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Larvicidal and IGR potential of *Ocimum tenuiflorum* and *Datura alba* leaf extracts against Malaria Vector

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ABSTRACT

In the present study, ethyl acetate leaf extracts of two medicinal plants Ocimum tenuiflorum and Datura alba were investigated for mosquitocidal potential including larvicidal and Insect Growth Regulatory (IGR) activities against 4th instar Anopheles larvae. Leaf extracts showed moderate to high larvicidal activity at 24hrs and 48hrs continuous exposure while their IGR activity was observed after 72hrs. At 24hrs continuous exposure the Lc_{50} value of O. tenuiflorum and D. alba were 44mg/L and 46.00mg/L respectively. Similarly at 48hrs Lc_{50} values of O. tenuiflorum and D.alba were 33.6mg/L and 30.25mg/L. These values were based on the concentration mortality data along with their regression equation. The test of significance with 95% confidence limit was determined by log probit regression analysis for the study. Moreover, wide range of morphogenetic deformities was observed and recorded in different categories including larval pupal mosaic, abnormal pupae and pupal adult mosaic according to stage of metamorphosis when death occurred due to abnormal growth and moulting. The study is of great importance in formulation of an effective vector control strategy based on environmental friendly alternative (plant origin) insecticides.

Key words: *Ocimum tenuiflorum*, *Datura alba*, Lc_{50}, Morphogenetic deformities.

INTRODUCTION

Mosquitoes have been regarded as important vectors for transmission of several diseases such as malaria, encephalitis and yellow fever. Malaria is a major public health concern in the various parts of India that is considered as to determinant for equitable socio-economic development of the region [1]. Malaria caused by *Plasmodium falciparum*, which causes human mortality and morbidity from infectious diseases predominantly in tropical and subtropical countries [2]. India is endemic for six major vector borne diseases namely malaria, dengue, chikungunya, filariasis, Japanese encephalitis and visceral leishmaniasis. Controlling of these vectors has been attempted for a long time using synthetic chemicals. But the chemicals cause environmental pollution and developing resistance among mosquito species [3]. These problems have highlighted the need for the development of new strategies for selective mosquito larval control [4]. The search for new strategies or natural products to control destructive insects and 144 vectors of disease I desirable, due to the prevalent occurrence of vector resistance to synthetic insecticide and the problem of toxic non biodegradable residues contaminating the environment and undesirable effects on non-target organisms [5]. Insecticidal applications although highly efficacious against the

target species vector control, in facing a threat due to the development of resistance to chemical insecticides, resulting in rebounding vectorial capacity [6]. Researchers have been done in this direction with different types of extracts from various plant and tree parts, shrubs and fruit etc. Toxicity tolerance towards specific chemical and leaf extracts has been tested on mosquito [7-9]. Several plants have been reported to possess mimics of insect ecdysones and juvenile hormone activity [10]. The methanol extract of *A. marmelos* was assayed for its toxicity against the early fourth instar larvae of *C. quinquefasciatus* [11]; evaluated the larvicidal activity and smoke repellent potential at different concentration against first to fourth instar larvae and pupae of *A. aegyptii* [12]. The ethyl acetate extract of *E. prostrata* and leaf extract of *A. paniculata* have the potential to be used against fourth instar larvae of *A. subpictus* and *C. tritaeniorhynchus* [13]. *Acronus calamus* extract induced malformations to a greater extent in *An. Stephensii* and to lesser extent in *C. quinquefasciatus* and *Ae. Ageyptii*. [14].

For these various reasons, interest in the screening of medicinal plants for their mosquito control remains of great scientific interest despite of extensive use of synthetic chemicals in modern clinical practices all over the world [15]. Increasing documentation of negative environmental and health impact of synthetic insecticides and increasingly stringent environmental regulation of pesticides have resulted in renewed interest in the developmental and use of botanicals insect management products for controlling mosquitoes [16].

