*Journal of Biological Control,* 40(2): 229-250, 2026, DOI: 10.18311/jbc/2026/55649




**Research Article**

# Phytochemical profiling and larvicidal assessment of *Tagetes patula* Linn. and *Phlogacanthus thyrsiformis* Nees. flower extracts against *Culex quinquefasciatus*

**Uma Dutta[1*], Nirodha Pegu[1], Sonali Dey[2], Shruti Baruah[1] and Nayanika Saikia[1]**

[1]*Cell, Molecular Biology and Toxicology Laboratory, Department of Zoology, Cotton University, Guwahati - 781001 Assam, India*

[2]*Department of Zoology, Goalpara College (Autonomous), Goalpara - 783101, Assam, India*

**Corresponding author E-mail*: umadutta1965@gmail.com

**ABSTRACT:** Vector-borne diseases are a significant global health concern, with millions of people affected every year. Mosquitoes remain a major carrier of parasites and pathogens that continue to affect humans. *Culex quinquefasciatus* is a well-known mosquito vector capable of causing a broad range of diseases, including filariasis. Due to the detrimental effects of chemical insecticides, eco-friendly plant-based chemicals have become a better alternative to control measures. The present research work was undertaken to study the efficacy of methanolic extracts of commonly available plants, *viz*. *T. patula* L., *Phlogacanthus thyrsiformis* Nees. and its combined formulation against the larvae of the *C. quinquefasciatus* at different sub-lethal concentrations, *viz*., 250 ppm, 500 ppm, 750 ppm, and 1000 ppm. The study was based on the behavioural changes, larval development, and mortality rate by the standard method. Phytochemical screening was performed, followed by GC-MS for bioactive components responsible for insecticidal activity. On exposure to the initial concentrations of the extract, various damages, including hyperactive wriggling movement, delayed hatching, and damaged bodies, were observed in the mosquito larvae. At higher concentrations of exposure, distorted bodies and delayed moulting were observed, which later led to the morbid condition. The mortality rate of the combined formulation applied to mosquito larvae is more compared to the individual extracts of the chosen plants. These findings demonstrate the pesticidal potential of *T. patula* L. and *P. thyrsiformis* Nees. extract against the *C. quinquefasciatus*, following its impact on physiological and morphological behaviour, suggesting the need for further research on control measures against the mosquito.

**KEYWORDS:** Detrimental effect, larvicidal activity, mosquito larvae, plant extracts, phytochemicals

**(Article chronicle:** Received: 01-04-2026; Revised:28-05-2026; Accepted: 03-06-2026)

## ABBREVIATIONS

AChE - Acetylcholinesterase; BOD - Biological Oxygen Demand; EI+ - Electron Impact Positive ionization; GC-MS - Gas Chromatography-Mass Spectrometry; $LC_{50}$ - Lethal concentration required to cause 50% mortality; LOD - Limit of Detection; m/z - Mass-to-charge ratio; MS - Mass Spectrometry; ppm - Parts per million; RT - Retention time; SEM - Standard Error of Mean; TIC - Total Ion Chromatogram; WHO - World Health Organization

## INTRODUCTION

Vector-borne diseases continue to pose a major threat to global public health, affecting millions of individuals each year. Among disease vectors, mosquitoes play a central role in transmitting a wide range of parasites and pathogens that severely impact human populations worldwide (Wang *et al.,* 2024). Their involvement in the spread of numerous infectious diseases accounts for millions of fatalities annually (Lee *et al.,* 2018). Mosquitoes are responsible

for transmitting several life-threatening illnesses, including malaria, yellow fever, dengue, chikungunya, filariasis, encephalitis, and West Nile virus infection, particularly across tropical and subtropical regions, as well as many other parts of the world (Ghosh *et al.,* 2012).

*Culex quinquefasciatus*, commonly referred to as the southern house mosquito, is a widely distributed subtropical species belonging to the *Culex pipiens* complex of the family Culicidae. This mosquito is considered one of the most prevalent and highly anthropophilic species globally (Negi & Verma, 2018). It functions as an efficient vector for a diverse range of pathogens, including arboviruses, filarial nematodes, and protozoan parasites (Bhattacharya *et al.,* 2016). Current mosquito management strategies largely rely on synthetic insecticides such as pyrethroids, neonicotinoids, organophosphates, carbamates, and chlorinated hydrocarbons (Karunaratne *et al.,* 2022). Although these chemical agents can be highly effective initially, their extensive and repeated application has resulted in widespread insecticide resistance, significantly diminishing their long-term effectiveness (Benelli, 2015). Insecticide resistance has emerged as a major challenge for public health authorities, as escalating chemical usage leads to environmental contamination and increased risks to human health. While chemical insecticides may provide short-term reductions in mosquito populations, their long-term ecological and health consequences often outweigh their immediate benefits (Shroff *et al.,* 2021).

Historically, herbs have played an important role in pest management and represent a valuable ecological reservoir for identifying compounds with larvicidal potential (Ghosh *et al.,* 2012). In recent years, there has been growing interest in plant-derived products as sustainable alternatives for mosquito control, owing to their environmental compatibility and long-term effectiveness. Floral extracts of several plant species are known to contain biologically active constituents such as flavonoids, alkaloids, and terpenoids that interfere with vital physiological processes in mosquito larvae, ultimately causing mortality (Ganesan *et al.,* 2023; Demirak *et al.,* 2022). Compared with synthetic pesticides, phytochemicals are generally less harmful to ecosystems, making plant-based larvicides an attractive and safer alternative. These natural products are biodegradable, readily available, and pose minimal risk to non-target organisms occupying the same ecological habitats (Dagar *et al.,* 2024).

In light of this background, the present study was undertaken to develop a combined formulation using crude flower extracts of *P. thyrsiformis* Nees and *T. patula* Linn., both locally available plant species, for the effective control of *C. quinquefasciatus* across different developmental stages. The selection of these herbs was guided by their traditional medicinal usage and documented biological properties, including anti-helminthic, antibacterial, and antifungal activities, as well as their characteristic strong pungent odour, which may contribute to insecticidal efficacy (Ali *et al.,* 2016; Ningombam *et al.,* 2013).

## MATERIALS AND METHODS

### Collection of plant materials

Flowers of *T. patula* L. and *P. thyrsiformis* Nees were harvested during their respective peak flowering periods. Both the flowering plant species are commonly found in the state of Assam. *T. patula* flowers were collected from the Cotton University campus, Guwahati, during the flowering season spanning December to March. Flowers of *P. thyrsiformis* were collected from the Indian Institute of Technology Guwahati campus, Assam, between February and April. Collected plant specimens were taxonomically identified and authenticated, following submission of a herbarium, at the Department of Botany, Gauhati University.

### Collection of mosquito larvae

Mosquito larvae were collected from multiple randomly selected sites within municipal areas of Guwahati, Assam, characterised by stagnant water bodies and drainage systems (Figure 1). Sampling was conducted during March 2025 from the following areas:

- Panbazar Area: Dighalipukhuri area (2611’16.8”N, 9145’03.7”E), Cotton University campus area (2618’74.05”N,9174’66.4”E)
- Guwahati Club Area: Silpukhuri area (26184’78.9”N, 9176’32.79”E)
- Uzan Bazaar Area: Jorpukhuri Area (2618’39.9”N, 9175’43.3”E)
- Bharalu River Area: (2615’23.69”N, 9175’79.07”E)
- Ulubari Foot Over Bridge near B. Borooah College in Guwahati, Assam: (2617’49.3”N, 9175’79.07”E)

**Identification of mosquito specimen**

Third- and fourth-instar mosquito larvae were collected from the selected sampling sites using insect nets and transported to the laboratory in plastic collection bags containing water from their original habitats. Preliminary identification to the genus level was carried out by examining larval resting posture relative to the water surface, following standard morphological characteristics described in entomological reference manuals (Becker *et al.,* 2020; Lancaster & Downes, 2013; Winsor, 1976). Species-level identification was subsequently confirmed using established taxonomic keys reported by Mathews *et al*. (2017), Snell (2005), Reuben *et al*. (1994), and Belkin (1976).

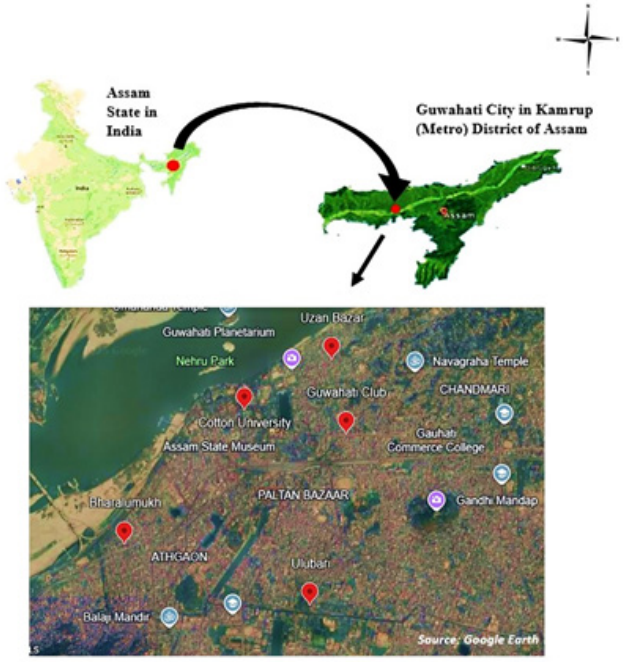



**Figure 1.** Map showing the collection sites of mosquito larvae from different locations in Guwahati, Kamrup Metro, Assam.

**Culture of mosquito larvae**

All experimental procedures were performed in accordance with established ethical guidelines and international standards governing animal research (World Health Organization, 2005). In the laboratory, collected mosquito specimens were maintained in water under controlled environmental conditions (26-28°C with a 12:12 h light-dark photoperiod) and reared until the pupal stage following standard rearing protocol (Das *et al.,* 2007). Upon adult emergence, mosquitoes were provided with a sugar solution and periodic blood meals to facilitate oviposition. The deposited eggs were transferred to trays containing 500 mL of water and allowed to hatch. Larval emergence was observed within 48 h, after which the larvae were fed with finely ground yeast and reared until they reached the third and fourth instar stages for experimental use (Figure 2).

