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Tagetes erecta Linn. and its mosquitocidal potency against *Culex quinquefasciatus*

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ABSTRACT

Objective: To investigate mosquitocidal effects of ethanolic extract of flowers of *Tagetes erecta* (*T. erecta*) and its chloroform and petroleum ether soluble fractions against the larvae of *Culex quinquefasciatus* (*Cx. quinquefasciatus*). **Methods:** The fresh flowers of *T. erecta* were extracted in cold with ethanol (5.0 L) and after concentration, the ethanol extract was fractionated with chloroform and petroleum ether to afford a brownish syrupy suspension of ethanol extract (50.0 g), petroleum ether soluble fraction (18.6 g) and chloroform soluble fraction (23.8 g). The larvicidal effect of ethanol extract and their solvent fractions were determined by the standard procedure of WHO against different instars of *Cx. quinquefasciatus*. **Results:** Among the tested samples the chloroform soluble fractions showed the highest toxicity and consequently, the lowest LC_{50} values (14.14 μg/mL, 17.06 μg/mL, 36.88 μg/mL and 75.48 μg/mL) for all the instars larvae of *Cx. quinquefasciatus*. The larvae showed comparative tolerance in the course of increasing age and time. **Conclusions:** It can be concluded that the flowers of *T. erecta* are very effective natural larvicide and could be useful against *Cx. quinquefasciatus*.

1. Introduction

Mosquitoes are one of the most medically significant vectors and they transmit parasites and pathogens, which continue to have devastating impact on human beings. The *Culex* genus of mosquito is found in tropical and warm temperate regions and can transmit several viral diseases[1]. Current control is based on the use of insecticides (chlorpyrifos, dichlorvos, cypermethrin) which have a potential toxic effect on public health and the environment. There is considerable international interest in developing benign natural products as an alternative to harmful synthetic pesticides to control invertebrate pests of medical and economic importance[2,3]. Botanical phytochemicals with mosquitocidal potential are now recognized as potent alternative insecticides to replace synthetic insecticides in mosquito control programs due to their excellent larvicidal, pupicidal and adulticidal properties[4-8]. Phytochemicals are less environmentally harmful than synthetic agrochemicals. It has renewed the interest in the research on these compounds, considering them as an ecologically safe alternative[9,10].

The main objective of the present study was to determine the effect of mosquitocidal properties of *Tagetes erecta* (*T. erecta*) Linn. (locally known as genda) against the first, second, third and fourth instar larvae of the mosquito *Culex quinquefasciatus* (*Cx. quinquefasciatus*) Say. This plant belongs to the family Asteraceae (Compositae). It is native to Mexico and widely distributed in South East Asia including Bangladesh and India. The flowers of *T. erecta* are used for the cure of eye diseases, colds, conjunctivitis, coughs and ulcers. Internally, it is employed to purify the blood and to cure the bleeding piles[11-13]. In our laboratory we have also found the antibacterial, antifungal and cytotoxic effects (against brine shrimp nauplii) of flowers of *T. erecta*[14].

2. Materials and methods

2.1. Plant materials

Fresh flowers of *T. erecta* were collected from the adjoining areas of Rajshahi University Campus, Bangladesh during the month of December to January and were

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identified by Professor Nadiruzzaman ATM, Department of Botany, University of Rajshahi, Bangladesh where a voucher specimen (No. J. Sultana 23, collection date 17.01.1994) has been deposited.

2.2. Extraction and fractionation

The fresh flowers of *T. erecta* were sun dried for 7 days and finally dried in an oven below 60 ℃. The dried plant materials (1 kg) were then extracted in cold with ethanol (5.0 L). After concentration, the ethanol extract was fractionated with chloroform and petroleum ether. The solvents were concentrated by rotary evaporator at 40 ℃ under reduced pressure to afford a brownish syrupy suspension of ethanol extract (50.0 g), petroleum ether soluble fraction (18.6 g) and chloroform soluble fraction (23.8 g).

2.3. TLC screening

All extracts were run on pre-coated silica gel plate using petroleum ether and ethyl acetate (9:1 and 7:5) as the mobile phase and vanillin-H_2SO_4 reagent was used as spray reagent. Ethanol extract of flower gave positive test for glycosides but the chloroform and petroleum ether soluble fractions mainly showed the presence of terpenoids and flavonoids[15].

2.4. Test insects

Larvae of the test mosquito were reared at (27±1) ℃, 40%-60% relative humidity and a 12:12 h light: dark photoperiod in laboratory. To rear larvae for toxicity assay, single egg rafts were placed in a number of 600 mL glass beakers containing 450 mL distilled water. The larvae were fed with powdered Brewer's yeast at 10, 20, 40 and 80 mg per beaker everyday for first, second, third and fourth instars larvae, respectively. Water was changed everyday to avoid scum formation, which might create toxicity.

