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Sumanta Chakraborty(1) Department of Zoology,
Sreegopal Banerjee College,
Hooghly, West Bengal, India(2) Department of Zoology,
Mosquito and Microbiology,
Nanotechnology Research Units,
Parasitology Laboratory,
The University of Burdwan,
Burdwan, West Bengal, India**Sunanda Burman**Department of Zoology,
Mosquito and Microbiology,
Nanotechnology Research Units,
Parasitology Laboratory,
The University of Burdwan,
Burdwan, West Bengal, India**Goutam Chandra**Professor, Department of
Zoology, Mosquito and
Microbiology, Nanotechnology
Research Units, Parasitology
Laboratory, The University of
Burdwan, Burdwan,
West Bengal, India**Corresponding Author:****Goutam Chandra**Professor, Department of
Zoology, Mosquito and
Microbiology, Nanotechnology
Research Units, Parasitology
Laboratory, The University of
Burdwan, Burdwan,
West Bengal, India

Repellent and oviposition-deterrent efficacies of *Cuscuta chinensis* on filarial vector *Culex quinquefasciatus*

Sumanta Chakraborty, Sunanda Burman and Goutam Chandra

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Abstract

In the laboratory, crude and chloroform: methanol extracts of *Cuscuta chinensis* seeds were evaluated for repellence and oviposition deterrence activities against adults of *Culex quinquefasciatus* at various concentrations.

Hundred percent protection was attained from a 99% concentration of chloroform: methanol extract for 120 minutes whereas from the crude extract for the first 90 minutes with a gradual decrease. Crude and chloroform: methanol extracts of seeds were found to be highly effective in preventing oviposition of adult mosquitoes. Furthermore, neither extract has any negative side effects such as allergy or other observable reactions on human skin during or after the exposure period. Oviposition activity index (OAI) was negative at all concentrations for tested plant extracts which indicated an inhibition of *Cx. quinquefasciatus* oviposition. So, against *Cx. quinquefasciatus* mosquito species, crude and chloroform: methanol seed extracts of *C. chinensis* may be utilized as a repellent and oviposition deterrent.

Keywords: *Cuscuta chinensis*, *Culex quinquefasciatus*, skin repellent, biting deterrent

1. Introduction

Mosquitoes are the most prevalent vectors of several communicable diseases, and they offer a greater health danger in tropical and subtropical climates, though this threat exists in every corner of the globe^[1].

Mosquitoes are the primary vectors of malaria and other vector-borne diseases in India, and they are responsible for a significant portion of the disease load. Lymphatic filariasis is a tropical disease that affects almost 120 million people worldwide, with 44 million experiencing persistent symptoms^[2].

In the Indian subcontinent, *Wuchereria bancrofti*, which is transmitted by female *Culex quinquefasciatus* mosquitos, is the causative agent of lymphatic filariasis^[3]. Although nature plays a role in limiting filarial transmission⁴, mosquito control and personal protection from mosquito bites are currently the most effective approaches to combat these infections. In many parts of the world, specific mosquito control measures have been implemented, such as mosquito population management using naturally existing mosquito larval predators such as fishes,^[5,6] insects^[7], and plant-derived substances^[8,9].

For pest specificity, easily biodegradability, and typically lack of toxicity to higher animals^[10], efficacy, and ease of availability, mosquito control through botanicals is a very desirable area of study. Not only mosquito, plant products have multidirectional activities against helminthes^[11,12], bacteria^[13,14,15], mollusca^[16] etc. Replacement of host plant may also reduce *Mansonia* population, vector of *Brugia*^[17]. Again, these are non-toxic, biodegradable, and environmentally friendly, as opposed to synthetic insecticides. The use of repellents is an obvious and cost-effective technique of minimizing disease transmission to humans^[18], and mosquito oviposition can be interrupted to reduce the population of mosquitos^[19]. The objective of the present study was to explore the skin repellent and oviposition-deterrent efficacies of seeds of *Cuscuta chinensis* against filarial vector, *Cx. quinquefasciatus*.

2. Materials and Methods

2.1 Collection of plant sample

Seeds of *C. chinensis* were gathered manually from natural population growing in Hooghly district, WB, India. Plant sample was authenticated by plant taxonomist Professor Dr. Ambarish Mukherjee, Botany Department, The University of Burdwan. The voucher specimen (GCSC05) was submitted in the herbarium of the Mosquito, Microbiology, and Nanotechnology Research Units, Parasitology Laboratory, Department of Zoology, The University of Burdwan (23.16°N, 87.54°E).

