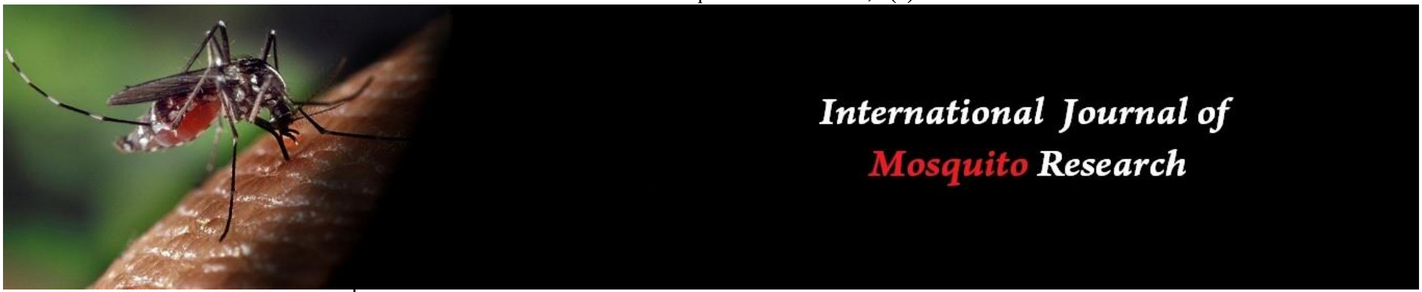

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Larvicidal efficacy of the leaf and flower extracts of *Tropaeolum majus* L. (Tropaeolaceae) against the vector of lymphatic filariasis

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Abstract
Billions of people face consequences throughout the world due to vector-borne diseases. Effective management of vector populations can reduce the burden of vector-borne diseases. The present study was conducted to assess the efficiency of *Tropaeolum majus* leaf and flower extracts on the Lymphatic Filariasis vector *Culex quinquefasciatus*. Crude extract of *T. majus* leaves and flowers was used for larvicidal bioassay against 3rd instar larvae. The effect of both the extracts on non-target organisms was also assessed. Log probit, regression, and ANOVA were carried out to find out the statistical significance. Crude extract of both leaf and flower showed significant larval mortality. 100% and 96% mortality were achieved within 72 h in 2% crude extracts of leaf and flower respectively. After 72 h treatment LC_{50} value was assessed as 0.56% and 0.78% in the case of leaf and flower extracts respectively, which were quite acceptable. ANOVA analysis established a significant relationship between exposure time and concentration of extracts in imposing mortality to the mosquito larvae. Though, the tests on the non-target organisms showed that those extracts were generally safe. The outcomes suggest that the extracts of *T. majus* leaves and flowers can be used as an eco-friendly mosquito larvicidal agent. The isolated compounds from this plant may also act as a potent larvicide and are worth further study.

Keywords: *Culex quinquefasciatus*, *Tropaeolum majus*, leaf, flower, larvicidal activity

1. Introduction

Mosquitoes are one of the most nuisance creatures of the world and are vectors of many diseases. They transmit malaria, dengue, chikungunya, West Nile, zika, filariasis, Japanese encephalitis, etc. throughout the earth, causing major health concerns and mortality. More than 3500 species of mosquito are distributed worldwide of which thousands feed on the blood of various animals and about 20% are the potential vector of diseases in humans and other animals [1, 2]. One of the best approaches to stop the spread of diseases by the mosquitoes is to kill the mosquitoes and disrupt their transmission. The mass killing of the larvae at the breeding c can be a strategy to reduce the vector mosquito population [3].

Due to harsh effects on the environment and expression of resistance among mosquitoes against synthetic chemical mosquitocides, there was an urge to discover newer eco-friendly insecticides. Our botanic diversity is a rich depot of phytochemicals, which can serve as a substitute for synthetic mosquitocides [4]. Phytochemicals from various plant parts, either obtained as a crude aqueous extract or by extraction with different solvents are reported to have mosquito control potential [5 - 7]. Phytochemicals with mosquitocidal properties should act as a healthier substitute for synthetic insecticides because they are physiologically and ecologically less harmful, biodegradable, and easy to use [8].

