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RESEARCH ARTICLE

Mosquito Larvicidal Efficacy of *Andrographis echioides* (Acanthaceae) Foliages Against Vector of Lymphatic Filariasis *Culex quinquefasciatus* Say (1823)

Moumita Das[1] · Aniket Singh[2] · Kuntal Bhattacharya[3] · Ambarish Mukherjee[1] · Goutam Chandra[2]

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Abstract Increasing resistance against synthetic insecticides, mosquito control is becoming serious problem. For an alternative measure novel botanical sources can be used as good insecticides with less toxic hazards to the environment. The present study estimated larvicidal activities of *Andrographis echioides* against the vector of lymphatic filariasis *Culex quinquefasciatus* under laboratory conditions. Crude extracts of *A. echioides* mature leaves were examined for mosquito larvicidal activity against all the larval instars of *Cx. quinquefasciatus.* Solvent extraction was carried out using three different solvents viz. petroleum ether, ethyl acetate and acetone. Dose dependent larvicidal bioassays were performed using the solvent extractives. LC_{50} and LC_{90} values were consummated through log-probit analysis. Regression and ANOVA analyses were done for statistical justification. The effects of crude and solvent extractives were tested on a non-target water fauna. Crude extract of *A. echioides* leaf exhibited 100% mortality in 1.00% concentration against 1st instars larvae after 72 h of exposure. Among three used solvent extracts ethyl acetate showed efficient larvicidal effect against target mosquito. In ethyl acetate extractive 100.00% mortality was noticed at 150 ppm concentration against 1st instars larvae after 48 h of exposure with LC_{50} and LC_{90} values of 32.96 ppm and 106.96 ppm respectively. Tested non target organism was completely safe both from crude and ethyl acetate extractives. This experiment was a pioneer attempt to establish *A. echioides* as a precious resource for production of mosquito larvicide against *Cx. quinquefasciatus.*

Keywords *Culex quinquefasciatus* · *Andrographis echioides* · Larvicidal activities · Non-target organism · Lymphatic filariasis

Introduction

Phytochemicals help in defense against insects, fungi and herbivorous mammals (Hassan Adeyemi 2010). These plant derived chemicals are established as insecticidal (Hossain et al. 2011) agents.

Diseases like chikungunya, dengue, filariasis, malaria, Japanese encephalitis and yellow fever etc. are some of the deadliest mosquito born diseases that cause millions of death worldwide (ICMR Bulletin 2013). Approximately over 3500 species of mosquito under 41 genera were distributed worldwide (Harbach 2011).

Culex quinquefasciatus, also well-known as southern house mosquito, belongs to the family culicidae is the chief vector of lymphatic filariasis (Hati et al. 1989). Filariasis, a parasitic disease caused by three nematode worms *Wuchereria bancrofti, Brugia timori and Brugia malayi.* It also transmits West Nil Virus in few areas around the world. Almost more than 1.2 billion people facing a threat across the globe due to lymphatic filariasis (Bockarie et al. 2015). In India approximately 21 million people with symptomatic filariasis and 27 million microfilaria carriers

✉ Goutam Chandra
goutamchandra63@yahoo.co.in

[1] Department of Botany, The University of Burdwan, Burdwan, West Bengal 713104, India

[2] Mosquito, Microbiology, Nanotechnology Research Units, Parasitology Laboratory, Department of Zoology, The University of Burdwan, Golapbag, Burdwan, West Bengal 713104, India

[3] Department of Zoology, Durgapur Government College, J.N. Avenue, Durgapur, West Bengal 713214, India

were estimated (Reddy et al. 2000). It usually breeds in polluted surface waters and water filled artificial containers, septic tanks etc. with high organic contains. It is an anthropophagic species and live close proximate to human. It is nocturnal bitter, peak biting hours are midnight to 3 a.m. (Chandra 1995). Their preferred biting site is legs, particularly below the knee (Chandra and Hati 1993).

Control of mosquitoes become very difficult due to the development of resistance against chemically synthesized insecticides. Besides, these insecticides have many harmful effects on the environment (Russell et al. 2009). To overcome these problems botanicals may be used as suitable alternative. A lot of researchers reported various plant sources from which novel insecticide may be derived (Singha et al. 2011a; Bhattacharya et al. 2014a; Pal et al. 2016; Singh et al. 2015).

