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TARGET SPECIFIC LARVICIDAL EFFECT OF *CAPPARIS ZEYLANICA* L. (CAPPARACEAE) FOLIAGES AGAINST FILARIAL VECTOR *CULEX QUINQUEFASCIATUS* SAY (1823)

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ABSTRACT

After prolonged use of the synthetic insecticides, novel insecticides of botanical origin are preferred for environment safety as well as human life. Present study was conducted to evaluate the larvicidal capability of *Capparis zeylanica* against *Culex quinquefasciatus.* Crude, cold and hot aqueous and ethyl acetate solvent extracts of foliages of *C. zeylanica* were tested against all instars larvae of *Cx. quinquefasciatus.* Lethal concentrations were determined. Effect on the non target water fauna was also tested. Cent percent mortality was found with 0.25% concentration of crude extracts against 1st and 2nd instar larvae. No larvicidal responses were found with cold and hot aqueous extracts. Highest mortality was found against 1st and 2nd instar larvae at 90 ppm concentration of ethyl acetate extracts at 72 h post exposure. Non-target populations were found non-responsive to active fraction. Secondary metabolites like Tannin, flavonoids and terpenoid were detected through preliminary qualitative phytochemical tests.

KEY WORDS: ***Capparis zeylanica, Culex quinquefasciatus, Non-target organisms, Larvicide.***

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INTRODUCTION

From pre-historical periods, human use various plant parts and plant products as natural insecticides. Plants are known as nature's chemical factories due to production of many chemicals having insecticidal, medicinal, esthetical and many other properties. Researchers are, nowadays, able to control a very minor number of pests or insects. Insecticides should be effectively harmless to living populations and environmentally safe. Many detoxifying mechanisms of chemical insecticides[1] disturbed the controlling process and at the same time synthetic insecticides have been accounted for genetic resistance against many mosquito species[2, 3]. Besides this, chemical insecticides may be harmful to non target species as well as human populations. So, plant derived products may fulfil the needs. Many phytochemical products like pyrethrum, quassia, derris, nicotine, azadirachtin, camphor, anabasine and terpenes have been used as pesticides. Till date more than 2000 plants have been reported to produce chemicals and metabolites responsible for insect mortality[4]. According to Sukumar *et al.* (1991)[5] around 344 plant species were accounted to use as an array of mosquitocidal agent. Plant parts were used to control agricultural pests and pests with human health concern. Phytochemicals derived from plants showed a positive effect on disease causing mosquitoes[6, 7, 8, 9]. Many plants belonging to the family Asteraceae, Labiateae, Miliaceae and Rutaceae possess different types of mosquitocidal activities[10]. Some common spices were also used to control mosquito with the help of derived phytochemicals[11]. In traditional health care system, use of medically important plants and plant products has opened a new branch of research in many countries. According to WHO, 80% population till depends on herbal medicines, so, antibiotics are also produced from plant based phytochemicals nowadays[12]. Mosquito can transmit various obnoxious diseases worldwide. In tropical country it is significantly correlated to the social impediment and poverty of mankind[13]. In warm and humid climate, *Culex quinquefasciatus* is known to carry and transmit the pathogen of bancroftian filariasis, *Wuchereria bancrofti,* responsible for lymphatic filariasis in India. It can also transmit avian malaria, and some arbovirus including St. Louis Encephalitis Virus, Western Equine Encephalitis Virus and West Nile virus. It breeds in septic tanks, muddy water and stagnant water bodies. Females lay eggs on nutrient rich water where larvae emerge into adults within eight days[14]. To diminish mosquito population it should be controlled at its larval stage because larvae are the most susceptible to insecticides and being a water breeder, the larval stages are favourable target of insecticides. *Capparis zeylanica* is a common climbing branched shrub mainly found in India, Bangladesh, some parts of China and several other parts of the world. This plant belongs to the order Brassicles and capparaceae family, known as Caper family which contains around 33 genera and 700 species. *Capparis* is the largest genus including 150 species. It is closely related to the Brassicaceae family because they both produce glucosinolate compounds[15]. Traditionally it was used as antidote of snake venom, cholera, small pox, neuralgia, pleurisy and sores[16]. Analgesic and antipyretic activity[17] was found from the Methanolic leaf extracts of this plant[18]. No such report was present about its mosquitocidal activity, so, the present study was carried out to examine the efficacy of this plant as a mosquitocidal agent.