Tropaeolum majus L. is an herbaceous annual plant included in the family Tropaeolaceae. The almost circular large leaves are green with a long petiole near the centre and several radiating veins. The flowers are with five petals, eight stamens, and a protracted nectar-spur at the end, colouration varies from red to orange to yellow. This plant is widely grown as an ornamental garden plant and above-ground parts are edible. It is reported to have several nutritional and medicinal properties [9 - 11].

Culex quinquefasciatus (Diptera) is an anthropophilic tropical mosquito species and in many parts of the world, they are a well-established vector of Lymphatic Filariasis [12, 13]. The object of the present study is to evaluate the crude extract of leaves and flowers of *T. majus* as a potential mosquito larvicide.

2. Materials and methods

2.1 Collection of plant materials

Fresh matured green leaves and flowers of *T. majus* were collected in January 2022, randomly from the gardens of Raniganj-Asansol (23.6234°N, 87.1143°E), West Bengal, India.

2.2 Rearing of test Mosquitoes

Larvae of *Cx. quinquefasciatus* for the bioassay were taken from the stock of the mosquito colony kept at the Epidemiology, vector biology, and environmental monitoring research unit, department of Animal Science, Kazi Nazrul University, West Bengal, India. The colony was well protected from insecticides, pathogens, and other harmful agents. The relative humidity was maintained at 80~85%, temperature at 27±2 °C, and a photoperiod of 13:11 (light: dark cycles). The larvae were provided with a diet of Brewer yeast, dog biscuits, and algae in the ratio of 3:1:1 [14].

2.3 Preparation of crude extract

Leaves and flowers were washed carefully with distilled water and wiped on paper towels. Leaves and flowers were grounded with a mechanical grinder separately to produce juice. The juices were filtered by Whatman No. 1 filter paper. Filtrates obtained were preserved in the freezer at 4°C and used as a stock solution (100% concentration) in bioassays later. For the experiments, distilled water was added to the stock solution to prepare the required concentrations (0.5%, 1%, 1.5%, and 2.0%).

2.4 Dose-response larvicidal bioassay

Protocol of World Health Organization (WHO) [15] was followed for the larvicidal assays with suitable modifications. 100 ml crude extracts of four different concentrations (0.5% to 2%) were taken in 150 ml volume sterilized glass Petri dishes. Twenty-five 3rd instar larvae of *Cx. quinquefasciatus* were introduced to respective dishes separately. Each experimentation was carried out in triplicate. A control set with water (without any extract) was also carried out. During the experiments, the relative humidity was 85±2%, temperature 28±2 °C, and photoperiod 13:11 (light: dark). After 24, 48, and 72 h exposure the number of dead larvae was recorded. The larvae were considered dead when they remained unmoved on probing.

2.5 Assessing the effect on the non-target population

Chironomid larvae share a similar habitat with mosquitoes and are hence selected as the non-target organisms for testing the consequence of phytochemicals under trial. Non-target organisms were exposed to a concentration equal to LC_{50} value (72 h of post-exposure) of 3rd instar *Cx. quinquefasciatus* larvae to observe the abnormal activities or death, if any.

Available data were subjected to appropriate statistical analyses [16 - 18].

3. Results

The crude extract of leaves and flowers of *T. majus* showed notable mortality against 3rd instar larvae of *Cx. quinquefasciatus*. After 72 h of exposure, in the crude extract of leaves 54.00%, 72.00%, 94.67%, and 100% mortality was observed in 0.5%, 1%, 1.5%, and 2% concentration respectively. Whereas after 72 h of exposure, in the crude extract of flowers, 28.00%, 61.33%, 74.67%, and 96.00% mortality was observed in 0.5%, 1%, 1.5%, and 2% concentration respectively (Table 1).