The objective of the present study includes the evaluation of larvicidal efficacy and insect growth regulatory (IGR) activity of ethyl acetate leaf extracts of *D. alba* and *O. tenuiflorum* on the fourth instar *Anopheles* larvae. The result of the present study would be useful in promoting research aiming and development of new agents for mosquito control based on bioactive chemical compounds from indigenous plant source.

MATERIALS AND METHODS

Collection and Identification of plant materials:
The fresh and green leaves of *D. alba* and *O. tenuiflorum* (Table I) were collected from Botanical garden, fort, Aligarh Muslim University. Taxonomic identification of these plants were made at Department of Botany, Aligarh Muslim University.

Preparation of Plant extracts:
The dried leaves (100g) were powdered mechanically using commercial electrical stainless steel blender and extracted with ethyl acetate (200ml, Merck) separately in a Soxhlet apparatus until exhaustion. Standard stock solution (1%) was separately by dissolving the residues in ethyl acetate for the assay.

Table I: List of medicinal plants tested for the larvicidal and IGR activity against larvae of *Anopheles.*

Botanical Name	Commom Name	Family	Medicinal Properties	Plant Parts Used
D. alba	Datura	Solanaceae	Anti-spasmodic Ache reliever Pain relief Relive the spasm of the Bronchitis in asthma	Leaf
O. tenuiflorum	Tulsi	Lamiaceae	Pain killer Antohyperlipidemic Cardioprotective	Leaf

Test organisms:
For the laboratory trial, identified early second, third and fourth instars larvae of *Anopheles* were obtained from section of Parasitology, Department of Zoology. The larvae were kept in plastic tray containing dechlorinated tap water. They were maintained and all the experiments were carried out at 28 ±2°C and 75-85% relative humidity, with a photoperiod of 14:10 light and dark cycles. Larvae were fed on yeast powder.

Larvicidal Bioassay:
One gram of each extract was first dissolved in 100ml of respective solvent, which was used for extraction. The larvicidal activity of the leaf extracts was evaluated as per the method recommended by the WHO technique [17] with slight modifications. A preliminary screening of different doses was performed on 4^{th} instars larvae to obtain 0% to 100% mortalities. Then 10,20,30,40 and 50mg/l ethyl acetate extract of plant leaves was used against 4^{th}

instars larvae. Immediately after moulting into 4th instars 25 larvae each were selected for the treatment of leaf extract at each dose in final volume of 250ml formulation in 500ml glass beaker. Three replicates for each dose and the control were tested for larval bioefficacy. No food was provided during the treatment. The larval mortality at different doses and in control was recorded after 24hrs and 48hrs continuous exposure.

Insect Growth Regulating Activity:
Leaf extracts of these medicinal plants were also tested for IGR activity against the mosquito larvae. Twenty five 4th instars larvae were transferred to beaker containing 250ml tap water. The plant extracts were tested at various concentrations viz: 10,20,30,40 and 50mg/l. Controls were also maintained side by side. For the accurate determination of the IGR activity, the deformities and the mortality rates were recorded every day until all treated larvae died at various stages or all adults emerged. Due to long duration of the test, larvae were provided with food at fixed intervals during the observation period. Morphological abnormalities in pupae, adults and partially emerged adults were recorded as larval-pupal mosaic and pupal-adult mosaic.

