Research Paper

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Leaf and seed extracts of *Abutilon indicum* against the fourth instar larvae of *Culex quinquefasciatus*

Zoology

KEYWORDS : Abnormal formation, Abutilon indicum, Petroleum ether, and Culex quinquefasciatus.

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ABSTRACT

Petroleum ether, chloroform and ethanol of leaf and seed extract of Abutilon indicum activity against the fourth instar larvae of Culex quinquefasciatus. Among the leaf and seed extracts, petroleum ether extracts of leaf and seed was effective compare to other extratcs. Abnormalities were observed in larval, pupal and adult stages in petroleum ether extract of leaf.

Introduction

Mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the world. WHO has declared the mosquitoes as "public enemy number one" (WHO, 1996). Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 700,000,000 people every year globally and 40,000,000 of the Indian population. They act as a vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, West Nile virus infection, *etc*., in almost all tropical and subtropical countries and many other parts of the world.

Culex spp. occurs in all climatic zones ranging from forest to semi desert zones. Altitude does not seem to limit its distribution since it is observed at elevations of 2,770 – 5,500 m in India and as well as in mines situated at depths of 1,250 m below the sea level (Bhat, 1975 ; Renapurkar *et al.,* 2001). At lower elevations, *C. quinquefasciatus* population occurs in high numbers with relatively little seasonal variation, although high numbers are found during the third quarter of the year. In contrast at middle (≈600 – 1000m) and high elevations (≈1,300 – 1,500m) mosquito densities are much lower with well pronounced seasonal variations (Lapointe, 2000).

C. quinquefasciatus is an obligatory ectoparasitic vector since it plays a major role in the transmission of the nocturnal periodic form of Bancroftian filariasis all over the world (WHO, 1972). It is the most common mosquito prevalent in both urban and rural India. As the vector of bancroftian filariasis it poses an important public health problem, particularly among low socio-economic communities in India. Though the disease is not fatal, it causes severe morbidity in affected communities. An integrated approach involving chemical treatments and suitable vector control measures can only check the growing population of filarial vectors.

Culex quinquefasciatus is probably the most abundant house mosquito in towns and cities of the tropical countries. *Culex spp.* develop in stagnant water bodies such as polluted ponds, marshes, tanks, street gutters and water barrels and develop mainly in highly polluted water rich in organic matter (David and William, 2000).

The control of these diseases is largely dependent on spraying of chemical insecticides to kill mosquito adults or larvae. Larvicidal is an effective method to reduce the mosquito densities before they emerge as adults and synthetic insecticides have been widely used for this purpose (Tiwary et al., 2007). The use of these synthetic pesticides have been found to have side-effects in non-targeted organisms (Bernadou et al., 2009)

Medicinal Plants

Natural products of plant origin with insecticidal properties have been tried in the recent past for control of variety of insect pest and vectors. Phytochemicals derived from plant sources can act as larvicide's, insect growth regulators and repellent and ovipositor attractant and have different activities (Venketachalam and Jebasan, 2001)

MATERIALS AND METHODS

Laboratory culture of larvae

Hay infusion method was adopted for culturing mosquito larvae. Hay was taken, cut into small pieces and boiled in 5 litres of water for 20 minutes. After cooling, this water was poured into buckets and kept in different areas where mosquitoes were abundant. After one or two days eggs were laid by female mosquitoes in clusters forming an egg raft. The egg rafts were collected and maintained in the laboratory. The third instar larvae were collected, reared in enamel trays containing culture medium and provided with powdered dog biscuits and yeast in the ratio of 3:1 as the nutrient source. Immediately after moulting, the fourth instar larvae were introduced into beakers containing 200 ml of water and used for the bioassay studies.

Preparation of leaf and seed extracts

Fresh leaves and seeds were collected, washed in water and air dried under shade. Dried leaves and seeds were powdered using an electric pulverizer. 10g of the seed powder was weighed and subjected to extraction with 500 ml of solvents such as petroleum ether, chloroform, and ethanol for 8h using a Soxhlet apparatus. Petroleum ether (60-80°C) extraction was followed by chloroform and ethanol extraction. The leaf and seed extract thus obtained was concentrated by distillation and dried by evaporation at 40°C.

Bioassay studies

A pilot study was conducted to find out the effective doses of both leaf and seed extracts that produced mortality in larvae of *C. quinquefasciatus.* After determining the effective doses, the detailed investigations were undertaken. Concentrations ranging from 20 – 120 ppm which produced larval mortality at or after 24 h treatment were used for the bioassay studies.

Experimental design

The experiment was laid down in a Completely Randomized Design (CRD). The experimental set up consisted of two treatments, each with three replicates, and one set for leaf extract and another for seed extract. Twenty newly emerged fourth instar larvae of *C. quinquefasciatus* were introduced into the beakers for bio-assay studies. Control group with three replicates were also maintained simultaneously. 250 ml beakers each containing 200ml of water were used for the experiments. The following parameters like larval mortality at 24, 48, 72 and 96 h of treatment, larval and pupal behaviour, pupal mortality, total developmental duration, morphological changes were observed to assess the effective dose. Incidences of malformations and adult emergence were also observed.

