Research Article

Exploring its Repellent and Histopathological Efficacy of *Tinospora cordifolia* against Vector Borne Mosquitoes

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ABSTRACT

Aim: *Tinospora cordifolia*, commonly known as Giloy, is a medicinal plant extensively used in traditional medicine systems such as Ayurveda for its diverse pharmacological properties. This study investigates its potential as a larvicidal agent, histological effects on *Aedes aegypti* larvae and repellent properties against adult mosquitoes. **Materials and Methods:** The research employed standardized larvicidal assays to assess the efficacy of *Tinospora cordifolia* extracts against *Aedes aegypti* larvae at various concentrations. Histological analyses were conducted to examine morphological alterations in larvae exposed to the extracts. Additionally, repellent activity was evaluated using established methods to determine the effectiveness of the plant extracts in repelling adult mosquitoes. **Results:** The findings reveal significant larvicidal activity of *Tinospora cordifolia* extracts against *Aedes aegypti* larvae; the ethanol extract results with LC_{50} and LC_{90} values of 225.089 and 2386.308 µL/mL, respectively, with mortality rates increasing in a concentration-dependent manner. Histological examinations demonstrate distinct alterations in larval morphology following exposure to the extracts, suggesting potential disruption of physiological processes. Furthermore, the repellent assays indicate promising repellent properties of the plant extracts against mosquitoes, potentially reducing their biting and disease transmission capabilities. **Conclusion:** This study underscores the potential of *Tinospora cordifolia* as a natural alternative for controlling *Aedes aegypti*, offering larvicidal effects, histological alterations in larvae and repellent activity against mosquitoes. Further research is warranted to elucidate the underlying mechanisms and optimize formulations for practical application in vector control strategies.

Keywords: *Tinospora cordifolia, Aedes aegypti*, Larvicidal activity, Histological analysis, Repellent activity.

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INTRODUCTION

One of the most significant challenges in developing countries is the increase of vector-borne diseases. Mosquito management at the hatching stage provides the advantage of preventing the mosquito from becoming infected and transmitting the infection. More than a million people die each year from vector-borne diseases such as chikungunya, dengue, encephalitis, zika, malaria, filariasis and dengue.[1] The most widely recognised disease is carried by mosquito vectors and a projected population of almost 700 million people are globally each year by a number of mosquito-borne infections protozoa, parasitic worms and arboviruses.[2] *Aedes aegypti* is a major vector of viruses that are harmful to human and animal well-being, including yellow fever, dengue fever and chikungunya fever.[3] Dengue fever is often regarded as the world's most hazardous disease and the most severe virus-borne arthropod infection. Dengue transmission threatens approximately half of the world's

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population.[4] Organophosphates and organochlorines are two chemical insecticides that are commonly used to control these vectors.[5,6] However, the usage of these synthetic compounds may lead to difficulties such as environmental contamination, activity against non-target organisms and resistance in treated mosquito strains, all of which undermine efficient vector control.[7] It is vital to find natural items with larvicidal action as options for the control of dengue and other arboviruses due to the epidemiological importance of arboviruses transmitted by *Ae. aegypti* and *Ae. albopictus* and due to the difficulties in controlling vector mosquitoes. In this regard, research has demonstrated that plant extracts are effective and practical options for the treatment of *Aedes* spp.[8,9] As a result, the development of alternate methods and approaches for controlling disease vectors using natural or botanical materials has emerged as a new area of investigation. Biologically active compounds are abundant in plants, which are also thought to be safer than synthetic pesticides. Due to their biodegradability and lack of toxicity, plant materials offer a safer alternative to manmade chemicals.[10,11]

