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Larvicidal activity of *Dregea volubilis* and *Bombax malabaricum* leaf extracts against the filarial vector *Culex quinquefasciatus*

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ABSTRACT

Objective: To analyze the larvicidal activity of two plant leaf powder and leaf-extracts, *Dregea volubilis* and *Bombax malabaricum* against *Culex quinquefasciatus*. **Methods:** The larvicidal bioassay was done for powdered leaves of *Dregea volubilis* and *Bombax malabaricum* individually and their methanol extracts against first-, second-, third- and fourth-instar larval forms of *Culex quinquefasciatus*. Mortality rate was recorded after 24, 48 and 72 h of post-exposure. LC_{50} and LC_{90} values were calculated at different time intervals for third instar larvae. **Results:** All the graded concentration (0.1%, 0.2%, 0.3%, 0.4%, 0.5%) of powdered leaves showed significant (P <0.05) larval mortality. The mortality rate was higher in 50 ppm doses of methanolic extracts of both the plants against *Culex quinquefaciatus*. The corresponding LC_{50} values were 56.97 ppm and 48.85 ppm, respectively after 24 h of exposure. There is no mortality of non-target organism such as *Chironomous circumdatus, Oreochromis niloticus niloticus* and *Diplonichus annulatum* within 72 h of post exposure to 0.1%, 0.2%, 0.3% crude powdered leaves and methanolic extracts of both the two individual plants under the laboratory condition. The results of preliminary qualitative phytochemical analysis of both the plants revealed the presence of many bioactive principles such as steroids, tannins, flavonoids, triterpenoids, saponins, etc. that may be responsible for their bio-control potentiality. **Conclusions:** The results have shown potential and eco-friendly use of both plant extracts against larva of *Culex quinquefasciatus* for the first time.

1. Introduction

Mosquitoes are well known group of harmful insects that belongs to the Order Diptera that are wide spread, causing serious health problems to human beings. Lymphatic filariasis is a widely distributed tropical disease, causing chronic manifestation in around 44 million people and infected around 120 million people worldwide[1]. One of the approaches, which has been achieved and practiced for a long time, using synthetic chemicals to control of this vector. But chemicals are non-biodegradable and cause pollution. Resistance against these chemicals grows among mosquito species quickly. Herbal products with proven potential as insecticides and repellent can play an important role in interruption of the transmission of vector borne diseases. Natural pesticides are effective in pest control, less toxic to non-target organisms and biodegradable at the same time and thus they may be safer than synthetic pesticides. Plant defense mechanism natural products often involves of a variety of toxins which make adaptation of the predator unfavorable[2].

Dregea volubilis (D. volubilis)(L.f.) Benth. ex Hook.f. (Family: Asclepiadaceae; Order: Gentianales) [Synonym: *Wattakaka volubilis* (L.f.) Stapf.; *Asclepias volubilis* L.f.] is a large twining shrub and is widely distributed in India, Sri Lanka, Myanmar, Indonesia, Thailand and China. It is a large twining shrub with woody vines, simple leaves, opposite and occasionally whorled and bisexual flowers [3]. In Ayurveda, *D. volubilis* is extensively used to treat inflammation, piles, leucoderma, asthma, tumors, urinary discharge *etc*[4]. Methanolic extract of the leaves also possess anti-inflammatory activity[5].

The plant, *Bombax malabaricum (B. malabaricum)* DC. [Synonym: *Bombax ceiba* L.; *Salmalia malabaricum* Schott

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& Endl.], belongs to the family Bombacaceae (Order: Malvales). It is a tropical tree which has a straight tall trunk and its leaves are deciduous in winter. Red flowers with 5 petals appear in the spring before the new foliage. It produces a capsule which, when ripe, contains white fibers like cotton. It possess specific role in economy for cotton and timber for its utilization to prepare match-sticks[6]. The leaves are used in inflammation and cutaneous trouble[6, 7]. The leaves extract exhibits significant antifungal activities against ringworm causing fungus. The bark gum contains catechutannic acid. Antioxidant and analgesic mangiferin was isolated from leaves of the plant[8].

