Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading

Evaluation of target specific larvicidal activity of the leaf extract of *Typhonium trilobatum* against *Culex quinquefasciatus* Say

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ARTICLE INFO

Article history:
Received 17 August 2011
Received in revised form 3 September 2011
Accepted 28 September 2011
Available online 15 October 2011

Keywords:
Culex quinquefasciatus
Larvicidal
Phytochemical
Methanol extract
Non-target organisms

ABSTRACT

Objective: To investigate the target specific larvicidal potential of an edible herb *Typhonium trilobatum* (*T. trilobatum*) (L.) Schott against mosquito *Culex quinquefasciatus* (*Cx. quinquefasciatus*) Say. **Methods:** Different concentrations of crude and methanol extract of *T. trilobatum* mature leaves were treated against *Cx. quinquefasciatus* larvae. LC_{50} concentration of crude extract on mosquito 3rd instar larvae was tested on *Chironomus circumdatus* and *Diplonychus annulatum* larvae. Preliminary phytochemical analysis was performed in search of plant's secondary metabolites. **Results:** 100% mortality of 1st instar mosquito larvae was recorded at 0.4% concentration after 72 h of exposure of crude extract. At 72 h 0.5% concentration produced 100%, 89.99% and 79.99% mortality of 2nd, 3rd and 4th instar larvae respectively. 50 ppm methanol extract showed 73.67% mortality of 3rd instar mosquito larvae at 72 h. 400 ppm concentration was responsible for 100% mortality in 24 h. Application of LC_{50} concentration (of 3rd instar mosquito larva) against non target organisms like *C. circumdatus* and *D. annulatum* larvae produced no significant mortality among them. Secondary metabolites like terpenoids and free glycoside bound anthraquinones were found. **Conclusions:** This experimental study was a pioneer attempt to establish *T. trilobatum* as a valuable resource of effective target specific mosquito larvicide.

1. Introduction

Amongst the arthropods, mosquitoes are perhaps regarded as the most redundant species to human beings because they transmit several deadly diseases mainly prevailing in the tropical countries around the globe, besides causing several physical nuisances. Malaria is the most prevalent among the lethal diseases spread by the vector of different species of *Anopheles* mosquitoes. Dengue fever, another illness of utmost concern is transmitted by *Aedes* mosquitoes. Human lymphatic filariasis also remains as one of the most alarming diseases in numerous countries spread by the *Culex* mosquitoes[1]. According to recent World Health Organization's report some 120 million people in 81 countries have been infected by filariasis and 1.34 billion lives are estimated to be at risk of infection. *Culex* mosquitoes transmit deadly diseases like St. Louis encephalitis, West Nile viral fever and Japanese encephalitis. To get rid of such kinds of diseases, the best way is to find a preventive measure that will effectively control the population of the vector species so that they cannot transmit the disease pathogens.

There have been widespread applications of various commercial chemical insecticides since few decades to combat these vector species. However, indiscriminate use of those synthetic chemicals unequivocally produced different adverse consequences on soil, water, air ecosystem, creating undesirable effects like toxicity to non-target organisms, human health and ultimately posing potential threat for global environment[2-4]. In addition, development of resistance in the vectors against these widespread chemical insecticides results in rebounding of vectorial capacity[5], thus reducing the effective efficacy of those chemicals.

From the prehistoric era, plant kingdom remained the ultimate supplier of food, clothes and shelter to human civilization. Thousands of life saving drugs and medicines mainly originated from plant sources are increasing our average life expectancy. Plants have also been proved to be useful solution to get relief from the detrimental insects. Botanically derived chemicals have been recognized as efficient armaments in mosquito control program as they have shown to function as general toxicant, repellents, growth inhibitors, reproductive inhibitors and oviposition deterrent[6]. Moreover, these plant derived solutions have advantages over synthetic poisonous chemicals as they are

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Foundation Project: This work was financially supported by University Grants Commission (UGC), New Delhi [grant No. F 17-8/08 (SA-I)].

readily biodegradable thus eco-friendly, less harmful to non-target organisms and comparatively cheap.