2.2 Preparation of crude extract

The crude extract was prepared by crushing the clean seeds (50 g) by using a mortar and pestle and the paste material was filtered using cheese cloth.

2.3 Preparation of solvent extract

Seeds were rinsed with tap water, dried in a well-ventilated area, and then ground in a mechanical grinder. A series of non-polar to polar solvents (petroleum-ether, hexane, chloroform: methanol (1:1 v/v), acetone, absolute alcohol, and distilled water) were used to extract powdered seeds (200 g) for 72 hours in a Soxhlet apparatus.

In a rotary vacuum evaporator, each extract was concentrated before being employed in the bioassays. The chloroform: methanol (1:1 v/v) extract was shown to have biological activity in preliminary study in the Mosquito, Microbiology and Nanotechnology Research laboratory, Department of Zoology, The University of Burdwan.

2.4 Mosquito culture

Egg rafts of *Cx. quinquefasciatus* were meticulously gathered with acute precession from nearby drains of Golapbag campus of University of Burdwan (23.16°N, 87.54°E) and reared in the laboratory at 27±2°C, 70-80% relative humidity, and a L: D 14:10 photoperiod [20]. Rainwater was used for larval hatching. Finely crushed brewer yeast and dog biscuits were mixed in a ratio of (3:1) and was given to the larvae as a

supplementary diet. Using a glass dropper, the transformed tumblers were physically separated in a 500 mL glass beaker filled with tap water. To promote mosquito adult emergence, the beaker was placed in cages. Adults were regularly given a 10% sucrose solution and a blood meal from restrained chicks (5-7 weeks old).

2.5 Repellency potentiality test against adult mosquitoes

The percentage of protection from mosquito bite in relation to dose dependent technique was used to examine the repellent activity of the crude seed extract and seed chloroform: methanol (1:1) extract [1]. Serial dilutions were made in ethanol at different percentage concentrations (1%, 25%, 50%, 75%, 99% W/V and the concentration of crude seed extract was considered as 100%). One hundred female mosquitoes (3-4 days old) which never received a blood meal and starved of their sugar diet for 12 h before the experiment were kept in net cage (45 x 30 x 45 cm) with a front access cotton stockinet sleeve. The forehands of the volunteer (self-volunteer) were cleaned with ethanol. All of the sample concentrations were examined in order, beginning with the lowest, and each concentration was assessed using a new batch of mosquitos. A 1mL aliquot of test solution was uniformly put to a forearm between the wrist and elbow using a pipette and allowed to dry for roughly 10 minutes while the remainder of the arm was covered with a glove. As a control, the other arm was given 1mL ethanol. At the same moment, the treated and control arms were inserted into the cage.

The numbers of bites were counted for 5 minutes at every 30 minutes from 6 p.m. to 6 a.m. next day. Before applying the next concentration, the arms were washed with unscented neutral soap, rinsed with tap water, and dried with a paper towel for 15 minutes. Again, the crude extract's repellency was measured by applying it directly on a forearm. To act as a control, the other forearm was cleaned with distilled water. Each experiment was repeated four times. The following formula was used to compute the percentage of protection [1].

$$\% \text{ of protection} = \frac{\text{Number of bites on control arm} - \text{Number of bites on treated arm}}{\text{Number of bites on control arm}} \times 100$$

2.6 Oviposition deterrent bioassay

The technique of Xue *et al.* (2001) [21] was adopted for oviposition deterrent bioassay against *Cx. quinquefasciatus*. Twenty gravid females of the *Cx. quinquefasciatus* (10 days old, four days after blood feeding) were introduced to oviposit in enclosed cages (45×38×38 cm) constructed of mosquito net with a muslin socket on the front side for access and a 10% glucose solution in a plastic cup. Acetone was used to make serial dilutions of leaf extract. Seed chloroform: methanol (1:1) extract was used to treat enamel bowls holding 100 mL of non-chlorinated tap-water to produce test solutions of 200, 300, 400, and 500 ppm. Two enamel bowls, each holding 100 mL of non-chlorinated tap-water, were kept diagonally at opposite corners of each cage, one with the test substance and the other with a solvent control containing 2% acetone. When the studies were repeated, the place of the test bowls was altered to rule out any effect of position of ovitrap on oviposition. Each bioassay had three replicates, with cages set side by side. All of the tests were conducted at room temperature (27±2°C) with a relative humidity of 70-80%.