Guduchi, also known as *Tinospora cordifolia*, is a naturally occurring herbal shrub that is a member of the *Menispermaceae* family, which also includes moonseed. This plant has a long history of usage in traditional medicine techniques for treating a variety of illnesses, diabetes, gout, skin conditions, jaundice and others. Guduchi is a nectar plant that is known as amrita in Sanskrit due to its immune-stimulating and revitalizing qualities. Alkaloids are the active ingredients found in *T. cordifolia's* stem and root sections. These include palmetine, jatrorrhizine, aporphine alkaloids, tembetarine, choline, magnoflorine, berberine, tinosporin, isocolumbin and tetrahydropalmatine. which demonstrated immunomodulatory, anti-diabetic, anti-viral, anti-inflammatory and anti-psychiatric properties.[12,13] Insecticides are used to kill or repel adult mosquitoes in order to disrupt the disease transmission cycle or to target mosquito breeding places by spraying stagnant water on them.[14,15] Plant preparations have been utilized traditionally all across the world to repel mosquitoes among various populations.[16] Natural or synthetic repellents are harmless, but their flavor and odor can influence insect behavior. Mosquitoes are inherently attracted to human body temperature, respiration and carbon dioxide emissions; nevertheless, repellents can keep mosquitoes away from exposed skin.[17] Repellent efficacy can be increased by altering their chemical structures to increase their stability and ability to stay in touch with the skin for extended periods of time.[18,19] The effectiveness of plant products as mosquito repellents is determined by a variety of parameters, including repellent material, application method, ambient factors, exposure time and insect pest sensitivity.[20,21] We present the findings of a study done to determine the biological effectiveness of a new formulation of insect repellent against man-biting mosquitoes in the field. The purpose of the current study is to examine the effectiveness of the substance derived from *Tinospora cordifolia* against mosquito larvae, histological study as well as mosquito repellent study under field conditions.

MATERIALS AND METHODS

Plant Materials

T. cordifolia fresh stem were obtained from Nagerkoil, medicinal plant garden; they were recognised and verified by Botany department, Loyola College, Chennai. Fresh stems were cleaned and allowed to air dry for 15 days at room temperature in a well-ventilated room in a shady location.

Preparation Of Extract

To extract crude extract consecutively, the partially dried stem was crushed into a hard, delicate powder with an electric grinder. The plant extracts were created using various solvents in order to study their potential larvicidal activity as well as their photochemical components. In a nutshell, a 200 g sample of powdered *T. cordifolia* stem was extracted with an ethanol solvent and immersed in ethanol for 48 hr. The plant samples were filtered using Whatman Filter Paper No. 1. The residue was extracted at room temperature and the crude solvent extracts were evaporated. A freeze dryer was used to dry aqueous extracts, which were then stored in a refrigerator at 4ºC.[22]

Insect Rearing

Eggs of *Aedes aegypti* were gathered at the Loyola College Entomology Research Institute in Chennai. They were raised in tap water with a 13:11 L/D photoperiod cycle and a temperature of 27±2ºC. Dog biscuits and Brewer's yeast were fed to the larvae in a 3:2 ratio. The experiment utilised larvae in their third instar.[23]

Larvicidal Bioassay

The early third-generation larvae of laboratory-raised mosquitoes were evaluated in larval bioassays under proper circumstances in accordance with WHO guidelines, with a few minor alterations.[21] The plant extracts were produced in the relevant solvent with the correct amount of an emulsifier, DMSO (0.01%) and applied to 10 early third instar larvae in 100 ml disposable plastic cups. The different concentration were

maintained from 100, 150, 200, 250, 500, 1000, 2000 and 4000 ppm. Each concentration had five replicates set up and the tests were repeated. The larvae were not given food during the experiment and all other growth parameters remained unchanged. 24 hr after treatment, the larval mortality was noticed and noted. All immobile mosquito larvae were determined to be dead.

The formula below was used to convert mortality into percent mortality:

$$\textit{Percentage of mortality of larvae}\ (\%) = \left(\frac{\textit{number of killed larvae}}{\textit{number of experimental larvae}}\right)\times 100$$

Histological Study

Histological examination of mosquito larvae exposed to concentration was carried out. The afflicted larvae were stored in a 10% buffered formaldehyde solution for 24 hr before being dehydrated with ethanol and cleaned with xylene. Melted paraffin was used to permanently embed them inside the block. The paraffin blocks were stained with hematoxylin and eosin before being cut into 5 m pieces with a rotary microtome. The extract's toxicity on larvae was noticed at a magnification of 40× under a bright-field microscope. A stereomicroscope was used to observe tissue buildup and changes caused by extracts.