**Life cycle**

The life cycle of *C. quinquefasciatus* comprises four sequential developmental stages: egg, larva, pupa, and adult. Under favourable environmental conditions, development from egg to adult typically occurs within 10-14 days; however, lower temperatures can extend this duration (Manimegalai & Sukanya, 2014). The eggs are

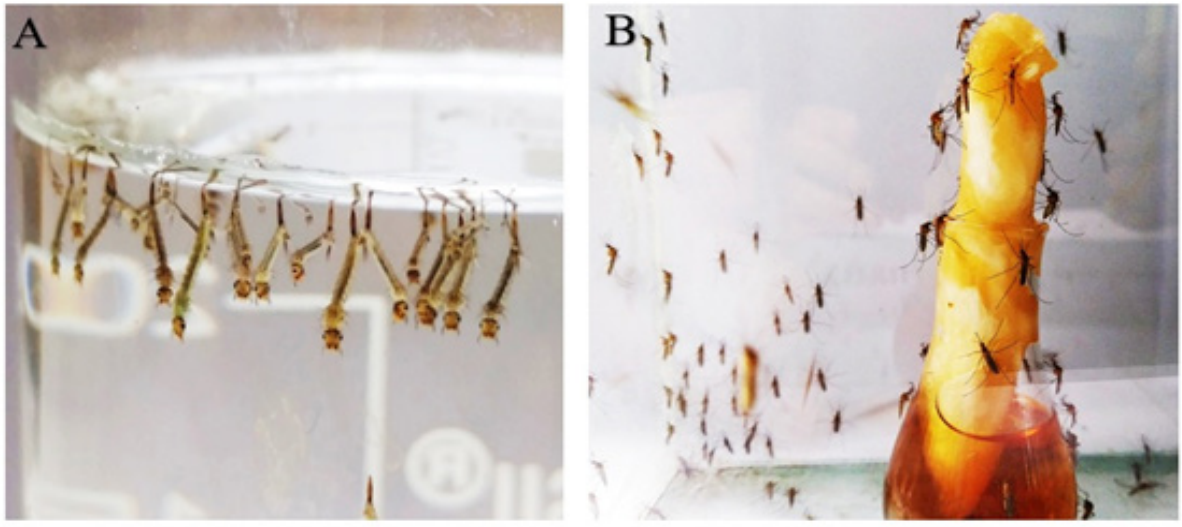



**Figure 2.** Rearing of mosquito larvae in laboratory; **(A).** Segregation of test larvae; **(B).** Adult mosquito in rearing box with sucrose meal.

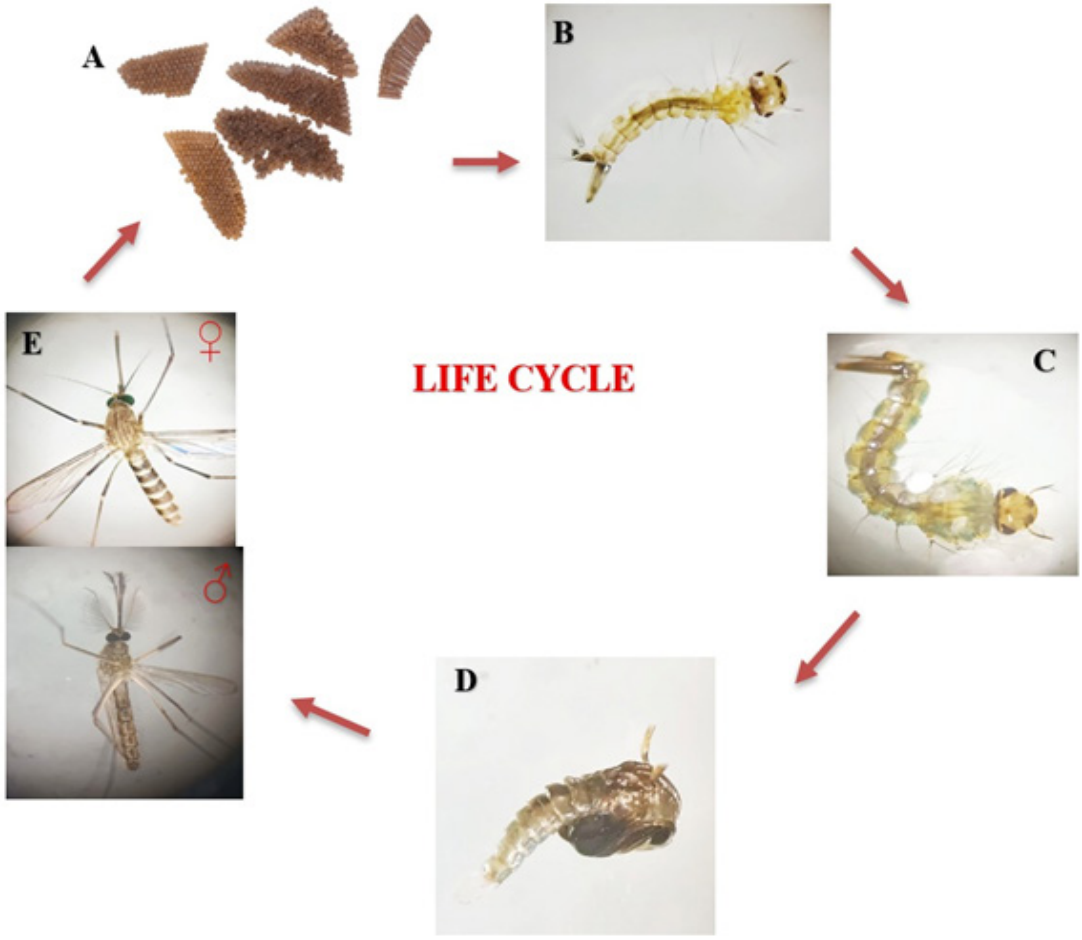



**Figure 3.** Photomicrographs showing different life cycle stages of *C. quinquefasciatus* under laboratory control conditions: **(A).** Egg, **(B).** Early larval stage, **(C).** Late larval stage, **(D).** Pupa, **(E).** Adult *C. quinquefasciatus* - Female (♀) and Male (♂).

dark brown in colour, measure approximately 0.6 mm in length, and possess desiccation tolerance, allowing them to remain dormant for extended periods. When suitable aquatic conditions are available, the eggs hatch to produce actively motile larvae, commonly referred to as wrigglers.

The larval phase progresses through four instars, during which larvae feed on organic matter present in their natural habitats; under laboratory conditions, yeast powder serves as a suitable food source. Following the completion of the fourth instar, larvae undergo moulting to form pupae, representing a non-feeding transitional stage. Adult mosquitoes emerge within 1-2 days of pupation. Newly emerged adults initially feed on sugar solutions, while females require a blood meal after mating to support egg development. Male *C. quinquefasciatus* generally have a shorter lifespan of approximately one to two weeks, whereas females can survive for up to two months (Manimegalai & Sukanya, 2014) (Figure 3).

**Flower extract preparation**

Fresh flowers of *T. patula* and *P. thyrsiformis* were carefully collected, washed to remove debris, and air-dried. The petals were separated, cut into smaller pieces, and shade-dried until complete removal of moisture. The dried floral material was then finely powdered using a grinder. Flower extracts were prepared using a standard maceration method with methanol. 10 mg of the powdered sample was dissolved in 70 mL of methanol and incubated overnight at 35°C in a BOD incubator, with intermittent shaking. The mixture was subsequently filtered through Whatman No. 1 filter paper, and the filtrate was collected in a conical flask. The remaining residue was re-extracted with fresh methanol under identical conditions. This extraction process was repeated several times to ensure maximum yield of

bioactive compounds. The combined filtrates were allowed to evaporate to reduce the volume to approximately half, followed by further evaporation in a petri dish to obtain the crude methanolic extract. The dried extract was sealed and stored under refrigerated conditions until further use.

**Qualitative phytochemical screening**

Qualitative phytochemical analysis of the prepared methanolic flower extracts was conducted using standard screening procedures described by Aguiar *et al.,* 2015; Tiwari & Chanda*,* 2016; Alamgir *et al.,* 2018; Shaikh & Patil, 2020. The extracts were tested for the presence of major phytochemical groups, including alkaloids, flavonoids, tannins, steroids, terpenoids, glycosides, and saponins, following classical protocols outlined by Harborne (1984).

**GC-MS analysis**

Gas chromatography-mass spectrometry analysis was performed using a PerkinElmer (USA) GC-MS system (Clarus 680 GC coupled with Clarus 600 MS) equipped with a liquid autosampler. Data acquisition and processing were carried out using TurboMass software version 6.1.2. High-purity helium (99.99%) served as the carrier gas at a constant flow rate of 1 mL/min. Samples were injected in splitless mode with an injection volume of 1 µL. The injector temperature was maintained at 280°C, while the ion source temperature was set at 180°C. The oven temperature programme initiated at 60°C (held for 1 min), followed by a temperature ramp of 7°C/min to 200°C (held for 3 min), and subsequently increased at 10°C/min to 300°C with a final hold of 5 min. The total run time was approximately 39 min, with a solvent delay of 8 min. Mass spectra were acquired in electron impact positive ($EI^+$) ionisation mode at 70 eV over a mass range of m/z 50-600 amu. Compound identification was achieved by comparing the obtained mass spectra with reference spectra available in the NIST 2014 library, enabling determination of compound names, molecular weights, and empirical formulas.

**Experimental design**

For larvicidal assays, a minimum of 25 identified third- and fourth-instar mosquito larvae were introduced into disposable containers containing 100 mL of clean, acclimatised water and maintained under standard laboratory conditions. The experimental setup comprised four groups, with Group I serving as the untreated control. Groups II, III, and IV were exposed to different concentrations of flower extracts, *viz*., 250, 500, 750, and 1000 ppm of *T. patula* and *P. thyrsiformis* and a combined extract of both in a 1:1 ratio, respectively. Each concentration was tested using at least 25 larvae, and all experiments were conducted in quadruplicate. The corresponding extract concentrations applied were 25, 50, 75, and 100 mg/mL. The prepared concentrations of flower extracts were applied once to each container, and observations were recorded at 1 h, 12 h, 24 h, 48 h, and 72 h post-treatment. In addition, larvae were monitored for up to one week to evaluate the effects of prolonged exposure. The larvicidal efficacy of the combined extract was compared with that of individual plant extracts at all observation intervals. Behavioural changes, morphological alterations, and mortality rates were documented across all experimental groups.

**Dose preparation for experimental studies**

Working concentrations were prepared by dissolving 25 mg, 50 mg, 75 mg, and 100 mg of the prepared methanolic flower extracts in 100 mL of distilled water. For the combined formulation, equal quantities of both extracts were mixed to achieve the desired concentrations. All prepared formulations were stored under refrigerated conditions and used as required for experimental assays.

**Larvicidal mortality bioassay**

Post-treatment, larval behaviour, including movement patterns and general activity, was closely monitored immediately and throughout the experimental period. Mortality assessments were carried out at 1 h, 12 h, 24 h, 48 h, and 72 h following treatment. Larvae exhibiting moribund conditions were considered dead, following the criteria described by Dutta and Dey (2023) and Ndione *et al*. (2007). Each treatment concentration was evaluated in four independent replicates to ensure reproducibility and reliability of the results.