Probit analyses of mortality rates of crude extracts of *T. majus* show LC_{50} and LC_{90} values as 1.19% and 7.27% in leaf extract and 1.97% and 8.48% in flower extract respectively against 3rd instars larvae within 24 h of exposure. Whare as after 72 h of exposure the calculated values of LC_{50} and LC_{90} were as lower as 0.56% and 1.06% for leaf extract and 0.78% and 1.8% for flower extract. Results of regression analysis revealed that the mortality rates (Y) were positively correlated with the concentration (X) having a Regression co-efficient value close to one in both leaf and flower extracts (Table 2).

Analysis of variance (ANOVA) revealed that the mortality rates of larvae were significantly influenced by the time and concentrations in both the leave (Table 3) and flower (Table 4) extracts tested. When interactions of the two factors (concentration and time) were taken into the account, no significant difference was noticed.

No abnormality and mortality were detected in the tested non-target organism.

Table 1: Mortality of 3rd instars *Culex quinquefasciatus* larvae against the crude extract of *Tropaeolum majus* leaves and flowers.

Plant Parts	Concentration (%)	Percentage Mortality (± SE)		
		24 h	48 h	72 h
Leaf	0.5	30.67 ± 0.67	44.00 ± 0.58	54.00 ± 0.67
	1.0	37.33 ± 0.88	58.67 ± 0.67	72.00 ± 0.58
	1.5	54.67 ± 0.67	81.33 ± 0.33	94.67 ± 0.88
	2.0	69.33 ± 0.67	94.67 ± 1.33	100.00 ± 0.00
	Control	0.00	0.00	0.00
Flower	0.5	13.33 ± 0.67	22.67 ± 0.88	28.00 ± 1.00
	1.0	22.67 ± 0.88	46.67 ± 0.88	61.33 ± 0.88
	1.5	41.33 ± 0.88	68.00 ± 1.00	74.67 ± 1.33
	2.0	53.33 ± 1.20	80.00 ± 0.58	96.00 ± 0.58
	Control	0.00	0.00	0.00

Table 2: Assessment of LC_{50} and LC_{90} values of crude extract of *T. majus* leaves and flowers by log-probit and regression analysis.

Plant Parts	Period of Exposure	LC_{50} (%)	LC_{90} (%)	Regression Equation	R^2 value
Leaf	24 h	1.19	7.27	y = 1.627x + 4.878	0.879
	48 h	0.56	1.85	y = 2.833x + 5.525	0.887
	72 h	0.56	1.06	y = 4.598x + 6.158	0.833
Flower	24 h	1.97	8.48	y = 2.018x + 4.406	0.964
	48 h	0.99	3.05	y = 2.625x + 5.007	0.992
	72 h	0.78	1.8	y = 3.550x + 5.374	0.92

Table 3: Two-way ANOVA analysis using Concentrations (Leaf extract) and Hours as the parameters.

Source of Variation	DF	Sum of Squares	Mean Squares	F value	P-value
Concentration (C)	11	9275.625	843.239	66.571	0.000
Hours (H)	1	75466.125	75466.125	5957.852	0.000
C * H	11	8575.625	779.602	61.548	0.000
Within	48	608	12.667		
Total	71	93925.375			

Table 4: Two-way ANOVA analysis using Concentrations (Flower extract) and Hours as the parameters.

