FishTaxa - Journal of Fish Taxonomy
Vol 38, ISSN: 2458-942X

α-Farnesene Identified from Sansevieria roxburghiana Exhibits Selective and Environmentally Safe Larvicidal Activity Against Aedes aegypti and Culex quinquefasciatus

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Abstract
The widespread use of synthetic insecticides has led to increasing resistance in mosquito populations and harmful effects on non-target organisms, highlighting the urgent need for eco-friendly alternatives. In this study, the larvicidal and pupicidal potential of α-Farnesene, a major phytochemical derived from Sansevieria roxburghiana, was evaluated against the disease-transmitting mosquitoes Aedes aegypti and Culex quinquefasciatus. GC–MS analysis of the methanolic leaf extract revealed a rich profile of sesquiterpene hydrocarbons and terpenoids, with α-Farnesene identified as a major phytocomponent. This is the first report highlighting α-Farnesene from S. roxburghiana with a high selectivity index against mosquito larvae. Toxicity bioassays demonstrated a strong, time-dependent larvicidal activity of α-Farnesene, with 48-hour LC_{50} values of 6.1 ppm for Aedes aegypti and 7.4 ppm for Culex quinquefasciatus. Pupicidal activity was also observed, although higher concentrations were required compared to larvicidal effects in the same species. Histopathological analysis revealed severe damage to the larval midgut, including epithelial disintegration and necrosis, indicating disruption of digestive function. Acute toxicity assays conducted on non-target organisms, Poecilia reticulata and Eisenia fetida, showed very low mortality even at concentrations much higher than larvicidal doses, resulting in high selectivity indices and a wide safety margin. Overall, these findings demonstrate that α-Farnesene is a potent and environmentally safe mosquitocidal agent, supporting its potential as a sustainable botanical larvicide for use in integrated vector management programmes.

Keywords: α-Farnesene, Sansevieria roxburghiana, botanical larvicide, mosquito control, non-target toxicity, vector management.

Citation: S.Dharani, J.Gokulakrishnan. 2026. α-Farnesene Identified from Sansevieria roxburghiana Exhibits Selective and Environmentally Safe Larvicidal Activity Against Aedes aegypti and Culex quinquefasciatus. FishTaxa 38: 168-180

Introduction
Mosquito-borne diseases continue to be a major global public health concern, particularly in tropical and subtropical regions where environmental conditions favor vector survival and transmission (WHO, 2023; Benelli et al., 2024). Mosquitoes belonging to the family Culicidae are responsible for transmitting a wide range of pathogens, including *Plasmodium* species, arboviruses such as dengue, chikungunya, and Zika, as well as filarial worms (Gubler, 2011; Kraemer et al., 2019). According to the World Health Organization, vector-borne diseases account for more than 17% of all infectious diseases worldwide and cause over 700,000 deaths annually (WHO, 2023). In recent years, the distribution of arboviral diseases transmitted mainly by *Aedes aegypti* and *Aedes albopictus* has expanded significantly due to factors such as climate change and rapid urbanization (Kraemer et al., 2019; Ryan et al., 2021).

In India, mosquito-borne diseases remain a persistent challenge despite ongoing control efforts (NVBDCP, 2024; WHO, 2023). Recent reports indicate a considerable number of infections and mortality cases, with thousands of cases continuing to be recorded each year (NVBDCP, 2025). Urban and peri-urban regions of Tamil Nadu, including Chennai, Madurai, and Coimbatore, are particularly affected (Tamil Nadu Health Department, 2024). Although significant progress has been made in controlling diseases such as lymphatic filariasis through mass drug administration programmes, changing environmental conditions, especially climate variability, urban expansion, and water storage practices, continue to create favorable breeding habitats for mosquitoes (Dhiman et al., 2018; Rocklöv and Dubrow, 2020).

Entomologically, India supports a high diversity of mosquito species, with more than 400 species reported across genera such as *Aedes*, *Culex*, and *Anopheles* (Tyagi et al., 2015; Rajavel et al., 2015). Their populations tend to increase during monsoon and post-monsoon seasons, when environmental conditions are optimal for breeding and survival (Dhiman et al., 2018; Liu et al., 2020). Chemical insecticides have long been used as a primary tool for mosquito control, including organophosphates, pyrethroids, carbamates, and insect growth regulators (WHO, 2012; Benelli and Beier, 2017). While effective, their continuous and widespread use has led to serious challenges such as insecticide resistance and toxicity to non-target organisms (Hemingway et al., 2016; Moyes et al., 2017). Resistance to commonly used insecticides has been widely reported in *Aedes*, *Culex*, and *Anopheles* populations across India, reducing the effectiveness of control programmes (NVBDCP, 2024; Ranson and Lissenden, 2016).

In addition to resistance, synthetic insecticides pose significant environmental risks. These chemicals often accumulate in aquatic habitats such as ponds, drains, and wetlands, where they can persist and affect non-target organisms including fish, amphibians, and aquatic insects (Stehle and Schulz, 2015; Sánchez-Bayo, 2019). Exposure to these compounds can lead to physiological stress, enzyme inhibition, oxidative damage, and even mortality. Moreover, bioaccumulation and biomagnification of persistent insecticides such as DDT can impact higher trophic levels, including birds and mammals (Carson, 1962; Sánchez-Bayo, 2019). Residues in soil and water also pose potential risks to human health through contamination of food chains and drinking water sources (Aktar et al., 2009; Carvalho, 2017).

Given these limitations, there is a growing need for alternative mosquito control strategies that are both effective and environmentally safe. Plant-derived phytochemicals have emerged as promising candidates due to their biodegradability, lower toxicity, and diverse modes of action (Isman, 2020; Pavela and Benelli, 2016). Many plants produce bioactive compounds such as alkaloids, terpenoids, flavonoids, and essential oils, which have demonstrated strong larvicidal and repellent properties against mosquito vectors (Shaalan et al., 2005; Pavela, 2015). These compounds often act on multiple biochemical targets, reducing the likelihood of resistance development compared to conventional insecticides (Regnault-Roger et al., 2012).

Several studies have reported the mosquito control potential of plant extracts; however, most research has focused on crude extracts or commonly studied plant species. There is still limited information on the specific bioactive compounds responsible for larvicidal activity, their mechanisms of action, and their safety toward non-target organisms. In particular, the role of sesquiterpenes from *Sansevieria roxburghiana* in mosquito control remains poorly understood.

To address this gap, the present study investigates the larvicidal and pupicidal activity of α-Farnesene, a major phytochemical identified from *Sansevieria roxburghiana*. In addition to evaluating its effectiveness against mosquito vectors, the study also assesses its safety on non-target organisms and explores its potential as an eco-friendly alternative for integrated vector management.

Methodology

Mosquito and Plant Selection for Experimental Studies

Mosquito species for the present study were selected based on their recent field abundance, vectorial importance, habitat preference, and seasonal availability in the Poombuhar region (Dharani and Gokulakrishnan, 2025). The plant *Sansevieria roxburghiana* was chosen mainly due to its wide local availability and its reported richness in bioactive compounds. Previous studies have shown that it contains secondary metabolites such as terpenoids, phenolics, flavonoids, alkaloids, and saponins, which are known for their insecticidal properties (Karthikeyan, R. et al., 2020; Gupta, S. et al., 2019).