The average percentage of mortality was calculated using the formula

$$\% \text{ of mortality} = \frac{Number\ of\ dead\ larvae}{Number\ of\ total\ larvae} \times 100$$

Mortality correction was done by following the formula given by Abbott (1925)

$$\text{Corrected \% of mortality} = \frac{1 - n\ in\ T\ after\ treatment}{n\ in\ C\ after\ treatment} \times 100$$

where, n = number of larvae, T = treated group, C = control group

**Statistical analysis**

Statistical analysis of larval mortality data, including calculation of mean ± SD, probit analysis,

$LC_{50}$ determination, regression analysis, and graphical representation, was performed using GraphPad Prism version 11.0.2 (GraphPad Software Inc., San Diego, CA, USA).

## RESULTS

### Detection of phytochemicals

The prepared extracts of *P. thyrsiformis* Nees and *T. patula* L flowers were subjected to different solvents to check the solubility and presence of different bioactive components. The methanolic extract showed the highest concentration of bioactive components, followed by the ethanol extract, and then the aqueous extract. The phytochemical screening of *P. thyrsiformis* Nees shows the presence of flavonoids, saponins, phenols, tannins, steroids, terpenoids, and triterpenoids; while the phytochemical analysis of *T. patula* L contains alkaloids, flavonoids, tannins, phenols, saponins, terpenoids, glycosides, steroids, and carbohydrates (Table 1). These results indicate that methanol is the most efficient extraction solvent for isolating both polar and semi-polar phytoconstituents in *P. thyrsiformis* and *T. patula* flowers, followed by ethanol, and then water.

### GC-MS analysis of bioactive constituents

The powdered flowers of *T. patula* and *P. thyrsiformis* were extracted with methanol and subjected to GC-MS

**Table 1.** Phytochemical analysis of *P. thyrsiformis* and *T. patula*

| Phytochemical | Aqueous extract | | Ethanolic extract | | Methanolic extract | |
|---|---|---|---|---|---|---|
| | ***P. thyrsiformis*** | ***T. patula*** | ***P. thyrsiformis*** | ***T. patula*** | ***P. thyrsiformis*** | ***T. patula*** |
| Alkaloids | - | - | +(*) | +(*) | - | +(**) |
| Flavonoids | +(*) | - | +(*) | - | +(*) | - |
| Tannins | +(*) | + | +(*) | + | +(*) | + |
| Steroids | - | - | + | - | +(*) | - |
| Terpenoids | - | +(*) | + | +(**) | + | +(***) |
| Anthraquinones | - | - | - | - | - | - |
| Glycosides | +(*) | - | +(*) | - | +(**) | - |
| Triterpenoids | - | - | + | +(*) | + | +(**) |
| Coumarins | - | - | + | + | + | + |
| Oils and Fats | - | - | +(*) | +(*) | +(**) | +(***) |
| Phenolic compounds | +(*) | - | +(*) | - | +(*) | - |
| Saponins | +(*) | - | +(*) | +(*) | +(***) | +(**) |

(+) Present, (-) absent, (*) Minimum intensity of reaction, (**) Moderate intensity of reaction and (***) Maximum intensity of reaction

GC-MS ***analysis of bioactive constituents***

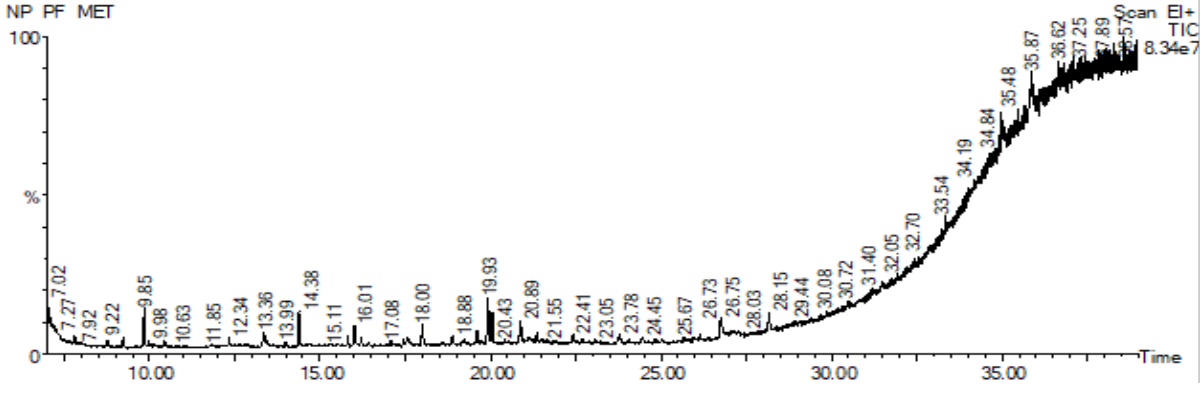



**Figure 4.** GC-MS chromatogram of the methanol extract of *P. thyrsiformis* Nees.

**Table 2.** The compounds present in the methanolic extract of *P. thyrsiformis* Nees

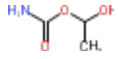
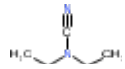
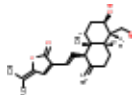
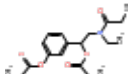
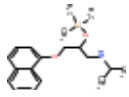
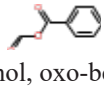
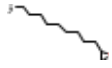
| RT value | Molecular formula | Molecular weight (g/mol) | Name of the compound | Bioactivity |
|---|---|---|---|---|
| 9.850 | $C_3H_7NO_3$ | 105 |  15 Carbamic acid, hydroxy-ethyl ester | Effective insecticides are so by virtue of their ability to inhibit acetylcholinesterase (AChE) in the nervous system. (WHO, EHC 64, 1989). |
| 13.361 | $C_6H_{13}NO_5$ | 179 |  D-Glucosamine | Glucosamine is part of the structure of two polysaccharides, chitosan and chitin. Effective as an insect growth regulator that prevents an insect from reaching maturity by interfering with the moulting process (Song *et al.,* 2017). |
| 14.381 | $C_5H_{10}N_2$ | 98 |  Diethylcyanamide | Binds to a specific receptor in muscles called the ryanodine receptor, which causes muscle cells to leak calcium. The muscles stop working normally. The insect is paralysed and dies. (Zheng *et al.,* 2025) |
| 15.822 | $C_6H_8O_3$ | 144 |  2,4-Dihydroxy-2,5-dimethylfuran-3-one | Acts as an insect attractant that can be used as poison bait or to attract beneficial insects. (Lorenzo *et al.,* 2014). |
| 16.017 | $C_{19}H_{27}NO_5$ | 349 |  Etilefrin-propionyl | Target α-adrenergic-like octopamine receptor found on insect immune cells and its role in mediating stress hormone effects on immune function (Xu *et al.,* 2022). |
| 18.008 | $C_{19}H_{29}SiO_2N$ | 373 |  Propranolol, TBDMS derivatives | Strong insecticidal activity, which could be a result of tyrosinase inhibition (Peng *et al.,* 2022). |
| 19.934 | $C_8H_6O_3$ | 150 |  Methanol, oxo-benzoate | Significantly increased the superoxide dismutase, catalase, α, β esterase, and Glutathione-S-transferase, which cause DNA damage as well as lead to shrunken body posture, damaged epithelial cells, and microvilli (Raguvaran *et al.,* 2022). |

**Table 2.** Continued

| | | | | |
|---|---|---|---|---|
| 20.059 | $C_5H_{12}Si$ | 100 |  1,1-dimethyl-1-silacyclobutane | They are abrasive and act as desiccants, removing the oily, protective film that covers insects' bodies, causing them to dry out and die. They also cause damage to the midgut epithelium, mainly through the detachment of epithelial cells from the basement membrane, as observed in larvae of the leaf miner (Alhousari & Greger, 2018; Olexa, 1992). |
| 20.889 | $C_{12}H_{24}O$ | 184 |  Oxirane | It is a sex pheromone that can significantly reduce successful mating attempts (Tobin *et al.,* 2013). |
| 21.359 | $C_8H_{14}O_2$ | 142 |  4-ethoxy-cyclohexanone | The differential modulation of GABA response by cyclohexanone isomers accounts for variable symptoms of poisoning in insects. They induce a profound increase in ionic permeability in lipid bilayers and uptake of calcium-dependent current. (Narahashi, 2010). |

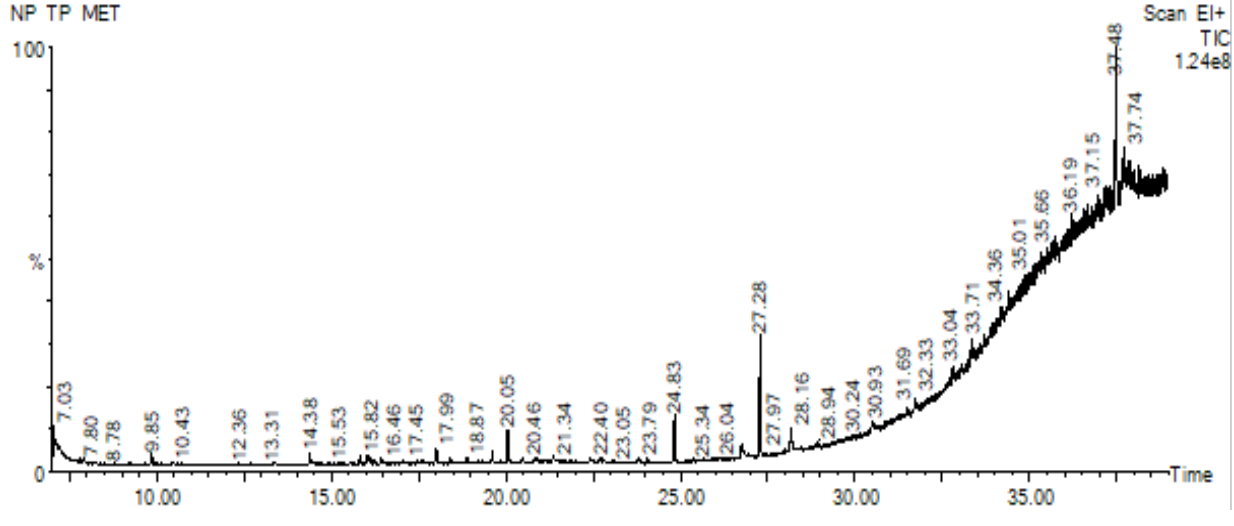



**Figure 5.** GC-MS chromatogram of a methanolic extract of *T. patula* L.

analysis. The resulting chromatographic peaks were analysed using the GC-MS library to identify the presence of bioactive compounds. The chemical structures of the identified constituents were subsequently illustrated using ChemDraw software (Tables 2, 3 and Figures 4, 5).

