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Larvicidal efficacy and histological evaluation of *Cyperus rotundus* extracts against disease-vectoring mosquitoes

[*Eficácia larvicida e avaliação histológica de extratos de* Cyperus rotundus *contra mosquitos vetores de doenças*]

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ABSTRACT

Mosquitoes are among the most significant disease vectors, transmitting dangerous illnesses such as malaria and dengue fever. With the increasing resistance of mosquitoes to chemical insecticides, there is a growing need for effective and environmentally safe natural alternatives. The larvicidal impact of *Cyperus rotundus* extracts was assessed against three disease-vector mosquito species: *Culex quinquefasciatus*, *Anopheles stephensi*, and *Aedes aegypti*, alongside morphological and histopathological alterations in larvae. Sequential extractions of *C. rotundus* using methanol, ethanol, chloroform, hexane, and aqueous solvents were tested at various concentrations (50–250 ppm). Amongst the extracts, methanol showed the highest efficacy, with LC_{50} values of 111.13 for *Ae. aegypti*, 108.61 ppm for *An. stephensi*, and 98.46 ppm for *Cx. quinquefasciatus*. Phytochemical analysis revealed that methanol and ethanol extracts were rich in bioactive compounds like tannins, flavonoids, alkaloids, and saponins, contributing to their larvicidal potency. Histological studies revealed significant midgut damage in treated larvae, including epithelial cell detachment, microvillus degradation, and peritrophic membrane disruption. These findings highlight the possible of *C. rotundus* extracts, predominantly methanol, as promising larvicidal agents for integrated mosquito management programs. Further investigation into the active compounds and their modes of action could contribute to developing eco-friendly, plant-based insecticides for controlling mosquito populations and mitigating disease transmission.

Keywords: *Ae. aegypti*, *A. stephensi*, *Cx. quinquefasciatus*, Methanolic extract, Phytochemicals

RESUMO

Os mosquitos estão entre os vetores de doenças mais importantes, transmitindo doenças perigosas como a malária e a dengue. Com o aumento da resistência dos mosquitos aos inseticidas químicos, há uma necessidade crescente de alternativas naturais eficazes e ambientalmente seguras. Este estudo avalia a eficácia larvicida dos extratos de Cyperus rotundus *contra* Aedes aegypti, Anopheles stephensi *e* Culex quinquefasciatus, *além de alterações morfológicas e histopatológicas nas larvas. Extrações sequenciais de* C. rotundus *usando metanol, etanol, clorofórmio, hexano e solventes aquosos foram testadas em várias concentrações (50-250 ppm). Entre os extratos, o metanol apresentou a maior eficácia, com valores de* LC_{50} *de 111,13 para* Ae. aegypti, *108,61 ppm para* An. stephensi *e 98,46 ppm para* Cx. quinquefasciatus. *A análise fitoquímica revelou que os extratos de metanol e etanol eram ricos em compostos bioativos como taninos, flavonoides, alcaloides e saponinas, contribuindo para sua potência larvicida. Estudos histológicos revelaram danos significativos ao intestino médio em larvas tratadas, incluindo descolamento de células epiteliais, degradação de microvilosidades e rompimento da membrana peritrófica. Essas descobertas destacam o potencial dos extratos de* C. rotundus, *especialmente o metanol, como agentes larvicidas promissores para programas integrados de controle de mosquitos. Uma investigação mais aprofundada sobre os compostos ativos e seus modos de ação poderia*

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contribuir para o desenvolvimento de inseticidas ecológicos à base de plantas para controlar as populações de mosquitos e mitigar a transmissão de doenças.

Palavras-chave: Ae. aegypti, A. stephensi, Cx. quinquefasciatus, *extrato metanólico, fitoquímicos*

INTRODUCTION

Vector control is an indispensable part of the global strategy for the effective and sustainable management of mosquito-borne diseases. Although the use of conventional insecticides such as organophosphates, organochlorines, pyrethroids, pyrethrins, carbamates, cyclodienes, and phenylpyrazoles has yielded positive results against disease-causing mosquito vectors, the indiscriminate and massive use of these insecticides has led to resistance among mosquito vectors and raised significant concerns about non-target effects on environmental safety and the human health (Benelli & Mehlhorn, 2016, Shroff *et al.*, 2020, Anoopkumar and Aneesh, 2022). This problem has prompted the development of a new generation of insecticides known as biorational insecticides. These products include analogs of juvenile hormones, insecticides that prevent molting, and biological insecticides (Picard *et al.*, 2021).

