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Original Research Article

Mosquito Larvicidal Potentiality of *Clematis gouriana* Against Filarial Vector *Culex quinquefasciatus*

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ABSTRACT:
Alternative biocontrol techniques based on botanicals are chosen over synthetic pesticides because botanicals are less prone to acquiring resistance and are more environmentally friendly. In this study, the effectiveness of *Clematis gouriana* (*C. gouriana*) against growing wrigglers of *Culex quinquefasciatus* (*Cx. quinquefasciatus*) was demonstrated. Leaves of *C. gouriana* were collected and fractionated using chloroform/methanol (1:1, v/v). In a dose-dependent technique, the crude and solvent extracts were tested for larvicidal activity for 72 hours. Cent percent larvicidal efficacy was found in a concentration of 5% crude and 250 ppm solvent extract, with the most effective LC_{50} value (22.78 ppm) against first instars. FT-IR spectrum indicated the presence of aromatics, alkanes, alcohols, amines, and amine salts in chloroform/methanol (1:1, v/v) extract. Overall, the present study demonstrated the potential of *C. gouriana* as a natural source for vector management programmes.

Keywords: *Clematis gouriana*, *Culex quinquefasciatus*, Bio-control, Elephantiasis, Mosquito-borne diseases

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INTRODUCTION

Mosquitoes spread a variety of diseases, including malaria, filariasis, dengue fever, and Japanese encephalitis, resulting in millions of fatalities each year. Lymphatic filariasis is the second most severe of the vector-borne infections, after malaria. In tropical and humid parts of the world, *Wuchereria bancrofti*, carried by *Culex quinquefasciatus*, is primarily responsible for filarial burden. In India, over 31 million people are filarial parasite carriers, and over 23 million persons suffer from filarial disease symptoms (World Health Organization, 2005a). Mosquito larvae are an interesting target for insecticides because they develop in water and are thus easier to control in this environment. The usage of traditional chemical pesticides has resulted in resistance (Severini et al., 1993; WHO, 1970), unfavourable impacts on non-target species, and environmental and human health problems (Forget O., 1989). Herbal treatments are one of the most effective ways to keep

mosquitoes at bay. For scientists working on alternate vector control, finding herbal formulations that have no negative effects on non-target species and are easily biodegradable remains a key research priority (Redwane et al. 2002).

Clematis gouriana is deciduous climber, capable of climbing up tall trees. The leaf and stem juices of *C. gouriana* are used by traditional medical practitioners in the Bhadra Wild Life Sanctuary, India, to cure infectious old wounds, psoriasis, dermatitis, blood illnesses, leprosy, and liver and cardiac ailments (Manjunatha et al., 2004). The leaf extract showed significant antioxidant, hepatoprotective, and antiproliferative properties (Naika et al., 2019).The roots of *C. gouriana* are used to cure malarial fever and headaches, while the root and stem paste are used to treat psoriasis, itching, and skin allergies (Harsha et al., 2003). However, little research has been done on anti-mosquito properties.

The rationale of the present trial was to explore the larvicidal properties of crude and chloroform/methanol (1:1, v/v) extracts of *C. gouriana* foliages against filarial vector *Cx. quinquefasciatus*. This study provides first ever report on the mosquito larvicidal activity of this plant leaf extract as a source of larvicidal agent against filarial vector *Cx. quinquefasciatus* as a target species under laboratory conditions.

MATERIALS AND METHOD

Preparation of crude extract

During the research period, fresh, mature, green leaves of *C. gouriana* were collected at random from medicinal plant Garden of Sreegopal Banerjee College. Initially, all of the leaves were washed in distilled water and dried on a paper towel. The crude extracts were made by pulverizing the plant material in a mortar and pestle and filtering it using Whatman No. 1 filter paper. The filtrate was kept as a stock solution (at 100% concentration) for future bioassays. The concentrations (0.5 percent, 0.4percent 0.3percent, 0.2 percent, 0.1 percent,) were made by diluting the stock solution with distilled water.