Test for larvicidal activity
The larval mortality in both treatment and control was recorded at 24h of treatment and the percentage of mortality was calculated using Abbott's formula (Abbott, 1925).

$$\% \text{ Mortality} = \frac{\text{Mortality in treatment (\%)} - \text{Mortality in control (\%)}}{100 - \text{Mortality in control (\%)}} \times 100$$

Statistical analysis
The data on bioassay studies were subjected to one way ANOVA as described by (Panse & Sukhatme, 1985). Further LC_{50}, LC_{70}, LC_{90}, Regression equation and 95 percent Upper Confidence Limit (UCL) and Lower Confidence Limit (LCL) and Chi-square values were calculated by using probit analysis (Finney, 1971).

Results
Leaf extracts
Larval mortality
Maximum mortality was obtained in 300 ppm concentration of petroleum ether extract (100%) at 48 h followed by chloroform leaf extract 300 ppm (90%). At 72 h 100% mortality was produced in chloroform leaf extract ranging from 260 ppm to 300 ppm followed by ethanol extract at 96 h showing 100% mortality (Table I).

LC_{50} value at 24 h in petroleum ether extract was 315.17 ppm with a regression equation of Y = -3.30 + 3.32X, 95% confidence limits UCL LC_{50} 363.14 ppm and LCL LC_{50} 273.54 ppm. Compared to this 24 h LC_{50} value of chloroform leaf extract was 322.97 ppm and ethanol leaf extract was 379.72 ppm (Table I).

Pupation, pupal mortality and adult emergence
In higher concentrations ranging from 260 to 300 ppm owing to 100% larval mortality, no pupation was observed. In the lower concentrations, the percentage of pupation ranged from 5 – 35 and the pupal mortality was 5 to 20%. Minimum adult emergence of 10% was recorded in all the three extracts in lower concentrations.

Seed extract
Larval mortality
In all the three treatments, 50% and >50% mortality was recorded only at 48 h. By 72 h and 96 h 100% mortality was observed in higher concentrations of petroleum ether and chloroform extracts (Table II). However, larval mortality of 90% only was recorded in 300 ppm of ethanol extract at 96 h.

24 h LC_{50} value of chloroform extract was 334.56 ppm, with a regression equation of Y=-3.49+3.36X, 95% confidence limits UCL LC_{50} 396.03 ppm and LCL LC_{50} 282.64 ppm. Ethanol and petroleum ether extracts were equally effective with 24 h LC_{50} value of 438.61 ppm and 335.13 ppm respectively (Table II).

Pupation, pupal mortality and adult emergence
Pupation was minimum (10%) in 200 ppm and 220 ppm of petroleum ether extracts and 200 and 240 ppm chloroform extracts. Pupal mortality ranged from 5 to 12% in these treatments. Adult emergence was totally arrested in petroleum ether extract as compared to the other treatments.

Abnormal Formations
Abnormalities were observed in larval, pupal and adult stages in both leaf and seed treatments. The dead larvae were found to be dark in colour and thoracic cuticle was split on the dorsal side. Abnormalities observed in pupal stages were the formation of non-melanized (Plate VIII A), a few partially melanized (Plate VIII B) and many hyper melanized pupae (Plate VIII C). Such abnormalities during metamorphosis could be due to an imbalance in the hormonal activity.

Discussion
Plants are rich sources of bioactive organic chemicals and synthesize a number of synthetic metabolites to serve as defense chemicals against insect attack. These chemicals may serve as insecticides, antifeedants, oviposition-deterrents, repellents, growth inhibitors, juvenile hormone mimics, moulting hormones, antimoulting hormones as well as attractants. They offer an advantage over synthetic pesticides as they are less toxic, less prone to the development of resistance and easily biodegradable (Ignacimuthu, 2000). Therefore the search for insecticides of plant origin has gained great impetus in recent times.

George and Vincent (2005) compared the individual and combined efficacy of the extracts of *A. squamosa* and *Pongamia glabra.* The syngeristic effect was more pronounced than individual effect. In the present work, higher concentrations of both leaf and seed extracts produced 100% larval mortality showing the individual efficacy of these extracts.In the present investigation several morphogenetic abnormalities such as formation of hypermelanized pupae, partially melanized pupae and non-melanized pupae (albino) were observed in treatment with all the leaf and seed extracts. Abnormal formations such as larval–pupal intermediates and pupal-adult intermediates were also seen. This corroborates with the findings of Saxena and Saxena (1992) who observed various defective stages in *C. quin-quefasciatus* in the treatment with *T. neriifolia* leaf and seed extracts. The present observation is also comparable to the description of Zebitz (1984).