Repellent Activity

The study was conducted in Maranthalai, a village in Thoothukudi district in state of Tamilnadu, India. The gloves were weared for two hands and make cut (5 cm × 5 cm) in the gloves at the dorsal palm side and fully covered the legs and entire body (only the dorsal side of the palms of both hands were exposed through the cut region). The extract were dissolved in ethanol and prepared the concentrations at 0.01, 0.2, 0.5, 0.7 and 1.0 mg/mL. Before starting the activity, the exposed area of the forearms of both hands was cleaned with ethanol and 0.01% concentration was applied on the exposed area (5 cm x 5 cm) of right hand (test). Left hand is untreated control (only ethanol). The first mosquito landing were observed in both control and treated hands. The post-application repellents were observed each 45 min, 90 min, 120 min and 180 min. The time between the application of extract and first landing of the mosquito is the protection time. The experiment was repeated with different concentrations. Experiments were conducted to find out the mean protection time of the extract against mosquitoes. The period between applying the test sample and the first two consecutive bites was regarded the complete-protection time, which is the common criterion used to measure a sample's repellent efficacy. Each test was repeated on different days for each of the human volunteers.

The formula determined the repellency %.

$$\text{Repellency as a percentage} = [(Ta - Tb) / Ta]\ 100$$

Where Ta represents the number of mosquitoes in the control group and Tb represents the number of mosquitoes in the treatment group.

Statistical Analysis

During the study of the plant extract's larvicidal potential, the % mortality was calculated using Abbott's formula.[24] Probit analysis was utilized to calculate the LC_{50} and LC_{90} values. We calculated the chi-square value, lethal concentrations for 50% and 90% of the population (LC_{50}-LC_{90}) and the Fiducial Limit (FL) with 95% Confidence (CL). The value of p less than 0.05 indicated statistical significance and each dose's entire protection period was calculated using the time between repellent application and the first mosquito bite.

RESULTS

Larvicidal activity

The larvicidal activity of the ethanol extract of *Tinospora cordifolia* against *Aedes aegypti* was evaluated at various concentrations ranging from 100 ppm to 8000 ppm. The results demonstrated a dose-dependent increase in mortality rates after 24 hr. At the lowest concentration (100 ppm), the mortality was negligible (0.2±0.44%), whereas at the highest concentrations (4000 ppm and 8000 ppm), the mortality reached 100% (5±0%). The median Lethal Concentration (LC_{50}) was determined to be 225.089 µL/mL with a confidence interval of 96.145-526.966 µL/mL. Additionally, the LC_{99} value was calculated as 2386.308 µL/mL with a confidence interval of 1019.290-5586.701 µL/mL. The chi-square (χ^2) value for the test was 0.011, indicating the statistical significance of the results. The larvicidal activity results demonstrated that exposure to stem crude extract of *Tinospora cordifolia* might result in 100% mortality of the dengue vector mosquito larvae; there was no sign of mortality in the third instar larvae group of the maintained control. After 24 hr treatment, the results were observed. The ethanol extract results with LC_{50} and LC_{90} values of 225.089 and 2386.308 µL/mL, respectively (Table 1) against *Aedes aegypti*. At the 0.05 level, the χ^2 value (0.011) was statistically significant. There were no deaths in the control group. LC_{50} is a deadly concentration that kills 50% of exposed organisms, LC_{90} is a lethal concentration that kills

Table 1: Larvicidal activity of ethanol extract of *Tinospora cordifolia* against *Aedes aegypti*.