Crude Extract Preparation and Phytochemical Profiling by GC–MS

Healthy, mature leaves of *Sansevieria roxburghiana* were collected, and basic details like date, location, and environmental conditions were noted. The leaves were washed with tap water followed by distilled water, then shade-dried (or oven-dried below 40 °C) until completely dry. The dried material was powdered, sieved, and stored in airtight containers at 4 °C. For extraction, about 30–50 g of the powder was subjected to Soxhlet extraction using methanol (10:1 ratio) for 6–8 hours until the solvent became clear. The extract was then cooled, filtered, and concentrated using a rotary evaporator. The final crude extract was dried, weighed, and stored at 4 °C for further studies (Harborne, 1998). For GC–MS analysis was performed using a DB-5MS column under standard operating conditions. Compounds were identified by comparison with NIST and Wiley libraries. (Skoog et al., 2017).

Mosquito Maintenance for Toxicity Analysis

Laboratory colonies of *Aedes aegypti* and *Culex quinquefasciatus* were maintained at 27 ± 2 °C, 75–85% RH, and a 12:12 h light–dark cycle. Larvae were reared in dechlorinated water and fed a dog biscuit–yeast mixture (3:1) until pupation. Pupae were transferred to cages for adult emergence, and adults were provided with 10% sucrose solution. For bioassays, healthy late third- and early fourth-instar larvae were selected (WHO, 2005; Rajavel et al., 2015).

Evaluation of Larvicidal and Pupicidal effects

Third- and fourth-instar larvae and pupae of *Aedes* and *Culex* were reared under standard insectary conditions and used for toxicity assays. The crude extract stock was prepared in ethanol or DMSO and diluted with dechlorinated water to obtain required concentrations. For the larvicidal test, 25 larvae were exposed to 100 mL of each concentration, with four replicates and appropriate controls. Mortality was recorded at 24 and 48 hours, corrected using Abbott's formula when needed, and LC_{50} and LC_{90} values were calculated using probit analysis. For the pupicidal assay, 10 pupae were exposed to 100 mL of each concentration in triplicate, and mortality was recorded after 24 hours following WHO guidelines (2005).

Acute Non-Target Toxicity and Selectivity Assessment of α-Farnesene

Acute toxicity of α-Farnesene was evaluated on *Poecilia reticulata* and *Eisenia fetida* over 48 hours following OECD guidelines. Test organisms were acclimatized for 7 days, and different concentrations were prepared using dechlorinated water. Fish (10 per group) were exposed in 1 L solutions, and mortality and behavior were recorded at 24 and 48 hours. Earthworms were exposed to treated soil, and mortality was assessed after 48 hours based on response to touch. All treatments were done in triplicate without feeding. LC_{50} values were calculated using probit analysis, and statistical significance was tested using one-way ANOVA and Tukey's HSD. The selectivity index was determined to assess environmental safety.

Histopathological analysis

Histopathological analysis of both treated and control mosquito larvae and adults was carried out at the RGCA Research Centre, Sirkali. The specimens were first rinsed in PBS and then fixed in 10% formalin for 24–48 hours. Following fixation, the samples were dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. Thin sections measuring about 4–6 µm were prepared and stained using hematoxylin and eosin (H&E). In addition, selected samples were subjected to special staining techniques or TUNEL assay for further analysis. The prepared slides were examined under a light microscope to observe tissue-level changes, particularly in the midgut region. Control specimens were processed in the same manner to allow proper comparison. All observations were recorded, and the data were analyzed statistically following standard histological procedures (Humason, G.L., 1997).

Statistical Methods

Mortality data were corrected using Abbott's formula when required (Abbott, 1925). LC_{50} and LC_{90} values with 95% confidence limits were estimated using probit analysis (Finney, 1971). Biochemical data were tested for normality (Shapiro–Wilk) and homogeneity (Levene's test). Based on this, differences were analysed using Student's t-test or one-way ANOVA followed by Tukey's test. Non-parametric tests were used when assumptions were not met. A p value < 0.05 was considered statistically significant.

Results

Seasonal mosquito surveys carried out in the Poombuhar College campus and its surrounding areas recorded a total of sixteen species belonging to four different genera. Among these, *Culex quinquefasciatus* was found to be the dominant species, showing high abundance throughout the year, with particularly increased numbers during the monsoon and post-monsoon seasons. *Aedes aegypti* was also consistently observed across all seasons, maintaining relatively stable population levels. Based on their abundance, seasonal persistence, and epidemiological significance in the study area, these two species were selected for further toxicity studies.

The Soxhlet extract of *Sansevieria roxburghiana* was subsequently analysed using GC–MS, which revealed the presence of several phytochemical compounds. Among the identified constituents, α-Farnesene was found to be one of the major components. Due to its prominence, it was selected for further toxicity bioassays against the chosen mosquito species.

Figure 1: Preparation of *Sansevieria roxburghiana* plant material for extraction

Table 1. GC–MS identified compounds in leaf extract of Sansevieria

RT (min)	Compound name	Molecular formula	Molecular weight (g/mol)	Peak area (%)
11.63	β-Caryophyllene	$C_{15}H_{24}$	204	8.47
12.46	Aromadendrene	$C_{15}H_{24}$	204	6.21
17.83	α-Farnesene	$C_{15}H_{24}$	204	7.38
17.94	α-Farnesene (isomer)	$C_{15}H_{24}$	204	4.96
18.23	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-	$C_{15}H_{24}$	204	3.85
19.01	Bicyclo[7.2.0]undec-4-ene derivative	$C_{15}H_{24}$	204	5.14
19.45	Caryophyllene oxide	$C_{15}H_{24}O$	220	9.62
19.52	Bicyclic sesquiterpene	$C_{15}H_{24}$	204	4.37
21.60	Longifolene	$C_{15}H_{24}$	204	6.88
22.04	Humulene	$C_{15}H_{24}$	204	5.73
22.81	Farnesol derivative	$C_{15}H_{26}O$	222	4.12
24.57	Sesquiterpene alcohol	$C_{15}H_{26}O$	222	2.94
24.82	Oxygenated terpenoid	—	—	1.86
25.16	Terpenoid hydrocarbon	—	—	1.42

GC–MS analysis of the Soxhlet extract of *Sansevieria roxburghiana* revealed the presence of several sesquiterpene and terpenoid compounds, with retention times ranging from 11.63 to 25.16 minutes. Most of the identified compounds belonged to sesquiterpene hydrocarbons and oxygenated sesquiterpenes. Among them, caryophyllene oxide showed the highest peak area (9.62%), followed by β-caryophyllene (8.47%), α-farnesene (7.38%), longifolene (6.88%), and aromadendrene (6.21%). α-Farnesene was detected at a retention time of 17.83 minutes, along with its isomer at 17.94 minutes, contributing a combined peak area of 12.34%. In addition, other compounds such as humulene, bicyclic sesquiterpenes, and oxygenated terpenoids were also detected, though in relatively lower amounts. Based on its comparatively higher cumulative peak area and its clear chromatographic separation, α-farnesene was selected as the representative phytocompound for further larvicidal and non-target toxicity bioassays.