Manimegalai *et al*., (2011) recorded that the leaf and seed extract of *Abrus precatorius* was found to be effective in controlling the larvae of culex quinquefasciatus. Similar observations were made in the present study in which at higher concentration of petroleum ether and chloroform extracts 100 percent larval mortality was observed in 72 h.

Manimegalai *et al*., (2011) observed that pupation was completely arrested in chloroform leaf extract of *calotropis procera*. This result is comparable with the persent study in which even at higher concentrations adult emergence was totally arrested in petroleum ether and chloroform extracts.

Annapoorani and Manimegalai (2013) examined abnormal formations and pupal adult intermediates in ethanol extract of leaf and seed. In the persent study also, abnormal formations were observed in petroleum ether extract of leaf.

Similar observations were recorded in the present study in which at higher concentration of both leaf and seed extracts, owing to 100% mortality no pupation and adult emergence was observed in the petroleum ether seed extract of *A.indicum*. This result is comparable with the present study in which even at lower concentration, no pupation and percent total mortality and total arrest of adult emergence was observed in petroleum ether seed extract of *A.indicum*.

lethal concentration of leaf extractS of *ABUTILON INDICUM* AGAINST *CULEX QUINQUEFASCIATUS*

Solvents used	Hours	LC_{50} (ppm)	LC_{70} (ppm)	LC_{90} (ppm)	Regression equation	95% confidence limits				X^2
						UCL (ppm)		LCL (ppm)		
						LC_{50}	LC_{90}	LC_{50}	LC_{90}	
Petroleum ether	24	315.17	453.45	766.61	Y=-3.30+3.32X	363.14	1384.71	273.54	424.41	0.38
	48	211.92	239.94	287.05	Y=-17.63+9.73X	219.95	304.29	204.19	270.78	2.89
	72	193.22	213.34	246.13	Y=-22.88+12.20X	204.14	261.72	182.89	231.47	2.50
	96	271.69	323.90	417.46	Y = -17.14+9.44X	283.03	479.31	260.79	363.60	5.27

Chloroform	24	322.97	435.12	669.06	Y=-5.17+1.78X	366.61	1035.08	284.53	432.46	4.06
	48	221.71	252.56	304.82	Y=-16.75+9.27X	229.07	321.90	214.58	288.65	5.27
	72	172.95	214.24	291.85	Y=-7.62+5.64X	211.95	375.10	141.12	227.08	0.37
	96	108.20	138.08	196.35	Y=-5.07+4.95X	285.17	232.47	41.05	165.84	0.46
Ethanol	24	379.72	506.17	766.48	Y=-5.84+4.20X	462.71	1284.24	311.61	457.46	2.57
	48	276.77	361.23	530.57	Y=-6.08+4.54X	295.54	711.24	259.19	395.80	2.02
	72	210.85	249.54	318.23	Y=-11.66+7.17X	221.69	344.45	200.53	294.01	2.18
	96	190.48	429.90	234.78	Y=- 27.18+14.12X	389.42	429.89	93.17	128.22	4.24

LETHAL CONCENTRATION OF SEED EXTRACT OF *ABUTILON INDICUM* AGAINST *CULEX QUINQUEFASCIATUS*

Solvents used	Hours	LC_{50} (ppm)	LC_{70} (ppm)	LC_{90} (ppm)	Regression equation	95% confidence limits				X^2
						UCL (ppm)		LCL (ppm)		
						LC_{50}	LC_{90}	LC_{50}	LC_{90}	
Petroleum ether	24	335.13	624.22	1532.09	Y = 9.61+1.94 X	447.20	7563.89	251.15	310.31	13.52
	48	193.02	238.48	323.64	Y = -8.05+5.71 X	239.46	420.84	155.58	248.89	12.43
	72	171.70	202.93	258.29	Y = -11.15+7.23X	193.17	274.36	152.63	243.17	3.61
	96	263.33	318.99	423.07	Y = -10.09+6.24 X	273.34	494.28	251.75	362.13	6.65
Chloroform	24	334.56	479.08	804.44	Y = -3.49+3.36 X	396.03	1489.79	282.64	434.37	0.71
	48	207.65	239.75	295.04	Y = -4.47+8.40 X	217.33	312.42	198.41	278.63	8.32
	72	150.76	194.56	281.16	Y = -5.32+4.74 X	884.58	781.05	25.70	101.21	14.77
	96	4.80	2.83	22.26	Y = -6.45+0.12 X	1.70	1.70	5.88	5.88	3.07
Ethanol	24	438.61	687.24	1314.16	Y = -2.11+2.69 X	651.05	4106.90	295.49	420.51	1.68
	48	242.10	312.40	451.38	Y = -6.30+4.74 X	254.55	564.10	230.26	361.18	5.03
	72	199.76	236.38	301.40	Y = - 11.51+7.18 X	212.39	323.07	187.88	281.18	0.21
	96	152.30	190.05	261.65	Y = -6.90+5.45 X	214.63	316.77	108.07	216.12	0.49

ABNORMAL FORMATIONS

a. Non melonized pupae b.Pupal adult intermediate

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