Traditionally, various plants and their products have been used to eliminate mosquitoes and other harmful agents (Said-Al Ahl *et al.*, 2017). The activity of secondary plant metabolites provides potential protection against herbivorous worms (Mohamed *et al.*, 2021). Many phytocompounds and mixtures derived from different plant families have been evaluated for their promising larvicidal activities. The insecticidal properties of approximately 2,000 species of dried plants have been reported with no harmful effects on the ecosystem (Raveen *et al.*, 2017). Among these plants is *C. rotundus*. The larvicidal and ovicidal activities of the essential oils from the rhizomes of this plant against the larvae of Aedes albopictus were reported (Kempraj and Bhat, 2008).

Recent discoveries have shown the potential effects of plant compounds such as saponins, alkaloids, steroids, essential oils, isoflavonoids, and tannins as larvicidal agents against mosquitoes. Plant secondary metabolites and their derivatives offer another source for mosquito control (Bekele, 2018).

Secondary metabolites synthesized by plants exert multifaceted effects on insects at the organismal, tissue, and cellular levels, owing to their broad-spectrum biological activity (Marciniak *et al.*, 2019, Adamski *et al.*, 2020, Selim *et al.*, 2021). Generally, these compounds interfere with physiological and cellular mechanisms essential for maintaining homeostasis, inducing sublethal alterations in various tissues and organs that may culminate in organismal death (Chowański *et al.*, 2016). The mode of action of natural insecticides is diverse; some primarily affect the nervous system, while others target the digestive system by disrupting the gut epithelium during transmembrane passage or by inducing genotoxic effects, leading to physiological and structural alterations in mosquitoes (Oguh *et al.*, 2019).

In certain cases, The detection of tissue damage induced by control agents is essential for elucidating their mechanisms of action and validating their efficacy in insect targets (Evans *et al.*, 2019) (Lahlali *et al.*, 2022). Allelochemicals are known to exert detrimental effects on the digestive epithelial cells and further decrease the survivability of the insect. According to Klowden (2013), The larval midgut serves as a key interface between the organism and its environment; therefore, understanding its morphology and physiology is crucial for evaluating the efficacy of control strategies.

The main objective of this study was to evaluate the larvicidal effectiveness of *C. rotundus* against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*, and to analyze the morphological and histopathological alterations it causes, highlighting the potential for its use in integrated mosquito control programs.

MATERIALS AND METHODS

Cyperus rotundus was obtained from the campus of Presidency College and its vicinity in Chennai, Tamil Nadu, India. The botanical specimen was recognized by Prof. P. Jayaraman, Plant Anatomy Research Centre (PARC), West Tambaram, Chennai-600 045, India. After

collection, the plant material was air-dried at ambient temperature to eliminate surplus moisture. After complete desiccation, the grass was pulverized into a fine powder utilizing a home blender and then subjected to sieving. A 100-gram sample of powdered grass was sequentially extracted using a Soxhlet apparatus with n-hexane, chloroform, ethanol, and methanol solvents, each for a period of 20 hours. An aqueous extract was obtained by immersing 100 g of dried grass powder in 200 ml of double-distilled water, refrigerating for 7 days, and subsequently filtering using Whatman No. 1 filter paper. All extracts were concentrated by a rotary evaporator and preserved in airtight containers at 5°C until subsequent use (Babu *et al.*, 2018).

The study used Senthilkumar & Reetha, (2009) and Nweze *et al.*, (2004) methodologies to analyze plant extracts for bioactive components. Different reagents were used to identify alkaloids, tannins, flavonoids, glycosides, saponins, steroids, terpenoids, and phenols,. Results were documented qualitatively.