Typhonium trilobatum (*T. trilobatum*) (L.) Schott, (family: Araceae) is a perennial herb, commonly known as Bengal Arum. It is endemic to tropical Asia, the South Pacific, and Australia. It grows wild in wet soils throughout India and has a range of traditional medicinal uses. The rhizome is effectively used for treating gastric ulcer, vomiting, cough, asthma, excessive expectoration, pyogenic sore throat, headache, abscess and snake-bite. The leaves and petioles are taken as vegetable food. *T. trilobatum* are also used by the Garos (a tribal community inhabiting the Madhupur forest region of Bangladesh) to treat diseases of cattle (cattle ulcer)[7]. It also has antibacterial activities against pathogenic bacteria[8]. Detailed literature survey by the authors revealed no scientific report of the mosquitocidal activity of *T. trilobatum* till date.

Our present study is the first attempt to evaluate the mosquito larvicidal efficacy of crude and methanol extracts of the mature leaves of the *T. trilobatum* against *Culex quinquefasciatus* (*Cx. quinquefasciatus*) Say. Simultaneously we examined the effect of lethal concentration 50 (LC_{50}) of crude extract on invertebrate non-target organisms like *Chironomus circumdatus* (*C. circumdatus*) larvae and *Diplonychus annulatum* (*D. annulatum*) larvae which live in the same aquatic habitat of immature mosquitoes. We extended our study upto search for some secondary biochemicals present in it.

2. Materials and methods

2.1. Collection and rearing of test mosquitoes

The larvae of filarial vector *Cx. quinquefasciatus* was collected during July, 2010 through dipping method from the natural breeding sites located within the campus of The University of Burdwan. They were kept in trays with sufficient water in stress free, pathogen free, hygienic condition in laboratory. Larvae were provided with finely ground dog biscuit. Colony adults were fed on 10% sucrose and 10% multivitamin syrup, and they were periodically blood-fed on restrained rats.

2.2. Collection of plant material

Fresh, mature, green leaves of *T. trilobatum* were gathered during June-July, 2010, from the plants growing within the university campus. The plant was identified properly and voucher specimens were deposited in the Mosquito and Microbiology Research Unit of The University of Burdwan, Burdwan (23° 16′ N, 87° 54′ E), West Bengal, India. Subsequent to collection, the leaves were initially rinsed with distilled water and dried on paper towel. Some of the plant leaves (wet weight quantities 50 g) were crushed with the help of mixer-grinder machine, and the plant juice was filtered by Whatman no. 1 filter paper. The clear filtrate was used as a stock solution (100% concentration of crude extract). By mixing up stock extract with variable amounts of sterilized distilled water required concentrations were prepared for bioassay experiments.

2.3. Preparation of plant extracts in methanol

Some of the mature leaves of *T. trilobatum* were dried in shed at room temperature. The dried 25 g leaves were put in a Soxhlet apparatus for preparation of plant extracts using 300 mL methanol (extraction period 48 h, temperature < 40 ℃). The extract was concentrated using a vacuum evaporator at 45 ℃ under low pressure. After complete evaporation of the solvent, the concentrated extract were collected and stored in a refrigerator.

2.4. Treatment of larvae with leaf extract: Dose-response larvicidal bioassay

For larvicidal bioassay study according to WHO protocol[9] thirty 1st, 2nd, 3rd and 4th instars of *Cx. quinquefasciatus* were separately introduced into a series of glass beakers (of 200 mL capacity) containing 100 mL of tap water and appropriate concentration of crude extract (0.1%, 0.2%, 0.3%, 0.4%, and 0.5%) of *T. trilobatum*. And the third instars of *Cx. quinquefasciatus* were tested against proper concentration (50, 100, 200, 400 ppm) of methonal extract of *T. trilobatum*. Dried yeast powder (20 mg) was added per glass beakers as food of larvae. The number of dead larvae (mortality rate) was counted at the end of 24 h, 48 h, and 72 h of post-exposure. Each experimental setup was performed thrice at three different days in association with control.

