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Phytochemical extractions from the leaves of *Ravenala madagasariensis* from Sundarban area and its effect on southern house mosquito (*Culex quinquefasciatus* Say 1823) larvae

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Abstract Vector control is a major issue mainly in this time when resistance to chemical insecticides leads to a greater problem. For an alternative measure novel botanical sources can be used as a good insecticide with less toxic hazards to the environment and public health. The present study estimated larvicidal activities of the crude and solvent extracts of *Ravenala madagasariensis* against the filarial vector *Culex quinquefasciatus* under laboratory conditions. Crude extracts of *R. madagasariensis* mature leaves were examined for larvicidal activity against all the larval instars (1st to 4th) of *Cx. quinquefasciatus*. Solvent extraction was carried out using three different solvents viz. petroleum ether, chloroform: methanol (1:1 v/v) and absolute alcohol. Dose dependent mortality assays were performed using the bioactive fractions of the solvent extracts. Further, determinations of LC_{50} and LC_{90} values were accomplished through log-probit analyses and regression analyses. The larvicidal activity of *R. madagasariensis* leaves were statistically justified through ANOVA analyses. Effects of the bio-active portions were examined on a non-target water fauna. After 72 h of exposure 78.67% mortality of 1st instars larvae was recorded at 0.5% concentration of crude extract. 150 ppm concentration of solvent extract showed 100% mortality against 1st instars larvae after 72 h of exposure. 200 ppm concentration was responsible for 100% mortality of 2nd instars larvae in 72 h. 250 ppm concentration showed significant mortality against 3rd and 4th instars larvae. *Chironomus circumdatus* larvae, non target organism, exhibited no significant mortality. This experimental study was an initiative to ascertain *R. madagasariensis* as a novel resource of target specific larvicide against *Cx. quinquefasciatus* larvae.
Keywords *Ravenala madagasariensis*; *Culex quinquefasciatus*; non-target organisms; larvicide

Introduction

Mosquitoes, well documented for their human health collision, act as the major vector of various infectious diseases (Tolle, 2009) like malaria, yellow fever, dengue, Lymphatic filariasis, chikungunya, Rift Valley fever, Japanese Encephalitis and so on. They can also transmit some other arbovirus born diseases viz. West Nile virus, Eastern equine encephalomyelitis virus in US, Saint Louis encephalitis virus etc. In India, mosquito can affect about 40 million people with such obnoxious diseases (Ghosh et al., 2012). Amongst the culicine mosquitoes, *Culex quinquefasciatus* is the major vector of lymphatic filariasis. *Cx. quinquefasciatus* also plays a major role to transmit avian malaria. It plentifully breeds in muddy water; septic tanks, stagnant drains etc. and complete its life cycle within 7 days. Filariasis is a parasitic disease caused by nematode worms, *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori*. Microfilariae, the larval forms of adult worm, are transmitted from one human host to another by mosquito bite. Filariasis is an endemic disease in tropical and subtropical countries and around 1.2 billion peoples are facing higher risk of this disease (Bockarie et al., 2009). To diminish the frequency of such socio-economic crisis, the primary step is to control the vector population (Aktar et al., 2009). But the vector control is facing a problem due to its highly increasing resistance phenomenon against the well branded chemical insecticides (WHO, 1992). Furthermore, synthetic insecticides depart a health hazard towards the non-target organisms and can put a negative impact on the environment due to less target specificity, non-biodegradability and non-eco-friendly nature (Wattal et al., 1981). At this situation, botanicals can nearly suffice the need of

controlling agent if proper isolation, categorization and utilization take place (Rawani et al., 2009; Chowdhury et al., 2007, 2009). With respect to synthetic insecticides, botanical insecticides are found to be cost effective, biodegradable and eco-friendly in nature (Rawani et al., 2010; Ghosh, 2012; Ray et al., 2014).

Ravenala madagasariensis, an endemic plant of Madagascar, known as traveller's palm or traveller's tree is not a true palm belonging to family Arecaceae, rather a member of family Strelitziaceae. Its four different forms have been distinguished. Paddle shaped leaves are oriented in a pattern like peacock tail (Figure 1).

Figure 1 *Ravenala madagasariensis*

R. madagasariensis is a traditional medicinal plant that is used in treatment of diabetes and kidney stone (Shakthi et al., 2010). Antiseptic activity of this plant (Jain, 2005) has also been reported. As per our literature review this is the first ever report on this plant as mosquito larvicide against *Cx. quinquefasciatus*, the vector of lymphatic filariasis.