MATERIALS AND METHODS

i) Plant material

Matured green leaves of *C. zeylanica*, commonly known as "Asarilata", were collected during September-October 2013, from the outskirts of Burdwan (23°16´N, 87°54´E), West Bengal, India. After identifying the plant a voucher specimen (GCZASR-07) was submitted as herbarium in the Department of Zoology, The University of Burdwan.

ii) Plant extracts procurements

Collected plant leaves were rinsed well in tap water followed by distilled water. Unspotted, clean leaves were grinded by using electric grinder. The juice was filtered through

Whatman's No.1 filter paper. Collected liquid was preserved as stock in refrigerator for further experiments. Required concentrations were prepared by adding distilled water with it.

iii) Hot aqueous extract

To prepare hot aqueous extract, 50 g of unspotted leaves were soaked in the 200 ml of tap water which was boiled for 30 minutes and put in a conical flask for 24 h without any disturbance. Juice was filtered with Whatman's No. 1filter paper and stored in another conical flask and subjected to water bath evaporation. Due to 100^0C boiling temperature, the aqueous solvent was evaporated. After evaporation, the standard extracts were stored at 4^0C in a refrigerator for future use[19].

iv) Cold aqueous extract

50 g of plant leaves were soaked in 200 ml of cold water in a conical flask with rubber cork as stopper. The set was left undisturbed for 24h. Next step was followed as depicted in hot aqueous extract preparation.

v) Preparation of solvent extracts with ethyl acetate

Leaves were shed dried for some days in room temperature. 200 gm of leaves were chopped into small pieces and put on a thimble of Soxhlet apparatus. 2 litre of ethyl acetate as solvent medium was added to the still pot. Extraction period was set for 72 h (maximum 8 h per day)[20]. Elute was collected on a beaker and intensified by evaporation with rotary evaporator.

vi) Mosquito culture

Larvae of *Cx. quinquefasciatus* were collected from the drains adjacent to University campus following the standard scooping and dipping method by Robert *et al.* (2012)[21] and maintained in the laboratory Mosquito, Microbiology and Nanotechnology Research Units, by using the protocol of Sharma and Saxena (1994)[22] with minute deviation. Larvae were transferred to a plastic tray filled with normal tap water and provided a complementary food, combination of dog biscuit, brewer yeast and algae in a ratio of 1:3:1[23]. Colony was maintained in 27 ± 2^0C and 80% RH in a photoperiod of 14:10 h light and dark cycle. Pupae were relocated to an insectary (45×45×40 cm) where adults emerged. Mosquitoes were identified by the keys provided by Barraud (1934)[24], Christophers (1933)[25] and Chandra (2000)[26]. Adults were regularly supplied 10% sucrose solution with multivitamin syrup with a moist cotton wick. An immobilized shaved pigeon was supplied for blood meal of adult females at the 5th day of rearing[27]. For oviposition of mosquitoes a Petri dish filled with water and crumpled filter papers were put on the insectary. Eggs were undisturbed and allowed to hatch under laboratory circumstances. The processes were continuously repeated until the establishment of a healthy mosquito colony[28].

vii) Dose dependent larvicidal bioassay

Maintaining the WHO protocol[29] larvicidal bioassay was performed in the laboratory. Twenty five larvae were transferred from the insectary to each of the glass Petridishes (150 mL) containing 100 mL of tap water. Hot and cold aqueous extracts of crude ranging from 0.06% to 0.16% were applied on all the different instars of *Cx. quinquefasciatus* larvae under laboratory condition. Ethyl acetate extract was applied from 50 ppm to 100 ppm concentrations against all larval instars. All experiments were done in triplicate with a set of control parallely having no leaf extractives. The Petridishes were kept at room temperature to observe the larval mortality after 24 h, 48 h and 72 h of exposure respectively. Larvae were assumes dead when they failed to reach water surface or unable to move after probing with a sharp needle to its siphon or cervical region[30].

viii) Phytochemical analysis of plant leaves

Phytochemical tests of *C. zeylanica* leaves were done according to the standard method of Sofowara (1993)[31], Trease and Evans (1989)[32] and Harborne (1973)[33] as follows

a) Tests for Alkaloid

Mayer's test and Wagner's test were done to determine the presence of alkaloid. At first glacial acetic acid was added to aqueous extracts then Mayer's reagent (1.36 g of $HgCl_2$ and 5 g of KI in 100 ml distilled water) was added to 1 ml of acidified aqueous extract. Formation of pale yellow precipitate

indicates the presence of alkaloids (Mayer's test). Few drops of Wagner's reagent (Iodine solution in potassium iodide) were added to 1 ml of acidified aqueous extract. Appearance of a reddish brown impetuous signifies the existence of alkaloids (Wagner's test).