Andrographis echioides (Acanthaceae) commonly known as False Water willow is an annual herb with hairy stems growing up to 45 cm tall, branched from the base. Leaves are oblong, long up to 7.5 cm, 2.4 cm in wide and hairy on both sides. Flowers are borne in spike-like racemes, long up to 2 cm. The stalk carrying the raceme is thickly hairy. Flowers are erect and sepal tube is 2 mm long. Stamen filaments are flattened. False Water willow is found in India and Sri Lanka. Flowering time is March–June and October–December. It has various medicinal activities like diuretic, antimicrobial, anthelmintic, hepatoprotective, antioxidant, anti-inflammatory, mosquito larvicidal and antiulcer activity (Mathivanan and Suseem 2015).

The purpose of the present experiment was to assess the lethal effect of crude and solvent extracts of *A. echioides* foliages against the *Cx. quinquefasciatus* larvae. This is the first ever report of this plant as a source of larvicidal agent under laboratory conditions against *Cx. quinquefasciatus.*

Materials and Methods

Collection of Plant Materials

Mature, fresh green foliages of *A. echioides* (Acanthaceae) were harvested randomly during March–April 2015 from the plants around the outskirts of Burdwan (23°44′41.46″N, 86°49′52.95″E), West Bengal, India. The plant was identified perfectly and a voucher specimen (Voucher No. GCZMD-11) was submitted at the Mosquito, Microbiology and Nanotechnology Research Units, Department of Zoology, The University of Burdwan.

Rearing of Larvae and Colony Set Up

By the help of standard scooping and dipping method (Robert et al. 2002) larvae of *Cx. quinquefasciatus* were collected from the adjacent drains of Burdwan University (23°16′N, 87°54′E). Larvae were kept in a plastic tray filled with dechlorinated tap water with proper sanitation to set up a larval colony. Larvae were fed with a composite diet of Brewer yeast, dog biscuits and algae in a ratio of 3:1:1 respectively (Kamaraj et al. 2009). Larvae became pupae within the tray and then those were shifted to an insectary (45 × 45 × 40 cm) for adult immergence. By using the mosquito identification key provided by Christophers (1933), Barraud (1934) and Chandra (2000) adult mosquitoes were identified. A multivitamin syrup and 10% sucrose solution were supplied to the adult mosquitoes for nutrition with a cotton wick in a container. Adult females were supplied a blood meal from a non-motile shaved rat on the 5th day of rearing. Petri dishes filled with 100 ml of tap water and wrinkled with filter paper were kept inside the cage for oviposition. Eggs were allowed to hatch under laboratory conditions. The colony was maintained at 80–85% relative humidity (RH) and 27 ± 2 °C temperature under the photo regime of 13:11 light-and-dark cycles. 1st generation larvae were used during bioassays.

Preparation of Crude Extract

At first the collected fresh and mature foliages were rinsed well in tap water followed by distilled water and soaked on a paper towel. Mature leaves were crushed with electrical grinder and the extract was filtered using Whatman No. 1 filter paper and the filtrate was used as a stock solution (100% concentration) for further bioassay experiments. The concentrations (0.6%, 0.7%, 0.8%, 0.9% and 1.0%) were prepared with addition of distilled water with the stock solution.

Preparation of Solvent Extract

The leaves of *A. echioides* were rinsed well in distilled water and socked by using paper towel. Leaves were dried for few days in shed at room temperature. The dried leaves were cut in tiny pieces and put (200 g) into the thimble of Soxhlet apparatus. Separately 2000 ml of each solvent was loaded on still pot one after another. 72 h of extraction period for each solvent (8 h maximum in a day) was fixed. Three different solvents petroleum ether, ethyl acetate and acetone were passed through the thimble one after another. Extracts were accumulated from the still pot and kept on separate beakers. The extractives were intensified through rotary evaporator. The concentrated extractives were preserved at 4 °C in a refrigerator for further work.

