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Mosquito larvicidal potentiality of wild turmeric, *Curcuma aromatica* rhizome, extracts against Japanese Encephalitis vector *Culex vishnui* group

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Abstract: One of the promising ways to reduce mosquito population is the use of larvicide. *Curcuma aromatica* is a cosmetic herb in South Asia. The present study was conducted to explore the larvicidal effect of *Curcuma aromatica* rhizome extracts against the *Culex vishnui* group. To assess the larvicidal activity, crude extracts of rhizome of *Curcuma aromatica* were prepared ranging from 0.02% to 0.1% concentrations against all the instars of *Cx. vishnui* larvae. Active fractions were obtained by using six different solvents i.e. petroleum ether, n-hexane, ethyl acetate, chloroform: methanol (1:1 v/v), acetone, and absolute alcohol from non-polar to polar fashion. All the solvent extractives were tested against all the larval instars with graded concentrations ranging from 20 ppm to 100 ppm. Through log–probit analyses, LC_{50} and LC_{90} values were determined. Afterwards the statistical justifications were done by ANOVA analyses with reference to time, concentrations and larval instars as three entirely randomized autonomous variables. Furthermore the bioactive fraction was tested against non-target organisms under laboratory conditions. Maximum mortality was recorded in 0.1% concentrations of crude rhizome extracts against all the instars. Ethyl acetate extractives were found to have good larvicidal effect only among all the solvent extractives. The LC_{50} and LC_{90} values of 3rd instars larvae were 17.25 ppm and 78.02 ppm respectively after 72 h of post exposure. The non-responsiveness of the non-target populations towards extractives was also noticed. The rhizome extracts of *Curcuma aromatica* can be used as an alternative larvicide against the JE vector *Cx. vishnui* group.

Keywords: *Curcuma aromatica*; Rhizome; *Culex vishnui*; larvicidal activity

Introduction

Mosquitoes, the perilous vectors of diseases, belong to the family of small, midge-like flies, the Culicidae. Although a few species are harmless or even helpful to mankind, most of them are considered as nuisances. Over 3,500 species of mosquitoes have been described to date around the globe. Some are vectors of a number of infectious diseases affecting millions of people per year (Leisnham, 2013). Among these, Japanese Encephalitis (JE) is a dreadful ailment caused by JE virus which is prevalent in South East Asia and Far East. The reservoirs of this virus are domestic pigs and some wild birds. Approximately 3 billion people of the humanity live in this affected area. In JE-endemic Indian sub-continent more than 50,000 cases per year occur and the mortality rate is near 30% (Kabilan et al., 2004). Pragmatically the populations of rural localities are at higher risk than urban regions.

Culex vishnui group, vectors of JE, preferably breeds in water with plentiful vegetation like paddy fields (Banerjee and Chandra, 2004), moreover shallow ditches, pools and rice cultivation sites.

Several synthetic insecticides have been applied but most of them are non-biodegradable and reported to be hazardous. Besides, the gradual increase of resistance towards chemical insecticides (WHO, 1992) led to the need of new insecticides from novel sources especially from botanical origin (Bhattacharya et al., 2014a, 2014b, Chowdhury et al., 2009).

Curcuma aromatica commonly known as wild turmeric is a member of the genus *Curcuma* belonging to the family Zingiberaceae. The perpetual shrubbery dies down in late autumn but the rhizomes remains latent. The inflorescence appears in early spring from the base of the rhizomes. Following immediate hot summer and rainy monsoon, the plant grows rapidly, upto 30-40 cm and is crowned with enlarged coloured bracts with pink tips. Leaves often appear subsequent to the flowers.

The rhizome extracts of *Curcuma aromatica* possess good anti-bacterial effect (Revathi et al., 2013); along with anti-inflammatory and wound healing activity (Kumar et al., 2009). It has mosquitocidal activity against *Aedes aegypti* (Choochote et al., 2005). However, documentation of its utilization as a larvicidal agent against *Cx. vishnui* group has yet not been put forwarded. This is the first ever report in this regard.

1 Materials and Methods:

1.1 Collection of plant material

The rhizome of *C. aromatica* was collected around the vicinity of Jaldapara (26^0 37'43" N, $89^0$22'39" E), West Bengal, India. Following proper identification of the plant, a voucher specimen (GCZSM-3) was acquiesced as a herbarium to the Mosquito, Microbiology and Nanotechnology Research Units, Department of Zoology, The University of Burdwan.