b) Tests for Flavonoids

10 drops of HCl (0.5 N) were added in 1 ml of aqueous extract, after that a small piece of zinc was added to it. Formation of reddish pink or pink colour precipitate indicates the presence of flavonoids (Zinc hydrochloride test) [31]. Few magnesium turnings were added to 1 ml of aqueous extract and then concentrated HCl (2 N) was added drop by drop. Emergence of pink scarlet or green colour indicates the presence of flavonoids (Shinoda test) [32]. 1.5 ml methanol (50%) solution was assorted with 4 ml of aqueous extract and then the mixture was warmed, 5-6 drops of concentrated HCl (2 N) with few metal magnesium turnings were added to it. The formation of red colour indicates the presence of flavonoids[34, 35].

c) Tests for Tannin detection

5-10 drops of $FeCl_3$ were added to 2 ml of aqueous extract. The manifestation of bluish black colour indicates the presence of tannins (Ferric chloride test) [31, 32, 33]. Few drops of bromine solution were implied to 1 ml of aqueous extract. Decolourization of the bromine water signifies the presence of tannins (Bromine water test) [32]. 4-6 drops NaOH (1 N) solution was added to 1 ml of aqueous extract. The quick appearances of yellow to red impetuous identify the presence of tannins (Alkaline reagent test) [31, 36].

d) Tests for terpenoid and steroid

Detection of terpenoid and steroid were done by following the standard protocol of Kantamreddi *et al.,* 2010[37]. 1 ml of aqueous extract was acidified with 1 ml of glacial acetic acid (1 N) and 1 ml of concentrated sulphuric acid (4 N) was added through the wall of the test tubes in ice chamber. Development of brown or green colour indicates the presence of terpenoids and steroids respectively.

ix) Test upon non-target organisms

Chironomus circumdatus larvae were selected as non-target organism as they share the similar habitat with mosquito larvae. Larvae were collected from field and maintained on the plastic trays in laboratory. Non-target organisms were exposed to the concentration level of LC_{50} of 3rd instar larvae to determine the effect on them after 72 h of post exposure.

Statistical analyses

Probit and regression analyses were performed to determine LC_{50} and LC_{90} values. Mortality rates were précised by Abbott's formula[38]. ANOVA analysis was done for further statistical justification. "MS Excel 2007" and "Stat Plus 2007 Professional" were used to complete statistical analyses.

RESULTS

Cold and hot aqueous extracts have no larvicidal activity. Larval mortality of all the instars is presented in Table1 using crude extract. The outcome of the study indicated that mortality of mosquito larvae against all the instars of *Cx. quinquefasciatus* were significantly higher at 0.25% of crude extract than mortality of 0.05%, 0.10%, 0.15% and 0.20% of crude concentrations at 24 h, 48 h and 72 h of post exposure. Larval mortality rates were also higher at 72 h than those at 24 h and 48 h of post exposure. Cent percent mortality of 1st and 2nd instar larvae were found at 0.20% and 0.25% of crude extract after 72 h of exposure respectively 90 ppm concentration of ethyl acetate extract showed 100% mortality against 1st and 2nd instar larvae of *Cx. quinquefasciatus* after 72 h. Mortality of the all instars of mosquito is presented in Table 2. In all the larval instar the mortality rate gradually increased with increasing time of post-exposure at each concentration. Result of log-probit analyses (at 95% confidence level) expressed that LC_{50} values gradually decreased with period of exposure (Table 3). From the result of regression analysis it can be concluded that mortality rate (Y) was positively correlated with time of exposure (X). The value of regression coefficient was close to one in each case (Table 3). After 72 h of exposure, lowest LC_{50} and LC_{90} values were monitored. After a

period of 24 h of exposure, the LC_{50} values of all the instars were 26.88, 30.02, 37.39, 139.11 ppm respectively (Table 3). Three-way factorial ANOVA established statistical significance of larval mortality ($p<0.05$) (Table 4) in terms of concentrations, instars and time of exposure collectively. Preliminary phytochemical analyses showed that the leaves of *C. zeylanica* contained tannin, flavonoids and terpenoid as secondary metabolites (Table 5).