2.5. Phytochemical analysis of the plant extracts

Phytochemical analysis of crude extract of the leaves of *T. trilobatum* was performed according to the methodologies of Harborne and Stahl[10,11]. In our study we searched for the presence or absence of some secondary biochemicals like tannin, saponins, terpenoids, alkaloid, steroids, flavonoids, cardiac glycosides and free glycoside-bound anthraquinones.

2.6. Effect on non-target organisms

To test the effect of crude plant extract on non-target organisms two invertebrate organisms *C. circumdatus* (3rd instar larval form) and *D. annulatum* (5th- instar larval form) were selected as they live in the same aquatic ecosystem with mosquito larvae. Ten healthy *C. circumdatus* and four *D. annulatum* were released into two separate glass bowls containing 500 mL of pond water along with plant extract that is similar to the LC_{50} values at 24 h on 3rd instar mosquito larvae. Effects were observed up to 72 h of post exposure for any type of physiological or behavioral abnormalities or for mortality. All the setups were replicated three times along with untreated controls.

2.7. Statistical analysis

The percentage of corrected mortality was calculated by

Abbott's formula[12]. Experimental data was statistically analysed by using the computer software "STAT PLUS 2007 (Trial version)" and MS EXCEL 2002 to find the LC_{50}, LC_{95}, regression equations (Y = mortality; X = concentrations), regression coefficient values and completely randomized three-way factorial ANOVA.

3. Results

Result of larvicidal bioassay with crude leaf extract of *T. trilobatum* is presented in Table 1 which shows that in case of 1st instar of *Cx. quinquefasciatus* 0.4% concentration exhibits 100% mortality at 72 h. For 2nd, 3rd, and 4th instars the concentration 0.5% shows highest mortality rate which increases with time of exposure (72 h > 48 h > 24 h). Mortality rate of 2nd instar larvae was higher than other larval stages at 24 h of exposure. Table 2 reveals significant difference in larval mortality ($P<0.05$) as the result of completely randomized three-way factorial ANOVA at different concentrations, different instars and hours as three variable. Table 3 presents the result of log-probit analysis (at 95% confidence level) where LC_{50} values were between 0.07% - 0.46%, 0.03% - 0.18%, and 0.05% - 0.11%, whereas range of LC_{90} values were 1.18% - 3.19%, 0.37% - 2.27% and 0.14% -1.66% concentrations of crude extract for 1st to 4th instar larvae after 24h, 48h, and 72h of exposure. From regression equation it was evident that for all four larval stages Y (mortality rate, dependent variable) was positively related to its corresponding X (dose, independent variable) and the value of R^2 in all cases were nearer to 1 which indicates that the rate of mortality linearly increases with the increasing dose. Table 4 shows the effect of methanol extract at different concentrations on 3rd instar larvae of *Cx. quinq uefasciatus* and the LC_{50} concentrations for 24 h, 48 h and 72 h were 99.24 ppm, 73.52 ppm, 19.87 ppm, respectively. The results reflected that methanol extract of this plant part produced mortality in immature mosquito in dose dependent manner. The result of qualitative phytochemical analysis of the crude extract of *T. trilobatum* showed the presence of terpenoid and free glycoside bound anthraquinones, and the absence of tannin, saponin, alkaloid, steroid, flavonoid, cardiac glycosides. In the toxicity test of *T. trilobatum* leaf extract on non-target organisms, LC_{50} concentration (0.41 ppm) of

Table 1
Mortality effect of *Cx. quinquefasciatus* exposed to different concentrations of crude extracts of *T. trilobatum*.