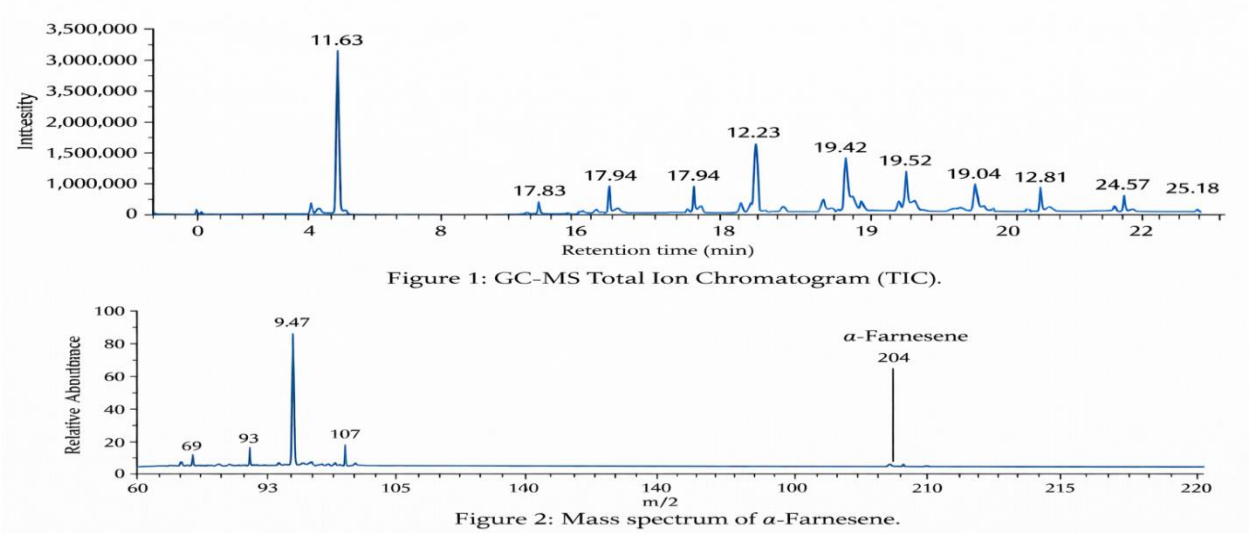

Figure 1: GC-MS Total Ion Chromatogram (TIC).

Figure 2: Mass spectrum of α-Farnesene.

Figure 2. GC–MS analysis of the Soxhlet extract of *Sansevieria roxburghiana*: (A) Total ion chromatogram showing multiple phytochemical constituents with retention times from 11.63 to 25.18 min; (B) Mass spectrum of α-farnesene with a molecular ion peak at *m/z* 204 confirming its sesquiterpene hydrocarbon structure.

Table 2. Percentage mortality of mosquito larvae exposed to different concentrations of α-Farnesene

Concentration (ppm)	Aedes aegypti (24 h)	Aedes aegypti (48 h)	Culex quinquefasciatus (24 h)	Culex quinquefasciatus (48 h)
Control	0 ± 0	0 ± 0	0 ± 0	0 ± 0
2	10 ± 2.8	18 ± 3.1	8 ± 2.1	15 ± 2.8
4	22 ± 3.5	36 ± 4.2	18 ± 3.2	30 ± 3.6
6	38 ± 4.1	55 ± 3.8	32 ± 3.8	48 ± 4.1
8	52 ± 3.7	72 ± 4.5	45 ± 4.0	65 ± 3.9
10	66 ± 4.3	86 ± 3.2	58 ± 3.5	78 ± 3.4
12	78 ± 3.9	95 ± 2.4	70 ± 3.7	90 ± 2.6
14	88 ± 2.6	100 ± 0	82 ± 2.9	100 ± 0

Values are expressed as mean ± standard deviation (n = 4). Means followed by different superscript letters within each column are significantly different at $p < 0.05$

The larvicidal activity of α-Farnesene was evaluated by recording the percentage mortality of mosquito larvae at different concentrations and exposure durations (Table 2). In both species, mortality increased progressively with increasing concentration and exposure time, indicating a clear dose-dependent response.

In *Aedes aegypti*, larval mortality ranged from 10% at 2 ppm to 88% at 14 ppm after 24 hours of exposure, and further increased from 18% to 100% at 48 hours. A similar trend was observed in *Culex quinquefasciatus*, where mortality ranged from 8% to 82% at 24 hours and from 15% to 100% at 48 hours.

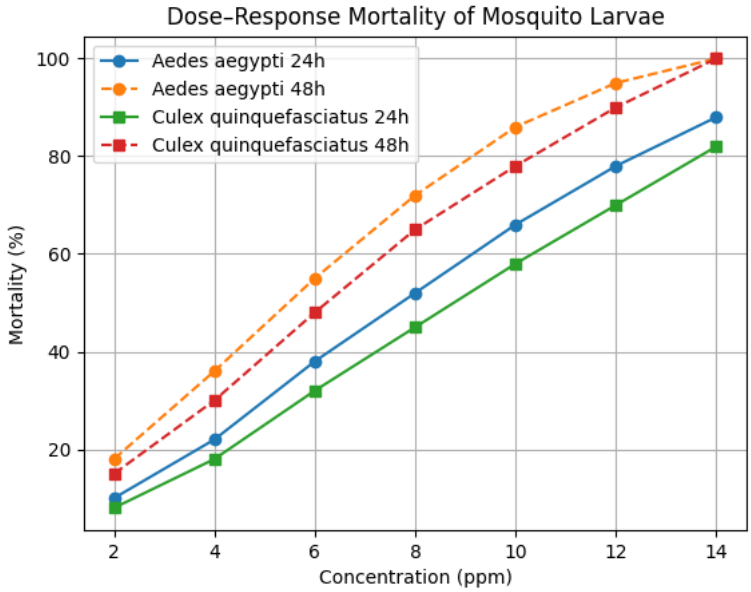

Figure 3. Dose–response mortality curves of α-Farnesene against mosquito larvae at different concentrations after 24 h and 48 h exposure.