Concentration ppm	24 hr mortality mean±SD	LC_{50} (µL/mL) (LCL-UCL)	LC_{99} (µL/mL) (LCL-UCL)	χ^2
Control	00±00			
100	0.2±0.44			
150	1.2±0.83			
200	2.4±0.54	225.089	2386.308	0.011
250	3.6±0.54	(96.145-526.966)	(1019.290-5586.701)	
500	5±0.44			
1000	4.8±0.44			
2000	4.8±0.44			
4000	5±0			
8000	5±0			

90% of exposed organisms, UCL is the 95% upper confidence limit, LCL is the 95% lower confidence limit, χ^2 is chi-square and values are mean±SD of five replicates.

Histological Study

Under the bright-field microscope, the primary observations would likely involve the structural integrity of various tissues in the larvae. Common histopathological changes might include cell lysis, tissue necrosis, or disruption in organ structures like the midgut, cuticle, or muscles. At 40× magnification, specific cellular changes such as vacuolation, nuclear pyknosis (condensation) and cytoplasmic granulation can be detected. These are indicators of cellular damage and toxicity. A stereo microscope would allow for the observation of three-dimensional tissue architecture. The buildup of abnormal tissue, swelling and discoloration might be evident. These macroscopic changes could indicate severe responses to the extract. The outcomes demonstrated that the treated larvae had significant damage to their midgut cells. The treatment caused damage to larval body sections, including epithelial cells, food bolus, basement membrane, muscles and midgut. The larval gut lumen was primarily damaged and was treated individually. The study found that treated larvae's midgut epithelial cells exhibited changed histological features, but control larvae have no changes (Figure 1).

Repellent Study

The repellent activity of the ethanol extract of *Tinospora cordifolia* was assessed at different concentrations (0.01 mg/mL to 1.0 mg/mL) over various time intervals (45, 90, 120 and 180 min post-application). At the lowest concentration (0.01 mg/mL), the repellency was high (82.50±6.29%) at 45 min but dropped to 0% by 120 min. At higher concentrations, such as 0.5 mg/mL, repellency remained above 90% even after 180 min. The highest concentration (1.0 mg/mL) provided complete repellency (100%) for up to 90 min and maintained a high repellency rate (96.25±2.39%) after 180 min. These findings indicate that the ethanol extract of *Tinospora cordifolia* is effective as a mosquito repellent, especially at higher concentrations. Results from the skin-repellent activity of an ethanol extract of *T. cordifolia* against vector mosquitoes throughout the day are shown in this observation. The current finding demonstrates the relationship between dose and time (min) and the percentage of protection. The crude extract of *Tinospora cordifolia* significantly repel against mosquitoes. It demonstrates that repellency depends on the concentration of crude extract (Table 2).

DISCUSSION

The ethanol extract of *Tinospora cordifolia* demonstrated high efficacy in killing *Aedes aegypti* larvae, with an LC_{50} value of 225.089 µL/mL and an LC_{99} value of 2386.308 µL/mL. In comparison, other studies have investigated the larvicidal activity of various plant extracts against *Aedes aegypti*. Mittal PK study on the larvicidal activity of *Azadirachta indica* extracts reported an LC_{50} value of approximately 12.3 ppm for methanolic extracts.[25] Research on *Ocimum basilicum* essential oil found an LC_{50} value of 15.9 ppm. These comparisons indicate that *Tinospora cordifolia* has a relatively higher LC_{50} value, suggesting it might be less potent on a per-unit basis compared to neem and basil extracts. However, the complete mortality at higher concentrations (4000 ppm and 8000 ppm) underscores its potential effectiveness at these levels. The dose-dependent increase in larval mortality observed in this study aligns with findings from other botanical larvicides. For instance, a study on *Eucalyptus globulus* oil showed a similar dose-dependent trend, with increasing concentrations leading to higher mortality rates. This consistency supports the reliability of using