1.2 Rearing of larvae and colony set up

Larvae of *Culex vishnui* group were collected from inundated rice fields of agriculture farm, The University of Burdwan (23°16 N, 87°54 E) by standard scooping and dipping method (Robert et al., 2002). They were kept in dirt-free plastic trays filled with tap water and were fed with a mixture of brewer yeast, dog biscuits and algae in 3:1:1 ratio (Kamaraj et al., 2011). Pupae were transferred from the trays to insectaries (45×45×40 cm). Adults were provided with 10% sucrose solution with multivitamin syrup in a container with a cotton wick. The mosquitoes were taxonomically identified with the help of the keys provided by Christophers (1933), Barraud (1934) and Chandra G (2000). On the day 5 of nurturing, a blood meal from a non-motile shaved pigeon was given to adults (females) overnight. Afterwards Petri dishes filled with 100 ml of tap water and wrinkled with filter paper were kept inside the cages for oviposition. The eggs were kept undisturbed and endorsed to hatch under laboratory conditions. By repeating this method, a laboratory reared colony of *Cx. vishnui* group was developed. The colony was set aside in a hygienic place and sustained at 30±2℃ temperature and 80–85% relative humidity (RH) under a 13:11 light-and-dark cycles.

1.3 Processing of crude extract

Collected fresh rhizomes of *C. aromatica* were firstly chopped to standard sizes and were rinsed in tap water followed by distilled water and soaked on paper towels. The dirt-free pieces of rhizomes were crushed by the electrical grinder and the fluid was filtered through Whatman no.1 filter paper. Thereafter the filtrate was used as a stock solution (100% concentration) and the desired concentrations (0.02%, 0.04%, 0.06%, 0.08% and 0.10%) were prepared for further bioassay experiments.

1.4 Solvent extraction

Cleaned rhizomes of *C. aromatica* were shed dried. 200 g dried rhizomes of *C. aromatica* were placed into the thimble of the Soxhlet apparatus and 2 litters solvent was loaded (1:10) into the still pot. Six different solvents viz. petroleum ether, n-hexane, ethyl acetate, chloroform: methanol (1:1 v/v), acetone and absolute alcohol from non-polar to polar fashion were passed through the column one after another with the same plant materials. The extraction period was fixed for 72 h (Ray et al., 2014, Bhattacharya and Chandra, 2014, 2013, Rawani et al., 2009) for each solvent with 8 h maximum a day. The extractives were collected from the still pot chamber and concentrated through evaporation. The fully concentrated extractives were kept at 4℃ in a refrigerator for further use.

1.5 Larvicidal bioassay

The larvicidal bioassays were executed following the standard protocol of World Health Organization (WHO, 2005) in the Mosquito, Microbiology and Nanotechnology Research Units, Parasitology Laboratory, Department of Zoology, The University of Burdwan. Twenty five larvae of each instar were put in sterilized glass Petri dishes (9 cm diameter/150 ml capacity) filled with 100 ml of distilled water. Crude extracts of 0.02%, 0.04%, 0.08% and 0.10% concentrations were added separately to different Petri dishes. From each extractive five different working solutions of 20, 40, 60, 80 and 100 ppm were prepared and applied on different Petri dishes for the assessment of the larvicidal property. Each experiment was triplicated (n=75) with 3 replicates of controls at laboratory condition. After 24 h, 48 h and 72 h of post exposures larval mortality were recorded.

1.6 Test on non-target organism

The effects of all the extracts were tested on *Chironomus circumdatus and Gambusia affinis*. Those were collected and maintained in cemented tanks in

the laboratory. The median lethal concentration of 3rd instar larvae after 72 h of exposure was applied on the non-target organism.

1.7 Statistical analyses

Abbott's formula (Abbott, 1925) was used to precise the percentage mortality (%M) throughout the observation. Estimations of LC_{50} and LC_{90} values through Log-probit analyses and regression analyses were carried out using the "STAT PLUS 2009 (Trial version)" and "MS EXCEL 2007" respectively. ANOVA analyses were also done for further statistical justifications.