The purpose of the present work was to investigate the mosquito larvicidal activities of powdered leaves and methanolic extracts of matured leaves of *D. volubilis* and *B. malabaricum*.

2. Materials and methods

2.1. Test mosquitoes

The present study was conducted at Burdwan (23°16′ N, 87°54′ E), West Bengal, India during March-April 2009. Larvae of all the instars of *Culex quinquefasciatus (Cx. quinquefasciatus)* were collected from cemented drains surrounding the University campus and kept in the plastic bucket (20 L) with addition of artificial foods (powdered mixture of dog biscuits and dried yeast powder in the ratio of 3:1).

2.2. Plant material

Fresh leaves of *D. volubilis* were collected from Ramchandrakhali, District of South 24 Parganas, West Bengal, India and identified by Botanical Survey of India, Howrah, India [Voucher specimen No. CNH/1-I (54/2004-Tech II/886)].

B. malabaricum leaves were collected from Azamgarh city, Uttar Pradesh, India and identified by Botanical Survey of India, Howrah, India [Voucher specimen No. CNH/1-Ⅰ(314/2009-Tech Ⅱ/356/346)]. Both the voucher specimens were deposited in the herbarium of Division of Pharmaceutical Chemistry and Medicinal Chemistry, Department of Pharmaceutical Technology, Jadavpur University, India for future reference.

The shade dried leaves of *D. volubilis* and *B. malabaricum* were powdered and stored in airtight containers.

2.3. Preparation of methanolic extract of both plants

The powdered leaves of *D. volubilis* and *B. malabaricum* were extracted individually in Soxhlet apparatus using methanol (SD Fine Chem, Mumbai, India). The solvent was removed under vacuum and the methanol extract of *D. volubilis* and *B. malabaricum* were stored separately in desiccator. The residues were weighed and dissolved in suitable volumes of distilled water to make different concentrations.

2.4. Dose-response larvicidal bioassay

The larvicidal bioassay was done followed the World Health Organization standard protocols [9] with suitable modifications. Required concentrations of powdered leaf (0.1%, 0.2%, 0.3%, 0.4%, and 0.5% w/v) and methanolic extract (10, 20, 30, 40, 50 ppm) were prepared through the mixing up of powdered leaf and the extract with variable amounts of sterilized distilled water. Each of the prepared concentrations of phyto-extract was transferred into the sterile glass petridishes (9 cm diameter/150 mL capacity). Ten first-, second-, third-, and fourth-instar larval forms of *Cx. quinquefasciatus* were separately introduced into different petridishes containing appropriate concentrations. Ten milligrams of larval food (dried yeast powder) was added per petridish. Mortality rate was recorded after 24, 48, and 72 h of post-exposure. The data of mortality in 48 and 72 h were expressed by the addition of the mortality at 24 and 48 h, respectively. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region. The experiments were replicated thrice on three different days (n= 3) and conducted at 25-30 ℃ and 80%-90% relative humidity. Similar types of bioassay were conducted with methanol extract (concentrations of 10, 20, 30, 40, and 50 ppm) of the leaves on first to fourth instar larval forms.

2.5. Phytochemical analysis of the plant extracts

Phytochemical analysis of both the plant extracts was carried out by suitable methodologies in search of active ingredient responsible for larval toxicity. The phytochemicals included under the study were saponins, terpenoids, alkaloid, steroids, tannin, flavonoids, cardiac glycosides and free glycoside bound anthraquinones *etc*. It was carried out according to the methodologies of Harborne [10] and Stahl[11].

2.6. Effect on non-target organisms

The effect of the crude extract of all the plant parts were tested against three non-target invertebrates e.g. *Diplonichus annulaum* (*D. annulaum*) (5th instar larval forms), *Oreochromis niloticus niloticus* (*O. n. niloticus*) and *Chironomous circumdatus* (*C. circumdatus*) present in the same habitat of the mosquito larvae. One *D. annulatum* and two *O. n. niloticus* were released into a glass beaker containing 500 mL of pond water. The insect was exposed to a concentration of plant extract that was similar to its LC_{50} values at 24 h and the insect was observed for mortality and other abnormalities up to 72 h of exposure. During the study with chironomid larvae, ten *C. circumdatus* was transferred into a sterile glass dish (9 cm diameter/150 mL capacity containing similar test concentration and studied up to 72 h for any mortality. Three replicates were performed for each non-target organism along with one replicate each of untreated controls.