Dose–Response Larvicidal Bioassay

The larvicidal bioassays were done at the Mosquito, Microbiology and Nanotechnology Research Units, Parasitology Laboratory, The University of Burdwan, according to the standard protocol of WHO (2005). Each larval instar of *Cx. quinquefasciatus* was tested against earlier prepared concentrations of crude and solvent extracts. Twenty five larvae of different instars (1st, 2nd, 3rd and 4th) were shifted into sterilized glass Petri dishes filled with 100 ml of distilled water. Crude extracts were applied in different Petri dishes in following grades viz. 0.6–1.0%. Likewise, graded concentrations of solvent extracts (from 50 to 150 ppm) were prepared and tested for their larvicidal potentiality against each instar stage. Each experiment was done in triplicate (n = 75) with a set (2 replicates) of controls. Petri dishes were placed at room temperature (30 ± 2° C) and 88 ± 2% relative humidity for 72 h of total observation. The percent mortality was recorded after 24 h, 48 h and 72 h of exposure. The larvae were assumed dead when they failed to move after probing with needle in the siphon or cervical area of it or when they were unable to reach the water surface (Pal et al. 2017).

Effect on Non-target Organism

The organisms who share the same aquatic habitat with mosquito larvae are the most vulnerable group. Impact of the plant extractives on non-target group was tested on *Diplonychys annulatum* nymph. They were exposed to the LC_{50} value of 3rd instar larvae for 72 h of crude and solvent extracts. The mortality or other abnormalities like sluggishness and reduced swimming activity were observed up to 72 h of exposure.

Statistical Analyses

The observed percent mortality (%M) was calculated by using Abbott's formula (Abbott 1925). Determination of LC_{50} and LC_{90} values of solvent extract was carried out through Log-probit and regression analyses (Y = mortality; X = concentration). For further statistical justifications three way ANOVA analyses was done by using different instars, hours and different concentrations as three random variables to validate the significance between the above parameters and larval mortality.

Result

A. echioides was found to have good mosquito larvicidal property against *Cx. quinquefasciatus* in laboratory observations. In crude extract of *A. echioides* leaf, 100% mortality was found at 1.00% concentration against 1st instars larvae after 72 h of exposure (Table 1). Among the three used solvent extracts, ethyl acetate showed most efficient larvicidal effect against the target mosquito. In ethyl acetate extractive 100.00% mortality was obtained at 150 ppm concentration against 1st instar larvae after 48 h of exposure (Table 2). With the increase in time of exposure mortality rate increased in each extract and in each instar. Results of log probit and regression analyses of larval mortality by ethyl acetate extractive are presented in Table 3. The results of log probit analysis (95% confidence level) states that LC_{50} and LC_{90} values gradually decreased with the increase of exposure period having the lowest value at 72 h of exposure in each instar. LC_{50} and LC_{90} value (95% confidence level) of ethyl acetate against 1st instars larvae at 72 h of exposure were 25.45 ppm and 70.25 ppm respectively which were significantly lower than all other instars. Regression analysis proved that mortality rate (Y) was positively correlated with time of exposure (X). The regression coefficient (R^2) value was close to one in each case. Using completely randomized three way ANOVA regarding hour (H), concentration (C) and instars (I) as three parameters proved statistical significance of the larvicidal effect of ethyl acetate extractive of leaf of *A. echioides* against *Cx. quinquefasciatus* (Table 4). Tested non target organism showed no abnormality or mortality when it was treated with crude and solvent extractives of plant.

Discussion

The present study reveals that leaf extract of *A. echioides* has effective larvicidal property against *Cx. quinquefasciatus* larvae. Both crude and solvent extracts showed mosquito larvicidal potentiality against all the larval instars of *Cx. quinquefasciatus*. In crude extract 96.00% mortality was obtained in 1.00% concentration against 3rd instar larvae after 72 h of exposure. Usually polar fractions of plant materials are proficiently extracted using polar solvents and non polar solvents take out non polar molecules. Petroleum ether is the most non polar solvent (polarity index of 0.1) that extracts essential oil, while ethyl acetate being moderately polar (polarity index of 4.4) mainly extracts steroids, alkaloids etc. (Ghosh et al. 2012). In the present experiment we got best larvicidal potentiality in ethyl acetate extractive which probably contains steroids and alkaloids or both as secondary phytochemical. In comparison to crude extract, in ethyl acetate solvent extractive cent percent mortality of 3rd instar larvae was obtained after 72 h at a very low dose rate i.e. only 150 ppm. Tested non target organism was completely

Table 1 Percent mortality of *Cx. quinquefasciatus* larvae using crude extract of mature leaf of *A. echioides*