**Table 3.** The compounds present in the methanolic solution of *T. patula*

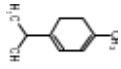
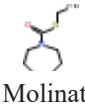
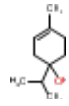
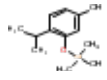
| RT value | Molecular formula | Molecular weight (g/mol) | Name of the compound | Bioactivity |
|---|---|---|---|---|
| 9.860 | $C_{15}H_{24}$ | 204 |  Cyprene | Known to affect the nervous system, especially by blocking the closure of sodium channels, causing repetitive after-discharges that result in hyperexcitation of the nervous system. This causes muscle spasms and inflicting paralysis. This creates an inability to function or eat, eventually leading to the pest's death (El-Sayed *et al.,* 2023; Narahashi *et al.,* 1992). |
| 14.381 | $C_{10}H_{14}O_2$ | 166 |  Piperitenone oxide | Known for larvicidal, ovicidal, oviposition-deterrent, developmental toxicity, and repellent properties against various insects. Also, piperitenone oxide also completely inhibited egg hatching at the dose of 75 µg/ml in ovicidal assay (Tripathi *et al.,* 2004). |
| 16.012 | $C_{10}H_{16}$ | 136 |  Alpha terpinene | A-T acts as a repellent at a minimum effective dosage as well as prompt olfactory responses (Khaleel *et al.,* 2018). Insect readily absorbs most alpha-terpenes offered and, due to their metabolic process, yield a product even more toxic to the insect than the original alpha-terpene, thus enhancing its toxicity (Rossi *et al.,* 2012; Scalerandi *et al.,* 2018). |
| 16.422 | $C_4H_7O_2N$ | 149 |  Azetidine-2-carboxylic acid | They lead to the production of unnatural proteins that cannot function properly. They presumably affect tyrosinase activity, which is important in the hardening of insect cuticle (Adeyeyé *et al.,* 1989). |
| 17.998 | $C_9H_{17}SON$ | 187 |  Molinate | Molinate is a cholinesterase inhibitor and elicits signs of neurotoxicity in insects by manipulating insect behaviour through the olfaction system (Stoker *et al.,* 2010). |

**Table 3.** Continued

| | | | | |
|---|---|---|---|---|
| 20.054 | $C_{10}H_{18}O$ | 154 |  Terpinen-4-ol | Terpinen-4-ol itself or with the combined effect of other ingredients has a variety of biological activities, such as attracting activity, repellent activity, fumigant toxicity, and growth inhibitory activity to insects, as well as antimicrobial activity, which brings broad prospects for its development and application in pesticides. (Govindarajan *et al.,* 2016). |
| 24.826 | $C_6H_9ON_3$ | 139 |  5-Ethyl-cytosine | Primary action is to lyse midgut epithelial cells in the target insect by forming pores in the apical microvilli membrane of the cells. Subsequently, cell lysis and disruption of the midgut epithelium release the cell contents, providing spores a germinating medium leading to severe septicaemia and insect death (Mandrioli & Manicar, 2015). |
| 27.281 | $C_{15}H_{24}$ | 204 |  Caryophyllene | They exhibit insecticidal and antifeedant activity. Caryophyllene acts as a nerve poison to pests via sodium channel modulators (Lima *et al.,* 2024). |
| 28.162 | $C_{12}H_{25}N$ | 368 |  Aziridine, 2-methyl-2-(2,2,4,4-tetramethylpentyl)- | Cause regurgitation and characteristic unnatural positioning of the abdomen, apparently resulting from unequal contraction of intersegmental muscles. They also have an inhibitory effect on larval growth and hence cause mortality (Holloway *et al.,* 1986). |
| 37.476 | $C_{13}H_{22}SiO$ | 264 |  Thymol, TBDMS derivative | Potentiate ligand-gated chloride channels in the insect nervous system and probably act as neurotoxic insecticides, and promising potential of thymol as ovicidal and larvicidal agents (Youssefi *et al.,* 2019). |

**Survey analysis**

A total of 4 species of mosquitoes were collected and identified from the selected sites. All the mosquitoes collected were of either the *Culex*, *Aedes,* or *Anopheles* genus (Table 4).

**Table 4.** Abundance of mosquito species in selected locations

| Area of Collection | Mosquito Species | | | |
|---|---|---|---|---|
| | ***C. quinquefasciatus*** | ***Culex vishnui*** | ***Anopheles culicifacies*** | ***Aedes aegypti*** |
| Dighalipukhuri area | +(***) | +(**) | + | + |
| Cotton University area | +(***) | +(**) | - | +(*) |
| Silpukhuri area | +(***) | +(*) | + | + |
| Jorpukhuri area | +(***) | +(*) | + | + |
| Bharalu River area | +(***) | +(**) | - | +(**) |
| Ulubari area | +(***) | +(**) | - | +(*) |

(+) Present, (-) Absent, (*) Low number of larvae, (**) Moderate number of larvae and (***) Highest number of larvae

**Behavioural change**

In the control group, no noticeable behavioural alterations were observed. In contrast, exposure to higher concentrations of the flower extracts rendered the eggs inactive. Larvae treated with varying dosages demonstrated delayed development and a marked reduction in adult emergence in a dose-dependent manner. During exposure, the larvae initially exhibited wriggling movements, descending to the bottom of the beaker and remaining there for approximately 2-5 min before ascending back to their normal position. At higher extract concentrations, the larvae became agitated and displayed intense, hyperactive wriggling. However, with prolonged exposure, their movements gradually slowed, and they appeared lethargic and motionless. Many larvae were observed positioning their mouthparts upward at the water surface, suggesting respiratory distress and an imminent death state. In addition, fourth-instar larvae showed delayed metamorphosis, and

**Table 5.** Comparative effect of methanolic extracts of *P. thyrsiformis*, *T. patula*, and their combined formulation on the life cycle of *C. quinquefasciatus*

| Life cycle stages | Dose concentration | ***P. thyrsiformis*** | ***T. patula*** | Combined formulation |
|---|---|---|---|---|
| Egg stage | 250 ppm | No change | Delayed hatching | Did not proceed to larval stage |
| | 500 ppm | Delayed hatching | Did not proceed to larval stage | Inactive (dead) |
| | 750 ppm | Did not proceed to larval stage | Inactive (dead) | Inactive (dead) |
| | 1000 ppm | Inactive (dead) | Inactive (dead) | Inactive (dead) |
| Early larval instar | 250 ppm | No change | Frequent wriggling movement | Hyperactive wriggling |
| | 500 ppm | Frequent wriggling movement | Damaged body | Delayed development |
| | 750 ppm | Did not proceed to the late larval stage | Did not proceed to the late larval stage | Did not proceed to the late larval stage and larval death |
| | 1000 ppm | Larval death | Larval death | Larval death |

**Table 4.** Continued

| | | | | |
|---|---|---|---|---|
| Late larval instar | 250 ppm | Hyperactive wriggling | Hyperactive wriggling | Hyperactive wriggling |
| | 500 ppm | Hyperactive wriggling | Distorted shape | Distorted body, delayed pupation |
| | 750 ppm | Delayed molting | Delayed pupation | Larval death |
| | 1000 ppm | Death | Larval death | Larval death |
| Pupa | 250 ppm | Normal shape | Hyperactive movement | Hyperactive movement |
| | 500 ppm | Normal shape | Delayed molting | Distorted body, delayed molting |
| | 750 ppm | Delayed molting | Pupal death | Pupal death |
| | 1000 ppm | Pupal death | Pupal death | Pupal death |
| Adult | 250 ppm | No change | No change | Irregular movement |
| | 500 ppm | No change | No change | Slow movement |
| | 750 ppm | No change | Slow movement | Slow movement |
| | 1000 ppm | Slow movement | Sluggish movement | Slow movement |

upon eventual adult emergence, the individuals were weak, unable to fly, and sluggishly rested on the water surface (Table 5).

**Morphological changes**

Exposure to the flower extract disrupted the normal growth and development of the larvae, frequently preventing them from attaining full adult size, particularly under treatment with the combined formulation. In certain cases, larvae were able to reach the pupal stage; however, their overall morphology was adversely affected (Figure 6). The dead larvae displayed intense pigmentation and pronounced structural abnormalities, including complete damage to the saddle, setae, siphon, and anal gills. The larval body appeared markedly shrunken, with evident impairment, destruction, and disorganisation of the gut. Furthermore, severe damage was observed in the external cuticular membrane, accompanied by slight shrinkage of the internal membrane of the anal papillae (Figure 7).

**Mortality rate**

The results revealed differential mortality responses in *C. quinquefasciatus* larvae depending on the type of flower extract used, its concentration, and the duration of exposure. The effects of the various flower extracts on larval mortality are presented in the corresponding Table 6. Larval mortality data were recorded from four independent replicates containing 25 larvae each (n = 100). Mean mortality and standard deviation (Mean ± SD) were calculated from replicate observations. Percentage mortality was calculated based on total larval death, while corrected mortality was determined using Abbott's formula to compensate for control mortality.

The methanolic extracts of *T. patula*, *P. thyrsiformis*, and their combined formulation exhibited a clear dose- and time-dependent larvicidal effect against *C. quinquefasciatus*. No mortality was observed at 1 h in any treatment of *T. patula* or *P. thyrsiformis*, except for minimal mortality (1%) at 1000 ppm of *P. thyrsiformis*. The combined formulation also showed low mortality at 1 h across all concentrations. However, mortality progressively increased with both rising concentrations and extended exposure durations. At 72 h, *T. patula* caused 65% (16.25 ± 0.96), 71% (17.75 ± 0.96), 76% (19.00 ± 0.82), and 93% (23.25 ± 1.71) mortality at 250, 500, 720, and 1000 ppm, respectively, with corrected