Larval instars	Concentrations (%)	Mortality rate (Mean ± SE)		
		24 h	48 h	72 h
First	0.1	57.78 ± 2.94	70.00 ± 1.92	82.22 ± 2.94
	0.2	61.11 ± 2.94	73.33 ± 1.92	93.33 ± 1.92
	0.3	68.88 ± 2.95	78.89 ± 1.11	98.88 ± 1.11
	0.4	73.33 ± 1.92	83.33 ± 1.92	100.00 ± 0.00
	0.5	80.00 ± 1.92	88.89 ± 1.11	100.00 ± 0.00
Second	0.1	63.33 ± 1.92	72.22 ± 1.11	76.67 ± 1.92
	0.2	72.22 ± 2.22	75.55 ± 2.94	85.55 ± 1.11
	0.3	75.55 ± 2.94	85.55 ± 2.22	96.67 ± 1.92
	0.4	80.00 ± 1.92	91.11 ± 1.11	98.89 ± 1.11
	0.5	84.44 ± 1.11	94.44 ± 1.11	100.00 ± 0.00
Third	0.1	16.67 ± 1.92	43.33 ± 1.92	53.33 ± 1.92
	0.2	28.89 ± 1.11	62.21 ± 2.94	66.66 ± 5.77
	0.3	41.11 ± 2.94	63.33 ± 1.92	76.66 ± 5.77
	0.4	46.44 ± 1.94	74.44 ± 4.00	81.11 ± 5.88
	0.5	58.89 ± 1.11	79.99 ± 1.92	89.99 ± 1.92
Fourth	0.1	16.67 ± 1.92	42.22 ± 4.00	52.22 ± 4.84
	0.2	27.22 ± 1.11	45.55 ± 4.00	54.44 ± 4.84
	0.3	39.99 ± 1.92	58.89 ± 2.94	65.22 ± 3.68
	0.4	44.42 ± 1.09	66.66 ± 1.92	73.77 ± 1.44
	0.5	53.33 ± 1.92	72.22 ± 1.11	79.99 ± 1.92

Table 2
Completely randomized three-way factorial ANOVA using different concentrations, different instars and hours as three variables.

Source of variation	SS	df	MS	*F* value	*P* value
Concentrations (C)	1610.589	4	402.647	222.321	0.000
Hours (H)	1973.411	2	986.706	544.807	0.000
Instars (I)	2863.261	3	954.420	526.981	0.000
C x H	12.811	8	1.601	0.884	0.532
C x I	115.989	12	9.666	5.337	0.000
H x I	192.589	6	32.098	17.723	0.000
C x H x I	39.411	24	1.642	0.907	0.593
Residual	217.333	120	1.811	–	–
Total	7025.394	179	–	–	–

Table 3
Log-probit analysis and regression analysis of larvicidal activity of *T. trilobatum* mature leaf extract against different larval forms (instars) of *Cx. quinquefasciatus* (mean of 3 experiments).

Larval instars	Period of exposure (h)	LC_{50} (%)	LC_{90} (%)	Regression equation	R^2 value
First	24	0.07	2.09	Y=56.66x + 51.22	0.99
	48	0.03	0.83	Y=47.78x + 64.55	0.99
	72	0.05	0.14	Y=42.23x + 82.22	0.77
Second	24	0.43	1.18	Y=60.00x + 59.50	0.95
	48	0.04	0.37	Y=60.00x + 65.77	0.67
	72	0.07	0.18	Y=60.00x + 73.56	0.88
Third	24	0.41	2.36	Y=101.99x + 7.80	0.99
	48	0.89	1.81	Y=64.45x + 53.88	0.92
	72	0.09	0.64	Y=87.77x + 47.22	0.97
Fourth	24	0.46	3.19	Y=89.97x + 9.44	0.98
	48	0.18	2.27	Y=81.11x + 32.77	0.97
	72	0.11	1.66	Y=74.87x + 42.67	0.97

Table 4
Result of larval mortality(%) of different concentration of methanolic extract of leaves of *T. trilobatum* on third instar of *Cx. quinquefasciatus* and determination of lethal concentration 50.