After 24 hours, the number of eggs placed in the treatment and control bowls were tallied.

The average number of eggs was computed, and the oviposition data was shown as a percentage of effective repellence (ER%) and an oviposition activity index (OAI). ER% was calculated with the following technique of Rajkumar and Jebanesan (2009) [22]. The Kramer and Mulla (1979) [23] formula was used to compute the oviposition activity index (OAI).

$$\text{Effective repellency (ER)} = \frac{\text{NC} - \text{NT}}{\text{NC}} \times 100\%$$

$$\text{Oviposition activity index (OAI)} = \frac{\text{NT} - \text{NC}}{\text{NT} + \text{NC}}$$

Where ER = percent effective repellency; NC = number of eggs in control; and NT = number of eggs in treatment.

2.7 Phytochemical analyses

Harbone [24] and Stahl [25] techniques were used to conduct brief phytochemical examinations of both immature and mature seeds.

2.8 Statistical analysis

For statistical justification paired t-test and Tukey's test of multiple comparisons were performed with the help of the statistical package SPSS.

3. Results

The results of the skin repellent ability of *C. chinensis* seeds in crude and chloroform: methanol solvent extracts against the biting activity of blood-starved adult female *Cx. quinquefasciatus* mosquitoes are summarized in Table 1. The results showed that the crude extract was cent percent protective against *Cx. quinquefasciatus* biting activity for the first 90 minutes, and then gradually decreased until it reached 79.00% after 150 minutes of application. After 30 minutes of application, 1% chloroform: methanol extract had 8.25% repellency, but after 90 minutes, it had none. Up to 30

minutes, 25% extract provided roughly 82.50% protection. While the 50% dose provided 94.25% protection for 30 minutes, the 75% and 99% concentrations provided cent percent protection for 2 hours. From a 99% concentration at 150 minutes following application, 94.50% protection was achieved.

The mean number of eggs laid in the chloroform-methanol extract at the four different concentrations viz., 200 ppm, 300 ppm, 400 ppm, and 500 ppm were 138.67 ± 8.95 , 92.66 ± 6.74 , 82.33 ± 5.81 eggs, and 66.66 ± 11.83 eggs per cup respectively while the corresponding figures for control cups were provided 316.66 ± 14.14 , 363.67 ± 20.73 , 373.67 ± 6.35 and 333.67 ± 17.52 eggs respectively (Table 2). For all concentrations examined, the number of eggs laid in the treated plastic cup was significantly different ($P < 0.05$) from the number of eggs laid in the corresponding control plastic cup. The container holding 500 ppm and 400 ppm extract solution had the highest ER% (i.e. 80% and 77% respectively), followed by 300 ppm (ER% = 74%) and 200 ppm (ER% = 56%). Chloroform: methanol extract of *C. chinensis* seeds contained several phytochemicals (Table 3).

Table 1: Dose dependent mosquito repellent activity of *Cuscuta chinensis* seed against *Culex quinquefasciatus*

Type of extract	Concentration (%)	Post application percentage repellency				
		30 min	60 min	90 min	120 min	150 min
Chloroform: methanol	1%	8.25±1.49	5.50±0.65	3.25±0.63	0.00±0.00	0.00±0.00
	25%	82.50±1.70	76.50±1.19	62.25±1.65	41.75±1.38	23.00±1.73
	50%	94.25±1.37	88.25±0.75	86.25±1.43	81.00±1.47	77.75±1.54
	75%	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	88.50±1.19
	99%	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	94.50±1.55
Crude	100%	100.00±0.00	100.00±0.00	100.00±0.00	82.75±2.95	79.00±2.48

Table 2: The oviposition deterrent activity of seed chloroform: methanol (1:1) extract of *Cuscuta chinensis* against *Culex quinquefasciatus* female mosquitoes

Concentration (ppm)	Number of eggs		Sig.*	ER (%) **	OAI
	Treated	Control			
200	138.67±8.95	316.66±14.14	0.001	56.23±0.67 ^a	-0.39
300	92.66±6.74	363.67±20.73	0.005	73.73±2.84 ^b	-0.59
400	82.33±5.81	373.67±6.35	0.000	77.87±1.29 ^b	-0.63
500	66.66±11.83	333.67±17.52	0.008	79.05±4.19 ^b	-0.66

* Number of eggs laid in the treated was significantly different ($P < 0.05$) from the number of eggs laid in control plastic cup for each concentration (paired t-test).