Mosquito species considered in this research included *Cx. quinquefasciatus*, *An. stephensi*, and *Ae. aegypti*. Late-instar (fourth) larvae of these species, with no prior exposure to insecticides or pathogens, were sourced from the Entomology Research Institute (ERI), Loyola College, Chennai, India. The larvae were housed in shallow, rectangular rearing trays made of plastic, filled with dechlorinated water to provide a suitable environment for growth. These trays were maintained at a controlled temperature of 25 to 29°C, with a 12:12 hour light-dark cycle to mimic natural conditions. To ensure optimal health, the water in the trays was regularly monitored and changed to prevent stagnation and contamination. The larvae were fed a diet of powdered dog biscuits and yeast mixed in a 3:1 ratio, providing essential nutrients for their growth and development. This feeding regimen was carried out daily, ensuring the larvae had access to a consistent food source. Once the larvae transformed into pupae, transferred to mesh-covered rearing boxes using a soft brush و allowing them to develop into adult mosquitoes.

Larval susceptibility assessments were conducted in accordance with the protocol established by the WHO (World Health Organization, 2024). The crude extract was initially dissolved in 1 mL of acetone to obtain a stock solution at a concentration of 1000 ppm followed by dilution to 100 ml with distilled water. Based on preliminary tests, fourth instar larvae were treated with concentrations of 50, 100, 150, 200, and 250 ppm, each in five replicates with 20 larvae per replicate. Larval bioassays were conducted in 500 mL beakers, each containing 250 mL of water and designated concentrations of the plant extracts. The beakers were maintained at a controlled room temperature of 28 ± 2 °C. A control group was prepared using 250 mL of water containing 0.1 mL of acetone. We documented the death of larvae subjected to all concentrations after 24 hours. Larvae were deemed deceased when they ceased active for an extended duration. The absence of larval movement upon gentle probing with a spatula was used to confirm mortality If the larva remains immobile despite gentle prodding, it is deemed deceased. LC_{50} and LC_{95} values were determined for each concentration at 24 hours utilizing Probit analysis in SPSS 28.

To investigate the impact of methanol extracts from *C. rotundus* on the gut morphology of *An. stephensi* larvae fourth instar larvae was exposed to LC_{50} concentration of 1 108.61, following the same protocol described above. Six live larvae were randomly selected from each treatment and control group after 24 hours of exposure and preserved in 10% formalin. Subsequently, they underwent a sequential dehydration process using escalating concentrations of ethyl alcohol (30%, 50%, 70%, 90%, and 100%), followed by immersion in xylene, and were subsequently embedded in paraplast at a temperature of 58–60 °C (MERCK). Tissue samples were sectioned at a thickness of 7 µm using a Leica RM223 rotary microtome, then stained with hematoxylin and eosin. The stained sections were examined and photographed under a Leica DF450 microscope to evaluate morphological alterations in the midgut.

The effects of different extracts on mosquito mortality rates were analyzed using ANOVA, Levene's test for homogeneity of variances, and Tukey's Honestly Significant Difference (HSD) test. Statistical significance was determined at $p < 0.05$. Mortality rates in treated groups were corrected for control mortality using Abbott's formula (Abbott, 1925)

RESULTS

The phytochemical screening of *C. rotundus* with different solvent extracts showed the presence of various phytocompounds in methanol and ethanol extracts. Methanol extract showed the strong presence of carbohydrates, tannins, saponins, flavonoids, alkaloids, anthocyanin, cardiac glycosides, terpenoids, triterpenoids, acids and steroids (Table 1).