Data management and statistical analysis
Mortality was calculated using Abbott's (1925) formula [18]. The LC_{50} values, 95% UCL and LCL and Chi-square were calculated according to probit analysis [19]. Results with $p<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Mosquitoes are the most deadly vectors for several of these diseases causing organisms. In recent scenario future of anti-malarial drugs is worrying. Recently, the malarial control programme focused more on the elimination of mosquitoes in larval stage with plant extract. The advantage of targeting larvae is that they cannot escape from their breeding sites until the adult stage and also reduce overall pesticide use in control of adult mosquitoes. The tested solvent plant extracts have exerted a promising activity. In the present investigation the toxicity of ethyl acetate leaf extracts of *O.tenuiflorum* and *D.alba* was tested against 4th instar larvae of *Anopheles* at 24hrs and 48hrs continuous exposure (Table II and Table III). The data were recorded and LC_{50} as well as t-test values were calculated. The LC_{50} values of *O.tenuiflorum* and *D.alba* at 24hrs were 44.00mg/L and 46.00mg/L. Maximum larvicidal activity was observed in *D.alba* in comparison to *O.tenuiflorum*. With 48hrs exposure of *O.tenuiflorum* and *D.alba* the LC_{50} values for the 4th instar *Anopheles* larvae were 33.06mg/L and 30.25mg/L. t-test values and 95% confidence limits were significant at P<0.05% level. The mortality values were significantly greater than that of control. The present study corroborate with earlier findings when LC_{50} values of petroleum ether extract of leaves and the chloroform extract of bark of *S.indica* were 228.0 and 291.5 ppm against larvae of *C. quinquefasciatus* [20]. In some other studies, the antifeedent and larvicidal activity of acetone, chloroform, ethyl acetate, hexane and methanol extracts of peel, leaf and flower extracts of *C. sinensis*, *O.tenuiflorum*, *O.sanctum* and *R.nestus* were studied using fourth instar larvae of *H.armigera*, *S. derogata* and *A. stephensi* [21]. The ethanolic and acetone extracts of *N.indicum* and *T. orientalis* have been studied against 3rd instar larvae of *A.stepensi* and *C. quinquefasciatus* [22].

Table II: Larvicidal activity of ethyl acetate leaf extracts of medicinal plants at various concentration after 24hrs.

Extract	Conc. (mg/l)	Larval Mortality (Mean±SE)	% Mortality	% Corrected Mortality	LC_{50} (mg/l)	Regression equation	t (df)	95% Confidence limit		Variance
								Lower	Upper	
O. tenuifloram	Control	1.00±0.58	4.00	20.77	44.00	y=0.8534x +11.194 R^2=0.9866	05.500(2)	0.798	6.535	352.274
	10	4.67±0.33	18.66	32.46			20.000(2)	5.232	8.100	
	20	7.67±0.88	30.66	37.66			08.000(2)	3.697	12.30	
	30	9.00±0.58	36.00	45.45			10.000(2)	5.697	14.30	
	40	11.00±0.58	44.00	55.83			19.000(2)	9.798	15.53	
	50	13.67±0.33	54.66							
D. alba	Control	0.33±0.33	1.33	20.77	46.00	y=0.9334x+ 8.528 R^2=0.9855	6.500(2)	1.464	7.201	445.187
	10	4.67±0.33	18.66	26.14			7.559(2)	2.872	10.46	
	20	7.00±0.58	28.00	32.87			12.50(2)	5.464	11.20	
	30	8.67±0.33	34.66	42.42			12.09(2)	6.872	14.46	
	40	11.00±0.58	44.00	56.16			14.00(2)	9.697	18.30	
	50	14.33±0.67	57.33							

*Lc50= lethal concentration that kill 50% of the exposed larvae,75 larvae (3 replicates of 25 each) were treated at each dose; * Significant at P<0.05%.*

Table III: Larvicidal activity of ethyl acetate leaf extracts of medicinal plants at various concentration after 48hrs.

Extract	Conc. (mg/l)	Larval Mortality (Mean±SE)	% Mortality	% Corrected Mortality	LC_{50} (mg/l)	Regression equation	t (df)	95% Confidence limit		Variance
								Lower	Upper	
O. tenuifloram	Control	0.66±0.33	2.66	25.67	33.6	y=1.1601x+ 13.195 R^2=0.983	10.393(2)	3.515	8.484	434.928
	10	6.66±0.33	26.66	35.14			12.500(2)	5.464	11.201	
	20	9.00±0.58	36.00	43.25			15.500(2)	7.464	13.201	
	30	11.00±0.58	44.00	60.81			16.630(2)	10.872	18.461	
	40	15.33±0.88	61.33	71.62			19.654(2)	13.538	21.127	
	50	18.00±0.58	72.00							
D. alba	Control	0.66±0.33	2.66	36.5	30.25	y=1.0664x+ 22.94 R^2=0.9345	26.00(2)	7.232	10.100	558.002
	10	9.33±0.33	37.34	43.25			11.71(2)	6.538	14.127	
	20	11.00±0.58	44.00	48.65			13.22(2)	13.229	15.461	
	30	12.33±0.66	49.33	62.16			13.03(2)	10.495	20.837	
	40	15.66±0.66	62.66	81.08			22.30(2)	15.872	23.461	
	50	20.33±0.66	81.33							