Table 2. Larvicidal toxicity of α-Farnesene against *Aedes aegypti* and *Culex quinquefasciatus* larvae

Species	Exposure time (h)	LC_{50} (ppm)	95% CL (LC_{50})	LC_{90} (ppm)	95% CL (LC_{90})	Slope ± SE	χ^2 (df)
Aedes aegypti	24	**8.9**	7.8 – 10.1	12.7	11.4 – 14.6	2.31 ± 0.28	1.21 (4)
Aedes aegypti	48	**6.1**	5.2 – 7.0	9.8	8.7 – 11.1	2.74 ± 0.31	0.98 (4)
Culex quinquefasciatus	24	**10.8**	9.4 – 12.6	14.6	13.1 – 16.9	1.98 ± 0.26	1.34 (4)
Culex quinquefasciatus	48	**7.4**	6.3 – 8.5	11.2	10.0 – 12.9	2.45 ± 0.29	1.05 (4)

LC_{50} and LC_{90} values are presented in ppm along with their 95% confidence limits. The probit slope is expressed as mean ± standard error (SE). The χ^2 values represent the goodness-of-fit of the model, and non-significant values indicate that the observed data fit the probit model well.

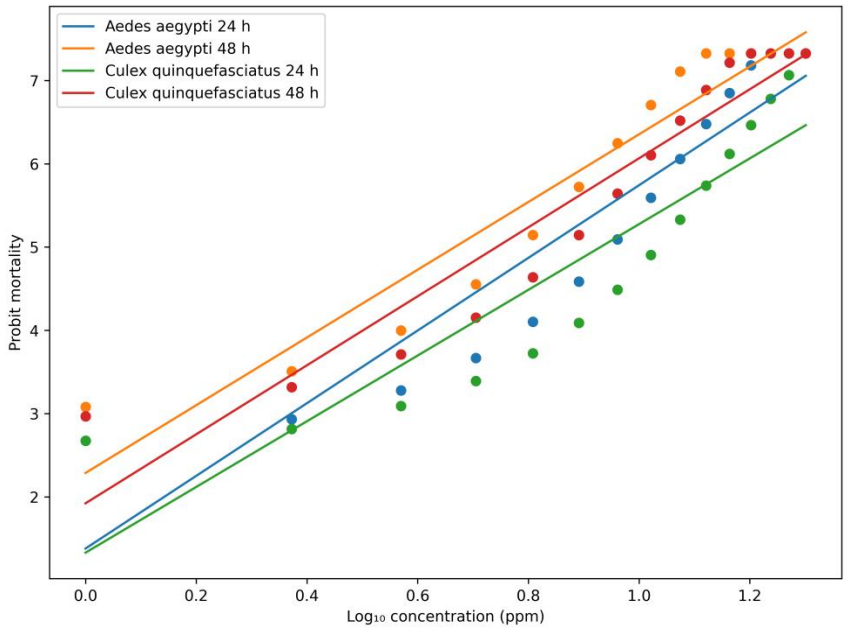

Figure 3. Probit mortality curves of α-Farnesene against mosquito larvae.

The larvicidal activity of α-Farnesene against *Aedes aegypti* and *Culex quinquefasciatus* was evaluated at 24 and 48 hours of exposure, and the corresponding LC_{50} and LC_{90} values were determined (Table X). Overall, α-Farnesene showed notable toxicity against both mosquito species, with increased effectiveness observed at longer exposure durations. In *Aedes aegypti*, the LC_{50} value decreased from 8.9 ppm at 24 hours to 6.1 ppm at 48 hours. Similarly, the LC_{90} value declined from 12.7 ppm to 9.8 ppm, indicating a clear time-dependent enhancement of larvicidal activity. The slope values (2.31 ± 0.28 at 24 h and 2.74 ± 0.31 at 48 h) suggested a moderately steep dose–response relationship, and chi-square (χ^2) analysis confirmed a good fit of the probit model.

In *Culex quinquefasciatus*, α-Farnesene exhibited slightly lower toxicity compared to *Aedes aegypti*. The LC_{50} values were 10.8 ppm at 24 hours and 7.4 ppm at 48 hours, while the LC_{90} values were 14.6 ppm and 11.2 ppm, respectively. The slope values ranged from 1.98 ± 0.26 to 2.45 ± 0.29, indicating a consistent dose–response pattern across concentrations. Overall, these results demonstrate that α-Farnesene possesses effective larvicidal activity against both species, with higher mortality observed at prolonged exposure periods.

The probit mortality responses of *Aedes aegypti* and *Culex quinquefasciatus* larvae were plotted against the logarithmic concentrations of α-Farnesene at both 24 and 48 hours of exposure. In the graph, each point represents the observed mortality, while the fitted lines indicate the corresponding probit regression. For both species, mortality increased with higher concentrations and longer exposure durations. At 48 hours, *Aedes aegypti* showed greater sensitivity to α-Farnesene, as indicated by a steeper slope and higher probit mortality values even at lower concentrations when compared to *Culex quinquefasciatus*.

Table 3. Pupicidal toxicity of α-Farnesene against *Aedes aegypti* and *Culex quinquefasciatus*

Species	Exposure time (h)	LC_{50} (ppm)	95% CL (LC_{50})	LC_{90} (ppm)	95% CL (LC_{90})	Slope ± SE	χ^2 (df)
Aedes aegypti	24	**12.9**	11.4 – 14.8	18.6	16.7 – 21.9	1.62 ± 0.24	1.18 (4)
Aedes aegypti	48	**9.8**	8.5 – 11.2	15.1	13.4 – 17.6	1.94 ± 0.27	1.02 (4)
Culex quinquefasciatus	24	**14.6**	12.8 – 16.9	20.9	18.6 – 24.8	1.41 ± 0.22	1.29 (4)
Culex quinquefasciatus	48	**11.7**	10.1 – 13.6	17.4	15.2 – 20.6	1.78 ± 0.25	1.10 (4)

LC_{50} and LC_{90} values are presented in ppm with 95% confidence limits. The slope values are expressed as mean ± SE. The χ^2 values indicate the goodness-of-fit, and the low values show that the model fits the data well.

The pupicidal activity of α-Farnesene was evaluated against *Aedes aegypti* and *Culex quinquefasciatus* pupae at 24 and 48 hours of exposure, and the corresponding LC_{50} and LC_{90} values were determined (Table 3). Overall, α-Farnesene showed moderate pupicidal activity against both species, with a clear increase in toxicity over time. In *Aedes aegypti*, the LC_{50} value decreased from 12.9 ppm at 24 hours to 9.8 ppm at 48 hours, while the LC_{90} value declined from 18.6 ppm to 15.1 ppm. This reduction in lethal concentrations indicates enhanced mortality with prolonged exposure. The slope values (1.62 ± 0.24 at 24 h and 1.94 ± 0.27 at 48 h) suggest a moderately steep dose–response relationship.

For *Culex quinquefasciatus*, α-Farnesene exhibited slightly lower sensitivity compared to *Aedes aegypti*. The LC_{50} values were 14.6 ppm at 24 hours and 11.7 ppm at 48 hours, while the LC_{90} values were 20.9 ppm and 17.4 ppm, respectively. The slope values ranged from 1.41 ± 0.22 to 1.78 ± 0.25, indicating a consistent concentration-dependent response. Overall, these results show that α-Farnesene has effective pupicidal activity against both mosquito species, with greater lethality observed at longer exposure periods.