2.7. Statistical analysis

The percentage mortality observed (% M) was corrected using Abbott's formula[12] during the observation of the larvicidal potentiality of the plant extract. Completely randomized three-way factorial ANOVA was carried out using different concentrations, different instars as variables to find the significance between the concentrations of the plant extract and mortality at different periods. Statistical analysis of the experimental data was performed using the computer software Stat plus 2006 and MS EXCEL 2003 to find the LC_{50}, regression equations (Y=mortality; X= concentrations) and regression coefficient values.

3. Results

The results of the present study indicated that the mortality rates at 0.5% concentration were highest amongst all concentrations of the crude powder tested for larval mortality and it was significantly higher ($P<0.05$) than the mortality rates at 0.1%, 0.2%, 0.3%, and 0.4% concentrations of crude plant powder at 24, 48 and 72 h of exposure (Table 1). The mortality rate was higher in 72 h bioassay than those in 24 and 48 h. The results of regression analysis of crude powder of mature leaves of *D. volubilis* and *B. malabaricum* revealed that the mortality rate (Y) is positively correlated with the concentration of exposure (X)

Table 1

Mean larval mortality out of 10 mosquito larvae of different instars of *Cx. quinquefasciatus* exposed to different concentration (in g) of powdered leaf of tested plants (mean of three experiments per plant) (Mean ± Standard error).

Name of plant	Instars	Concentration (g/100 mL)	Mortality		
			24 h	48 h	72 h
D. volubilis	1st	0.1	3.00 ± 0.58	3.33 ± 0.88	5.00 ± 0.58
		0.2	4.00 ± 0.58	4.33 ± 0.33	5.66 ± 0.66
		0.3	3.00 ± 0.58	3.66 ± 0.66	4.00 ± 0.58
		0.4	4.00 ± 0.58	4.66 ± 0.66	5.00 ± 0.58
		0.5	5.00 ± 0.58	5.66 ± 0.33	7.00 ± 0.58
	2nd	0.1	2.00 ± 0.58	3.00 ± 0.58	3.67 ± 0.33
		0.2	2.33 ± 0.88	3.67 ± 0.88	4.33 ± 0.67
		0.3	3.00 ± 0.58	4.00 ± 0.58	5.00 ± 0.58
		0.4	3.67 ± 0.33	4.67 ± 0.33	6.00 ± 0.58
		0.5	5.00 ± 0.58	5.33 ± 0.33	6.67 ± 0.33
	3rd	0.1	1.33 ± 0.88	2.67 ± 0.88	4.00 ± 0.58
		0.2	3.00 ± 0.58	4.00 ± 0.58	4.66 ± 0.33
		0.3	3.67± 0.33	5.00 ± 0.58	6.00 ± 0.58
		0.4	5.00 ± 0.58	6.00 ± 0.58	7.00 ± 0.58
		0.5	5.33 ± 0.33	6.33 ± 0.33	7.00 ± 0.67
	4th	0.1	2.00 ± 0.58	3.00 ± 0.58	4.33 ± 0.33
		0.2	2.33 ± 0.33	3.33 ± 0.33	5.00 ± 0.58
		0.3	3.00± 0.58	3.66 ± 0.33	5.33 ± 0.33
		0.4	4.00 ± 0.58	4.66 ± 0.88	6.00 ± 0.58
		0.5	4.33 ± 0.33	5.00 ± 0.58	6.66 ± 0.33
B. malabaricum	1st	0.1	2.66 ± 0.33	4.00 ± 0.58	4.66 ± 0.33
		0.2	3.00 ± 0.58	4.33 ± 0.33	5.33 ± 0.33
		0.3	3.33 ± 0.33	4.66 ± 0.33	5.33 ± 0.33
		0.4	5.00 ± 0.58	5.66 ± 0.33	7.33 ± 0.33
		0.5	5.33 ± 0.33	6.66 ± 0.33	7.66 ± 0.33
	2nd	0.1	3.00 ± 0.58	4.00 ± 0.58	5.00 ± 0.58
		0.2	4.00 ± 0.58	4.33 ± 0.33	6.00 ± 0.58
		0.3	4.66 ± 0.33	6.00 ± 0.58	6.66 ± 0.58
		0.4	5.00 ± 0.58	6.33 ± 0.33	7.00 ± 0.33
		0.5	5.33 ± 0.33	6.66 ± 0.66	7.66 ± 0.33
	3rd	0.1	3.33 ± 0.66	4.33 ± 0.66	5.66 ± 0.88
		0.2	4.33 ± 0.33	5.00 ± 0.58	6.33 ± 0.66
		0.3	4.66 ± 0.88	5.33 ± 0.33	6.66 ± 0.33
		0.4	5.00 ± 0.33	5.66 ± 0.33	7.33 ± 0.33
		0.5	5.33 ± 0.33	6.33 ± 0.33	7.66 ± 0.33
	4th	0.1	3.00 ± 0.58	3.66 ± 0.66	5.00 ± 0.58
		0.2	3.33 ± 0.33	4.00 ± 0.58	5.66 ± 0.33
		0.3	4.00 ± 0.58	5.33 ± 0.33	6.33 ± 0.33
		0.4	4.66 ± 0.88	5.66 ± 0.66	7.33 ± 0.33
		0.5	5.00 ± 0.58	6.33 ± 0.33	7.66 ± 0.33