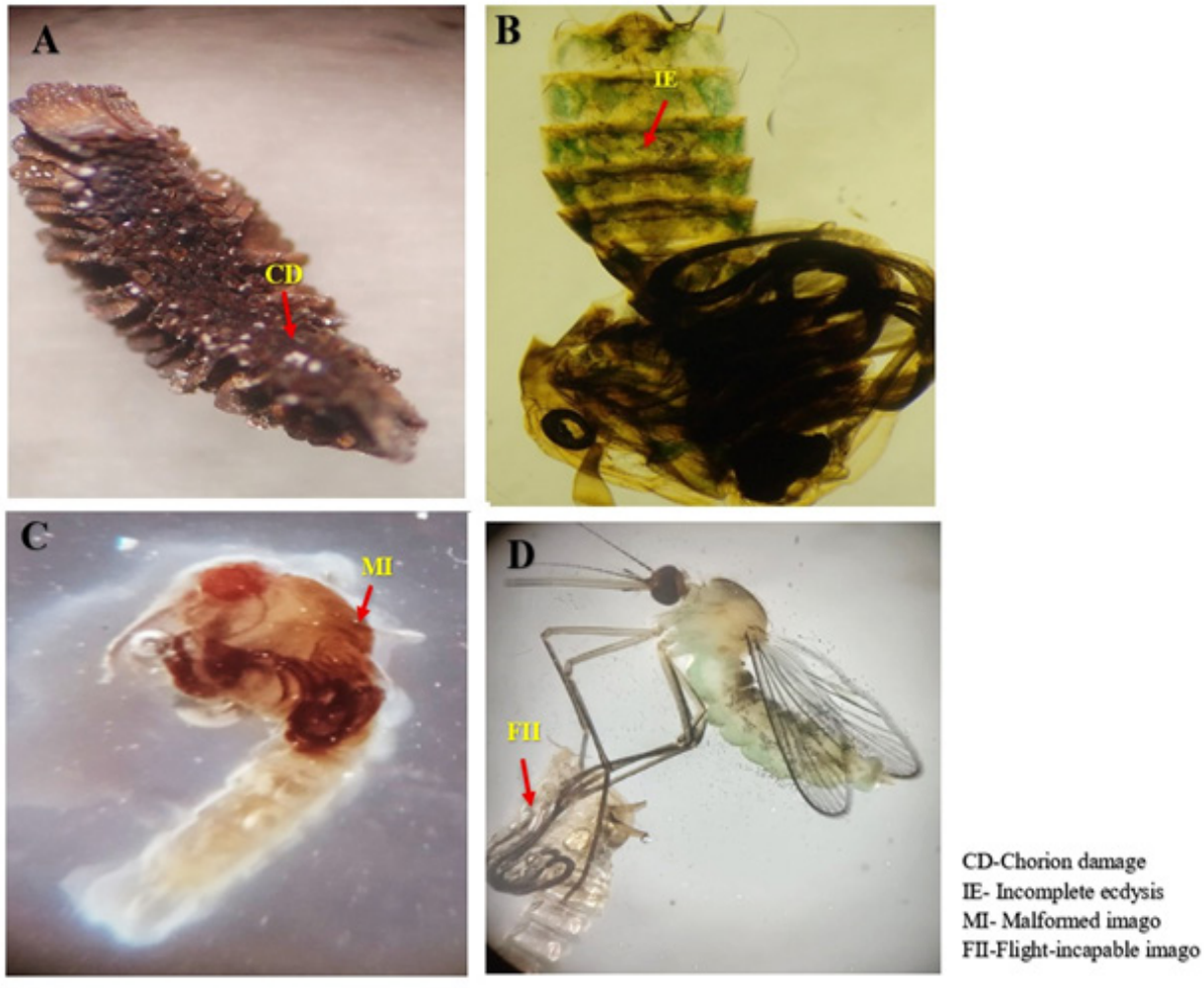



**Figure 6.** Photomicrographs showing morphological alterations in different developmental stages of treated *C. quinquefasciatus*. **(A).** Egg raft showing severe damage to the chorion. **(B).** Pupa exhibiting incomplete ecdysis along with malformation of abdominal segments. **(C).** Imago partially emerged, characterised by reduced antennal and labial structures, with appendages retained within the pupal cuticle. **(D).** Fully emerged imago incapable of flight, as the hind and mid legs remain entrapped in the pupal exuviae.

mortalities of 64.30%, 70.40%, 75.51%, and 92.86%. In comparison, *P. thyrsiformis* induced 52% (13.00 ± 0.82), 61% (15.25 ± 0.96), 67% (16.75 ± 0.96), and 81% (20.25 ± 0.96) mortality at the same concentrations, with corrected mortalities of 51.02%, 60.20%, 66.33%, and 80.61%, respectively. The combined formulation proved most effective, achieving 86% (21.50 ± 1.29) mortality at both 250 and 500 ppm, 99% (24.75 ± 0.50) at 720 ppm, and complete mortality (25.00 ± 0.00) at 1000 ppm, with corrected values of 85.71%, 85.71%, 98.97%, and 100%, respectively. Regression analysis demonstrated a strong positive correlation between concentration and corrected larval mortality (following 72h exposure), with $R^2$ values ranging from 0.80 to 0.98 (Figure 8).

The larvicidal activity of methanolic extracts of *P. thyrsiformis*, *T. patula*, and their combined formulation against *C. quinquefasciatus* increased progressively with increasing concentration and exposure duration. Among the tested samples, the combined formulation exhibited the highest larvicidal efficacy, achieving 100% mortality at 1000 ppm after 72 h of exposure. *T. patula* showed greater larvicidal activity compared to *P. thyrsiformis* at corresponding concentrations and exposure periods.

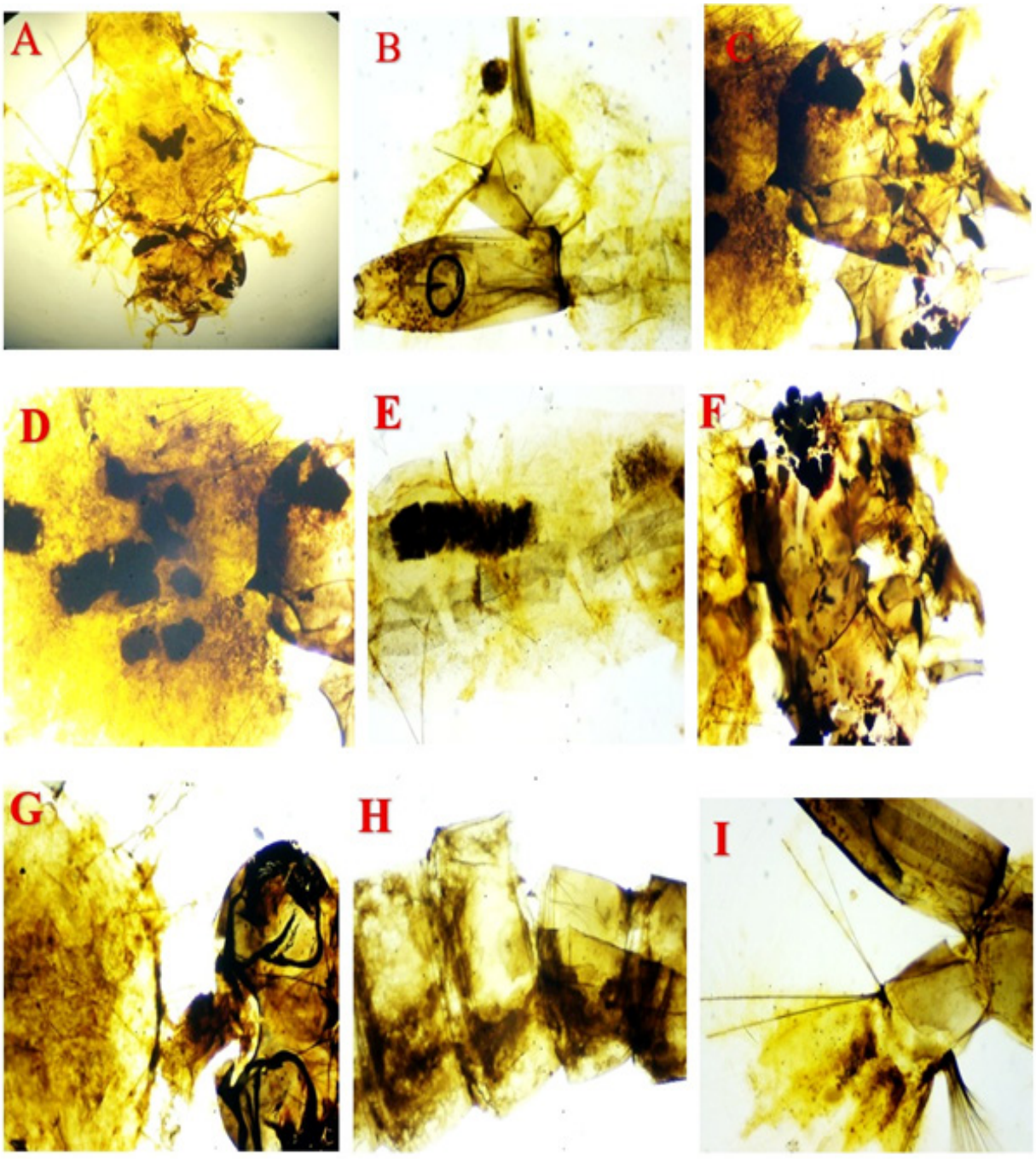



**Figure 7.** Photomicrographs showing morphological alterations observed in the larvae of *C. quinquefasciatus*, following treatment: **(A).** Hyperpigmented body, **(B).** Damaged siphon, **(C).** Broken mouthparts, **(D).** Granulation in the prothoracic region, **(E).** Disrupted midgut, **(F).** Distorted compound eye, **(G).** Detachment of the neck region, **(H).** Damaged cuticular membrane, **(I).** Distorted anal papillae.

Mortality was comparatively low during the initial exposure period but increased substantially after 24-72 h, indicating a clear dose- and time-dependent toxic effect of the extracts.

Comparative analysis of larval mortality across treatments showed the following order of efficacy:

Combined formulation > *T. patula* > *P. thyrsiformis*

**Table 6.** Comparative dose- and time-dependent larval mortality of *C. quinquefasciatus* following treatment with methanolic extracts of *P. thyrsiformis*, *T. patula*, and their combined formulation

| Treatment | Dose (ppm) | Time (h) | Mean Mortality ± SD | % Mortality (n=100) | Corrected % mortality |
|---|---|---|---|---|---|
| *P. thyrsiformis* | 250 | 24 | 6.00 ± 0.82 | 24 | 24 |
| | | 48 | 10.00 ± 1.41 | 40 | 40 |
| | | 72 | 13.00 ± 0.82 | 52 | 51.02 |
| | 500 | 24 | 8.50 ± 1.29 | 34 | 34 |
| | | 48 | 11.25 ± 0.96 | 45 | 45 |
| | | 72 | 15.25 ± 0.96 | 61 | 60.2 |
| | 720 | 24 | 9.75 ± 0.96 | 39 | 39 |
| | | 48 | 13.75 ± 0.96 | 55 | 55 |
| | | 72 | 16.75 ± 0.96 | 67 | 66.33 |
| | 1000 | 24 | 12.25 ± 0.96 | 49 | 49 |
| | | 48 | 16.25 ± 0.96 | 65 | 65 |
| | | 72 | 20.25 ± 0.96 | 81 | 80.61 |
| *T. patula* | 250 | 24 | 9.00 ± 0.82 | 36 | 36 |
| | | 48 | 12.50 ± 0.58 | 50 | 50 |
| | | 72 | 16.25 ± 0.96 | 65 | 64.3 |
| | 500 | 24 | 9.75 ± 0.96 | 39 | 39 |
| | | 48 | 15.00 ± 0.82 | 60 | 60 |
| | | 72 | 17.75 ± 0.96 | 71 | 70.4 |
| | 720 | 24 | 10.75 ± 0.50 | 43 | 43 |
| | | 48 | 17.25 ± 0.96 | 69 | 69 |
| | | 72 | 19.00 ± 0.82 | 76 | 75.51 |
| | 1000 | 24 | 15.50 ± 0.58 | 62 | 62 |
| | | 48 | 20.00 ± 0.82 | 80 | 80 |
| | | 72 | 23.25 ± 1.71 | 93 | 92.86 |
| Combined formulation | 250 | 24 | 12.75 ± 1.26 | 51 | 51 |
| | | 48 | 16.00 ± 0.82 | 64 | 64 |
| | | 72 | 21.50 ± 1.29 | 86 | 85.71 |

