. Earlier studies

have similarly documented strong larvicidal activity of *Mentha* species against mosquito larvae, highlighting the insecticidal potential of mint-derived essential oils and phytochemicals (Manzoor et al., 2013; Kumar et al., 2024).

High larvicidal activity was also observed with *A. indica*, which caused 93.33% mortality in third instar larvae at the highest concentration after 48 h exposure. Neem-based products are among the most extensively studied botanical insecticides because of the presence of biologically active limonoids, particularly azadirachtin, which acts as an insect growth regulator, feeding deterrent, and physiological disruptor. Similar larvicidal activity of neem extracts against *Culex* mosquitoes has been reported by Zareen et al. (2016) and other researchers investigating plant-derived alternatives to synthetic insecticides. The effectiveness of neem-based products against mosquito vectors has contributed substantially to their incorporation into eco-friendly mosquito management approaches (Ghosh et al., 2012).

Substantial mortality was also recorded in larvae exposed to *E. globulus* extract. The larvicidal activity of eucalyptus-based products has frequently been attributed to eucalyptol (1,8-cineole) and related terpenoid compounds that possess insecticidal, fumigant, and repellent properties. Previous studies have likewise reported significant larvicidal activity of eucalyptus extracts and essential oils against *Aedes*, *Anopheles*, and *Culex* mosquitoes, supporting the present observations (Govindarajan & Benelli, 2016; Rani et al., 2024).

Although comparatively lower mortality was observed with *C. citratus* and *O. sanctum*, both botanicals exhibited appreciable larvicidal activity, particularly at higher concentrations and longer exposure durations. Interestingly, *C. citratus* exhibited a distinct larvicidal profile compared with the other botanical extracts, showing comparatively greater activity against first instar larvae and causing 80.00% mortality at 0.9% concentration after 48 h exposure. This acute effect on early larval stages may be associated with the presence of citral, citronellal, and related terpenoid compounds, together with the greater vulnerability of younger larvae to phytochemical penetration and toxicity. The effectiveness of lemon-grass-derived products has been linked to the presence of citral, citronellal, and related volatile constituents with known insecticidal activity. The enhanced susceptibility of first instar larvae may also be associated with their relatively thinner cuticle and less developed detoxification system, which could facilitate greater penetration and action of bioactive phytochemicals. Previous investigations have similarly reported significant larvicidal effects of *Cymbopogon*-based extracts against mosquito vectors (Goselle et al., 2017). Likewise, the larvicidal activity of *O. sanctum* may be associated with phenolic compounds

and essential oil constituents such as eugenol, which have been reported to exert toxic effects on mosquito larvae.

Larval susceptibility varied among developmental stages, with third instar larvae generally exhibiting the highest mortality across most botanical treatments. Differences in stage-specific susceptibility have been reported previously in mosquito bioassays involving botanical extracts and essential oils (Ebe et al., 2014; Roba et al., 2015). Such variation may be related to differences in feeding behaviour, metabolic activity, body size, cuticle development, and detoxification capacity among larval instars. In contrast, fourth instar larvae generally exhibited comparatively lower mortality under similar treatment conditions, possibly because of increased physiological robustness and a more developed cuticular barrier that may reduce penetration of toxic compounds.

The growing interest in botanical insecticides reflects increasing concern regarding environmental contamination and resistance development associated with prolonged use of conventional insecticides. Plant-derived larvicides have gained considerable attention because they are biodegradable, renewable, and often contain multiple bioactive compounds with diverse modes of action (Benelli et al., 2017; Sá et al., 2023). Recent reviews have emphasized that botanical insecticides may serve as promising components of integrated vector management programmes because their complex phytochemical composition may reduce the likelihood of rapid resistance development compared with conventional synthetic insecticides. Furthermore, botanical products have demonstrated activity against several medically important mosquito genera, including *Aedes*, *Anopheles*, and *Culex*, highlighting their broader applicability in vector control programmes.

The present findings are consistent with recent global efforts aimed at developing sustainable and environmentally safer mosquito management strategies. Similar efforts have been reported from tropical regions such as Northeast Brazil, where locally available botanical resources have been investigated as sustainable alternatives for the management of *Aedes*, *Culex*, and *Anopheles* mosquitoes, highlighting the broader applicability of plant-based vector control strategies across the Global South. Plant-derived larvicides continue to attract considerable interest because of their local availability, lower environmental persistence, and compatibility with integrated pest and vector management approaches (Hillary et al., 2024). In tropical and subtropical regions where mosquito-borne diseases remain major public health concerns, botanical formulations may offer practical alternatives or complementary tools for mosquito control programmes.

Although the results demonstrated promising larvicidal activity of aqueous botanical extracts, the study

was conducted under laboratory conditions using crude aqueous preparations. Therefore, further investigations involving phytochemical characterization, isolation of active constituents, formulation development, evaluation against field populations, and semi-field or field validation are necessary before large-scale application can be recommended. Such studies would help clarify the mechanisms underlying larval mortality and facilitate the development of effective botanical larvicides for sustainable mosquito management.

Conclusion

The present study demonstrated that aqueous extracts of *Mentha arvensis*, *Azadirachta indica*, *Eucalyptus globulus*, *Cymbopogon citratus*, and *Ocimum sanctum* possess appreciable larvicidal activity against different larval instars of *Culex quinquefasciatus*. Among the tested botanicals, *M. arvensis* exhibited the greatest potential, followed by *A. indica* and *E. globulus*. The observed variation in susceptibility among larval instars highlights the importance of considering developmental stage when evaluating botanical larvicides. Given their effectiveness, local availability, and environmentally compatible nature, these botanical extracts may serve as promising components of sustainable mosquito management programmes. Further research focusing on phytochemical characterization, formulation development, and field validation is warranted to facilitate their practical application in vector control strategies.