Table 2

Log-probit analysis and regression analysis of larvicidal activity of tested plant parts (leaf) against different instar larval forms of *Cx. quinquefasciatus* (n=3 experiments/trial).

Name of plants	Instar	Time of exposure	LC_{50}	LC_{90}	Regression equation	r^2 value
D. volubilis	1st	24 h	0.93	183.65	Y=4.00x+2.60	0.76
		48 h	0.56	74.55	Y=4.99x+2.83	0.87
		72 h	0.16	479.70	Y=3.34x+4.33	0.48
	2nd	24 h	0.74	10.56	Y=7.34x+0.99	0.97
		48 h	0.49	14.79	Y=5.66x+2.44	0.99
		72 h	0.25	2.97	Y=7.67x+2.83	0.99
	3rd	24 h	0.43	2.02	Y=10.00x+0.66	0.98
		48 h	0.29	1.63	Y=9.32x+2.01	0.98
		72 h	0.19	1.97	Y=8.34x+3.23	0.97
	4th	24 h	0.78	12.67	Y=6.33x+1.23	0.98
		48 h	0.68	41.97	Y=5.33x+2.33	0.98
		72 h	0.17	8.02	Y=5.66x+3.76	0.99
B. malabaricum	1st	24 h	0.45	4.23	Y=7.34x+1.66	0.95
		48 h	0.26	8.64	Y=6.65x+3.07	0.97
		72 h	0.14	1.39	Y=8.00x+3.66	0.94
	2nd	24 h	0.39	9.84	Y=5.66x+2.70	0.97
		48 h	0.19	2.78	Y=7.32x+3.23	0.95
		72 h	0.11	2.00	Y=6.32x+4.57	0.98
	3rd	24 h	0.37	20.09	Y=4.67x+3.13	0.97
		48 h	0.18	12.15	Y=4.66x+3.93	0.98
		72 h	0.07	1.89	Y=5.00x+5.23	0.99
	4th	24 h	0.59	31.25	Y=5.33x+2.39	0.99
		48 h	0.26	3.02	Y=7.00x+2.89	0.98
		72 h	0.12	1.39	Y=6.99x+4.29	0.99

with a regression coefficient close to 1 in each case (Table 2). The results of log probit analysis (95% confidence level) revealed that LC_{50} and LC_{90} values gradually decreased with the exposure periods with the lowest value at 72 h of exposure to third instar larvae, followed by first and fourth instar larvae (Table 2). The result of the three-way factorial ANOVA of crude powder of mature leaves of *D. volubilis* and *B. malabaricum* revealed significant difference in larval mortality (P<0.05). The mortality of the all instar larval form with methanol solvent extracts was presented in Table 3. The highest mortality was observed for third instar larvae at 50 ppm at 24 h and it was significant higher than 10, 20, 30 and 40 ppm concentrations(P<0.05). Results are similar both at 48 and 72 h of exposure (Table 3). LC_{50} and LC_{90} values of methanol solvent extract of mature leaves of *D. volubilis* and *B. malabaricum* after 24 h of exposure period against third instar larval form presented in Table 4. The regression equation of methanol solvent extract also revealed that mortality rates (Y) was positively correlated with the concentration of exposure (X) with a regression coefficient close to one (Table 4).