Concentration (ppm)	Mortality rate (%) (Mean ± SE)		
	24 h	48 h	72 h
50	32.67±1.20	54.33±0.88	73.67±1.20
100	45.33±1.20	68.67±1.20	82.00±0.58
200	62.33±1.45	72.33±1.45	85.00±0.58
400	100.00±0.00	100.00±0.00	100.00±0.00

the methanol extract for 3rd instar mosquio larvae was tested on *C. circumdatus* and *D. annulatum*. Only 6.66% mortality was observed against *C. circumdatus* at 72 h, whereas at that concentration in case of *D. annulatum* no mortality had been observed.

4. Discussion

Before invention of synthetic insecticides, plant derived materials have been used to control harmful insects since time immemorial. Then in hallow of apparent quick success of chemically synthesized insecticides natural insecticides got less preference. But over past few decades plant-based insecticides have renewed their importance in mosquito control. Several plant species have been established to have mosquitocidal potentiality[13-16]. Results of this study shows that the mortality of mosquito larvae of *Cx. quinquefasciatus* exposed to the plant extracts of *T. trilobatum* increased with concentration of extracts as well as time of exposure which is supported by the report of Obomanu *et al*[17]. The increased mortality with time may be due to individual or synergistic effect of several factors like time provides better chance for accumulation of active moiety of the compound in the larval body or with time the active compound turns into more toxic substance for the larvae of mosquitoes[18]. Several authors reported that methanol extract of plant parts act as mosquito larvaicide[19,20]. Bansal *et al*[21] found that methanol extracts of different parts of *Solanum xanthocarpum* is of higher larvicidal efficacy than its aqueous extract. Our study also shows good efficacy of methanol extract against *Culex* larvae. 50 ppm concentration exhibited 73.67% mortality at 72 h of post exposure. 85% larval death occurred in 72 h at 200 ppm concentration and 100% mortality was achieved at 400 ppm concentration in 24 h. LC_{50} concentrations were 99.24 ppm, 73.52 ppm, 19.87 ppm for 24 h, 48 h and 72 h of post exposure respectively. These results are similar to the report by Rahuman *et al*[22] which stated that the methanol extract of *Cedrus deodara* Roxb. Stem bark is effective against *Cx. quinquefaciatus* with LC_{50} 95.19 ppm.

Plants use their secondary metabolites in defense mechanisms. These secondary metabolites like steroids, alkaloids, triterpenes, phenolics, essential oils, saponins *etc* can be used to control mosquito vectors[23-31]. Our study identified the presence of only two secondary metabolites like terpenoids and free glycoside bound anthraquinones which indicates that any one or both of them might be responsible for larval death.

In our present study the plant *T. trilobatum* have been proved as a potent, fast acting botanical in the field of mosquito control. Methanol extract of mature leaves have great impact on mortality of larval form. LC_{50} concentration of this plant extract has no significant effect on mortality of tested non-target organisms.

Several plant species and their derivatives have been described as mosquito larvicides but there are ample scopes for discovery of more effectual plant products. Present finding is encouraging as the plant extract seems to be target specific, effective at low dose, biodegradable and easily available. Undoubtedly further research is required to improve formulations with enhanced activity so that it may become an effective replacement of chemical insecticides and environmentally acceptable. So it could be concluded that the use of mature leaf extract of *T. trilobatum* have some scientific justification for further research in the arena of botanical control of mosquitoes at larval forms.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors acknowledge with thanks the help of Dr. Ambarish Mukherjee, Professor of Botany, The University of Burdwan for identification of the plant. Authors also gratefully acknowledge University Grants Commission (UGC), New Delhi, India for financial support and KM Haldar acknowledges UGC for her fellowship [No. F 17-8/08 (SA-I)].

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