2.5. Mosquitocidal bioassay

The larvicidal effect of crude ethanol extracts and their solvent fractions were determined by the standard procedure of World Health Organization (WHO)[16]. The stock solutions were prepared by dissolving extracts and fractions (10 mg of each) in 1 mL of dimethyl sulphoxide (DMSO). After that thirty laboratory reared first, second, third and fourth instars larvae were released into 100 mL glass beakers separately, containing 50 mL of distilled water to which 50, 100, 200 and 400 μL of each stock solutions were added using capillary micro-pipettes to get the desired test concentrations (w/v), *viz*, 10, 20, 40 and 80 μg/mL. Three types of control were maintained: i) distilled water; ii) distilled water plus food medium and iii) distilled water plus solvent (DMSO). Three replicates were made for each concentration and the experiment was performed under laboratory conditions at (27±1) ℃ and 40%-60 % relative humidity. Brewer´s yeast was supplied as a larval food during the test periods for larval feeding.

2.6. Statistical analysis

The mortality data were then subjected to Probit analysis for the determination of LC_{50} values[17,18] using the computer software SPSS of 14 version. Results with $P<0.05$ were considered to be statistically significant.

3. Results

The crude ethanol extract of flowers of *T. erecta* and its chloroform and petroleum ether soluble fractions were highly effective against the first, second, third and fourth instars larvae of *Cx. quinquefasciatus* (Table 1). The chloroform soluble fractions showed the highest toxicity than the other samples and consequently, the lowest LC_{50} values (14.14 μg/mL, 17.06 μg/mL, 36.88 μg/mL and 75.48 μg/mL) in all instar larvae. The larvae showed comparative tolerance with the increase of their age and time, and LC_{50} values of all samples increased in all the instars tested (Table 1). Against first, second, third and fourth instar larvae, the lowest LC_{50} value for ethanol extract was found to be 113.91, 113.14, 256.67 and 1 023.04 μg/mL after 48 h of exposure whereas petroleum ether fraction had lowest LC_{50} (59.55 μg/mL) value against first instar larvae after 24 h ($P<0.05$) (Table 1).

Table 1
Mosquitocidal activity of flower of *T. erecta* against *Cx. quinquefasciatus* larvae.

Plant extracts	Larval stage	Exposure time (h)	LC_{50} (μg/mL)	*Chi*-squre value (χ^2)
Ethanol extract	1st instar	12	330.89	0.175 7*
		24	202.67	0.206 4*
		48	113.91	0.235 8*
	2nd instar	24	320.63	0.001 0
		48	113.14	0.010 3
	3rd instar	24	918.62	0.033 5
		48	256.67	0.037 1
	4th instar	24	3 783.33	0.089 8
		48	1 023.04	0.032 3
Petroleum ether fraction	1st instar	6	121.08	0.388 3
		12	74.19	0.830 4
		24	59.55	2.170 1*
	2nd instar	12	167.89	0.123 5
		24	114.64	0.154 0
		48	77.24	0.302 1*
	3rd instar	24	403.58	0.199 1
		48	142.52	0.122 5*
	4th instar	24	829.90	0.227 4
		48	386.42	0.331 8
Chloroform fraction	1st instar	3	28.59	0.675 9*
		6	19.95	0.744 2*
		12	14.14	1.533 0*
	2nd instar	6	37.72	0.326 8*
		12	24.71	0.277 2*
		24	17.06	1.936 5*
	3rd instar	12	75.20	0.269 8*
		24	36.88	0.3644*
	4th instar	12	118.08	0.320 7
		24	75.48	0.335 9*

Values were based on four concentrations with 30 insects each. # Control groups showed no mortality. *Significant at $P<0.05$ level.

4. Discussion

The increase in mortality during the course of exposure period could be due to several factors, which may be acting separately or jointly. For example, the uptake of the active moiety of the compound could be time dependent, leading to a progressive increase in the titer of the plant-derived compounds tested and its effect on the larval body, the active moiety of the compound could get converted into more toxic metabolites in the larval integument and alimentary canal, resulting in time-dependent effects.

Similar results were obtained from the latex and stem of *Euphorbia tirucalli*, extracellular secondary metabolites of different fungi and various Euro-Asiatic plants against *Cx. quinquefasciatus* larvae[19-22]. The extracts from *T. erecta* had larvicidal activity against *Aedes aegypti* and against the larvae of *Meloidogyne incognita*, respectively[23-25] but till now there is no report on the toxicity of *T. erecta* against *Cx. quinquefasciatus* Say.

The findings of the present study suggested that flowers of *T. erecta* may be explored as potential natural mosquitocidal agent. The future potential use of extracts from genus *Tagete*s as botanical insecticides will require their phytochemical analysis and examination of insecticidal activity of the individual biochemically characterized components.

Conflict of interest statement

We declare that we have no conflict of interest.

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