Table 1. Phytochemical analysis of *C. rotundus* grass weed extracts

S. No	Secondary metabolites	Methanol	Hexane	Ethanol	Aqueous	Chloroform
1	Alkaloids	+++	-	+	-	+
2	Anthocyanin	+++	++	+++	+	-
3	Cardiac glycosides	+++	-	+	++	+
4	Carbohydrate	+++	+++	+++	+++	+++
5	Coumarins	-	-	-	-	+
6	Fatty acids	+++	+	++	+	-
7	Flavonoids	+++	-	+	-	-
8	Glycosides	-	-	-	-	-
9	Phenols	++	+	-	+	+
10	Protein	-	-	-	-	-
11	Quinones	-	+	-	+	-
12	Saponins	+++	+	+++	++	+
13	Steroids	+++	-	+++	++	-
14	Tannins	+++	-	+++	+	-
15	Terpenoids	+++	-	+++	+	-
16	Triterpenoids	+++	-	+++	++	+

+++ Strongly positive ++ Positive + Trace amount - Absent

The study evaluated the larvicidal effects of five extracts from *C rotundus* aqueous, chloroform, ethanol, hexane, and methanol against three mosquito species: *Cx. quinquefasciatus An. stephensi*, and *Ae. aegypti*. Significant differences in mortality rates and lethal doses (LC_{50} and LC_{95}) were observed across the extracts and mosquito species (Tables 2 and 3).

The methanol extract was the most effective overall, achieving 100% mortality at lower concentrations compared to the other extracts. For *Ae. aegypti* and *An. stephensi*, the methanol extract caused 100% mortality at 200 ppm, with LC_{50} values of 111.13 ppm and 108.61 ppm, respectively, and LC_{95} values of 226.39 ppm and 223.88 ppm. Meanwhile, *Cx. quinquefasciatus* exhibited 100% mortality at 250 ppm, with LC_{50} and LC_{95} values of 98.46 ppm and 213.73 ppm, respectively.

The ethanol extract ranked second in effectiveness, with LC_{50} values ranging from 83.85 ppm for *An. stephensi* to 123.20 ppm for *Cx. quinquefasciatus*. The LC_{95} values for ethanol ranged from 202.45 ppm to 241.79 ppm. The chloroform extract also exhibited strong larvicidal activity, achieving LC_{50} values of 159.93 ppm for *Ae. aegypti*, 117.14 ppm for *An. stephensi*, and 149.62 ppm for *Cx. quinquefasciatus*. The LC_{95} values for chloroform were 306.44 ppm, 263.64 ppm, and 296.12 ppm, respectively, demonstrating good efficacy but requiring higher concentrations than methanol and ethanol.

Table 2. Effects of different concentrations of *C. rotundus* extracts on mortality rates of *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus* (mean ± standard deviation) 24 hours post-treatment

Mosquito Species	Mortality (Mean ± SD)					
	Concentration (ppm)	Aqueous	Chloroform	Ethanol	Hexane	Methanol
Ae. aegypti	50	17 ± 2.74	15 ± 3.54	22 ± 2.74	10 ± 3.54	28 ± 5.70
	100	32 ± 2.74	29 ± 2.24	48 ± 2.74	21 ± 4.18	59 ± 4.18
	150	51 ± 5.48	47 ± 2.74	71 ± 4.18	34 ± 2.24	90 ± 3.54
	200	67 ± 2.74	63 ± 2.74	89 ± 4.18	53 ± 2.74	100 ± 0.0
	250	86 ± 2.24	79 ± 5.48	100 ± 0.0	66 ± 4.18	100 ± 0.0
An. stephensi	50	23 ± 2.74	13 ± 4.47	30 ± 3.54	15 ± 3.54	36 ± 2.24
	100	52 ± 4.47	39 ± 4.18	73 ± 2.74	37 ± 2.74	78 ± 2.74
	150	70 ± 3.54	63 ± 2.74	94 ± 4.18	51 ± 4.18	100 ± 0.0
	200	89 ± 4.18	88 ± 5.70	100 ± 0.0	67 ± 2.74	100 ± 0.0
	250	100 ± 0.0	100 ± 0.0	100 ± 0.0	84 ± 2.24	100 ± 0.0
Cx. quinquefasciatus	50	12 ± 2.74	14 ± 4.18	18 ± 2.74	12 ± 2.74	21 ± 2.24
	100	31 ± 2.23	33 ± 5.70	39 ± 4.18	26 ± 4.18	47 ± 2.74
	150	48 ± 4.47	52 ± 4.47	64 ± 4.18	41 ± 4.18	69 ± 4.18
	200	66 ± 2.24	68 ± 2.74	88 ± 5.70	55 ± 3.54	98 ± 2.74
	250	82 ± 4.47	84 ± 2.24	100 ± 0.0	69 ± 4.18	100 ± 0.0