Larval instars	Concentration (%)	Percent mortality (Mean ± SE)		
		24 h	48 h	72 h
First	0.6	66.67 ± 0.54	68.00 ± 0.33	77.33 ± 0.33
	0.7	72.00 ± 0.00	74.67 ± 0.33	82.67 ± 0.54
	0.8	76.00 ± 0.33	81.33 ± 0.67	86.67 ± 1.20
	0.9	82.67 ± 0.67	88.00 ± 0.33	93.33 ± 0.33
	1.0	89.33 ± 0.54	94.67 ± 0.54	100.00 ± 0.00
Second	0.6	62.67 ± 0.33	66.67 ± 0.54	72..00 ± 0.54
	0.7	68.00 ± 0.00	73.33 ± 0.88	80.00 ± 0.33
	0.8	72.00 ± 0.00	80.00 ± 0.00	85.33 ± 0.88
	0.9	78.67 ± 0.54	86.67 ± 0.33	96.00 ± 0.00
	1.0	86.67 ± 0.33	93.33 ± 0.33	98.67 ± 0.67
Third	0.6	60.00 ± 0.00	64.00 ± 0.88	68.00 ± 0.33
	0.7	65.33 ± 0.88	70.67 ± 0.33	77.33 ± 0.54
	0.8	68.00 ± 0.33	73.33 ± 0.33	84.00 ± 0.33
	0.9	77.33 ± 0.33	84.00 ± 0.00	89.33 ± 0.67
	1.0	84.00 ± 0.33	89.33 ± 0.54	96.00 ± 0.00
Fourth	0.6	28.00 ± 0.00	37.33 ± 0.67	44.00 ± 0.33
	0.7	33.33 ± 0.54	40.00 ± 0.00	49.33 ± 1.20
	0.8	41.33 ± 0.54	45.33 ± 0.54	54.67 ± 0.33
	0.9	48.00 ± 0.00	53.33 ± 0.33	60.00 ± 0.00
	1.0	54.67 ± 0.33	56.00 ± 0.00	62.67 ± 1.20

Table 2 Percent mortality of *Cx. quinquefasciatus* larvae using ethyl acetate solvent extracts of *A. echioides*

Larval instars	Concentration (ppm)	Mortality rate (Mean ± SE)		
		24 h	48 h	72 h
1st	50	64.00 ± 0.00	69.33 ± 0.67	84.00 ± 0.00
	75	69.33 ± 0.88	78.67 ± 0.33	88.00 ± 0.00
	100	76.00 ± 0.58	84.00 ± 0.00	94.67 ± 0.67
	125	82.67 ± 0.33	93.33 ± 0.58	100.00 ± 0.00
	150	89.33 ± 0.33	100.00 ± 0.00	100.00 ± 0.00
2nd	50	56.00 ± 0.00	68.00 ± 0.33	73.33 ± 0.33
	75	61.33 ± 0.54	74.67 ± 0.33	80.00 ± 0.00
	100	68.00 ± 0.00	82.67 ± 0.58	90.67 ± 0.88
	125	73.33 ± 0.88	89.33 ± 0.33	100.00 ± 0.00
	150	80.00 ± 0.00	94.67 ± 0.33	100.00 ± 0.00
3rd	50	41.33 ± 0.54	54.67 ± 0.33	62.67 ± 0.67
	75	50.67 ± 0.88	61.33 ± 0.33	73.33 ± 0.88
	100	60.00 ± 0.00	68.00 ± 0.33	80.00 ± 0.00
	125	65.33 ± 0.67	78.67 ± 0.33	94.67 ± 0.58
	150	74.67 ± 0.33	88.00 ± 0.00	100.00 ± 0.00
4th	50	26.67 ± 0.54	30.67 ± 0.33	33.33 ± 0.33
	75	32.00 ± 0.33	36.00 ± 0.00	38.67 ± 0.67
	100	38.67 ± 0.57	44.00 ± 0.00	48.00 ± 0.00
	125	44.00 ± 0.00	50.67 ± 0.33	58.67 ± 0.33
	150	49.33 ± 0.33	56.00 ± 0.00	65.33 ± 0.88

secured from the effect of crude and solvent extractives of the plant understudy.