**Table 6.** Continued

| | | | | | |
|---|---|---|---|---|---|
| Combined formulation | 500 | 24 | 13.25 ± 0.96 | 53 | 55 |
| | | 48 | 19.00 ± 0.82 | 76 | 76 |
| | | 72 | 21.50 ± 1.29 | 86 | 85.71 |
| | 720 | 24 | 15.25 ± 1.26 | 61 | 61 |
| | | 48 | 21.00 ± 0.82 | 84 | 84 |
| | | 72 | 24.75 ± 0.50 | 99 | 98.97 |
| | 1000 | 24 | 17.00 ± 0.82 | 68 | 68 |
| | | 48 | 22.00 ± 0.82 | 88 | 88 |
| | | 72 | 25.00 ± 0.00 | 100 | 100 |
| Statistical analysis demonstrated significant variation in larval mortality among treatments, concentrations, and exposure durations ($p < 0.05$) | | | | | |

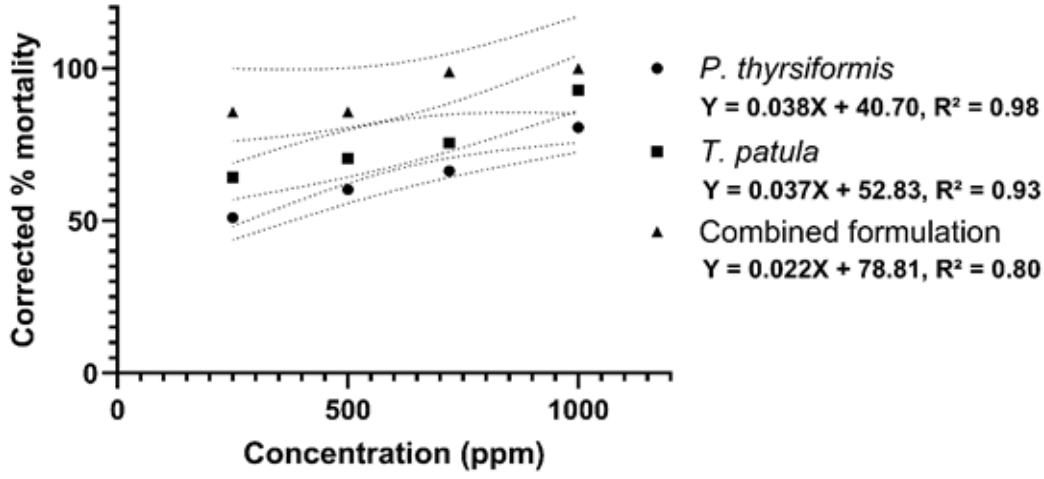



**Figure 8.** Linear regression analysis showing the dose-dependent corrected larval mortality of *C. quinquefasciatus* following treatment with methanolic extracts of *P. thyrsiformis*, *T. patula*, and their combined formulation after 72 h exposure.

**Probit analysis**

The probit analysis results for the larvicidal efficacy of the different plant extracts against *C. quinquefasciatus* larvae are presented in Table 7. The combined formulation demonstrated the highest larvicidal potency, exhibiting an $LC_{50}$ value of 426.58 ppm at 72 h. This was followed by *T. patula* with an $LC_{50}$ of 489.78 ppm, while *P. thyrsiformis*

**Table 7.** Probit analysis of larvicidal efficacy of different plant extracts against *C. quinquefasciatus*

| Flower extract | $LC_{50}$ at 24 | $LC_{50}$ at 72 | Regression equation | $R^2$ |
|---|---|---|---|---|
| T. patula | 1258.9 | 489.78 | y = 0.037x + 52.83 | 0.93 |
| P. thyrsiformis | 1548.8 | 1047.1 | y = 0.038x + 40.70 | 0.98 |
| Combined extract | 831.76 | 426.58 | y = 0.022x + 78.81 | 0.80 |

showed comparatively lower efficacy with an $LC_{50}$ of 1047.1 ppm at 72 h. At 24 h, the $LC_{50}$ values were higher for all treatments, indicating time-dependent toxicity. The combined extract recorded an $LC_{50}$ of 831.76 ppm, whereas *T. patula* and *P. thyrsiformis* showed $LC_{50}$ values of 1258.9 ppm and 1548.8 ppm, respectively. These findings confirm that larvicidal activity increased with exposure duration, with the combined formulation exhibiting superior efficacy compared to the individual extracts.

## DISCUSSION

The present study evaluated the larvicidal potential of methanolic flower extracts of *T. patula* and *P. thyrsiformis* against *C. quinquefasciatus*. Unlike conventional synthetic insecticides, botanical larvicides possess biodegradable and eco-friendly characteristics with reduced environmental persistence. In addition, the complex phytochemical composition of plant extracts may delay resistance development compared to single-target synthetic compounds. Both extracts produced significant larvicidal toxicity, as evidenced by behavioural alterations, morphological deformities, and increased mortality across developmental stages. These findings reinforce the growing interest in plant-based biocontrol agents as environmentally sustainable alternatives to synthetic insecticides. *C. quinquefasciatus* is one of the most abundant and anthropophilic mosquito species (Lopes *et al.,* 2019) and serves as a vector for a wide range of pathogens, including arboviruses, filarial worms, and protozoans (Naik, 2018). Its high prevalence is often linked to organically enriched water bodies that provide suitable breeding habitats. In contrast, botanical insecticides are biodegradable and consist of complex blends of bioactive compounds that act synergistically on behavioural and physiological pathways of the vector, reducing the likelihood of resistance development (Ghosh *et al.,* 2012). Plant extracts have long been used traditionally to control insect pests and vectors and have demonstrated promising toxicity against mosquito species (Shaalan *et al.,* 2005).

Phytochemical screening of *T. patula* and *P. thyrsiformis* revealed the presence of flavonoids, saponins, phenols, tannins, steroids, and terpenoids, compounds widely reported for their insecticidal activity. Terpenoids, in particular, have well-established insecticidal properties (Andrade-Ochoa *et al.,* 2018). Phenolic compounds such as tannins and flavonoids are known to function as mitochondrial toxins in insects (Mann & Kaufman, 2012). Other constituents, including alkaloids, saponins, and steroids, further enhance larvicidal efficacy (Shaalan *et al.,* 2005). GC-MS analysis identified several bioactive molecules associated with insecticidal activity. In *P. thyrsiformis*, carbamic acid derivatives were detected, which are known inhibitors of acetylcholinesterase (AChE) in the nervous system (Georgiev *et al.,* 2022). Inhibition of AChE disrupts synaptic transmission and leads to severe physiological abnormalities (Ghosh *et al.,* 2012). In *T. patula*, compounds such as thymol (Pavela *et al.,* 2009), cyprene (El-Sayed *et al.,* 2023), molinate (Stoker & Kavlock, 2010), and caryophyllene (Govindarajan *et al.,* 2016) were identified, all of which are reported to affect insect nervous systems and contribute to larvicidal activity.

Exposure to the extracts induced marked behavioural and morphological changes in *C. quinquefasciatus* across life stages. Early exposure resulted in hyperactivity, followed by sluggishness and immobility. Larvae frequently adopted an upward mouth posture at the water surface, indicating respiratory distress. Delayed development and impaired metamorphosis were observed, with some adults unable to fly, possibly due to growth-inhibitory compounds such as terpinen-4-ol (Govindarajan *et al.,* 2016). Histological observations revealed gut pigmentation, structural disorganisation, and midgut damage. Similar midgut epithelial disruption, vacuolization, and degeneration following botanical exposure have been documented in mosquito larvae treated with essential oils and plant-derived compounds (Lim *et al.,* 2023). Since the larval midgut plays a central role in digestion and nutrient absorption, structural damage to this tissue can severely impair larval survival and development. Additional abnormalities, including cuticular damage and impairment of siphon, setae, and anal papillae, may be attributed to hormonal disruption or interference with chitin synthesis (Soonwera & Phasomkusolsil, 2016). All treatments exhibited concentration-dependent larvicidal activity. Regression and probit analyses further confirmed a strong positive dose-response relationship between extract concentration and larval mortality. The high $R^2$ values obtained from regression analysis indicate a strong correlation between extract concentration and larval mortality. The combined formulation exhibited the lowest $LC_{50}$ values at both 24 h and 72 h exposure periods, indicating greater larvicidal potency compared to the individual extracts. At 72 h exposure, the $LC_{50}$ value of the combined formulation (426.58 ppm) was considerably lower than those of *T. patula* (489.78 ppm) and *P. thyrsiformis* (1047.1 ppm), further demonstrating its enhanced larvicidal potency. The decrease in $LC_{50}$ values with increasing exposure duration suggests progressive toxic effects of the phytochemicals on larval physiology. Among the individual

extracts, *T. patula* demonstrated greater efficacy, likely due to its higher terpenoid content. Similar larvicidal efficacy of Tagetes species against mosquito vectors has been documented previously. Nikkon *et al.* (2011) demonstrated significant mosquitocidal activity of *Tagetes erecta* against *C. quinquefasciatus*, attributing the effect to phytochemical-rich floral extracts. The presence of thymol in *T. patula* may contribute to its enhanced larvicidal efficacy, as thymol-rich essential oils have previously demonstrated strong toxicity against *C. quinquefasciatus* larvae (Pavela, 2009). The combined formulation outperformed both individual extracts, achieving over 50% mortality at 24 h and complete mortality at 72 h. The superior efficacy of the combined formulation compared to the individual extracts indicates possible synergistic interactions among phytochemicals. Synergistic botanical combinations are known to enhance larvicidal activity by simultaneously targeting multiple physiological pathways, including neurotransmission, respiration, feeding behaviour, and cuticle integrity (Scalerandi *et al.,* 2018). Such synergism may also reduce the likelihood of resistance development in mosquito populations.

The present study is among the few reports evaluating the combined larvicidal efficacy of *T. patula* and *P. thyrsiformis* against *C. quinquefasciatus*, integrating phytochemical profiling, histological alterations, regression analysis, and probit-based toxicity assessment. The findings highlight the potential of indigenous medicinal plants as sustainable alternatives for mosquito vector management. Although the laboratory findings are promising, field-based evaluations are necessary to determine the influence of environmental factors on efficacy. Comprehensive ecotoxicological assessments are also required before large-scale application. Future research should focus on formulation development, molecular mechanism studies, and integration into sustainable vector management strategies. Overall, methanolic extracts of *T. patula* and *P. thyrsiformis*, individually and in combination, demonstrate significant potential as plant-based larvicides, supporting their potential development as eco-friendly botanical larvicides for sustainable mosquito vector management.