Table 3. Lethal concentrations (LC_{50} and LC_{95}) of *C. rotundus* extracts against *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus* with 95% confidence intervals after 24 hours of treatment

Extracts	Mosquito Species	LC_{50} (ppm)	95% Confidence Interval (Lower - Upper)	LC_{95} (ppm)	95% Confidence Interval (Lower - Upper)
Aqueous	*Ae. aegypti*	148.09	137.01 – 159.15	297.67	280.42 – 317.98
	An. stephensi	97.24	84.76 – 109.07	246.81	231.19 – 264.88
	Cx. quinquefasciatus	156.72	145.67 – 167.85	306.30	288.70 – 327.06
Chloroform	*Ae. aegypti*	159.93	149.15 – 170.84	306.44	289.31 – 326.54
	An. stephensi	117.14	105.47 – 128.46	263.64	247.84 – 281.96
	Cx. quinquefasciatus	149.62	138.74 – 160.49	296.12	279.43 – 315.66
Ethanol	*Ae. aegypti*	118.28	108.68 – 127.66	236.87	223.89 – 251.83
	An. stephensi	83.85	73.13 – 94.02	202.45	190.07 – 216.46
	Cx. quinquefasciatus	123.20	113.61 – 132.61	241.79	228.65 – 256.95
Hexane	*Ae. aegypti*	226.85	208.53 – 247.90	461.85	420.82 – 515.67
	An. stephensi	222.42	204.32 – 243.10	457.42	416.82 – 510.66
	Cx. quinquefasciatus	196.83	179.84 – 215.47	431.83	393.85 – 481.53
Methanol	*Ae. aegypti*	111.13	101.55 – 120.42	226.39	214.00 – 240.57
	An. stephensi	108.61	98.95 – 117.98	223.88	211.49 – 238.05
	Cx. quinquefasciatus	98.46	88.45 – 108.09	213.73	201.41 – 227.74

In contrast, the hexane extract showed the lowest mortality rates at the lowest concentrations across all mosquito species. For *Ae. aegypti*, the hexane extract caused only 10% mortality at 50 ppm, which was the lowest among all the extracts. Similarly, in *An. stephensi*, the mortality rate was 15% at 50 ppm, and for *Cx. quinquefasciatus*, the mortality rate was 12% at 50 ppm. The hexane extract also had higher LC_{50} and LC_{95} values across all mosquito species, with LC_{50} values reaching 226.85 ppm and LC_{95} values reaching 461.85 ppm, indicating lower potency.

A two-way ANOVA confirmed that extract type ($F = 55.511$, $p < 0.001$) and concentration ($F = 214.172$, $p < 0.001$) had significant effects on mosquito mortality. The interaction between extract and concentration was also significant ($F = 2.816$, $p = 0.006$), while the interactions between mosquito species and extract, as well as

between mosquito species and concentration, were not statistically significant.

In summary, methanol was the most potent extract across all mosquito species, followed by ethanol and chloroform. The hexane extract required the highest concentrations to achieve mortality, making it the least effective. These findings highlight the potential of methanol, ethanol, and chloroform extracts of *C. rotundus* as effective larvicidal agents for controlling *Cx. Quinquefasciatus, An. Stephensi* and *Ae. Aegypti*.

The whole-body morphology of the control fourth instar larvae of *Cx. quinquefasciatus*, *An. stephensi* and *Ae. Aegypti* exhibited well-defined head, thorax, and abdomen (Fig 1 A, B and C). However, after a 24-hour exposure to methanolic extract of *C. rotundus* at LC_{50} concentrations the treated fourth instar larvae displayed severe morphological deformities in the head, thorax, and abdominal segments. The midgut region was ruptured, with the midgut contents visibly oozing out. Damages were also evident in the anal gills, where the outer cuticular membrane was ruptured and the anal gills appeared indistinct. In *Cx. quinquefasciatus*, the larvae showed swelling of the siphon, with damage to the epithelial layer of the outer cuticle, and a pronounced loss of external hairs in the abdominal segments. Additionally, the respiratory tube was narrowed and damaged at the posterior end, leading to the siphon (Fig1 D, E and F).