1 Materials and Method

1.1 Collection of plant material

Fresh mature leaves of *R. madagasariensis* were harvested during January-February, 2014 from some part of Sundarban (21^0 56'59" N, 89^0 10'59.988" E), West Bengal, India. After proper identification of the plant a voucher specimen (GCP-14) was submitted as a herbarium to the Department of Zoology, The University of Burdwan.

1.2 Rearing of larvae and colony set up

From the drains adjacent to Burdwan University (23°16′N, 87°54′E), larvae of *Culex quinquefasciatus* were collected with the help of standard scooping and dipping method (Robert et al., 2002). To set up a larval colony larvae were kept in a plastic tray filled with dechlorinated tap water with proper hygiene. Larvae were fed with a mixture diet of Brewer yeast, dog biscuits and algae in a ratio of 3:1:1 respectively (Kamaraj et al., 2011). Within the tray, larvae became pupae, and then those were transferred to an insectary (45×45×40 cm) for adult immergence. Adult mosquitoes were identified with the help of the key provided by Barraud (1934), Christophers (1933) and Chandra (2000). Adult mosquitoes were supplied a nutrition of a multivitamin syrup and 10% sucrose solution with a cotton wick in a container.

On the 5^{th} day of rearing, adult females were supplied a blood meal from a non-motile shaved rat. Petri dishes filled with 100 ml of tap water and wrinkled with filter paper were kept inside the cage for oviposition. Eggs were undisturbed and allowed to hatch under laboratory conditions. The colony was maintained at 27±2℃ temperature and 80–85% relative humidity (RH) under the photo regime of 13:11 light-and-dark cycles.

1.3 Processing of crude extract

Collected mature leaves of *R. madagasariensis* were washed well with tap water and soaked on a paper towel. Green leaves were crushed with the help of an electrical grinder and the juice was filtered with Whatman's no-1 filter paper and stored at 4℃ as a stock solution of 100% concentration for further bioassay experiments.

1.4 Solvent extraction

Unspotted and unsoiled mature leaves were dried in shed for a few days. 200 g of dried leaves were taken in the column of the Soxhlet apparatus and 2 lit of solvent were put into the solvent chamber. Three different solvents in a non-polar to polar fashion viz. petroleum ether, chloroform: methanol (1:1 v/v) and absolute alcohol were passed through the column one after another. 72 hours of extraction period was fixed

for each solvent with 8 hours maximum a day. Elutes were collected from the solvent chamber and concentrated through evaporation in a rotary evaporator. The extractives were preserved at 4℃ in a refrigerator for further bioassay experiments**.**

1.5 Larvicidal bioassay

The larvicidal activity of the crude extract against *Cx. quinquefasciatus* were estimated at room temperature under laboratory condition as per the standard protocol (WHO, 2005). Twenty five larvae were relocated from the larval colony to the glass Petri dish (150 ml). 0.1% to 0.5% concentrations of the crude extractives were applied to all the larval instars (1^{st}, 2^{nd}, 3^{rd} and 4^{th}) in different Petri dishes. Solvent extracts were also provided to other Petri dishes with a concentration of 50 ppm to 250 ppm. During the total observation period of 72 hours, the relative humidity was maintained at 88±2 %. Mortality rate was calculated after 24 h, 48 h and 72 h respectively; mortality rate of 48 h and 72 h were calculated with addition of mortalities of 24 h and 48 h. Larvae were assumed dead when they failed to move after pricking with a sharp needle to the cervical or siphon region of the larvae or when they failed to reach the water surface **(**Macedo et al., 1997). 3^{rd} instars larvae were chosen to examine the larvicidal potentiality of all three solvent extractives and then the best active fraction was examined against all the larval instars.

1.6 Effect on non-target organism

Effect of crude and solvent extracts were examined against the non-target insect larvae of *Chironomus circumdatus*. They were exposed to different concentration of crude and solvent extractives and after 72 h of observation no abnormalities like reduced swimming activity, sluggishness or mortality were found.

1.7 Statistical analysis

The percentage of corrected mortality was calculated following Abbott's formula (Abbott WS, 1925). To find the LC_{50}, LC_{90} values, regression equations (Y=mortality; X=concentrations) and regression coefficient values, experimental figures were statistically analyzed by using the computer software "STAT PLUS 2007 (Trial version)" and MS excel 2007. 95% confidence levels were calculated following the method proposed by (Zar, 2008).