## CONCLUSION

The findings of the present study demonstrate that the tested flower extracts vary in their effectiveness in suppressing mosquito larval growth and interfering with their progression to adult stages. The larvicidal activity was dose-dependent, with differential effects on moulting and developmental transitions. The combined formulation significantly exhibited greater potency than the individual extracts, causing targeted mortality of more than 50% of larvae within 24 hours of exposure. Among the treatments evaluated, the combined extract showed the highest larvicidal efficacy, followed by *T. patula* and *P. thyrsiformis*. The results suggest that these botanical formulations, particularly when used in combination, effectively disrupt larval moulting processes and inhibit successful metamorphosis into pupal and adult stages. The utilisation of native plant resources for mosquito larval control represents a sustainable and eco-friendly alternative to conventional synthetic insecticides. Botanical insecticides offer advantages such as reduced environmental toxicity, lower risk of resistance development, and cost-effectiveness in vector management programs. Furthermore, integrating such herb-based formulations into community-driven mosquito control initiatives could contribute significantly to reducing vector populations and disease transmission, thereby improving public health outcomes in endemic regions.

## ACKNOWLEDGEMENT

The authors sincerely acknowledge the Department of Zoology, Cotton University, for providing the necessary laboratory facilities and administrative support to carry out this research work.

## FUNDING

The first and second author gratefully acknowledge the financial support provided by the Assam Science Technology and Environment Council (ASTEC) under the Student Science Project scheme (Award No.: ASTEC/S&T/206/2023-24/2053–2057), which facilitated the successful completion of this work.

## REFERENCES

Adeyeyé, O. A., and Blum, M. S. 1989. Inhibition of growth and development of *Heliothis zea* (Lepidoptera: Noctuidae) by a nonprotein imino acid, L-azetidine-2-carboxylic acid. *Environ Entomol*, **18**(4): 608-611. https://doi.org/10.1093/ee/18.4.608

Aguiar, R. W. S., dos Santos, S. F., da Silva Morgado, F., Ascencio, S. D., de Mendonça Lopes, M., Viana, K. F., ..., Ribeiro, B. M. 2015. Insecticidal and repellent activity of *Siparuna guianensis* Aubl. (Negramina) against Aedes aegypti and *Culex quinquefasciatus*. *PloS One*, **10**(2): Article e0116765. https://doi.

org/10.1371/journal.pone.0116765 PMid:25646797 PMCid:PMC4315403

Alamgir, A. N. M. 2018. Methods of qualitative and quantitative analysis of plant constituents. In Therapeutic use of medicinal plants and their extracts: volume 2: Phytochemistry and bioactive compounds (pp. 721-804), Springer International Publishing, Cham. https://doi.org/10.1007/978-3-319-92387-1_9 PMCid:PMC7123938

Alhousari, F., and Greger, M. 2018. Silicon and mechanisms of plant resistance to insect pests. *Plants*, **7**(2): Article 33. https://doi.org/10.3390/plants7020033 PMid:29652790 PMCid:PMC6027389

Ali, A., Tabanca, N., Amin, E., Demirci, B., and Khan, I. A. 2016. Chemical composition and biting deterrent activity of essential oil of *Tagetes patula* (Marigold) against *Aedes aegypti*. *Nat Prod Commun*, **11**(10):1535-1538. https://doi.org/10.1177/1934578X1601101028

Andrade-Ochoa, S., Correa-Basurto, J., Rodríguez-Valdez, L. M., Sánchez-Torres, L. E., Nogueda-Torres, B., and Nevárez-Moorillón, G. V. 2018. *In vitro* and *in silico* studies of terpenes, terpenoids and related compounds with larvicidal and pupaecidal activity against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Chem Cent J*, **12**(1): Article 53. https://doi.org/10.1186/s13065-018-0425-2 PMid:29748726 PMCid:PMC5945571

Becker, N., Petrić, D., Zgomba, M., Boase, C., Madon, M. B., Dahl, C., and Kaiser, A. 2020. Mosquitoes: Identification, ecology and control. Springer Nature. pp 570.

Belkin, J. N. 1976. Mosquitoes of Middle America. https://apps.dtic.mil/sti/html/tr/ADA035346/ Accessed on 12 December 2025.

Benelli, G. 2015. Research in mosquito control: Current challenges for a brighter future. *Parasitol Res*, **114**(8): 2801-2805. https://doi.org/10.1007/s00436-015-4586-9 PMid:26093499

Bhattacharya, S., Basu, P., and Bhattacharya, C. S. 2016. The southern house mosquito, *Culex quinquefasciatus*: Profile of a smart vector. *J Entomol Zool Stud*, **4**(2): 73-81.

Dagar, P., and Ramakrishna, W. 2024. Plant based and synthetic products as mosquito repellents: Effects, target sites and their mechanism of action on mosquitoes. *Int J Trop Insect Sci*, **44**(4):1509-1530. https://doi.org/10.1007/s42690-024-01329-y

Das, S., Garver, L., and Dimopoulos, G. 2007. Protocol for mosquito rearing (*A. gambiae*). *J Vis Exp*, (5): Article 221. https://doi.org/10.3791/221

Dutta, U., and Dey, S. 2023. Bioassay of larvicidal efficacy of selected plant extracts against mosquito larvae *Anopheles Culicifacies* and *Aedes Aegypti* L. *Bull Pure Appl Sci Zool*, **42A**(1): Article 170. https://doi.org/10.48165/bpas.2023.42A.1.16

El-Sayed, M. H., Ibrahim, M. M., Elsobki, A. E., and Aioub, A. A. 2023. Enhancing the toxicity of cypermethrin and spinosad against *Spodoptera littoralis* (Lepidoptera: Noctuidae) by inhibition of detoxification enzymes. *Toxics*, **11**(3): Article 215. https://doi.org/10.3390/toxics11030215 PMid:36976980 PMCid:PMC10058348

Ganesan, P., Samuel, R., Mutheeswaran, S., Pandikumar, P., Reegan, A. D., Aremu, A. O., and Ignacimuthu, S. 2023. Phytocompounds for mosquito larvicidal activity and their modes of action: A review. *S Afr J Bot*, **152**: 19-49. https://doi.org/10.1016/j.sajb.2022.11.028

Georgiev, B., Nikolova, M., Aneva, I., Dzhurmanski, A., Sidjimova, B., and Berkov, S. 2022. Plant products with acetylcholinesterase inhibitory activity for insect control. *BioRisk*, **17**: 309-315. https://doi.org/10.3897/biorisk.17.77052

Ghosh, A., Chowdhury, N., and Chandra, G. 2012. Plant extracts as potential mosquito larvicides. *Indian J Med Res*, **135**(5): 581-598.

Govindarajan, M., Rajeswary, M., Hoti, S. L., and Benelli, G. 2016. Larvicidal potential of carvacrol and terpinen-4-ol from the essential oil of *Origanum vulgare* (Lamiaceae) against *Anopheles stephensi*, *Anopheles subpictus*, *Culex quinquefasciatus* and *Culex tritaeniorhynchus* (Diptera: *Culicidae*). *Res Vet Sci*, **104**: 77-82. https://doi.org/10.1016/j.rvsc.2015.11.011 PMid:26850541

Harborne, J. B. 1984. Methods of plant analysis. In Phytochemical methods. Springer, Dordrecht (pp. 1-36). https://doi.org/10.1007/978-94-009-5570-7_1

Holloway, S. J., Scott, J. G., Casida, J. E., and Ruzo, L. O. 1986. Bioactive aziridine derivatives of chrysanthemate insecticides. *J Agric Food Chem*, **34**(6): 1057-1060. https://doi.org/10.1021/jf00072a031

Karunaratne, S. H. P. P., and Surendran, S. N. 2022. Mosquito control: A review on the past, present and future strategies. *J Natl Sci Found Sri Lanka*: Article **50**. https://doi.org/10.4038/jnsfsr.v50i0.11244

Khaleel, C., Tabanca, N., and Buchbauer, G. 2018. α-Terpineol, a natural monoterpene: A review of its biological properties. *Open Chemistry*, **16**(1): 349-361. https://doi.org/10.1515/chem-2018-0040

Lancaster, J., and Downes, B. J. 2013. Aquatic entomology. OUP Oxford. pp. 304.

Lee, H., Halverson, S., and Ezinwa, N. 2018. Mosquito-borne diseases. *Prim Care*, **45**(3): 393-407. https://doi.org/10.1016/j.pop.2018.05.001 PMid:30115330

Lim, H., Lee, S. Y., Ho, L. Y., and Sit, N. W. 2023. Mosquito larvicidal activity and cytotoxicity of the extracts of aromatic plants from Malaysia. *Insects*, **14**(6): Article 512. https://doi.org/10.3390/insects14060512 PMid:37367328 PMCid:PMC10299645

Lima, S. C., de Oliveira, A. C., Tavares, C. P., Costa, M. L. L., and Roque, R. A. 2024. Essential oil from *Piper tuberculatu*m Jacq.(Piperaceae) and its majority compound β-caryophyllene: Mechanism of larvicidal action against *Aedes aegypti* (Diptera: Culicidae) and selective toxicity. *Environ Sci Pollut Res*, **31**(23): 33454-33463. https://doi.org/10.1007/s11356-024-33416-5 PMid:38684608

Lopes, R. P., Lima, J. B. P., and Martins, A. J. 2019. Insecticide resistance in *Culex quinquefasciatus* Say, 1823 in Brazil: A review. *Parasit Vect*, **12**(1): Article 591. https://doi.org/10.1186/s13071-019-3850-8 PMid:31852489 PMCid:PMC6921570

Lorenzo, M. G., Vidal, D. M., and Zarbin, P. H. 2014. Control of neglected disease insect vectors: Future prospects for the use of tools based on behavior manipulation-interference. *J Braz Chem Soc*, **25***:*1799-1809. https://doi.org/10.5935/0103-5053.20140202

Mandrioli, M., and Manicardi, G. C. 2015. Cytosine methylation in insects: New routes for the comprehension of insect complexity. *AIMS Biophys*, **2**(4): 412-422. https://dx.doi.org/10.3934/biophy.2015.4.412

Manimegalai, K., and Sukanya, S. 2014. Biology of the filarial vector, *Culex quinquefasciatus* (Diptera: Culicidae). *Int J Curr Microbiol Appl Sci*, **3**(4): 718-724.