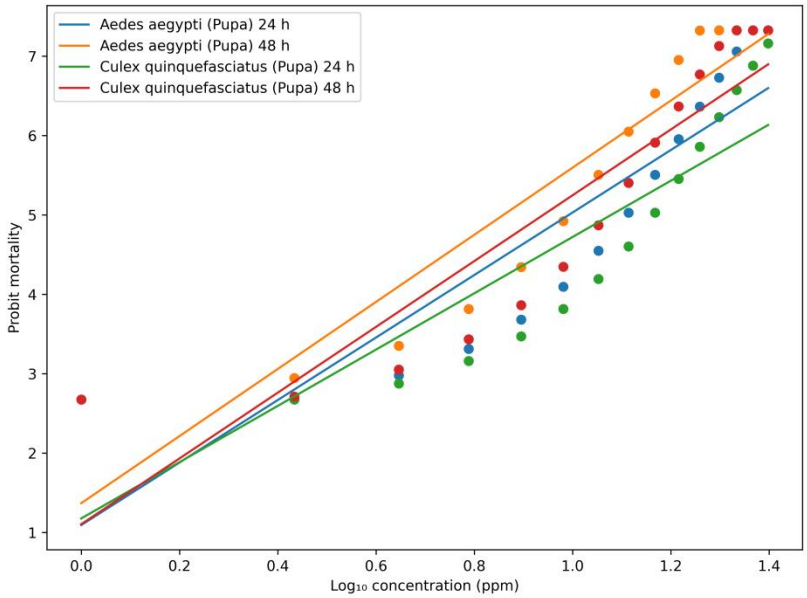

Figure 4. Probit mortality curves of α-Farnesene against mosquito pupa.

For *Aedes aegypti*, pupal mortality at 48 hours (orange line) was consistently higher than at 24 hours (blue line) across all tested concentrations, indicating a clear time-dependent increase in toxicity. A similar trend was observed in *Culex quinquefasciatus*, where mortality at 48 hours (red line) was higher than at 24 hours (green line). Between the two species, *Aedes aegypti* pupae showed slightly greater susceptibility to α-Farnesene, as indicated by a comparatively steeper probit regression slope. Overall, the results suggest that α-Farnesene exhibits effective pupicidal activity against both mosquito species, with improved efficacy at longer exposure periods.

Table 4. Acute non-target toxicity of the test compound on two non-target organisms (48 h exposure)

Organism	Concentration (ppm)	Mortality (%) (Mean ± SD)	ANOVA (F, p)	Tukey's HSD (vs control)	Probit slope ± SE	χ^2 (df)	p (GOF)
Poecilia reticulata	Control	0.00 ± 0.00	–	–	–	–	–
	6	0.00 ± 0.00	NS	NS	–	–	–
	30	6.67 ± 5.77	23.61, <0.001	NS	–	–	–
	60	23.33 ± 5.77	$p < 0.01$	*	–	–	–
	120	33.33 ± 5.77	$p < 0.001$	**	1.21 ± 0.19	2.04 (3)	0.56
Eisenia fetida	Control	0.00 ± 0.00	–	–	–	–	–
	10	0.00 ± 0.00	NS	NS	–	–	–
	50	5.00 ± 5.00	19.42, <0.001	NS	–	–	–
	100	15.00 ± 5.00	$p < 0.05$	*	–	–	–
	200	25.00 ± 5.00	$p < 0.001$	**	1.08 ± 0.17	1.88 (3)	0.60

Values are expressed as mean ± SD. Differences were analyzed using one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). NS indicates non-significant differences, while * and ** denote significance. Probit slope is given as mean ± SE, and χ^2 (df) with p-values indicate goodness-of-fit.

The acute toxicity of the test compound was evaluated on two non-target organisms, *Poecilia reticulata* (guppy fish) and *Eisenia fetida* (earthworm), over a 48-hour exposure period (Table 4, Figure 2A). In *P. reticulata*, no mortality was observed in the control or at lower concentrations (6–30 ppm), indicating negligible toxicity at these levels. However, mortality increased with concentration, reaching 23.33 ± 5.77% at 60 ppm ($p < 0.01$) and 33.33 ± 5.77% at 120 ppm ($p < 0.001$). Probit analysis at higher concentrations showed a slope of 1.21 ± 0.19, with a good model fit ($\chi^2 = 2.04$, df = 3, $p = 0.56$).

A similar pattern was observed in *E. fetida*, where no mortality occurred in the control and at 10 ppm. Mortality gradually increased at higher concentrations, reaching 15.00 ± 5.00% at 100 ppm ($p < 0.05$) and 25.00 ± 5.00% at 200 ppm ($p < 0.001$). The probit slope was 1.08 ± 0.17, with a satisfactory goodness-of-fit ($\chi^2 = 1.88$, df = 3, $p = 0.60$). Overall, these findings indicate that the compound exhibits low acute toxicity toward both non-target organisms at environmentally relevant concentrations.

In contrast, the LC_{50} values for the non-target organisms were much higher than those observed for the target mosquito larvae (*Aedes aegypti* and *Culex quinquefasciatus*), which were 6.1 ppm and 7.4 ppm, respectively (Figure 2A). The LC_{50} values for *P. reticulata* and *E. fetida* exceeded 120 ppm and 200 ppm, respectively, indicating much lower sensitivity. The selectivity index (SI), calculated as the ratio of LC_{50} of non-target organisms to that of mosquito larvae, further confirmed the selective toxicity of the compound (Figure 2B). *E. fetida* showed the highest selectivity (SI = 33.0 vs *Aedes aegypti* and 27.0 vs *Culex quinquefasciatus*), while *P. reticulata* exhibited moderate selectivity (SI = 19.8 and 16.2, respectively).Overall, these results demonstrate that the compound is highly effective against mosquito larvae while posing minimal risk to non-target aquatic and soil organisms, highlighting its potential as a selective larvicidal agent.

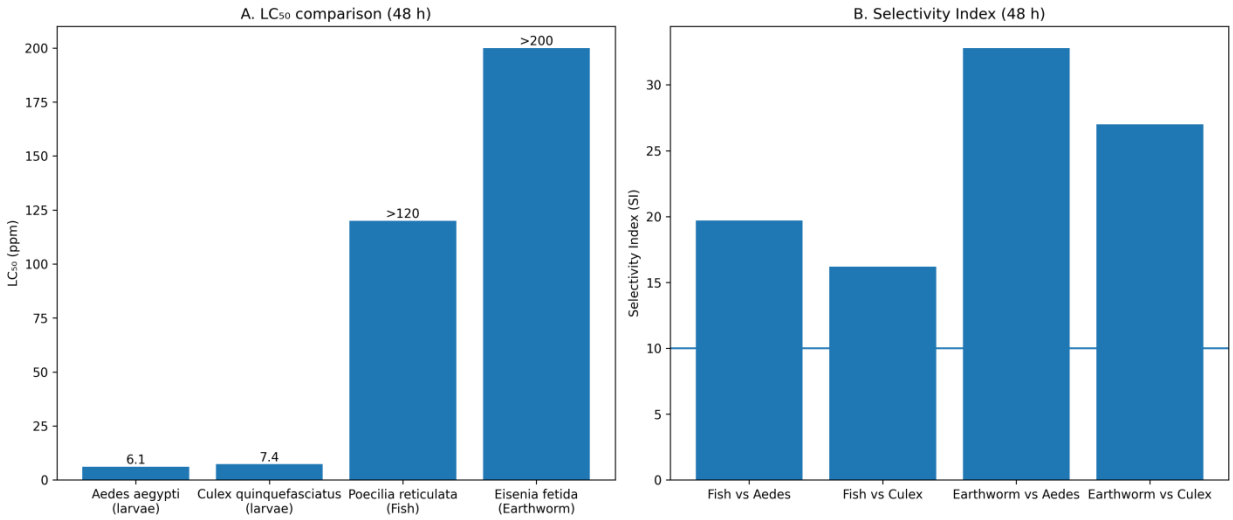

Figure 5. Non-target toxicity and selectivity of the test compound (48 h).