2 Results and Discussion

R. madagasariensis was found to have remarkable mosquitocidal property against *Cx. quinquefasciatus* in the present laboratory observation. Considerable 78.67% mortality was recorded against 1^{st} instars larvae at 0.5% concentration of crude extract after 72 h of exposure (Table 1). The mortality rate gradually increased in all instars with increase in time of exposure for every working concentration. It was

Table 1 Percent mortality of *Cx. quinquefasciatus* larvae using crude extract of *R. madagasariensis* leaves

Larval Instars	Concentration (%)	Percent Mortality (Mean ±SE)		
		24h	48h	72h
First	0.1	30.67 ±1.25	45.33 ±0.82	53.33 ±0.94
	0.2	38.67 ±1.63	53.33±1.41	60.00 ±0.82
	0.3	49.33 ±2.05	62.67 ±0.00	66.67 ±0.94
	0.4	58.67 ±1.63	69.33 ±0.94	72.00 ±1.47
	0.5	65.33 ±0.80	73.33 ±0.65	78.67 ±0.00
Second	0.1	24.00 ±0.94	32.00 ±0.82	36.00 ±1.70
	0.2	34.67 ±2.05	40.00 ±1.63	42.67 ±0.65
	0.3	52.00 ±2.16	52.00 ±2.05	60.00 ±0.82
	0.4	56.00 ±1.70	61.33 ±0.82	66.67 ±1.25
	0.5	62.67 ±1.41	66.67 ±0.47	72.00 ±0.00
Third	0.1	22.67 ±0.00	29.33 ±0.80	36.00 ±1.47
	0.2	32.00 ±0.94	37.33 ±1.25	41.33 ±2.45
	0.3	49.33 ±0.00	53.33 ±1.41	57.33 ±0.00
	0.4	53.33 ±1.94	58.67 ±1.25	72.00 ±1.41
	0.5	56.00 ±1.63	62.67 ±0.00	74.67 ±0.00
Fourth	0.1	0.00 ±0.00	8.00 ±1.25	24.00 ±0.00
	0.2	2.67 ±1.25	9.33 ±0.47	30.67 ±0.00
	0.3	6.67 ±0.00	13.33 ±2.87	34.67 ±0.47
	0.4	9.33 ±0.47	17.33 ±1.45	36.00 ±0.82
	0.5	13.33 ±1.28	24.00±1.63	41.33 ±2.16

highest in 72 h of exposure and lowest in 24 h of exposure. Collected fractions through petroleum ether and absolute alcohol did not cause any larval mortality at all and those data were excluded. In case of chloroform: methanol (1:1 v/v) extract 1st instars larvae exhibited 100% mortality at 150 ppm concentration onwards at 72 h of exposure (Table 2). Cent percent mortalities were found at 200 ppm concentrations onwards at 72 h of exposure for 2nd instars larvae. In case of 3rd and 4th instars larvae maximum mortalities (>86%) were detected at 250 ppm concentration after 72 h of exposure in the laboratory (Table 3). The non-target organism was entirely non-responsive to any kind of extract throughout the experiment.

Table 2 Percent mortality of *Cx. quinquefasciatus* larvae using bio-active fraction of chloroform: methanol (1:1 v/v) extract of *R. madagasariensis* leaves

Larval Instars	Concentration (ppm mg/mL)	Mortality Rate (Mean ±SE)		
		24h	48h	72h
1st	50	56.00 ±0.00	68.00 ±2.12	80.00 ±0.00
	100	61.33 ±1.67	74.67 ±0.49	86.67 ±1.23
	150	76.00 ±1.89	80.00 ±0.33	100.00 ±0.00
	200	82.67 ±1.44	86.67 ±1.77	100.00 ±0.00
	250	88.00 ±0.00	93.33 ±1.89	100.00 ±0.00
2nd	50	49.33 ±0.78	57.33 ±1.79	68.00 ±1.44
	100	54.67 ±1.23	67.67 ±2.78	77.33 ±1.73
	150	62.67 ±1.45	72.00 ±0.00	85.33 ±2.56
	200	69.33 ±2.12	77.33 ±1.82	100.00 ±0.00
	250	77.33 ±0.36	86.67 ±0.67	100.00 ±0.00
3rd	50	36.00 ±0.00	41.33 ±1.44	61.33 ±2.44
	100	41.33 ±1.39	58.67 ±0.49	74.67 ±1.48
	150	48.00 ±2.11	66.67 ±1.52	80.00 ±0.00
	200	66.67 ±1.91	73.33 ±1.67	89.33 ±1.73
	250	76.00 ±0.00	81.33 ±1.45	98.67 ±1.79
4th	50	32.00 ±0.00	38.67 ±1.89	57.33 ±1.79
	100	37.33 ±1.77	53.33 ±1.73	70.67 ±1.35
	150	46.33 ±0.78	58.67 ±0.36	77.33 ±0.67
	200	64.00 ±0.00	65.33 ±0.78	82.67 ±1.33
	250	70.67 ±1.44	73.33 ±2.12	86.67 ±2.78