2 Results

The mortality percentages for all the instars with crude extracts of different concentrations were presented in Table 1. Maximum mortality was recorded at 0.1% concentration of the crude extracts. Among all the solvent media ethyl acetate showed positive result only. After 72 h of exposure, 1st, 2nd and 3rd instars larvae exhibited 100% mortality at 60, 80

Table 1 Dose response larvicidal bioassay using crude extract of *C. aromatica* rhizome against *Cx. vishnui* group

Larval Instars	Concentration (%)	Percent Mortality rate (Mean ±SE)		
		24h	48h	72h
First	0.02	29.33 ±0.54	46.33 ±0.88	50.67 ±0.33
	0.04	38.67 ±0.58	53.33±1.20	60.00 ±0.00
	0.06	48.00 ±0.33	61.33 ±0.33	78.67 ±0.89
	0.08	58.67 ±0.67	69.33 ±0.67	90.67 ±0.33
	0.10	62.67 ±0.33	72.00 ±0.00	100.00 ±0.00
Second	0.02	22.67 ±0.33	32.00 ±0.00	34.67 ±0.33
	0.04	34.67 ±0.58	40.00 ±0.33	41.33 ±0.33
	0.06	50.67 ±1.45	52.00 ±0.00	60.00 ±0.33
	0.08	53.00 ±0.00	61.33 ±0.88	65.33 ±1.20
	0.10	60.00 ±0.88	66.67 ±0.33	80.00 ±0.00
Third	0.02	21.33 ±0.67	28.00 ±0.89	34.67 ±0.33
	0.04	30.67 ±0.88	37.33 ±0.33	45.33 ±0.67
	0.06	48.00 ±0.00	52.00 ±0.33	58.67 ±0.33
	0.08	53.33 ±0.33	58.67 ±1.20	60.00 ±0.00
	0.10	56.00 ±0.00	61.33 ±0.33	65.33 ±0.33
Fourth	0.02	16.00 ±0.00	20.00 ±0.00	22.67 ±0.33
	0.04	20.00 ±1.20	22.67 ±0.33	30.67 ±0.33
	0.06	25.33 ±0.33	26.67 ±0.33	34.67 ±0.67
	0.08	29.33 ±0.33	33.33 ±0.67	41.33 ±0.33
	0.10	37.33 ±1.45	41.33±0.33	48.00 ±0.00

Table 2 Dose response larvicidal bioassay using ethyl acetate extract of *C. aromatica* rhizome against *Cx. vishnui* group

Larval Instars	Concentration (ppm)	Percent Mortality Rate (Mean±SE)		
		24h	48h	72h
First	20	54.67±0.58	68.00±0.00	80.00±0.00
	40	61.33±0.88	74.67±0.33	86.67±0.88
	60	76.00±0.58	80.00±0.00	100.00±0.00
	80	82.67±0.33	86.67±0.58	100.00±0.00
	100	88.00±0.00	100.00±0.00	100.00±0.00
Second	20	49.33±1.45	57.33±0.33	68.00±0.00
	40	54.67±1.20	67.67±1.20	77.33±0.33
	60	62.67±0.88	72.00±0.58	85.33±0.88
	80	69.33±0.88	77.33±0.33	100.00±0.00
	100	77.33±0.33	92.00±0.00	100.00±0.00
Third	20	36.00±0.00	41.33±0.33	61.33±0.67
	40	41.33±0.88	58.67±0.33	74.67±0.88
	60	48.00±0.00	66.67±0.33	80.00±0.00
	80	66.67±0.67	73.33±0.33	89.33±0.58
	100	73.00±0.00	81.33±0.33	100.00±0.00
Fourth	20	41.33±0.33	52.00±0.00	65.33±0.33
	40	48.00±0.00	61.33±0.67	70.67±0.58
	60	56.00±0.00	68.00±0.00	78.67±0.67
	80	65.33±0.33	74.67±0.33	84.00±0.00
	100	74.67±0.33	80.00±0.88	90.67±0.33

and 100 ppm concentrations respectively. For 4th instar larvae, 90.67% mortality was recorded in 100 ppm following 72 h of post exposure (Table 2). LC_{50} and LC_{90} values (at 95% level of confidence) were given in Table 3 which was noticed to decrease along time (from 24 h to 72 h).

The mortality (Y) was positively concurrent with the concentration of exposure (X) with regression coefficient (R^2) close to 1 in each case (Table 3). However the non-target populations were fairly nonresponsive throughout the observations. The larvicidal activity was found statistically significant ($p < 0.05$) through completely randomized ANOVA analyses (Table 4).