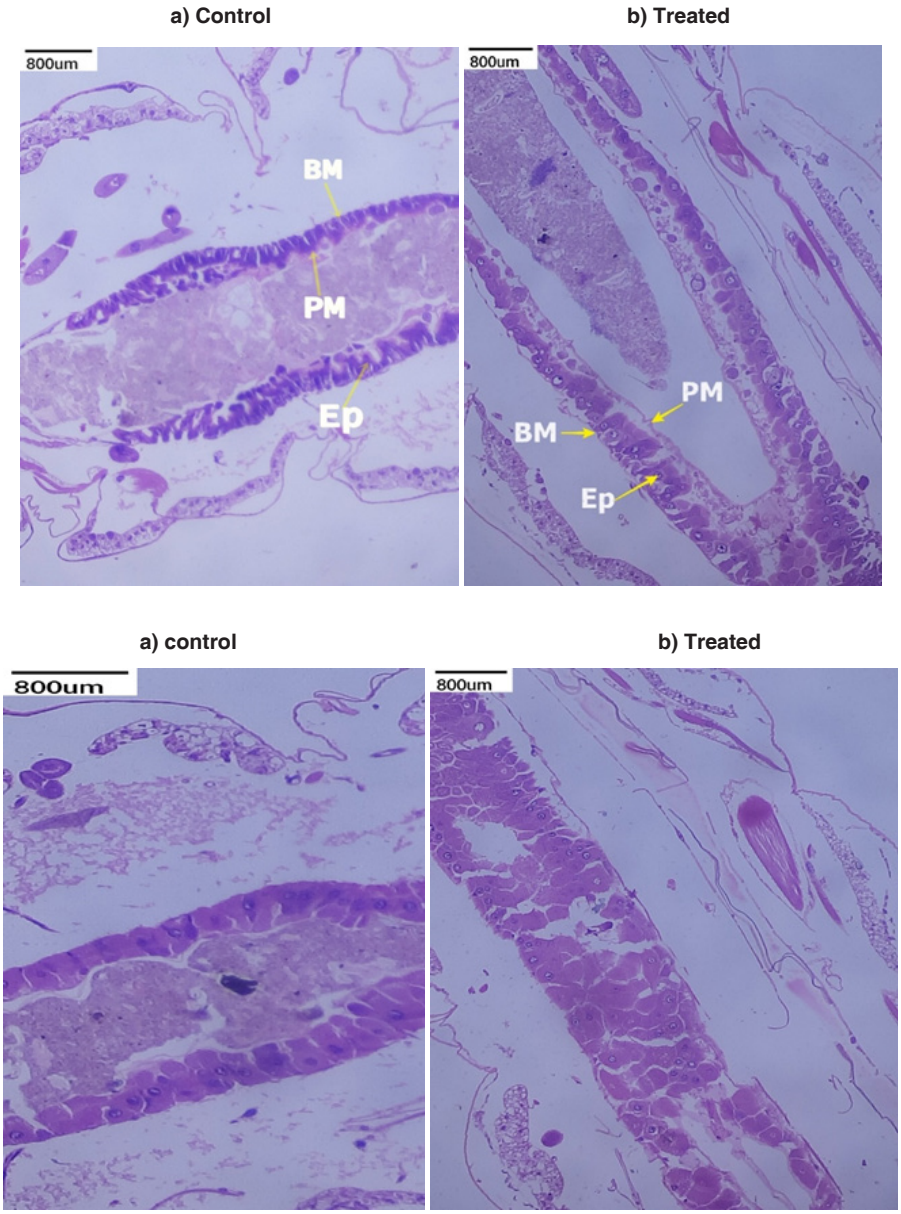

Figure 1: a) Histological studies on gut of control shows intact normal epithelial cells b) Third- instar larvae of gut region of treated larvae shows vacuolization and disintegration of gut epithelial cells. BM-Basement Membrane; PM-Peritrophic Membrane;
Ep-Epithelial cells (Gut epithelial cells); FM-Food material.

Table 2: Repellent activity of ethanol extract of *Tinospora cordifolia* against the mosquitoes in the field.