Table 3 Assessment of LC_{50} and LC_{90} values of chloroform: methanol (1:1 v/v) extract of *R. madagasariensis* through log-probit and regression analyses

Larval Instars	Period of Exposure	LC_{50}	LC_{90}	Regression	R^2- value
1st	24	44.65	466.43	0.04 x + 11.93	0.96
	48	23.49	315.52	0.03 x + 15.56	0.99
	72	25.41	90.98	0.03x + 19.13	0.90
2nd	24	70.71	758.98	0.04x + 10.36	0.99
	48	40.95	593.06	0.03x + 12.93	0.99
	72	32.96	175.13	0.04 x + 14.89	0.96
3rd	24	115.90	908.67	0.05x + 5.77	0.95
	48	77.67	566.48	0.05x + 8.97	0.96
	72	39.07	241.46	0.04x + 13.86	0.97
4th	24	137.75	1126.53	0.03x + 1.90	0.99
	48	97.92	1026.96	0.03x + 5.44	0.99
	72	38.46	457.29	0.02x + 8.27	0.99

The result of three-way randomized factorial ANOVA, considering different concentrations, spans of exposures and different instars as three independent parameters, statistically justified the larvicidal potentiality of *R. madagasariensis* using chloroform: methanol (1:1 v/v) extract. The results of regression analyses revealed that the mortality (Y) was positively correlated with the concentration (X) having a regression coefficient (R^2) close to 1 in each case (Table 4). The results of log probit analyses (95% confidence level) revealed that LC_{50} and LC_{90} values gradually decreased with the increase in post-exposure period having the lowest value at 72 h of exposure to first instars larvae followed by second, third and fourth instars larvae. LC_{50} and LC_{90} values of 1st instars larvae at 72 h of exposure were 25.41 and 90.98 ppm respectively. The preliminary qualitative phytochemical analyses revealed that tannins and steroids were predominantly present in the leaves of *R. madagasariensis*.

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
Hours (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	----	---

The secondary metabolites of plants do possess significant mosquito larvicidal property. These are especially useful due to easy availability, cost effectiveness, biodegradability, eco-friendliness and target specificity. Amongst all the life stages of mosquitoes, larvae are most susceptible. Different plant extractives have been used as good larvicidal agents (Bhattacharya and Chandra 2013, 2014, Kundu et al., 2013, Chakraborty et al., 2013, Singha et al., 2011) against various mosquito species. Chowdhury et al., (2008) reported that chloroform: methanol extract of *Solanum villosum* berry showed the highest mortality (76.66%) against 3rd instars larvae of *Stegomyia aegypti*. Chloroform: methanol (1:1 v/v) extract of some common spices and vegetable wastes showed very promising effect as mosquito larvicide in a very low concentration (Singha and Chandra, 2011). Rahuman et al. (2008) reported that methanol extract of *Cedrus deodara* stem bark was effective against *Culex quinquefasciatus* with the LC_{50} value of 95.19 ppm. In another work, Rawani et al., (2013) showed 80% mortality against 3rd instars larvae of *Culex quinquefasciatus* with the 200 µg/ml concentration of chloroform: methanol extract of *Solanum nigrum* berry.

The present study showed that the leaves of *R. madagasariensis* exhibited significant mortality of *Culex quinquefasciatus* larvae. Therefore, it can be concluded that further studies on leaves of *R. madagasariensis* may fulfil the search of establishing a new bio-insecticide.

Conflict of interest statement

The authors have no conflict of interest.

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