*Lc50= lethal concentration that kill 50% of the exposed larvae,75 larvae (3 replicates of 25 each) were treated at each dose; * Significant at P<0.05%*

In addition to larvicidal toxicity, the IGR effects of the leaf extracts of these two indigenous medicinal plants were also observed when the treated 4th instar larvae moulted to pupae and adult stages. Different stages of delayed toxic effect was observed and recorded in different categories according to the stage of metamorphosis reached when death occurred (Table IV).

Table IV: The percent mortality values and morphological deformaties of mosquito larvae after 72h.

Extract	Conc (mg/l)	% of Mortality after 72h	% of Larval-Pupal Mosaic	% of Pupal-Adult mosaic	% live pupae after 72h
O.tenuiflorum	10	59.33	18.33	13.00	09.33
	20	64.33	15.33	11.33	09.00
	30	85.33	09.66	02.00	03.00
	40	89.00	08.33	-	2.66
	50	93.33	06.66	-	-
D.alba	10	61.33	17.33	12.00	09.33
	20	69.33	13.33	09.33	08.00
	30	75.66	12.33	06.66	05.33
	40	92.66	05.00	01.33	01.00
	50	95.00	03.00	02.00	-

The IGR activity was generally apparent after 72hrs of treatment and exhibited by the appearance of larval pupal mosaic and pupal adult mosaic of various categories (Fig 1-2). At various doses of *O.tenuiflorum* leaf extract larval pupal mosaic formation occurred resulting in death at an early stage of pupation and late stage (Fig 1A-1D). Morphological deformaties due to *D.alba* leaf extracts resulted in apperance of larval-pupal mosaic and pupal-adult mosaic at various doses. In case of larval-pupal mosaic, death has occurred at an early stage of pupation.

Fig. IA

Fig. IB

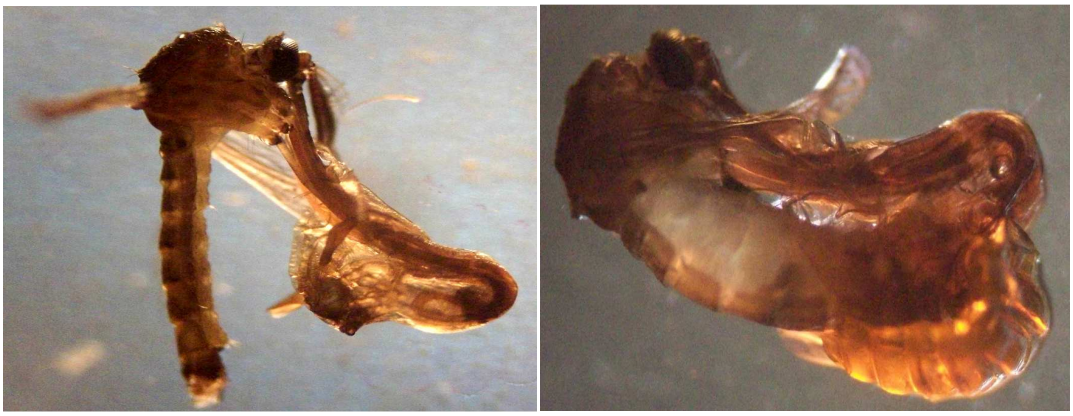

Fig. IC **Fig. ID**

FIG I: Morphological deformaties due to *O.tenuiflorum* leaf extract after 72hrs. (A-D)