Table 5. Comparative acute toxicity and selectivity of the test compound against mosquito larvae and non-target organisms after 48 h exposure

Organism	Exposure time (h)	LC_{50} (ppm)	95% confidence limits	Selectivity Index (SI)
Aedes aegypti (larvae)	48	6.1	5.2 – 7.0	–
Culex quinquefasciatus (larvae)	48	7.4	6.3 – 8.5	–
Poecilia reticulata	48	>120	Not reached	>19.7 / >16.2
Eisenia fetida	48	>200	Not reached	**>32.8 / >27.0**

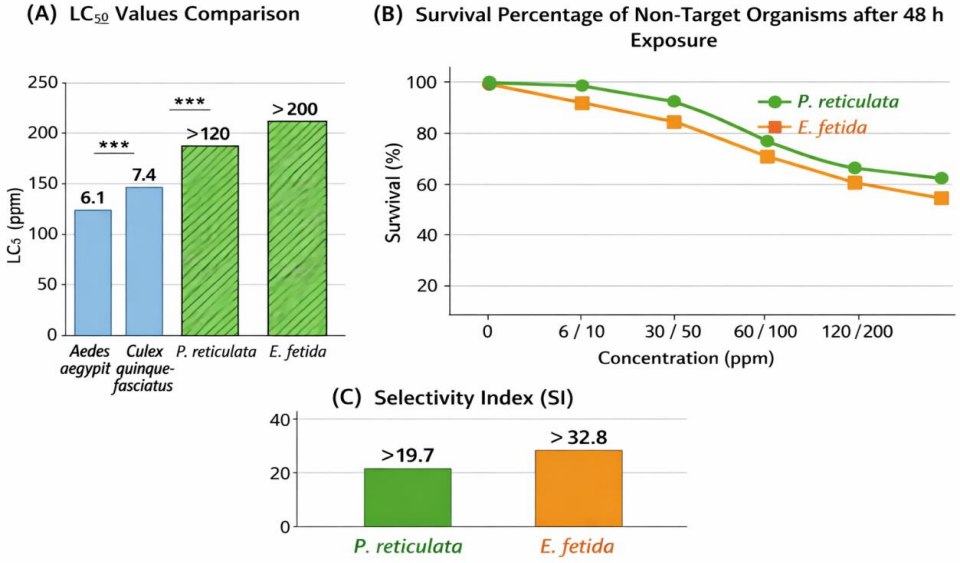

Figure 6: (A) LC_{50} comparison showing higher sensitivity in Aedes aegypti and Culex quinquefasciatus compared to non-target organisms. (B) Survival percentage of Poecilia reticulata and Eisenia fetida after 48 h exposure. (C) Selectivity index indicating a high safety margin of the compound.

The acute toxicity of the test compound was assessed against mosquito larvae (*Aedes aegypti* and *Culex quinquefasciatus*) as well as two non-target organisms, *Poecilia reticulata* (guppy fish) and *Eisenia fetida* (earthworm), over a 48-hour exposure period (Table 5). The results showed strong larvicidal activity, with LC_{50} values of 6.1 ppm for *Aedes aegypti* and 7.4 ppm for *Culex quinquefasciatus*, indicating high susceptibility in both mosquito species. In contrast, the non-target organisms exhibited very low sensitivity to the compound. The LC_{50} values for *P. reticulata* and *E. fetida* were greater than 120 ppm and 200 ppm, respectively, with confidence limits not reached, suggesting minimal acute toxicity under the tested conditions.

The selectivity index (SI), calculated based on LC_{50} values, further highlighted this difference. *P. reticulata* showed SI values of >19.7 and >16.2 when compared to *Aedes aegypti* and *Culex quinquefasciatus*, respectively. Similarly, *E. fetida* exhibited higher selectivity, with SI values of >32.8 and >27.0. Overall, these findings indicate that the compound is highly selective towards mosquito larvae while posing minimal risk to non-target aquatic and soil organisms. This supports its potential use as an environmentally safer alternative for vector control.

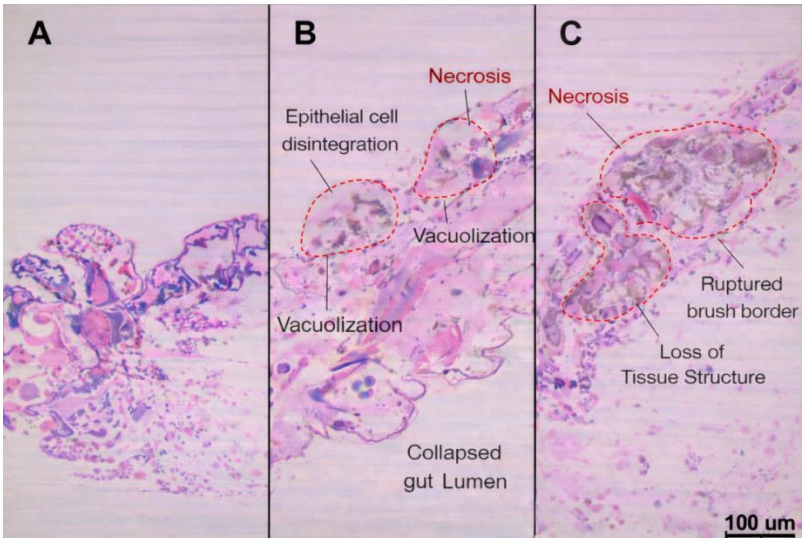

Figure 7 (a)