Mosquito control mainly depends on control of larvae and adults. Due to larval low mobility and confinement to

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

Larval instars	Period of exposure	LC_{50}	LC_{90}	Regression	R^2-value
1st	24	38.22	218.66	0.064x + 12.66	0.99
	48	32.96	106.79	0.076x + 13.66	0.99
	72	25.45	70.25	0.044x + 18.93	0.94
2nd	24	43.17	373.81	0.06x + 10.93	0.99
	48	37.96	150.74	0.068x + 13.66	0.99
	72	31.91	86.99	0.073x + 14.86	0.94
3rd	24	73.68	402.85	0.081x + 6.46	0.99
	48	52.40	217.03	0.084x + 9.13	0.98
	72	45.98	113.46	0.096x + 10.93	0.98
4th	24	178.05	1433.47	0.057x + 3.80	0.99
	48	126.57	911.94	0.065x + 4.33	0.99
	72	102.61	536.60	0.084x + 3.80	0.98

Table 4 Completely randomized three way ANOVA analysis using concentration (C), hour (H) and instars (I) as three parameters

Source of variation	Sum of squares (SS)	Degree of freedom (*df*)	Mean of squares (MS)	F value	*p* level
Instars (I)	2711.80	3	903.93	716.77	0.00
Hours (H)	568.48	2	284.24	225.39	0.00
Conc. (C)	1136.24	4	284.06	225.25	0.00
I × H	77.65	6	12.94	10.26	0.00
I × C	30.51	12	2.54	2.02	0.028
H × C	16.36	8	2.04	1.62	0.126
I × H × C	35.96	24	1.50	1.19	0.267
Within groups	151.33	120	1.26	–	–
Total	4728.33	179	26.42	–	–

water bodies, larval control is easier than control of adults. Many researchers reported a lot of plants which showed effective mosquito larvicidal (Singha and Chandra 2011; Singh Ray et al. 2014; Bhattacharya and Chandra 2013, 2015; Mondal et al. 2016) repellent, smoke toxicity, pupicidal and adulticidal (Singha et al. 2011b; Bhattacharya and Chandra 2014; Rawani et al. 2012) properties. Rawani et al. (2010) published that ethyl acetate extract of *Solanum nigrum* showed potent larvicidal property against *Cx. quinquefasciatus* and the LC_{50} value was 17.04 ppm after 24 h of exposure. Singha Ray et al. (2015) reported that ethyl acetate extractive of *Capparis zeylanica* leaf showed larvicidal efficiency against *Cx. quinquefasciatus* with lowest LC_{50} and LC_{90} values of 12.44 ppm and 33.88 ppm respectively. According to Kundu et al. (2013) ethyl acetate solvent extractive of seed coat of *Cassia sophera* showed potent larvicidal property against *Cx. quinquefasciatus*. In comparison, we found lowest LC_{50} and LC_{90} values 25.45 ppm and 70.25 ppm respectively. Bhattacharya et al. (2014b) reported that chloroform: methanol (1:1 v/v) extractives of *Ravenala madagascariensis* leaves showed larvicidal property against the same mosquito species. The LC_{50} and LC_{90} values were 25.41 ppm and 90.98 ppm respectively against 1st instar larvae of *Cx. quinquefasciatus* after 72 h of exposure that is higher dose in comparison to our study. In another work, Rawani et al. (2013) reported that chloroform: methanol (1:1 v/v) extract of *Solanum nigrum* berry showed effective larvicidal property against *Cx. quinquefasciatus*. Hexane flower extract of *Nerium oleander* (Raveen et al. 2014), Acetone extractives of leaf of *Nicotiana plumbaginifolia* (Singh et al. 2016), petroleum ether and N-butanol extract of *Cassia occidentalis* (Kumar et al. 2014) also showed potent larvicidal effect against *Cx. quinquefasciatus.*

The above study showed that the foliages of *A. echioides* exhibited significant mortality of *Cx. quinquefasciatus* larvae. Therefore, it can be concluded that further detail studies on foliages of *A. echioides* may fulfill the search of establishing a novel bio-insecticide.

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Author Contribution The screening was done and the plant having mosquitocidal activity was selected by Moumita Das. Rearing of mosquito larvae and colony set up was done by Moumita Das. Extract preparation and laboratory bioassay was carried out by Moumita Das and Aniket Singh. Manuscript preparation was done by Kuntal Bhattacharya. Plant identification was done by Professor Ambarish Mukherjee. Professor Goutam Chandra designed the study and supervised the whole work. All authors read and approved the final version of the manuscript.

Compliance with Ethical Standards

Conflict of interest The authors have no conflict of interest.

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