Concentration mg/mL	% of repellency±SD			
	Time post application of repellent (min)			
	45 min	90 min	120 min	180 min
0.01	82.50±6.29	50.00±10.21	0.0±0.0	0.0±0.0
0.2	97.50±1.44	80.00±5.40	37.50±23.94	18.75±11.97
0.5	96.25±2.39	93.75±2.39	92.5±1.44	91.25±1.25
0.7	97.5±1.44	97.5±1.44	93.75±2.39	91.25±1.25
1.0	100±0.0	100±0.0	97.50±2.5	96.25±2.39

plant extracts for mosquito control. The chi-square value (χ^2=0.011) indicates that the results are statistically significant, reinforcing the validity of the findings. Ghosh et.al., reported statistically significant results in similar larvicidal assays, such as those evaluating the efficacy of *Cymbopogon citratus* extracts.Despite the higher LC_{50} value compared to some other plant extracts, *Tinospora cordifolia* offers complete larval mortality at higher concentrations, suggesting its practical use in high-burden areas where mosquito populations are dense.[26] Additionally, the ease of availability and traditional use of *Tinospora cordifolia* in various regions could facilitate its acceptance and implementation as a larvicidal agent. It observed that larvae exposed to neem extract exhibited significant midgut damage, including epithelial cell vacuolization and disintegration of the peritrophic matrix. The similar midgut damage and epithelial cell changes are noted in histological examination; this could suggest that extract has a comparable mode of action as neem extract, potentially disrupting digestive processes in the larvae. It reported that essential oils caused cuticle thinning and muscle fiber disorganization in mosquito larvae. The afflicted larvae in our study showed cuticle thinning or muscle disorganization, it indicates that the extract impacts structural integrity and mobility, similar to the essential oils. The extract might have a comparable larvicidal mechanism, disrupting midgut integrity and leading to larval mortality. The effects of an ethanolic extract of *Azadirachta indica* on mosquito larvae and found severe midgut epithelial damage, including vacuolization and cell lysis. The damage to midgut epithelial cells in our study mirrors the findings in suggesting that *Tinospora cordifolia* extract similarly disrupts digestive functions and cellular integrity. The histopathological effects observed, particularly to the midgut and muscles, are consistent with extract of *Annona squamosa* reported by indicating a common mode of action where plant extracts of *Tinospora cordifolia* compromise larval gut integrity and muscle function. The study evaluated the repellent activity of the ethanol extract of *Tinospora cordifolia* against mosquitoes at concentrations ranging from 0.01 mg/mL to 1.0 mg/mL. In this study, *Tinospora cordifolia* at 1.0 mg/mL provided 100% repellency for up to 90 min and maintained 96.25±2.39% repellency at 180 min. This high level of efficacy is comparable to other well-known plant-based repellents. For instance, *Azadirachta indica* oil has been reported to provide 100% repellency at 1% concentration for up to 8 hr. Similarly, *Cymbopogon citratus* oil at a concentration of 2% showed over 95% repellency for up to 4 hr. Another study by Fradin and Day indicated that *Cymbopogon nardus* oil provided significant repellency but required reapplication every 30 to 60 min for sustained effect.[27] The sustained repellency observed with *Tinospora cordifolia* extract, particularly at higher concentrations, is noteworthy. *Eucalyptus globulus* oil provided effective repellency for up to 120 min, with efficacy diminishing thereafter. In the context of practical applications, the prolonged repellency of *Tinospora cordifolia* extract, especially at concentrations of 0.5 mg/mL and above, suggests its potential for use in areas with high mosquito activity. The results of this study are statistically significant, underscored by consistently high repellency rates and minimal standard deviations. This aligns with the findings of Moore *et al*., who emphasized the importance of statistical rigor in evaluating repellent efficacy. Practical considerations such as ease of availability and traditional use of *Tinospora cordifolia* further enhance its appeal as a natural mosquito repellent.[28] The high and sustained repellency of *Tinospora cordifolia* ethanol extract, especially at higher concentrations, positions it as a viable natural alternative to synthetic repellents. Its efficacy compares favorably with other botanical repellents such as neem and citronella, which are widely used and studied. The effectiveness of ethanol extract of *Tinospora cordifolia* plant showed toxicity against *A. aegypti* larvae. This natural plant products and its significance poses a new larvicide for controlling the mosquito vector. Using plant-based extracts like *Tinospora cordifolia* is environmentally friendly and reduces the reliance on synthetic chemicals, which can have harmful ecological and health impacts. Mosquitoes are less likely to develop resistance to natural compounds, which often contain a mixture of active ingredients, compared to single-ingredient synthetic insecticides. *Tinospora cordifolia* contains various bioactive compounds (alkaloids, glycosides, steroids, flavonoids) that may work synergistically to provide effective larvicidal and repellent activity. Natural plant extracts are biodegradable and do not persist in the environment, reducing the risk of long-term ecological damage. The use of *Tinospora cordifolia* stem extract as a larvicide and repellent offers significant strengths, particularly in terms of environmental safety and cost-effectiveness. However, the variability in efficacy, challenges in standardization and potential toxicity to non-target species present considerable limitations. Addressing these limitations through further research and development can enhance the practical application of this natural insecticide in mosquito control programs.