Table 3 Assessment of LC_{50} and LC_{90} values of ethyl acetate extract of *C. aromatica* rhizome through log-probit and regression analyses

Larval Instars	Period of Exposure	LC_{50}	LC_{90}	Regression	R^2- value
1st	24	21.31	145.21	0.11x + 11.53	0.97
	48	12.38	89.75	0.078 x + 15.43	0.99
	72	11.22	33.61	0.066 x + 19.33	0.80
2nd	24	27.65	492.06	0.088 x + 10.36	0.99
	48	18.49	160.25	0.086 x + 12.79	0.99
	72	15.02	54.62	0.108 x + 15.03	0.95
3rd	24	47.69	438.70	0.124 x + 5.785	0.96
	48	31.07	226.59	0.118 x + 8.91	0.96
	72	17.25	78.02	0.115 x + 13.36	0.99
4th	24	38.15	498.01	0.21 x + 5.862	0.99
	48	20.01	331.96	0.17 x + 9.864	0.99
	72	11.65	159.76	0.16 x + 13.06	0.99

Note: x = concentration of ethyl acetate extractives (in ppm)

Table 4 Completely randomized three way ANOVA analyses 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)	1079.91	3	359.97	285.44	0.00
Time (H)	670.08	2	335.04	265.67	0.00
Conc. (C)	1223.02	4	305.76	242.45	0.00
I ×H	50.99	6	8.50	6.74	0.00
I ×C	31.42	12	2.62	2.08	0.02
H ×C	15.31	8	1.91	1.52	0.15
I ×H ×C	20.51	24	0.85	0.68	0.86
Within groups	151.33	120	1.26	---	---
Total	3242.57	179	18.12	----	---

3 Discussions

Owing to hazardous impact of synthetic insecticides on public health, employment of ecological and recyclable natural insecticides of plant origin has been given importance. Larval control is regarded as the best approach to lessen up the mosquito populations at very early stage. Hence, management of mosquito populations is predominantly inclined at wrigglers' control and only against matures when essential. Miscellaneous plant ingredients were reported to be larvicidal (Bhattacharya et al., 2014a, 2014b, Ray et al., 2014), pupicidal (Rawani et al., 2012), adulticidal, smoke toxic and repellent (Chowdhury et al., 2007) against different species of mosquitoes.

Present study well documented the bio-activity of ethyl acetate extracts of wild turmeric against *Cx. vishnui* group. The larvicidal activity of crude extract of leaves of *Swietenia mahagoni* against *Cx. vishnui* group was reported by Adhikari and Chandra, 2012 where cent percent mortality of 3rd instar was recorded at of 0.4% concentration after 72 h of post exposure. In the current

study 84% mortality of 3rd instars were noticed after 72 h of post exposure with 0.1% concentration only. The biocontrol efficacy of ethyl acetate extract of seed coat of *C. sophera* against *Cx. quinquefasciatus* was established by Kundu et al., 2013 with 100% mortality at 480 ppm concentration against 1st instar larvae after 72 h of exposure. The present study relates 100% mortality of 1st instar larvae at 60 ppm concentration only after 72 h of post exposure. So, the larvicidal effect of the *C. aromatica* was considerably higher ($p < 0.05$) at relatively lower concentration of the active fraction than their work. Elango et al., (2009) accounted that ethyl acetate extract of the leaves of *Aegle marmelos* (L) displayed larvicidal properties against 4th instar *Anopheles subpictus* and *Culex tritaeniorhynchus* with the LC_{50} values of 167.00 and 99.03 ppm, respectively after 72 h of exposure. The LC_{50} value of the 4th instars larvae was found 39.13 ppm after 72 h of post exposure in this present study (Table 3) against *Cx. vishnui* using ethyl acetate extracts of *C. aromatica* rhizome.

Concisely, the findings of the present study disclosed that the rhizome of *C. aromatica* exhibit notable larvicidal activity against *Cx. vishnui* group. Further investigations are needed to enlight the actual chemical amalgam accountable for larvicidal activity, to identify the chemical individuality of the active constituent and to fruitfully make use of, if feasible, by articulating a trade product.

Conflict of interest statement

We declare that we don't have any conflict of interest.

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