Phytoextract preparation

Fresh, mature leaves of *C. gouriana* were washed with distilled water, soaked on paper towel and dried in shed with good air draft. The dried leaves were finely ground in an electric blender. Furthermore, powdered leaves (25 g) were decocted in stopper container with 250 ml chloroform/methanol (1:1, v/v) for 3 weeks with frequent agitation. The extracts were then filtered with Whattman grade filter paper and the solvent was evaporated. The solid residues of evaporated solvent extract was mixed with a set volume of double distilled water to make working concentration gradients ranging from 50 ppm (w/v) to 250 ppm (w/v).

Collection and maintenance of larvae

To establish the colony, egg strips of *Cx. quinquefasciatus* were initially collected with acute precision from adjoining drains of Sreegopal Banerjee college campus (22°59' N, 88°22'E) and were grown at the research laboratory, Department of Zoology, Sreegopal Banerjee College, West Bengal, India. Colonies were maintained following the technique of Sharma and Saxena (1994), with slight change and were kept in insectaries (45 cm×30 cm×10 cm) at 27±1°C , 80±2 percent relative humidity with a photoperiod of 13:11 hour light and dark cycles. Larvae were fed a supplemental meal of finely crushed brewer yeast and dog biscuits (3:1) at a regular interval, and laboratory conditioning of the raised larvae was performed for further evaluation.

Dose dependent larvicidal bioassay

The larvicidal bioassay was carried out at the Research Laboratory, Department of Zoology, Sreegopal Banerjee College following the World Health Organization protocol (WHO, 1981) with minor changes. The extractive gradients (0.1 percent, 0.2 percent, 0.3 percent, 0.4 percent, and 0.5 percent) were evaluated on all instars of *Cx. quinquefasciatus* larvae. Each experiment was replicated five times, with control set up consisting of distilled water and no extractive. Twenty five larvae of a specific instar were placed in plastic cup (150 ml capacity) and were selectively filled with 100 ml of distilled water. This process was repeated for a variety of combinations, including different concentration gradients (0.1 percent to 0.5 percent) and instars (first to fourth). Same procedure was followed and repeated for a variety of combinations,

including different concentration gradients (50 ppm to 250ppm) and instars (first to fourth). The larvae were counted as dead, when the larvae failed to move after being poked by a sharp needle in the siphon or cervical area, or when they were unable to reach the water surface (Macêdo et al. 1997). The number of dead larvae was counted every 24 hours for up to 72 hours, and the percentage mortality was calculated using the mean average of five replicates. The 48-hour and 72-hour mortality figures were derived by summing the 24-hour and 48-hour death rates, respectively.

Effects on non-target organism

The non-targeted risk group includes the tiniest animals that share the same ecological milieu. The phyto-extractive susceptibility of these species was tested using *Chironomus circumdatus* larvae (insect) as a non-targeted population representative. They were subjected to leaf extracts at LC_{50} concentrations for 24 hours of 3rd instar larvae to examine mortality and sluggish swimming activity for up to 72 hours. Each test concentration had five replicates, as well as five replicates of untreated controls.

Statistical calculations

Several statistical components including LC_{50}, regression equations (Y=mortality, X=concentrations), regression coefficients were figured out using statistical tools such as "Stat Plus 2007 (Trial Version)" and MS Excel 2003. Statistical software SPSS was considered for computing a completely randomized three-way ANOVA to explain the differences in terms of concentration, instars, and exposure hours, as well as their interactions. Results with a P value of less than 0.05 were considered statistically significant.

RESULTS

In present study, *C. gouriana* was found to have strong mosquito larvicidal properties against *Cx. quinquefasciatus*. After 72 hours of exposure to a crude extract of *C. gouriana* leaf, 100 percent death was observed amongst first instar larvae at a concentration of 0.5 percent (Table 1). Chloroform/methanol (1:1, v/v) extract also had the larvicidal efficacy action against the target insect. After 72 hours of exposure to chloroform/methanol (1:1, v/v) extractive, 1st instar larvae died at a rate of cent percent (Table 2). In each extract and instar, the percentage mortality increased as the exposure period increased. The findings of log probit and regression analyses of larval mortality induced by chloroform/methanol (1:1, v/v) extract are presented in Table 3.The computation of log probit analysis (at a 95% confidence level) show that LC_{50} values dropped with increasing exposure time, with the lowest value at 72 hours in each instar. At 72 hours after exposure, the LC_{50} values for chloroform/methanol (1:1, v/v) extract against 1st instar larvae were 22.78 ppm, which were substantially lower than all subsequent instars. Regression analysis indicated a positive correlation between mortality rate (Y) and the time of exposure (X). The results of a completely three-way ANOVA with mortality as a fixed factor and instars (I), solvent extract concentrations (C), and hours (H) as three parameters are shown in Table 4. Individual components, as well as all conceivable parameter combinations, were shown to have a significant relationship with larval mortality ($P<0.05$), with the exception of the combination of three parameters. FT-IR spectrum indicated the presence of aromatics, alkanes, alcohols, amines, and amine salts in chloroform/methanol (1:1, v/v) extract (Fig. 1). When treated with crude and solvent extractives of the plant, non-target organisms showed no abnormalities or death.