The abdomen has retracted to at least halfway along the larval abdominal skin and adopted the characteristic pupal shape as shown in Fig 2A. In pupal-adult mosaics death occurred after complete moulting from pupal skin but some part remained attached to the pupal exuviae as shown in Fig 2B-2D. Larval pupal mosaics were obtained at 10mg/L, 20mg/L and 30mg/L whereas pupal-adult mosaic appeared at 30mg/L and 40mg/L. Based on aforementioned data, the leaf extract *D.alba* seems to have more IGR activity than *O.tenuiflorum*, as it caused more profound harmful effects in larvae and pupae during moulting.

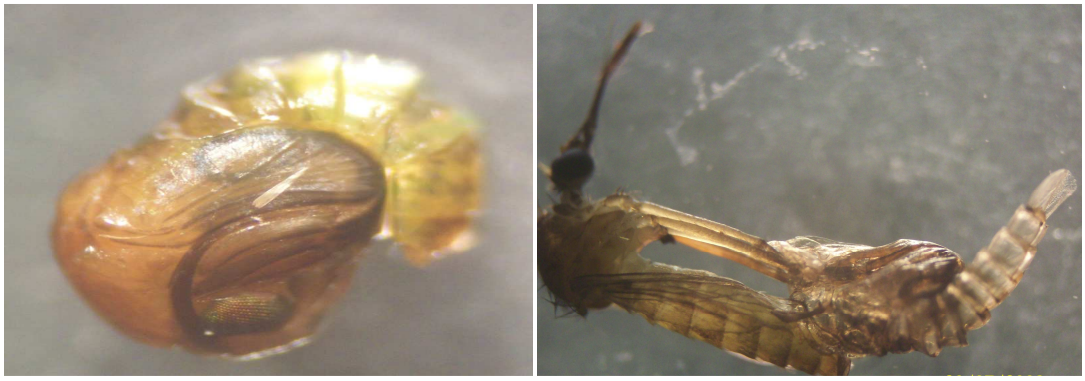

Fig. IIA **Fig. IIB**

Fig. IIC **Fig. IID**

FIG II: Morphological deformaties due to *D.alba* leaf extract after 72hrs. (A-D).

However, from the experiments it is difficult to contemplate on the genuine mode of action of these leaf extracts; but it is interesting to note that there was apparent difference in the nature and extent of deleterious effects on growth,

moulting and metamorphosis. At high dose, the death occurred in mostly larval stage whereas low concentration stimulated the morphological abnormalities to the larvae, pupae and adult. Some scientists reported that plant resources can act as larvicides, insect growth regulators, repellents and ovipositional attractant having detterent observed by different researches [23-25]. The methanolic extracts of *Solanum suratence*, *Azadirachta indica* and *Hydrocotyl javanica* exhibited larvicidal activity against *C. quinquefasciatus* [26]. The acetone extract of *N. indicum* and *T. orientalis* have been studied with LC_{50} values of 200.87, 127.23,209.00 and 155.97 ppm against third instar larvae of *A. stephensi* and *C. quinquefasciatus* [27]. Nevertheless, this system has proved to be much more effective over other plant systems reported earlier [28-29] where significantly higher doses of plant extracts were required for achieving 100% lethality against *C. quinquefasciatus* (LC_{50}= 41.41 and 129.24ppm) and *A. stephensi* (LC 50= 16 and 79.58 ppm) larvae from *Vitex*, *Azadirachta* and *Feronia* plant extracts respectively. The acetone leaf extract of *C.fistula* and *S.indica* exhibited significant mortality and also showed morhogenetic effects in 4^{th} instar larvae of *Anopheles* and *Culex* [30].

The study concludes that these two leaf extracts has potent larvicidal property along with morphogenetic effects that actually exhibited IGR activity pertaining to its effect on growth and development. So, these extracts can be used as a solution for mosquito problem in the developing countries without damaging the environment.

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