DISCUSSION

Researchers followed a new trend to control the vector mosquito through phytochemicals. It is a well established trend from old days. Many phytochemicals based research works have been successfully published in many well established journals[39, 40, 41]. Gaining of much importance is due to an immense reason of not building up any non-native chemical component in earth soil. In spite of all these, vector control remains a challenge today. An easy step to mosquito control is to kill their larvae, because this stage is more susceptible than adult or pupae. Although, pupae or adult mosquito control was also done previously [42, 43]. Rawani *et al*. (2010) [44] reported that 50 ppm concentration of ethyl acetate extract of *Solanum nigrum* can kill 100% larval instars of *Cx. quinquefasciatus* and the LC_{50} value was 17.04 ppm after 24 h of exposure. In a piece of work, Kamaraj *et al*. (2010)[45] stated that, ethyl acetate extract of *Annona squamosa* bark had a good larvicidal effect against 4th instar larvae of *An. stephensi*., LC_{50} and LC_{90} values were 25.18 and 94.04 ppm after 24 hours of post exposure. Bagavan *et al.* (2008) [46] reported that 100% larval mortality was found against *Cx. quinquefasciatus* with 1000 ppm concentration of *Acyranthes aspera* leaf ethyl acetate extracts. Kundu *et al.* (2013) [47] showed that 1% concentration of crude extract was able to kill all the larval instars and 480 ppm concentration of ethyl acetate solvent extract was responsible for 100% larval death of *Cx. quinquefasciatus.* Remarkable mosquitocidal activity was found from ethyl acetate extract of *Ocimum sanctum* leaves against *Aedes aegypti* and *Cx. quinquefasciatus* with LC_{50} values of 425.94 ppm and 592.60 ppm respectively (Anees, 2008)[48]. The present study was well established the target specific insecticidal nature of leaf extracts of *C. zeylanica.* In another research, Bhattacharya *et al.* (2014)[49] reported that ethyl acetate extracts of *Ravenala madagascariensis* leaf exerted 100% mortality at 200 ppm and 250 ppm concentrations against 1st and 2nd instar larvae of *Cx. vishnui.* In comparison, we found 100% mortality of 1st instar larvae at very low concentrations (0.20% of crude and 90 ppm in case of solvent extracts) after 72 h of exposure. Preliminary observations are indications that active principle responsible for larval death may be tannin, flavonoids or terpenoids. In an experiment of Haldar *et al.* (2012)[50] terpenoid and glycoside bound anthraquinone were found from *Typhonium trilobatum.* Phyto extracts are considerably cost effective, safe for environment and can easily apply in the field where mosquito larvae are tolerant to chemical as well as microbial larvicides. But after applying on the field, many more research work should be done regarding its efficacy, environmental acceptability and toxicology towards field application.

Table 1
Larvicidal bioassay using crude extract of Capparis zeylanica leaves against Culex quinquefasciatus larvae

Larval Instars	Concentration (%)	Percent Mortality (Mean±SE)		
		24h	48h	72h
First	0.05	54.67 ± 0.00	64.00 ± 0.33	70.67 ± 0.33
	0.10	61.33 ± 0.33	70.67 ± 0.00	80.00 ± 0.54
	0.15	68.00 ± 0.33	77.33 ± 0.67	90.67 ± 1.20
	0.20	73.33 ± 0.00	90.67 ± 0.00	100.00 ± 0.00

	0.25	82.67 ± 0.54	97.33 ± 0.00	100.00 ± 0.00
Second	0.05	49.33 ± 0.33	58.67 ± 0.33	66.67 ± 0.54
	0.10	54.67 ± 0.00	65.33 ± 0.88	77.33 ± 0.33
	0.15	62.67 ± 0.00	76.00 ± 0.00	86.67 ± 0.88
	0.20	69.33 ± 0.54	86.67 ± 0.33	93.33 ± 0.67
	0.25	80.00 ± 0.33	92.00 ± 0.00	100.00 ± 0.00
Third	0.05	46.67 ± 0.00	54.67 ± 0.88	64.00 ± 0.33
	0.10	53.33 ± 0.88	61.33 ± 0.00	70.67 ± 0.54
	0.15	56.00 ± 0.33	68.00 ± 0.33	77.33 ± 0.00
	0.20	65.33 ± 0.33	74.67 ± 0.00	84.00 ± 0.00
	0.25	74.67 ± 0.33	81.33 ± 0.54	90.67 ± 0.00
Fourth	0.05	18.67 ± 0.67	29.33 ± 0.00	36.00 ± 0.33
	0.10	24.00 ± 0.00	32.00 ± 0.00	41.33 ± 1.20
	0.15	30.67 ± 0.00	40.00 ± 0.00	48.00 ± 0.00
	0.20	37.33 ± 0.33	46.67 ± 0.33	54.67 ± 0.00
	0.25	44.00 ± 0.33	53.33 ± 0.67	60.00 ± 1.20