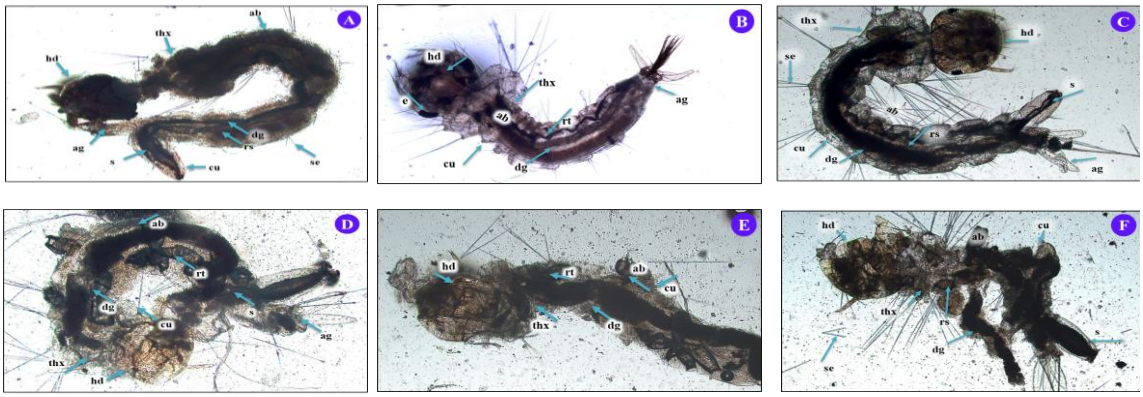


Figure 1. Light micrographs of (A, b and c) control and (D, E and F) treated Late-instar (fourth) larvae of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* (From left to right) with methanolic extract of *C. rotundus* after 24h of exposure (200x magnification) hd- head, thx- thorax, se- siphon, ab- abdomen, dg- digestive gland, rt- respiratory tract, cu- cuticle, ag- anal gill, e- eye

The histological examination of the midguts of *An. stephensi* 4th instar larvae treated with the methanol extract of *C. rotundus* reveals significant structural alterations in comparison to the untreated larvae. In the untreated group (Figure 2A), the midgut appears to maintain its typical architecture, displaying well-organized components. The microvilli, essential for nutrient absorption, are intact and aligned, while the epithelial cells, peritrophic membrane, and nuclei show no signs of damage or disruption. Additionally, the longitudinal muscles surrounding the gut appear normal, reflecting the healthy state of the untreated larvae.

In stark contrast, the midgut of the larvae treated with *C. rotundus* methanol extract (Figure 2B) exhibits considerable damage after 24 hours of exposure. There are marked signs of degeneration throughout the midgut tissue. The microvilli, critical for digestion and nutrient absorption, appear heavily degraded, indicating that the extract has compromised the larvae's ability to process food. Similarly, the epithelial cells, which form the lining of the gut and play a protective role, are degenerating. The nuclei within these cells show clear signs of degradation, suggesting cellular death or severe dysfunction. Moreover, the peritrophic membrane, which typically serves as a protective barrier between the gut contents and the epithelial cells, is also in a state of degradation.