The results of preliminary qualitative phytochemical analysis of both the plants revealed the presence of many bioactive principles that may be responsible for their bio-control potentiality. Methanol extract of *D. volubilis* leaves have shown positive test for saponins, steroids and cardiac glycosides whereas tannins, steroids, flavonoids, triterpenoids were found in methanol extract of *B. malabaricum* leaves.

No changes in the survival rate and swimming activity of the non-target organisms *ie*. *D. annulatum*, *O. n. niloticus* and *C. circumdatus* were observed within 72 h of post exposure to the plant extract with the concentration equals to their respective LC_{50} values at 24 h.

4. Discussion

As synthetic insecticides are hazardous for both aquatic and terrestrial ecosystem it is also responsible for biological magnification through food chain. Larval control can be an effective control tool due to the low mobility of larval mosquitoes, especially where the principal breeding habitats are manmade and can be easily identified. The secondary phytochemicals of plants are a vast repository of compounds with a wide range of biological activities. The larvicidal activity of many of the secondary compounds, such as saponins [13,14], phenolics[15], isoflavonoids[16], essential oil[17,18], alkaloids[19,20], and tannin compounds[21], were well documented. However, several authors also reported significant result by applying moderately polar solvents such as chloroform and methanol[22-28]. Most studies reported active compounds responsible for mosquito larvicidal property as steroidal saponins. Wiesman and Chapagain[13] revealed that saponin extracted from the fruit of *Balanites aegyptica* showed 100% mortality against larvae of *Stegomyia aegypti*. It is also reported that ethyl acetate solvent extract of *Solanum nigrum* shows highest mortality against *Cx. quinquefasciatus* at 50 ppm[29].

During the present study the bio-control potentiality of crude extracts and methanol solvent extract of both plants *D. volubilis* and *B. malabaricum* against *Cx. quinquefasciatus*

Table 3

Larval mortality of instar of *Cx. quinquefasciatus* treated by different concentration of methanolic extract of leaves of *D. volubilis* and *B. malabaricum* (Mean ± Standard error).