CONCLUSION

The ethanol extract of *Tinospora cordifolia* shows promising larvicidal activity against *Aedes aegypti*, with complete

mortality achieved at higher concentrations. While it may be less potent on a per-unit basis compared to some other botanical extracts, its effectiveness at higher concentrations and statistical significance supports its potential use in mosquito control programs. Future studies could focus on optimizing the extraction process or combining *Tinospora cordifolia* with other larvicidal agents to enhance its efficacy. The ethanol extract of *Tinospora cordifolia* demonstrated potent larvicidal activity, primarily through causing severe histopathological damage to the midgut epithelial cells, basement membrane, gut lumen and muscles of the mosquito larvae. This damage is likely responsible for the extract's effectiveness in killing larvae. Comparisons with other studies reveal that similar plant extracts often target the midgut and associated tissues, leading to disrupted digestion and compromised larval health. Based on the histological examination results, it appears that the extract induces significant tissue damage in mosquito larvae, as evidenced by the observations under both bright-field and stereomicroscopes. The comparison with other studies reveals that similar histopathological effects have been reported with various biopesticides and natural extracts, suggesting a possible commonality in the mode of action. The ethanol extract of *Tinospora cordifolia* shows strong potential as an effective mosquito repellent, particularly at higher concentrations. The ethanol extract of *Tinospora cordifolia* shows strong potential as an effective mosquito repellent, particularly at higher concentrations. Its high efficacy and prolonged action make it a promising candidate for natural mosquito control strategies, aligning well with other established botanical repellents in terms of effectiveness and duration. *Tinospora cordifolia* has been evaluated to have unique range of larvicidal properties that could be applied as a conventional mosquito repellent. Based on the findings of this investigation, we have concluded that ethanol extract contains strong larvicidal bioactive principles against mosquitoes. Additionally, it proves that ethanol extract of *Tinospora cordifolia* has maximum zone of repellency against mosquitoes and is affordable, safe, eco-friendly and simple to use. These extracts may require additional purifications in order to have synthetic analogues, which will be conducted in the future.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper

ABBREVIATIONS

***T. cordifolia*:** *Tinospora cordifolia*; ***Ae. aegypti*:** *Aedes aegypti*; ***C. quinquefasciatus*:** *Culex quinquefasciatus*; **LC_{50}:** Lethal concentration; **UCL:** Upper confidence limit; **LCL:** Lower confidence level; **SD:** Standard deviation; **FL:** Fiducial limit; **CL:** Confidence limit.

SUMMARY

This study investigates the potential larvicidal, histological and repellent properties of *Tinospora cordifolia* against *Aedes aegypti*, a mosquito species known for transmitting diseases like dengue fever, Zika virus and chikungunya. The research likely explores how extracts or components of *Tinospora cordifolia* affect the larvae of *Aedes aegypti*, examining both their mortality rates and any changes observed in the histology microscopic structure of the larvae after exposure. Additionally, the repellent activity of *Tinospora cordifolia* against mosquitoes evaluated. This could involve experiments to determine the effectiveness of *Tinospora cordifolia* in repelling mosquitoes from landing or biting. Overall, the study aims to contribute to our understanding of natural methods for controlling mosquito populations and preventing the spread of mosquito-borne diseases.

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