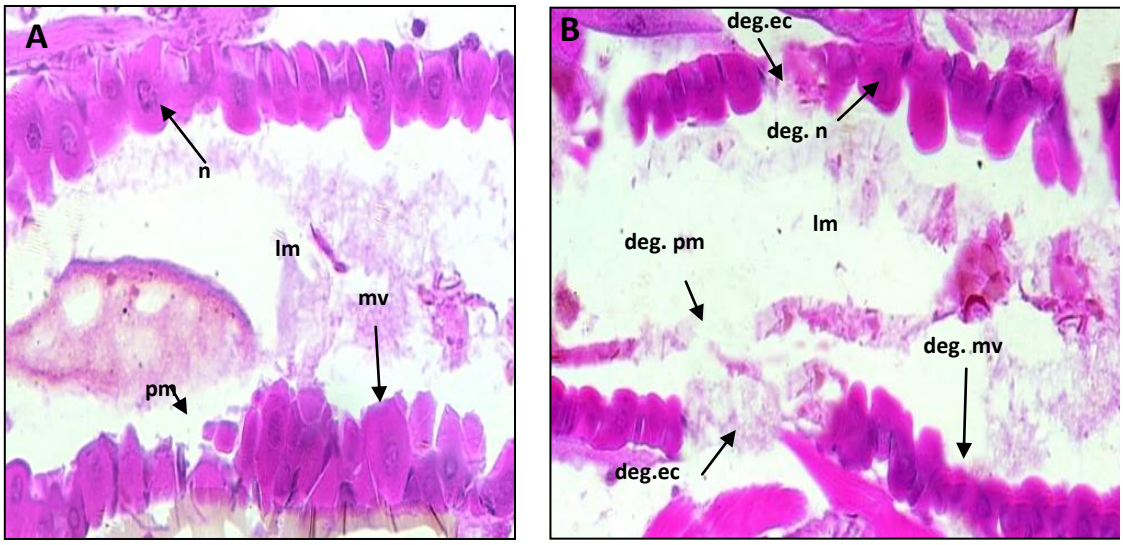


Figure 2. Photomicrographs of midgut cross-sections from fourth-instar *An. stephensi* larvae at 24 hours post-treatment with the methanol extract of *C. rotundus*. (A) Midgut of untreated larvae showing normal morphology. (B) Midgut of treated larvae showing structural damage, including degraded microvilli (deg. mv), degenerating epithelial cells (deg. ec), degenerating peritrophic membrane (deg. pm), and degenerating nuclei (deg. n)

These results collectively suggest that the methanol extract of *C. rotundus* causes significant disruption to the midgut's cellular structures, potentially leading to impaired digestion and physiological functions in *An. stephensi* larvae. This damage likely contributes to the high mortality observed in larvae treated with the extract, demonstrating its potential as an effective larvicidal agent.

DISCUSSION

The methanol extract of *C. rotundus* demonstrated significant larvicidal activity against *Ae. aegypti*, *An. stephensi*, and *Cx. quinquefasciatus*. This aligns with a study showing that methanolic extracts of *C. rotundus* caused 60% mortality in first instar larvae at 50 ppm of *An. gambiaeand* 100% mortality at 350 ppm after 24 hours of exposure (Théophile *et al.*, 2020). Another study found that methanol extracts of *C. rotundus* reached 95% mortality in *Cx pipiens* within 24 hours at an LC50 of 235.41 ppm (Baz *et al.*, 2024) The larvicidal activity of *C. rotundus* extracts was also confirmed in a study where the petroleum ether extract caused 98% mortality in *Ae. aegypti* larvae at 1,000 ppm, and the ethyl alcohol extract resulted in 97% mortality at the same concentration. In our study, methanol extract proved to be the most effective, with lower lethal concentrations compared to these other solvents (Imam *et al.*, 2013).

Further chemical analysis of *C. rotundus* essential oil revealed presence of various phytocompounds, including hydrocarbons, alcohols, aldehydes, and phenols, supporting its potential for insecticidal activity. Similarly, another studies identified a significant number of phenolics, fatty acids, terpenes, and alkaloids in *Cyperus esculentus*, which may explain the larvicidal properties (Jingjing *et al.*, 2024, Bezerra *et al.*, 2023). The observed larvicidal activity of *C. rotundus* may be attributed to the presence of alkaloids, terpenoids, triterpenoids and which may jointly or independently contribute to the bioactivity (Hostettmann and Potterat, 1997).