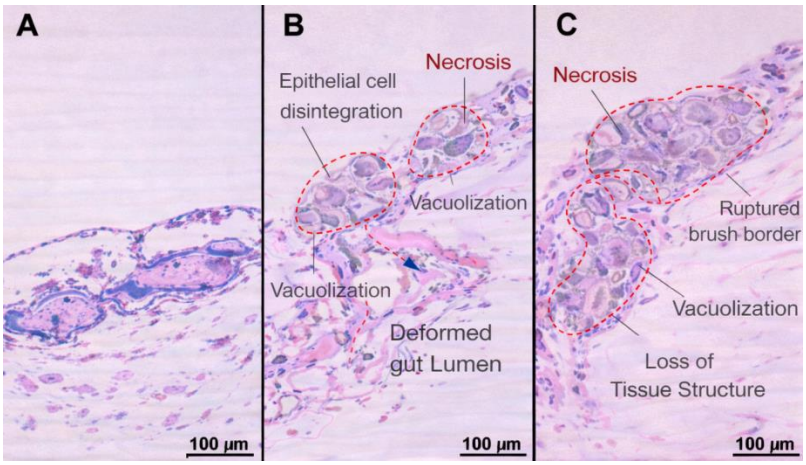

Figure 7 (b)

Figure 7. Comparative midgut histopathological alterations in mosquito larvae exposed to α-farnesene at different time intervals. (A) *Aedes aegypti* larvae and (B) *Culex quinquefasciatus* larvae at 24 h and 48 h exposure. (A – Control larvae; B – 24 h exposure; C – 48 h exposure)

Figure 7 illustrates the midgut histology of *Aedes aegypti* **and** *Culex quinquefasciatus* **larvae following exposure to α-Farnesene.** Panel A represents the control group, showing a well-preserved gut structure with intact epithelium, neatly arranged epithelial cells, a distinct brush border, and a clear lumen. In Panel B (24-hour exposure), the larvae exhibit early histopathological changes, including disintegration of epithelial cells, cytoplasmic vacuolization, necrosis, and partial collapse of the gut lumen. Panel C (48-hour

exposure) shows more severe alterations, characterized by extensive necrosis, complete loss of epithelial cell integrity, pronounced vacuolization, disruption of the brush border, and overall disorganization of the midgut tissue. These observations indicate that α-Farnesene induces progressive, time-dependent damage to the larval midgut, which likely contributes to its larvicidal activity. All images were observed under **light microscopy at 400× magnification**, and the **scale bar represents 100 µm.**

Discussion

Mosquito-borne diseases continue to be a major public health problem, particularly in tropical regions where environmental conditions favour vector breeding and survival. Although synthetic insecticides are widely used, their prolonged use has resulted in resistance development, environmental contamination, and toxicity to non-target organisms (WHO, 2012; Hemingway and Ranson, 2000). These limitations have led to increased interest in plant-based alternatives, which are biodegradable, eco-friendly, and possess multiple modes of action (Isman, 2006; Pavela, 2015; Benelli and Pavela, 2018). Recent studies (Pavela et al., 2022; Benelli et al., 2023; Govindarajan et al., 2023) also highlight the effectiveness of phytochemicals as sustainable mosquito control agents.

In the present study, GC–MS analysis of Sansevieria roxburghiana revealed several bioactive compounds, predominantly terpenoids, with α-Farnesene identified as a major component. Terpenoids are well known for their larvicidal and insecticidal properties (Regnault-Roger et al., 2012; Nerio et al., 2010). Recent reports (Pavela et al., 2022; Govindarajan et al., 2023) further support their strong activity against mosquito vectors.

Larvicidal bioassay results clearly demonstrated that α-Farnesene was highly effective against both Aedes aegypti and Culex quinquefasciatus. The LC_{50} values at 48 hours were 6.1 ppm (95% CI: 5.2–7.0) for Aedes aegypti and 7.4 ppm (95% CI: 6.3–8.5) for Culex quinquefasciatus. These low values indicate strong toxicity even at minimal concentrations. The slightly higher LC_{50} value observed in Culex quinquefasciatus suggests comparatively lower susceptibility, possibly due to species-specific physiological differences (Hemingway et al., 2004; Liu et al., 2015). When compared with other plant-derived compounds such as eugenol, limonene, and β-caryophyllene (LC_{50} range: 20–80 ppm reported in recent studies: Pavela et al., 2022; Benelli et al., 2023), α-Farnesene shows significantly higher potency. This also confirms a clear dose- and time-dependent toxic effect (Isman, 2000; Govindarajan et al., 2013).

The pupicidal activity, although comparatively lower than larvicidal activity, still showed measurable mortality, indicating that α-Farnesene can act across multiple developmental stages. This observation is consistent with earlier findings that pupae are generally less sensitive due to their non-feeding stage and thicker cuticle (WHO, 2005; Benelli, 2015).

Histopathological observations revealed severe damage to the larval midgut, including epithelial disintegration, cytoplasmic vacuolization, necrosis, and disruption of the gut lumen. Such damage directly affects digestion and nutrient absorption, ultimately leading to larval mortality (Clements, 1992; Billingsley and Lehane, 1996). Similar midgut alterations have been reported in recent studies (Pavela et al., 2022; Benelli et al., 2023), supporting this mode of action.

The non-target toxicity results further strengthen the environmental safety profile of α-Farnesene. In Poecilia reticulata, no mortality was observed at 6 ppm (0.00 ± 0.00%), while only 6.67 ± 5.77%, 23.33 ± 5.77%, and 33.33 ± 5.77% mortality were recorded at 30, 60, and 120 ppm, respectively. Statistical analysis showed that significant mortality occurred only at higher concentrations ($p < 0.01$ and $p < 0.001$).

Similarly, in Eisenia fetida, no mortality was observed at 10 ppm (0.00 ± 0.00%), whereas 5.00 ± 5.00%, 15.00 ± 5.00%, and 25.00 ± 5.00% mortality were recorded at 50, 100, and 200 ppm, respectively. Significant effects were observed only at higher concentrations ($p < 0.05$ and $p < 0.001$). The probit slope values (1.21 ± 0.19 for fish and 1.08 ± 0.17 for earthworms) and low χ^2 values (2.04 and 1.88; $p > 0.05$) indicate a good fit of the model.

Importantly, the LC_{50} values for non-target organisms were not reached even at the highest tested concentrations (>120 ppm for Poecilia reticulata and >200 ppm for Eisenia fetida), which is in contrast to mosquito larvae where LC_{50} values were below 10 ppm. This demonstrates a wide safety margin. The selectivity index values were also high, with >19.7 and >16.2 for fish, and >32.8 and >27.0 for earthworms when compared with Aedes aegypti and Culex quinquefasciatus, respectively (Su and Mulla, 1998; Benelli and Pavela, 2018). Recent studies (2022–2024) also report similar high selectivity indices for plant-based larvicides (Pavela et al., 2022; Benelli et al., 2023).

Overall, the findings clearly demonstrate that α-Farnesene is a potent and selective larvicidal compound. Its very low LC_{50} values (6.1 and 7.4 ppm) against mosquito larvae, combined with very high LC_{50} values (>120 and >200 ppm) for non-target organisms,

highlight both its effectiveness and environmental safety. Compared to many plant-derived compounds reported in recent literature, α-Farnesene shows superior larvicidal activity.

However, the study has certain limitations. All experiments were conducted under laboratory conditions, and field validation is necessary to confirm its effectiveness under natural conditions. Further studies on formulation development, stability, and large-scale application are also required. Despite these limitations, the present results strongly suggest that α-Farnesene can be considered a promising eco-friendly alternative for mosquito control and can be effectively incorporated into integrated vector management programmes.