Table 1: Efficacy of different concentrations of *Clematis gouriana* on larval instars of *Culex quinquefasciatus*

Instars	Concentrations	% Mortality (Mean ± standard error)		
		24h	48h	72h
First	0.1	50.40±0.98	53.60±0.98	66.40±2.40
	0.2	61.60±2.40	63.20±3.20	72.80±2.33
	0.3	69.60±3.00	70.40±3.48	73.60±5.60
	0.4	86.40±3.70	87.20±3.88	88.80±4.27
	0.5	90.40±1.60	92.80±0.80	100.00±0.00
	Control	0.00±0.00	0.00±0.00	0.00±0.00
Second	0.1	48.80±1.49	49.60±0.98	62.40±5.31
	0.2	50.40±1.60	59.20±1.85	67.20±0.80
	0.3	68.80±3.44	69.60±2.99	72.80±3.20
	0.4	69.60±0.98	74.40±2.40	81.60±2.99
	0.5	85.60±1.60	90.40±2.71	98.40±2.71
	Control	0.00±0.00	0.00±0.00	0.00±0.00
Third	0.1	46.40±1.60	48.80±0.80	55.20±2.33
	0.2	51.20±3.20	53.60±1.60	58.40±3.71
	0.3	66.40±1.60	67.20±1.49	69.60±0.98
	0.4	67.20±0.80	69.60±0.98	79.20±0.80
	0.5	74.40±2.40	84.80±2.65	89.60±2.99
	Control	0.00±0.00	0.00±0.00	0.00±0.00
Fourth	0.1	44.80±1.96	47.20±1.96	52.80±1.49
	0.2	49.60±1.60	52.80±1.49	56.80±3.88
	0.3	58.40±2.04	62.40±2.71	67.20±1.96
	0.4	63.20±1.95	68.80±1.50	71.20±2.65
	0.5	69.60±2.40	75.20±2.33	81.60±3.25
	Control	0.00±0.00	0.00±0.00	0.00±0.00

Table 2: Efficacy of different concentrations of chloroform/methanol (1:1, v/v) extracts of *Clematis gouriana* on larval instars of *Culex quinquefasciatus*

Instars*	Concentrations (ppm)	% Mortality (Mean ± standard error)		
		24h	48h	72h
First[(a)]	50	49.60±2.03	52.80±1.50	72.80±3.88
	100	64.00±3.79	66.40±4.49	74.40±3.25
	150	71.20±4.08	72.80±4.08	76.80±4.45
	200	84.00±4.19	84.80±4.63	85.60±4.83
	250	86.40±2.99	93.60±0.98	100.00±0.00
	Control	0.00±0.00	0.00±0.00	0.00±0.00
Second[(b)]	50	50.40±0.98	51.20±1.50	65.60±6.01
	100	53.60±1.60	57.20±2.15	66.40±0.98
	150	63.20±1.50	64.80±1.50	71.20±2.65
	200	71.20±1.50	76.80±4.27	80.80±4.08
	250	83.20±2.65	93.60±0.98	100.00±0.00
	Control	0.00±0.00	0.00±0.00	0.00±0.00
Third[(c)]	50	46.40±1.60	50.40±0.98	52.80±1.50
	100	53.60±2.71	55.20±1.96	58.40±2.04
	150	60.80±2.33	62.40±0.98	65.60±0.98
	200	65.60±0.98	68.80±1.50	77.60±1.60
	250	73.60±3.25	86.40±1.60	86.40±1.60
	Control	0.00±0.00	0.00±0.00	0.00±0.00

Early Fourth[(d)]	50	46.40±1.60	46.40±1.60	52.00±2.19
	100	52.80±1.96	53.60±2.03	54.40±1.60
	150	57.60±1.60	59.20±0.80	64.00±1.79
	200	62.40±1.60	67.20±1.49	68.80±1.50
	250	72.80±4.45	78.40±3.24	78.40±3.24
	Control	0.00±0.00	0.00±0.00	0.00±0.00

*Values of mean of different letters are significantly different at $p<0.05$ level (Tukey's test of multiple comparison).