Source of Variation	DF	Sum of Squares	Mean Squares	F value	P-value
Concentration (C)	11	11654.958	1059.542	51.825	0.000
Hours (H)	1	43956.125	43956.125	2150.028	0.000
C * H	11	10830.958	984.633	48.161	0.000
Within	48	981.333	20.444		
Total	71	67423.375			

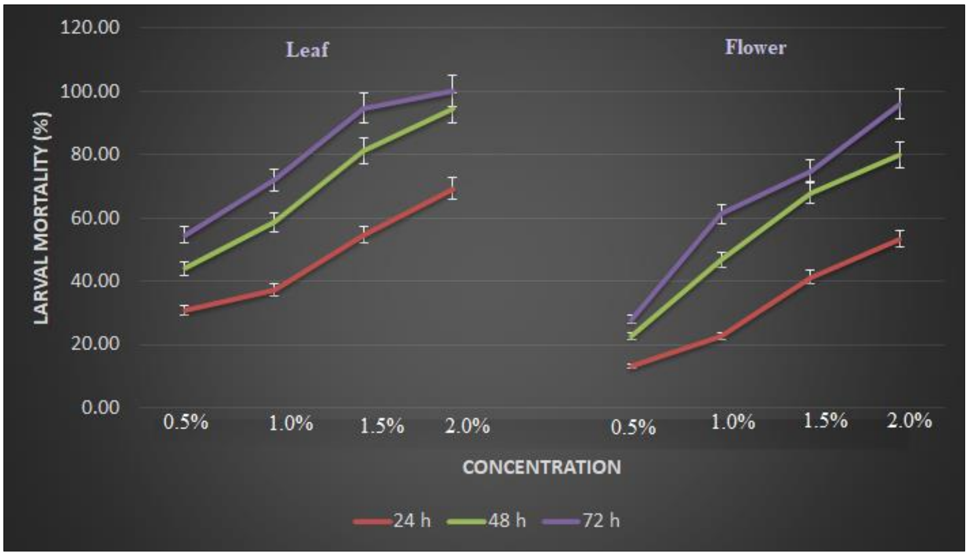

Fig 1: Mortality of 3rd instars *Cx. quinquefasciatus* larvae against the crude extracts of *T. majus* leaf and flower.

4. Discussion

Plants generate various secondary metabolites in their body in order to defend against predators which could be used as mosquito larvicidal agents. These bioactive compounds can be used as a substitute for synthetic insecticides as they are biodegradable, comparatively safe, low-cost, and often readily available all over the globe [19]. The larvicidal action of the plant extracts may be due to alkaloids, flavonoids, polyphenols, steroids, terpenoids, etc. synthesized to a variable extent in different parts of plants [20]. The strategy of using phytochemicals for mosquito control has opened up novel prospects for large-scale extraction of phytochemicals. Screening of available plants for mosquitocidal efficacy is the first step in this regard. The present study assessed the toxicity of crude extracts of leaves and flowers of *T. majus* against 3rd instars larvae of *Cx. quinquefasciatus*.

Crude extract from different parts of plants was shown to have mosquito larvicidal activity which was due to the presence of secondary metabolites [5, 21]. This is the first-ever toxicity report of extracts of leaves and flowers of *T. majus* against mosquito larvae. At a 2% concentration, 100% death was detected in the 3rd instar larvae of *Cx. quinquefasciatus* in crude leaf extract while crude flower extract showed 96% mortality within 72 h (Fig 1). One or more of the bioactive compounds or synergetic effects of the bioactive compounds present in the leaf and flower of *T. majus* may be responsible for larval mortality.

5 Conclusion

Reduction and elimination of the problem of vector-borne diseases are essential at the present time. Currently, WHO (2017) emphasizes a flexible vector management approach that may be developed locally with locally available resources beyond the use of only available control interventions [22]. The plant *T. majus* is grown all over the world, readily available, easy to cultivate, traditionally used for various purposes, and even known to be eaten raw by humans and other animals and hence should be safe to use. Present trial with crude extracts of leaves and flowers of the plant shows a good prospect of using the plant as a mosquito larvicidal agent for the first time and may be helpful in the mosquito control program in the future. However, further studies to elucidate the effeactivity against other species of mosquitoes, identify the active compounds, and assess the effectiveness in field trials are essential and it is undergoing.

6. Conflict of interests
The author declares that they have no conflict of interests.

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