** Each effective repellency represented by (Mean ± SE) uses mean of three values. Values of mean of different letters are significantly different at $p < 0.05$ level (Tukey's test of multiple comparison).

Table 3: Qualitative analysis of phytochemicals of immature and mature seeds of *Cuscuta chinensis*

Seed Sample	Alkaloids	Terpenoids	Phenolics	Flavonoids	Tannins	phytosterols	Cardiac glycosides	Saponin
Immature	+	+	+	+	+	+	-	-
Mature	+	+	+	+	+	+	-	-

4. Discussion

Future mosquito control efforts may benefit from chemicals produced from plants. Phytochemicals have the potential to be used as growth and reproduction inhibitors, repellents, and oviposition deterrents in addition to being used as general toxicants against many phases of mosquito life.

Several research opportunities exist in the identification and characterization of novel plant compounds, as well as the assessment of their ecological, evolutionary, and physiological implications. The use of phytochemicals to control this medically important sporadic pest is of utmost importance, since biting deterrence can minimize mosquito-borne illnesses, resulting in a low disease transmission rate. In

current scenario, both the crude part and chloroform: methanol extract was cent percent protective against *Cx. quinquefasciatus* biting activity for certain period. Notably, tested extract is not responsible for allergy symptoms or other observable reactions during or after the exposure period.

According to Xue *et al.* (2001) [1], oviposition is one of the most essential stages in a mosquito's life cycle. The mosquito life cycle is interrupted and population expansion is inhibited if oviposition is avoided. The seed extracts of *C. chinensis* function as oviposition deterrents, indicating that *Cx. quinquefasciatus* mosquitoes are intensely sensitive to phytochemical stimuli and respond to the odour of the leaf extract. According to Davis and Bowen (1994) [26],

mosquitoes use chemical signals received by sensory receptors on the antenna to choose or reject certain oviposition locations. The strong odour created by larger concentrations of tested seed extracts provides the most efficient oviposition repellency. The oviposition activity index (OAI) was negative at all concentrations for tested plant extracts which indicated an inhibition of *Cx. quinquefasciatus* oviposition.

Single plant species may contain active principles with a number of efficacies, for example, *Azadirachta indica* extractive showed antifeedant, oviposition deterrent, skin repellent and growth regulating activities [27]. In a laboratory experiment, Bhattacharya and Chandra [28] reported on phagodeterrence, larvicidal and oviposition deterrent activities of root extractives of *Tragia involucrata* L. (Euphorbiaceae) against *Cx. quinquefasciatus*. The ethyl acetate extract of *Annona reticulata* prevented oviposition in adults of *Cx. quinquefasciatus*, according to Mallick and Chandra (2015) [29]. In their research, Soonwera *et al.* (2017) [30] discovered that 10% essential oil of *Zanthoxylum limonella* showed 99.53% repellency for *Cx. quinquefasciatus*.

The results of this study demonstrate that *C. chinensis* seed extract has oviposition deterrent and skin repellent properties against *Cx. quinquefasciatus*. A range of chemicals in this plant, including phenolics, terpenoids, and alkaloids, may be responsible for the biological activity of the plant extract. These chemicals may work as oviposition deterrence and skin repellence against *Cx. quinquefasciatus*, either together or separately. More research is needed to determine the active volatile compound(s) responsible for its effectiveness as well as to evaluate the effect of *C. chinensis* extracts on a wider range of mosquito species.

According to Rosell *et al.* (2008) [31] direct toxicity, oviposition, repellency, and attraction all appear to be caused through interactions of phytochemicals with the insect nervous system, either through acetylcholinesterase inhibition or octopamine receptor antagonism. Though the route of action in our study was not determined, it is possible that the phytochemicals in *C. chinensis* seed extract have a similar impact on *Cx. quinquefasciatus*.

Findings of the present study imply that botanical biopesticides can be used alone or in combination to provide effective protection against mosquito bites, as well as to reduce mosquito reproduction as part of an integrated vector control programme in a number of circumstances. They also offer a low-cost alternative to synthetic compounds, hazardous to the environment that people and communities can access.

5. Acknowledgment

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6. Conflict of Interest

Authors declare that there is no conflict of interest pertaining to publication of this manuscript in your esteemed Journal.

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