Name of methanolic plant extract	Instars	Concentration(ppm)	Mortality		
			24 h	48 h	72 h
D. volubilis	1st	10	3.67 ± 0.67	4.67 ± 0.67	5.00 ± 0.87
		20	4.33 ± 0.88	5.00 ± 0.58	6.33 ± 0.33
		30	5.00 ± 0.58	5.33 ± 0.33	6.33 ± 0.33
		40	5.33 ± 0.88	6.00 ± 0.58	7.00 ± 0.58
		50	5.67 ± 0.33	5.67 ± 0.88	7.33 ± 0.58
	2nd	10	2.00 ± 0.58	2.67 ± 0.33	4.67 ± 0.67
		20	3.67 ± 0.58	4.67 ± 0.88	5.67 ± 0.33
		30	4.33 ± 0.33	5.00 ± 0.67	6.33 ± 0.58
		40	4.67 ± 0.88	5.33 ± 0.33	6.67 ± 0.33
		50	5.00 ± 0.33	5.67 ± 0.33	7.67 ± 0.33
	3rd	10	2.66 ± 0.88	3.33 ± 0.88	4.33 ± 0.33
		20	3.00 ± 0.58	3.66 ± 0.67	4.66 ± 0.33
		30	4.66 ± 0.33	5.33 ± 0.33	6.33 ± 0.33
		40	6.00 ± 0.58	6.66 ± 0.66	7.66 ± 0.33
		50	8.66 ± 0.33	9.33 ± 0.33	9.66 ± 0.33
	4th	10	2.33 ± 0.67	3.00 ± 0.58	4.00 ± 0.58
		20	2.67 ± 0.33	3.33 ± 0.88	4.33 ± 0.33
		30	3.33 ± 0.88	4.33 ± 0.33	6.00 ± 0.58
		40	4.00 ± 0.67	4.67 ± 0.88	6.67 ± 0.58
		50	4.67 ± 0.33	5.33 ± 0.88	7.00 ± 0.67
B. malabaricum	1st	10	3.66 ± 0.33	4.33 ± 0.33	5.33 ± 0.33
		20	4.00 ± 0.58	4.67 ± 0.88	5.67 ± 0.33
		30	5.33 ± 0.33	6.33 ± 0.33	7.33 ± 0.33
		40	6.00 ± 0.58	6.67 ± 0.67	7.67 ± 0.33
		50	6.33 ± 0.33	7.33 ± 0.33	8.00 ± 0.58
	2nd	10	3.33 ± 0.33	4.00 ± 0.58	4.67 ± 0.333
		20	4.00 ± 0.58	4.67 ± 0.88	5.33 ± 0.88
		30	5.00 ± 0.58	5.33 ± 0.67	6.00 ± 0.58
		40	4.67 ± 0.33	5.67 ± 0.33	6.33 ± 0.33
		50	6.33 ± 0.33	6.67 ± 0.33	8.00 ± 0.58
	3rd	10	3.33 ± 0.33	5.00 ± 0.58	5.66 ± 0.66
		20	3.66 ± 0.66	4.33 ± 0.33	5.66 ± 0.33
		30	4.33 ± 0.33	6.00 ± 0.58	6.66 ± 0.33
		40	7.00 ± 0.58	7.33 ± 0.33	8.33 ± 0.33
		50	7.60 ± 0.33	8.66 ± 0.33	9.33 ± 0.33
	4th	10	2.00 ± 0.58	2.33 ± 0.67	3.00 ± 0.58
		20	2.33 ± 0.33	3.33 ± 0.88	3.67 ± 0.68
		30	3.00 ± 0.88	3.67 ± 0.67	4.67 ± 0.67
		40	3.67 ± 0.88	4.67 ± 0.88	5.00 ± 0.58
		50	4.33 ± 0.88	5.00 ± 0.58	5.67 ± 0.67

Table 4

Log-probit analysis and regression analysis of larvicidal activity of methanolic solvent extract of tested plant parts against 3rd instar larval forms of *Cx. quinquefasciatus* (n=3 experiments/trial).

Name of plant	Time of exposure	LC_{50}	LC_{90}	LCL-UCL	Regression equation	r^2 value
D. volubilis	24 h	56.97	218.40	32.67-105.67	Y=0.07x+0.49	0.97
	48 h	42.02	142.67	19.59-62.59	Y=0.17x+1.16	0.97
	72 h	31.29	117.73	7.34-47.46	Y=0.06x+2.43	0.98
B.malabaricum	24 h	48.85	264.22	10.13-112.39	Y=0.06x+1.59	0.95
	48 h	28.89	199.94	0.01-51.13	Y=0.05x+3.17	0.93
	72 h	23.55	100.34	0.94-38.58	Y=0.07x+4.13	0.96

are well established in the laboratory condition. Highest mortality was recorded in *B. malabaricum* mature leaf extract. The phytochemical analysis of the plant extracts reveals the present of several bioactive secondary metabolites that singly or in combinations may be responsible for the larval toxicity. As no mortality occurs in the non target organisms it can be assumed that all the plant extracts are safe to use in the aquatic ecosystem. However further studies are necessary to reveal the structure of the bioactive principles and their mode of action in the target species.

In conclusion, crude extracts of *D. volubilis* and *B. malabaricum* can be recommended in large scale field trials and can be effectively used as a potent larvicide in mosquito control programmes. The extract from the plant could be used in stagnant water bodies which are known to be the breeding grounds for mosquitoes. However, further studies on the identification of the active principals involved and their mode of action and field trials are needed to recommend *D. volubilis* and *B. malabaricum* as an anti-mosquito product used to combat and protect from mosquitoes in a control program.

Conflict of interest statement

We declare that we have no conflict of interest.

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