Table 2

Larvicidal bioassay using Ethyl acetate solvent extract of Capparis zeylanica leaves against Culex quinquefasciatus larvae

Larval Instars	Concentration (%)	Percent Mortality (Mean±SE)		
		24h	48h	72h
1st	30	58.67±0.33	80.00±0.00	88.00±0.00
	60	64.00±0.00	89.33±0.33	97.33±0.54
	90	73.33±0.58	96.00±0.00	100.00±0.00
	120	80.00±0.00	100.00±0.00	100.00±0.00
	150	90.67±0.33	100.00±0.00	100.00±0.00
2nd	30	54.67±0.58	70.67±0.33	84.00±0.00
	60	62.67±0.67	81.33±1.20	93.33±0.33
	90	68.00±0.00	88.00±0.00	100.00±0.00
	120	74.67±0.33	100.00±0.00	100.00±0.00
	150	82.67±0.33	100.00±0.00	100.00±0.00
3rd	30	50.67±0.33	62.67±0.58	74.67±0.67
	60	57.33±0.33	72.00±0.33	84.00±0.00
	90	64.00±0.00	81.33±0.33	90.67±0.54
	120	70.67±0.67	96.00±0.00	100.00±0.00
	150	77.33±0.33	100.00±0.00	100.00±0.00
4th	30	29.33±0.54	34.67±0.88	41.33±0.33
	60	34.67±0.33	41.33±0.33	48.00±0.00
	90	40.00±0.00	49.33±0.54	56.00±0.67
	120	49.33±0.33	56.00±0.00	65.33±0.33
	150	56.00±0.00	66.67±0.33	72.00±0.00

Table 3

Assessment of LC_{50} and LC_{90} values through log-probit and regression analyses using ethyl acetate extract of Capparis zeylanica leaves

Larval Instars	Period of Exposure	LC_{50}	LC_{90}	Regression	R^2- value
1st	24	26.88	250.69	0.066x + 12.33	0.99
	48	14.84	51.03	0.042x + 19.46	0.89
	72	12.44	33.88	0.022x + 22.26	0.67
2nd	24	30.02	497.94	0.056x + 12.03	0.99
	48	22.06	73.42	0.064x + 16.20	0.95
	72	14.36	40.74	0.032x + 20.96	0.76
3rd	24	37.39	685.29	0.055x + 11.00	0.99
	48	26.64	107.49	0.078x + 13.30	0.99
	72	18.75	68.44	0.055x + 17.46	0.94
4th	24	133.11	2250.37	0.056x + 5.366	0.99
	48	87.03	1125.56	0.065x + 6.50	0.99
	72	57.22	747.01	0.065x + 8.23	0.99

x = concentration of ethyl acetate extractives (in ppm)

Table 4

Completely randomized three way ANOVA analyses of the larvicidal activity using concentration (C), hour (H) and instars (I) as three independent parameters

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean of squares (MS)	F value	p-level
Instars (I)	392.77	3	130.92	0.40	0.75
Hours (H)	1,342.74	2	671.37	2.08	0.15
Conc. (C)	993.86	4	248.46	85.43	0
I × H	1,935.08	6	322.51	146.88	0
I × C	37.03	12	3.08	1.41	0.23
H × C	16.14	8	2.02	0.92	0.51
I × H × C	52.70	24	2.20	3.32	0
Within groups	79.33	120	0.66	---	---
Total	4,849.66	179	27.09	-----	---

Table 5

Result of qualitative analyses of phytochemicals from crude leaf extracts of Capparis zeylanica

Name of the Plant	Plant Part	Tannin	Saponin	Steroid	Flavonoid	Terpenoid
Capparis zeylanica	Leaves	+ +	_ _	_ _	+ +	+ +

+ + ... Present
_ _ ..... Absent

CONCLUSION

The presents study reveals the mosquito larvicidal activity of *C. zeylanica* leaves extractives against the malarial vector *An. stephensi.* It can be a very useful larvicide in near future but the work is on its preliminary stage. It needs a continuous effort to isolate the active ingredients with required chemical precision.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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