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Author contributions

SS conducted the experimental trials, recorded observations and drafted the whole manuscript. KS designed the study, supervision of the work, and made availability of resources. VM carried out statistical analysis, designed some figures and revised the manuscript. SC reanalysed, drafted and revised the manuscript.

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Data availability

The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare no competing interests.

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References

- Barbosa, J. D., Costa, E. N., Rodrigues, K. A. F., Silva, W. M., Américo, M. A., Carvalho, M. G., Souza, A. B., & Moura, A. A. N. (2014). Larvicidal activity of seed extracts from Brazilian plants against *Aedes aegypti* (Diptera: Culicidae). *Parasitology Research*, 113, 3347–3354.
- Benelli, G., Pavela, R., Canale, A., Cianfaglione, K., Ciaschetti, G., Conti, F., Nicoletti, M., Senthil-Nathan, S., Mehlhorn, H., & Maggi, F. (2017). Botanical insecticides in the twenty-first century: fulfilling their promise? *Annual Review of Entomology*, 62, 233–249. <https://doi.org/10.1146/annurev-ento-031616-035044>
- Corzo-Gómez, J. C., Espinosa-Juárez, J. V., Ovando-Zambrano, J. C., Briones-Aranda, A., Cruz-Salomón, A., & Esquinca-Avilés, H. A. (2024). A review of botanical extracts with repellent and insecticidal activity and their suitability for managing mosquito-borne disease risk in Mexico. *Pathogens*, 13(9), 737.
- Ebe, T. E., Akpan, C. E., & Usip, L. P. E. (2014). Larvicidal activities of *Nymphaea lotus*, *Nymphaea pubescens* and *Nelumbo nucifera* against mosquito larvae. *International Journal of Mosquito Research*, 1(2), 15–19.
- Gerberg, E. J., Barnard, D. R., & Ward, R. A. (1994). *Manual for Mosquito Rearing and Experimental Techniques*. American Mosquito Control Association Bulletin No. 5, Lake Charles.
- Ghosh, A., Chowdhury, N., & Chandra, G. (2012). Plant extracts as potential mosquito larvicides. *Indian Journal of Medical Research*, 135, 581–598.
- Goselle, O. N., Onwuliri, C. O. E., Akele, R. Y., & Mbah, A. N. (2017). Larvicidal efficacy of selected plant extracts against mosquito larvae. *Journal of Entomology and Zoology Studies*, 5(3), 123–129.
- Govindarajan, M., & Benelli, G. (2016). Eco-friendly larvicides from Indian plants: effectiveness of *Eucalyptus* and related botanicals against mosquito vectors. *Parasitology Research*, 115, 1967–1978.
- Hillary, V., Pradeep, S., Kumar, R., & Singh, A. (2024). Efficacy of plant products in controlling disease-vector mosquitoes: a review. *Entomologia Experimentalis et Applicata*, 172, 1–18. <https://doi.org/10.1111/eea.13401>
- Kamaraj, C., Rahuman, A. A., Bagavan, A., Elango, G., Zahir, A. A., & Santhoshkumar, T. (2008). Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchus*. *Indian Journal of Medical Research*, 134, 101–106.
- Kumar, P., Sharma, R., & Singh, N. (2024). Botanical phytochemicals with mosquito-cidal potential: recent advances and future prospects. *Journal of Vector Borne Diseases*, 61, 45–58.
- Manzoor, F., Naz, S., & Malik, S. A. (2013). Larvicidal activity of plant extracts against mosquito larvae under laboratory conditions. *Asian Pacific Journal of Tropical Biomedicine*, 3(6), 466–470.
- Meier, C. J., Cano, M. A., Bond, J. G., Martinez-Torres, D., Basáñez, M. G., & Vázquez-Prokopec, G. M. (2022). Chemical control of mosquitoes and the pesticide treadmill: a case for photosensitive insecticides as larvicides. *Insects*, 13(12), 1093.
- Rani, P., Meena, K., & Choudhary, R. (2024). Evaluation of insecticidal efficacy of various botanical extracts against mosquito larvae. *International Journal of Tropical Insect Science*, 44, 231–240.
- Roba, A. A., Gemechu, F., & Beyene, D. (2015). Comparative susceptibility of mosquito larval instars to plant-derived larvicides. *International Journal of Entomological Research*, 3(2), 55–61.
- Sá, R. A., Bezerra, J. W. A., Costa, A. R., Silva, M. F. A., & Coutinho, H. D. M. and collaborators. (2022). Arbovirus vector insects: are botanical insecticides an alternative for their management? *Journal of Pest Science*, 96, 1125–1142.
- Sá, R. A., Bezerra, J. W. A., Costa, A. R., Silva, M. F., & Coutinho, H. D. M. (2023). Arbovirus vector insects: are botanical insecticides an alternative for their management? *Journal of Pest Science*, 96, 1125–1142.
- World Health Organization (WHO). (1997). *Guidelines for Laboratory and Field Testing of Mosquito Larvicides*. WHO Communicable Disease Control, Prevention and Eradication, WHO Pesticide Evaluation Scheme (WHOPES).

Zareen, N., Ahmed, S., & Khan, R. R. (2016). Larvicidal activity of neem-based formulations against *Culex quinquefasciatus* under laboratory conditions. *Journal of Vector Borne Diseases*, 53, 164–170.

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