Mann, R. S., and Kaufman, P.E. 2012. Natural product pesticides: Their development, delivery and use against insect vectors. *Mini Rev Org Chem*, **9**(2): 185-202. https://doi.org/10.2174/157019312800604733

Mathews, G., Derraik, J. G., Walker, M., Knox, R., and Barraclough, R. K. 2017. Morphological variation in invasive mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae) larvae from an urban site in Auckland, New Zealand. *N Z J Zool*, **44**(4): 342-353. https://doi.org/10.1080/03014223.2017.1342665

Naik, B. 2018. Biological Control of *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae), the ubiquitous vector for Lymphatic Filariasis: A review. In: Tyagi, B. (eds). Lymphatic Filariasis: Epidemiology, treatment and prevention - The Indian perspective, (pp. 281-292). https://doi.org/10.1007/978-981-13-1391-2_22

Narahashi, T. 2010. Neurophysiological effects of insecticides. In Hayes' handbook of pesticide toxicology (pp. 799-817). Academic Press. https://doi.org/10.1016/B978-0-12-374367-1.00031-8

Narahashi, T., Frey, J. M., Ginsburg, K. S., and Roy, M. L. 1992. Sodium and GABA-activated channels as the targets of pyrethroids and cyclodienes. *Toxicol Lett*, **64**: 429-436. https://doi.org/10.1016/0378-4274(92)90216-7 PMid:1281937

Ndione, R. D., Faye, O., Ndiaye, M., Dieye, A., and Afoutou, J. M. 2007. Toxic effects of neem products (*Azadirachta indica* A. Juss) on *Aedes aegypti* Linnaeus 1762 larvae. *Afr J Biotechnol*, **6**(24): 2846-2854.

Negi, C., and Verma, P. 2018. Review on *Culex quinquefasciatus*: Southern house mosquito. *Int J Life-Sci Sci Res*, **4**: 1563-1566. https://doi.org/10.21276/ijlssr.2018.4.1.9

Nikkon, F., Habib, M. R., Saud, Z. A., and Karim, M. R. 2011. *Tagetes erecta* Linn. and its mosquitocidal potency against *Culex quinquefasciatus*. *Asian Pac J Trop Biomed*, **1**(3): 186-188. https://doi.org/10.1016/S2221-1691(11)60024-5 PMid:23569756 PMCid:PMC3609190

Ningombam, D. S., and Singh, P. K. 2013. Ethnobotanical study of *Phologacanthus thyrsiformis* nees: A conserved medicinal plant of Manipur, Northeast India. *Int J Herb Med,* **1**(5): 10-14.

Olexa, M. T. 1992. Pesticide use and impact: FIFRA and related regulatory issues. *NDL Rev*, **68**: Article 445.

Pavela, R., Vrchotová, N., and Tříska, J. 2009. Mosquitocidal activities of thyme oils (*Thymus vulgaris* L.) against *Culex quinquefasciatus* (Diptera: Culicidae). *Parasitol Res*, **105**(5): 1365-1370. https://doi.org/10.1007/s00436-009-1571-1 PMid:19653003

Peng, Z., Wang, G., Zeng, Q. H., Li, Y., Liu, H., Wang, J. J., and Zhao, Y. 2022. A systematic review of synthetic tyrosinase inhibitors and their structure-activity relationship. *Crit Rev Food Sci Nutr*, **62**(15): 4053-4094. https://doi.org/10.1080/10408398.2021.1871724 PMid:33459057

Raguvaran, K., Kalpana, M., Manimegalai, T., Kalaivani, S., Devapriya, P., Siddharthan, N., ... & Maheswaran, R. 2022. Larvicidal, antioxidant and biotoxicity assessment of (2-(((2-ethyl-2 methylhexyl) oxy) carbonyl) benzoic acid isolated from *Bacillus pumilus* against *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus*. *Arch Microbiol*, **204**(10): Article 650. https://doi.org/10.1007/s00203-022-03264-3 PMid:36173486

Reuben, R., Tewari, S. C., Hiriyan, J., and Akiyama, J. 1994. Illustrated keys to species of Culex (Culex) associated with *Japanese encephalitis* in Southeast Asia (Diptera: Culicidae). *Mosq Syst*, **26**(2): 75-96.

Rossi, G. D., Santos, C. D., Carvalho, G. A., Alves, D. S., Pereira, L. L. S., and Carvalho, G. A. 2012. Biochemical analysis of a castor bean leaf extract and its insecticidal effects against *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae). *Neotrop Entomol*, **41**(6): 503-509. https://doi.org/10.1007/s13744-012-0078-0 PMid:23949676

Scalerandi, E., Flores, G. A., Palacio, M., Defagó, M. T., Carpinella, M. C., Valladares, G., ... & Palacios, S. M. 2018. Understanding synergistic toxicity of terpenes as insecticides: Contribution of metabolic detoxification in Musca domestica. *Front Plant Sci*, **9**: Article 1579. https://doi.org/10.3389/fpls.2018.01579 PMid:30420868 PMCid:PMC6218333

Demirak, M. Ş. S., and Canpolat, E. 2022. Plant-based bioinsecticides for mosquito control: Impact on insecticide resistance and disease transmission. *Insects*, **13**(2): Article 162. https://doi.org/10.3390/insects13020162 PMid:35206735 PMCid:PMC8878986

Shaalan, E. A. S., Canyon, D., Younes, M. W. F., Abdel-Wahab, H., and Mansour, A. H. 2005. A review of botanical phytochemicals with mosquitocidal potential. *Environ Int*, **31**(8): 1149-1166. https://doi.org/10.1016/j.envint.2005.03.003 PMid:15964629

Shaikh, J. R., and Patil, M. 2020. Qualitative tests for preliminary phytochemical screening: An overview. *Int J Chem Stud*, **8**(2): 603-608. https://doi.org/10.22271/chemi.2020.v8.i2i.8834

Shroff, S., Mir, S., Naik, B., Baitharu, I., and Behera, A. K. 2021. Chemical methods for control of mosquito vector. In Molecular identification of mosquito vectors and their management (pp. 35-50). Springer, Singapore. https://doi.org/10.1007/978-981-15-9456-4_3 PMid:32031712

Snell, A. E. 2005. Identification keys to larval and adult female mosquitoes (Diptera: Culicidae) of New Zealand. *N Z J Zool*, **32**(2): 99-110. https://doi.org/10.1080/03014223.2005.9518401

Song, Y., Villeneuve, D. L., Toyota, K., Iguchi, T., and Tollefsen, K. E. 2017. Ecdysone receptor agonism leading to lethal molting disruption in arthropods: Review and adverse outcome pathway development. *Environ Sci Technol*, **51**(8): 4142-4157. https://doi.org/10.1021/acs.est.7b00480 PMid:28355071 PMCid:PMC6135102

Soonwera, M., and Phasomkusolsil, S. 2016. Effect of *Cymbopogon citratus* (lemongrass) and *Syzygium aromaticum* (clove) oils on the morphology and mortality of *Aedes aegypti* and *Anopheles dirus*

larvae. *Parasitol Res*, **115**(4): 1691-1703. https://doi.org/10.1007/s00436-016-4910-z PMid:26796022

Stoker, T. E., and Kavlock, R. J. 2010. Pesticides as endocrine-disrupting chemicals. In Hayes' handbook of pesticide toxicology, (pp. 551-569). Academic Press. https://doi.org/10.1016/B978-0-12-374367-1.00018-5

Tiwari, R. K., and Chanda, S. 2016. Quantitative analysis of secondary metabolites in aqueous extract of *Clerodendrum serratum*. *Int Res J Pharm,* **7**(12): Article 61-65. https://doi.org/10.7897/2230-8407.0712148

Tobin, P. C., Onufrieva, K. S., and Thorpe, K. W. 2013. The relationship between male moth density and female mating success in invading populations of *Lymantria dispar*. *Entomol Exp Appl*, **146**(1): 103-111. https://doi.org/10.1111/j.1570-7458.2012.01299.x

Tripathi, A. K., Prajapati, V., Ahmad, A., Aggarwal, K. K., and Khanuja, S. P. 2004. Piperitenone oxide as toxic, repellent, and reproduction retardant toward malarial vector *Anopheles stephensi* (Diptera: Anophelinae). *J Med Entomol*, **41**(4): 691-698. https://doi.org/10.1603/0022-2585-41.4.691

Wang, X., Lu, H., and Li, J. 2024. Mechanisms of mosquito-mediated pathogen transmission to humans. *J Mosq Res*, **14**(4): 172-183. https://doi.org/10.5376/jmr.2024.14.0017

WHO, G. 1989. Geographical distribution of arthropod-borne diseases and their principal vectors. https://iris.who.int/handle/10665/60575

Winsor, M. P. 1976. The development of Linnaean insect classification. *Taxon*, **25**(1):57-67. https://doi.org/10.2307/1220406

World Health Organization. 2005. Guidelines for laboratory and field testing of mosquito larvicides, pp. 1-39. https://www.who.int/publications/i/item/WHO-CDS-WHOPES-GCDPP-2005.13

Xu, G., Zhang, Y. Y., Gu, G. X., Yang, G. Q., and Ye, G. Y. 2022. Molecular and pharmacological characterization of β-adrenergic-like octopamine receptors in the endoparasitoid *Cotesia chilonis* (Hymenoptera: Braconidae). *Int J Mol Sci*, **23**(23): Article 14513. https://doi.org/10.3390/ijms232314513 PMid:36498840 PMCid:PMC9740559

Youssefi, M. R., Tabari, M. A., Esfandiari, A., Kazemi, S., Moghadamnia, A. A., Sut, S., ... & Maggi, F. 2019. Efficacy of two monoterpenoids, carvacrol and thymol, and their combinations against eggs and larvae of the West Nile vector *Culex pipiens*. *Molecules*, **24**(10): Article 1867. https://doi.org/10.3390/molecules24101867 PMid:31096594 PMCid:PMC6572342

Zheng, L., Xu, H., Wen, Q., Zhou, N., and Zheng, X. 2025. Toxicity and sublethal effects of calcium cyanamide against susceptible strains of *Aedes albopictus*. *Chin J Schistosomiasis Control,* **37**(2): 196-200, 222.

## AUTHOR CONTRIBUTION

Uma Dutta contributed to the conceptualization and design of the study, project design and submission for obtaining financial assistance, supervision of the research work, execution of experimental work, photography and documentation of all experimental procedures, data collection, material preparation, manuscript drafting, editing, proofreading, and overall coordination of the study. Nirodha Pegu contributed to funding acquisition, execution of experimental work in replicates, data collection, and review and editing of the manuscript. Sonali Dey contributed to manuscript drafting, literature review, statistical data analysis, preparation of figures and tables, and critical review and editing of the manuscript. Shruti Baruah and Nayanika Saikia contributed to the literature search, compilation of relevant references, and proofreading of the manuscript. The first draft of the manuscript was prepared by Uma Dutta, Nirodha Pegu, and Sonali Dey. All authors reviewed and commented on previous versions of the manuscript. All authors read and approved the final manuscript.