Conclusion

The present study highlights the effectiveness of α-Farnesene, a major sesquiterpene identified from *Sansevieria roxburghiana*, against the mosquito vectors *Aedes aegypti* and *Culex quinquefasciatus*. The compound exhibited strong larvicidal and pupicidal activity, with toxicity increasing over time. It also caused pronounced histopathological damage to the larval midgut, ultimately reducing mosquito survival even at relatively low concentrations. Importantly, α-Farnesene showed very low acute toxicity toward non-target organisms such as *Poecilia reticulata* (aquatic) and *Eisenia fetida* (soil), resulting in high selectivity indices and a wide safety margin. Overall, these findings indicate that α-Farnesene has strong potential as a selective, eco-friendly, and sustainable alternative to conventional synthetic larvicides. The study further supports the use of plant-derived phytochemicals in integrated mosquito management strategies aimed at effective vector control with minimal environmental impact.

Reference

1. Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology, 18*, 265–267.
2. Aktar, M. W., Sengupta, D., & Chowdhury, A. (2009). Impact of pesticides use in agriculture: Their benefits and hazards. *Interdisciplinary Toxicology, 2*(1), 1–12.
3. Ali, U., Syed, J. H., Malik, R. N., et al. (2021). Environmental toxicology of insecticides. *Environmental Research, 194*, 110664.
4. Benelli, G. (2015). Research in mosquito control: Current challenges for a brighter future. *Parasitology Research, 114*, 2801–2805.
5. Benelli, G., & Beier, J. C. (2017). Current vector control challenges in the fight against malaria. *Acta Tropica, 174*, 91–96.
6. Benelli, G., & Pavela, R. (2018). Repellence of essential oils and selected compounds against mosquitoes. *Acta Tropica, 179*, 119–128.
7. Benelli, G., Pavela, R., & Rakotosaona, R. (2024). Botanical insecticides in mosquito control: Advances and future perspectives. *Industrial Crops and Products, 203*, 117134.
8. Benelli, G., Pavela, R., Canale, A., & Mehlhorn, H. (2017). Tick repellents and botanical insecticides: A green roadmap to control vector-borne diseases. *Parasitology Research, 116*, 881–896.
9. Billingsley, P. F., & Lehane, M. J. (1996). Structure and ultrastructure of the insect midgut. In *Biology of the Insect Midgut* (pp. xx–xx). Chapman & Hall.
10. Carson, R. (1962). *Silent spring*. Houghton Mifflin.
11. Carvalho, F. P. (2017). Pesticides, environment, and food safety. *Food and Energy Security, 6*, 48–60.
12. Clements, A. N. (1992). *The biology of mosquitoes: Vol. 1. Development, nutrition and reproduction*. Chapman & Hall.
13. **Dharani, S., & Gokulakrishnan, J. (2025).** *Ecological insights on mosquito diversity and distribution at Poompuhar College, Melaiyur, Mayiladuthurai district, Tamil Nadu, India.* **Journal of Experimental Zoology, India, 28**, 0000–0000. https://doi.org/10.51470/jez.2025.28.1.0000
14. Dhiman, R. C., Pahwa, S., Dhillon, G. P. S., & Dash, A. P. (2018). Climate change and vector-borne diseases in India. *Parasitology Research, 117*, 3509–3525.
15. Ellman, G. L., Courtney, K. D., Andres, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology, 7*, 88–95.
16. Finney, D. J. (1971). *Probit analysis* (3rd ed.). Cambridge University Press.
17. Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry, 226*, 497–509.
18. Goulson, D., Nicholls, E., Botías, C., & Rotheray, E. L. (2015). Bee declines driven by pesticides. *Journal of Applied Ecology, 52*, 316–325.
19. Govindarajan, M., Sivakumar, R., Rajeswary, M., & Yogalakshmi, K. (2013). Chemical composition and larvicidal activity of *Eclipta prostrata* essential oil. *Parasitology Research, 112*, 3753–3764.
20. Gupta, S., et al. (2019). Larvicidal effects of *Sansevieria* and related *Dracaena* species against mosquitoes. *Parasitology Research, 118*, 345–354.

21. Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S-transferases. *Journal of Biological Chemistry, 249*, 7130–7139.
22. Harborne, J. B. (1998). *Phytochemical methods: A guide to modern techniques of plant analysis* (3rd ed.). Springer.
23. Hemingway, J., & Ranson, H. (2000). Insecticide resistance in insect vectors of human disease. *Annual Review of Entomology, 45*, 371–391.
24. Hemingway, J., Hawkes, N. J., McCarroll, L., & Ranson, H. (2016). The molecular basis of insecticide resistance in mosquitoes. *Insect Biochemistry and Molecular Biology, 73*, 13–22.
25. Humason, G. L. (1997). *Animal tissue techniques* (5th ed.). W. H. Freeman.
26. Isman, M. B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture. *Annual Review of Entomology, 51*, 45–66.
27. Isman, M. B. (2020). Botanical insecticides in the twenty-first century. *Insects, 11*, 332.
28. Karthikeyan, R., et al. (2020). Phytochemical analysis and insecticidal activity of *Sansevieria roxburghiana*. *Journal of Medicinal Plants Research, 14*, 87–95.
29. Kraemer, M. U. G., et al. (2019). Past and future spread of *Aedes aegypti* and *Aedes albopictus*. *Nature Microbiology, 4*, 854–863.
30. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.
31. Moyes, C. L., et al. (2017). Contemporary status of insecticide resistance. *PLoS Medicine, 14*, e1002346.
32. OECD. (2016). *Test No. 207: Earthworm, acute toxicity tests*. OECD Publishing.
33. OECD. (2019). *Test No. 203: Fish, acute toxicity test*. OECD Publishing.
34. Pavela, R. (2016). History, presence and perspective of using plant extracts as commercial botanical insecticides. *Plants, 5*, 28.
35. Pisa, L. W., et al. (2021). Effects of pesticides on non-target organisms. *Environmental Science and Pollution Research, 28*, 5400–5418.
36. Rajavel, A. R., Natarajan, R., & Tyagi, B. K. (2015). Maintenance of mosquito colonies and bioassay methods. *Indian Journal of Medical Research, 141*, 45–52.
37. Regnault-Roger, C., Vincent, C., & Arnason, J. T. (2012). Essential oils in insect control. *Annual Review of Entomology, 57*, 405–424.
38. Su, T., & Mulla, M. S. (1998). Comparative toxicity of mosquito larvicides to aquatic organisms. *Journal of the American Mosquito Control Association, 14*, 215–220.
39. World Health Organization. (2005). *Guidelines for laboratory and field testing of mosquito larvicides*. WHO.
40. World Health Organization. (2017). *Global vector control response 2017–2030*. WHO.
41. World Health Organization. (2023). *Vector-borne diseases*. WHO.
42. Zar, J. H. (2010). *Biostatistical analysis* (5th ed.). Pearson Education.