In comparison, a study found that essential oil extracted with *C. rotundus* using diethyl ether was more toxic to first instar larvae of *Ae. aegypti* than ethanol extract, though ethanol proved more effective in adult mosquitoes (Al-Massarani *et al.*, 2016). Additionally, *C. rotundus* methanolic root extracts were found to have significant insecticidal activity against pests like *Aphis craccivora* and *Planococcus lilacinus* (Singh *et al.*, 2024). Another related study

showed that *Eragrostis repens* methanol extracts had significant larvicidal activity with LC_{50} values ranging from 43,173 ppm to 58,234 ppm for mosquito species (Prakash *et al.*, 2024), emphasizing the lower lethal concentrations of *C. rotundus* extracts.

The LC_{50} of the methanolic extract of *C. rotundus* revealed significant histological alterations in the midgut of fourth instar larvae of *An. stephensi*. Notably, these alterations included the detachment of epithelial cells from the basement membrane, often resulting in pronounced elongations into the lumen and disruption of the brush border. The most severe effects were observed as increasing damage to the midgut epithelium, characterized by cell vacuolization, ruptured epithelial walls, microvillus damage, and leakage of cellular contents into the midgut lumen (Manimegalai *et al.*, 2020, Javed *et al.*, 2019, Gomes *et al.*, 2017). Additionally, alterations in the anterior and posterior regions of the midgut were noted, such as detachment of epithelial cells and damage to the peritrophic membrane, leading to compromised intestinal tissue integrity. These disruptions may facilitate the mixing of gut cell contents with hemolymph, potentially contributing to larval mortality (Fouzi *et al.*, 2024, Sadaqat *et al.*, 2021, Napoleão *et al.*, 2019). Observations of damage to the peritrophic membrane and epithelial cells were also reported in studies involving other plant extracts, further emphasizing the consistency of these effects across different treatments (Al-Mekhlafi, 2018). The severe damage observed in midgut cells suggests a significant disruption of their normal function, ultimately leading to larval death.

The histopathological effects observed in *Ae. aegypti* larvae treated with plant extracts like Charantagenin D further illustrate the cytopathological impacts on the midgut epithelium and associated structures (Rodrigues *et al.*, 2021, Raguvaran *et al.*, 2023). Additional studies have documented severe tissue damage in the gut and surrounding areas after treatments with various essential oils, reinforcing the notion of similar adverse effects caused by different phytochemicals (Zayed *et al.*, 2012, Medhi *et al.*, 2010). This underscores the potential of phytochemicals in inducing significant disruptions in the gut architecture of mosquito larvae. Moreover, the findings regarding the effects of the methanolic extract of *C. rotundus* on the midgut of *An. stephensi* larvae are consistent with results from studies on Culex pipiens, where similar histopathological changes were noted (Abutaha *et al.*, 2022) (Hussein *et al.*, 2018). Research indicates that treatments with certain extracts result in swollen epithelial cells and alterations in the peritrophic membrane (Shalaby *et al.*, 2022, Hashem *et al.*, 2018). The mechanisms by which phytochemicals exert these effects include interference with mitochondrial function, specifically at proton transforming sites, as well as direct impacts on the midgut epithelium, gastric caeca, and Malpighian tubules in mosquito larvae (Menegazzi *et al.*, 2020). Compounds like flavonoids disrupt the respiratory system and inhibit electron transport, leading to reduced ATP production and oxygen consumption (Souto *et al.*, 2021, Ikram *et al.*, 2021). Additionally, tannins can inactivate crucial enzymes and proteins within the larvae, further contributing to their mortality (Leona *et al.*, 2021).

CONCLUSION

This study demonstrated that *C. rotundus* extracts possess significant larvicidal activity against the larvae of *Ae. aegypti*, *An. stephensi*, and *Cx quinquefasciatus*. The methanolic extract, in particular exhibited the highest larvicidal efficacy, suggesting its potential as a natural alternative to chemical insecticides. The presence of bioactive compounds, such as phenols and flavonoids, in the extract likely contributed to the observed toxic effects on mosquito larvae. These findings underscore the importance of exploring plant-based insecticides as eco-friendly strategies in mosquito control programs. Further research is recommended to isolate the specific active compounds and assess their mechanisms of action, as well as evaluate their safety for non-target organisms and the environment.

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