Table 3: Log probit and regression analyses of larvicidal activity of chloroform/methanol (1:1, v/v) extract of *Clematis gouriana* on *Culex quinquefasciatus*

Instar	**Exposure**	**LC_{50} (ppm)**	**Confidence interval (95%)**		**Linear regression**	**R^2**
			Lower	**Upper**		
First	24h	55.99	1.83	93.86	Y=0.187x+42.96	0.76
	48h	51.79	10.72	80.52	Y=0.2x+44.08	0.80
	72h	22.78	0.88	586.45	Y=0.131x+62.24	0.55
Second	24h	62.72	0.26	106.18	Y=0.166x+39.36	0.89
	48h	61.20	23.32	160.61	Y=0.2084x+37.4	0.87
	72h	36.38	4.10	322.93	Y=0.166x+51.84	0.65
Third	24h	70.18	57.03	81.71	Y=0.132x+40.08	0.80
	48h	62.56	23.70	165.15	Y=0.171x+38.96	0.88
	72h	54.85	1.29	92.62	Y=0.172x+42.24	0.92
Fourth	24h	73.92	17.36	111.23	Y=0.124x+39.68	0.74
	48h	71.78	13.37	109.84	Y=0.155x+37.68	0.87
	72h	55.99	1.83	93.86	Y=0.134x+43.36	0.81

Table 4: Completely randomized three-way factorial ANOVA related to mortality of *Culex quinquefasciatus* using different larval instars (I), different concentrations (C) and different hours (H) as three variables

Source of variation	**Sum of squares**	**df**	**Mean squares**	**F value**	**P value**
Instar (I)	9445.693	3	3148.564	89.754	<0.001
Concentration (C)	40421.013	4	10105.253	288.063	<0.001
Hour (H)	4251.707	2	2125.853	60.600	<0.001
I × C	843.840	12	70.320	2.005	0.025
I × H	492.507	6	82.084	2.340	0.032
C × H	726.827	8	90.853	2.590	0.010
I × C× H	850.560	24	35.440	1.010	0.454
Residual	8419.200	240	35.080		
Total	65451.347	299			

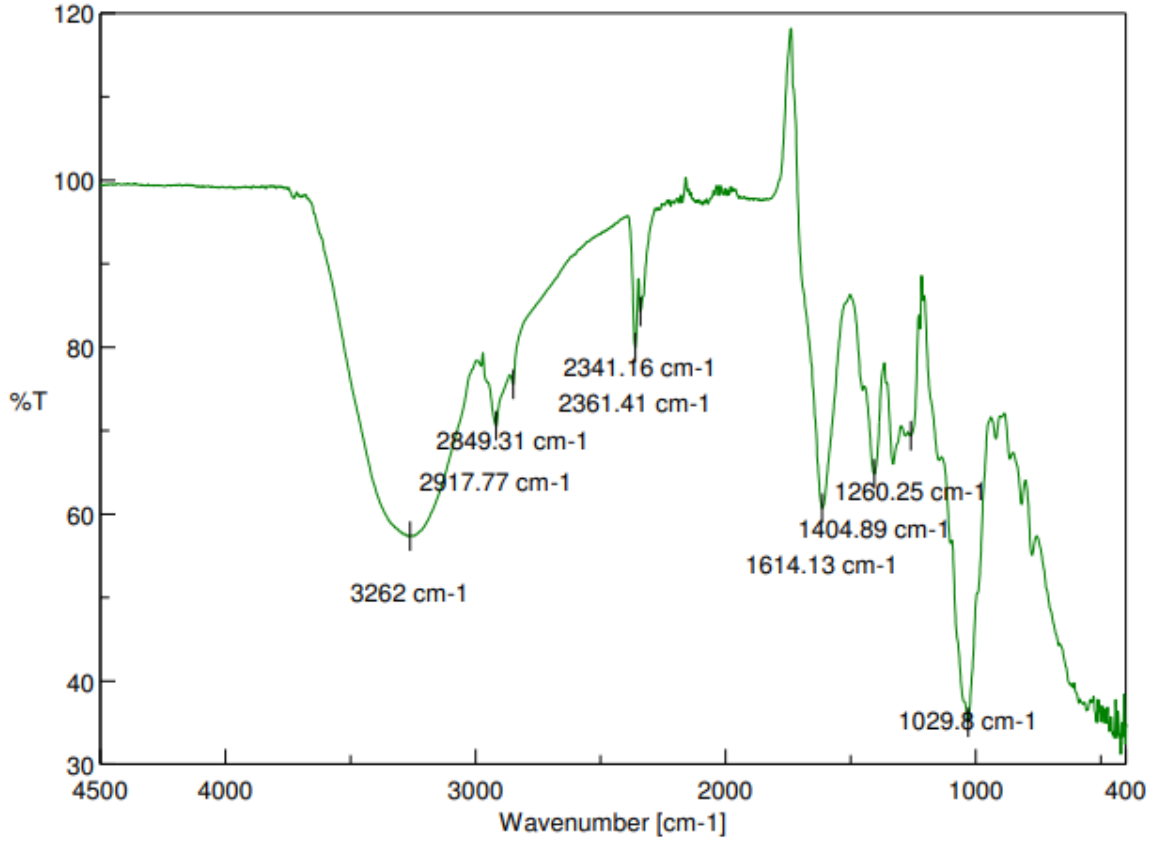

Figure 1: IR spectra of chloroform/methanol (1:1, v/v) extract of *Clematis gouriana*.

DISCUSSION

Because of the disadvantages of synthetic chemical pesticides, such as environmental impact and toxicity to non-target creatures, including people, as well as the development of resistance in targeted insect populations, interest in producing natural pesticides has surged in recent years (Jaswanth et al. 2002). Mosquito larvae are most susceptible when they are confined to water sources and have a limited rate of dispersion; larval management is the greatest technique for reducing mosquito populations at an early stage (Howard et al, 2007). Several plants have been discovered to have mosquito larvicidal potential and are environmentally beneficial (Ghosh et al, 2008). Herein, the larvicidal activity of *C. gouriana* leaf extract against *Cx. quinquefasciatus* has been investigated.

The larvicidal efficiency of the tested extract in this investigation was dose-dependent. The presence of numerous bioactive phytomolecules contained in the plant is thought to be responsible for the biological activity of plant extracts (Vindhya et al, 2014). Several plant extracts have been investigated as mosquito larvicide (Ghosh et al, 2012). *Cayratia trifolia* was tested for its larvicidal activities against *Cx. quinquefasciatus* at various doses, where absolute mortality was achieved by using mature leaves at 0.4% crude concentration and above (0.5%, 0.6%) after 72 h of exposure (Chakraborty et al., 2013). Similarly, another study found that a leaf crude extract (1 %) of *Andrographis echioides* had a 100 percent mortality rate against first instar larvae after 72 hours of exposure, and an ethyl acetate extractive of *A. echioides* foliage had a 100.00 percent mortality rate after 48 hours at 150 ppm concentration, with an LC_{50} value of 32.96 ppm for the first instars (Das et al., 2019). In contrast to previous findings, the current study found 100% mortality in first instars after 72 hours of exposure to 0.5 percent crude foliage extract of *C. gouriana*, with lowest LC_{50} values of chloroform/methanol (1:1, v/v) extractive of 22.78 ppm, 36.38 ppm, 54.85 ppm, and 55.99 ppm for the first, second, third, and fourth instars larvae of *Cx. quinquefasciatus* respectively.

Again, the current investigation demonstrates that the foliage extract of *C. gouriana* is an effective larvicide for *Cx. quinquefasciatus* wrigglers and might be a viable alternative to synthetic pesticides in mosquito control. So, after probable identification of the active principle is causing the observed larvicidal action and histological investigations confirming how the active material operates on the targeted larvae, it may contribute in the development of a novel domestic bio-insecticide against wrigglers of *Cx. quinquefasciatus*.

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