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MOSQUITO LARVICIDAL AND CHEMOSTERILANT ACTIVITY OF *AGERATUM CONYZOIDES* AGAINST *CULEX QUINQUEFASCIATUS*

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Abstract: Mosquito are the vector for various diseases including malaria, filaria, yellow fever, encephalitis and chickungunea. Mosquito have developed resistance to the chemical pesticides. Farther the mosquito pesticidal compounds caused several ill effects to the human being. The present problem was proposed to be undertaken to provide suitable alternatives to control vector mosquito using biological control methods. Ageratum conyzoides whole plant of family Asteraceae was collected locally from Vidisha district of M.P. The crude extracts were isolated and purified using column chromatography, TLC, acid hydrolysis, methylation and other chemical methods. Alkaloid isolated from *Ageratum conyzoides* have shown larvicidal, growth-regulating and chemosterilant activities against *Culex quinquefasciatus* at concentration of 50 to 250 ppm. Developmental defect due to chloroform extract which was a dose dependent. Adults exposed as larvae to different treatments showed reduced fecundity and fertility in females. Mortality in the larvae, pupae and adults produced about a 20-96% decrease in the laboratory experiment. The total developmental period was slightly reduced from the control. Treatment with the alkaloids had a significant effect on the mortality, emergence and reproductive physiology of *Culex quinquefasciatus.*

Keywords: Chemosterilent, Fecundity, Fertility, larvicidal, alkaloid, *Culex quinquefasciatus*, *Ageratum conyzoides*

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INTRODUCTION

Human beings have suffered from the activities of mosquito since time immemorial. It is believed that mosquitoes are ranked as the most important human health pests. They scourge him with their vicious biting and continuous singing, but most seriously they transmit malaria, filaria, japanese encephalitis, chikungunya and dengue fever to human beings. These diseases devastate Indian economy every year (Jaswanth et al. 2002)[1]. Worldwide, mosquito transmits diseases to more than 70, 00, 00, 000 people annually and are responsible for one death for every seventeen people currently alive.

Since, prevention is better than cure, a major strategy of malaria control is to attack the vector with insecticides (Rajkumar and Jabanesan 2009)[2]. The control of mosquito at the larval stage is necessary and efficient in integrated mosquitos management. During the immature stage, mosquito are relatively immobile remaining more concentrated than they are in the adult stage (Rutledge et al. 2003)[3]. Since the discovery of DDT, mosquito control approach has been almost completely based on synthetic organic insecticides. But the extensive use of synthetic organic insecticides during the last five decades have resulted in environmental pollution and also in the development of physiological resistance in major vector species in addition to the increased costs of insecticides. This has necessitated the need for search and development of environmentally safer, low cost, indigenous methods for vector control (Chakkarvarthy et al. 2010)[4].The present paper report the analysis of larvicidal and chemosterilant activity in an alkaloid compound from *Ageratum conyzoides* of Astereaceae against filarial vector.

MATERIAL AND METHOD

Plant material

Ageratum conyzoides native of South America now well naturalized throughout India. Very comman in waste land, road sides and cultivated fields, quite abundant in moist situations used as cattle fidder. Its roots and leaves are used as antihelithic and stypic respectively. It shows many biological activities such as insecticidal, anti fungal and antimicrobial. The collected whole plant was shade dried at room temperature in laboratory keeping for one day at 100^0c in oven. A voucher specimen of the plant has been preserved in our herbarium record in Pest Control and Ayurvedic Drug Research Lab. Vidisha for future reference.

Extraction method

The extraction of whole plant of *Ageratum conyzoides* shade dried powder material (40-60) mesh size was carried out separately by soxhlet apparatus in the laboratory using different solvents in increasing order of polarity. The extraction procedure adopted as given by Harborne (1984)[5].

Laboratory colonized *Culex quinquefasciatus* second and fourth instar larvae were used for the experimental bioassay. Larval study was conducted according to standard of procedure WHO (1981)[6].

Experimental bioassay

For experimental bioassay, 25 second and fourth instar larvae of *Culex quinquefasciatus* were kept in 500 ml glass beakers containing 249ml of distilled water and 1ml alkaloids. Acetone was used as solvent to dilute the compound to an appropriate test concentration. The treatments were replicated three times. Each replicate set contains one control, which received 1 ml of 50% acetone and 249 ml of distilled water and one untreated, which contained only 250 ml of distilled water. The number of dead larvae, pupae and adults were recorded. Mortality was corrected according to Abbott formula (1925)[7].

Fertility and fecundity was observed in the adult mosquitoes which were emerged from treated and untreated sets (Saxena et al. 1993)[8]. To study these experiments, equal number of female and male mosquitoes emerged from the treated and untreated larvae were mated in cages (32x32x32 cm) in the following four groups.

A. Treated females x Treated males (T♀ x T♂)

B. Treated females x Untreated males (T♀ x UT♂)

C. Untreated females x Treated males (UT♀ x T♂)

D. Untreated females x Untreated males (UT♀ x UT♂)

Three days after blood meal eggs were collected daily from the ovitraps placed in the cages. Just after hatching larvae were transferred to the enamel trays (30x25x5cm.) containing distilled water. Number of females and males died in the cages, oviposition day after blood meal, number of eggs, and percentage of hatching and subsequent larval mortalities were observed regularly.

$$\text{Sterility Index (S.I.)} = 100 - \frac{\text{Test no. of eggs x \% hatching}}{\text{Control no. of egg x \% hatching}} \times 100$$

$$\text{Growth Index G.I.} = \frac{a}{b}$$

Where,

a = percentage adult emergence

b = average developmental period (days)

Statistical evaluation of data were carried out by probit analysis (Finney 1971)[9] and fecundity and fertility experiments, level of significance was calculated by ' Duncans ' multiple range test (Duncan, 1963)[10].

Table -1 Effect of chloroform extract of *Ageratum conyzoides* on development, moulting and metamorphosis of *Culex quinquefasciatus.*

Concentration (ppm)	Average larval period (days)	Average pupal period (days)	Pupal mortality (%)	Adult emergence (a) (%)	Average developmental period (b) (days)	Fall in vector population (%)	Growth index* (a/b)
50	6.5	2.0	16	22	8.5	78	2.58
100	6.0	2.0	14	18	8.0	82	2.25
150	6.0	1.5	12	16	7.5	84	2.13
200	6.0	1.5	10	10	7.5	90	1.33
250	5.5	1.5	08	08	7.0	92	1.14
Control	7.0	2.5	00	98	9.5	02	10.31
Untreated	7.0	2.5	00	99	9.5	02	10.42

25 each second instar larvae were taken in four replicates.

***Values are significantly different from control (Duncan's multiple range test, p< 0.05)**

Table -2

Fecundity and fertility effect of *Ageratum conyzoide* chloroform extract on adults of *Culex quinquefasciatus* from treated larvae.

Treated group	Female dead (%)	Percentage of biting	Oviposition day after blood meal	Average no. of eggs obtained	Hatching (%)	Larval mortality (%)	Adult emergence (%)	Sterility index (%)
T♀ X T♂	40.0	33.3	5	82	62	66	20	74.06
T♀ XUT♂	33.3	33.3	4	120	74	58	28	54.69
UT♀ X T♂	13.3	86.6	3	190	86	08	78	16.63
UT♀XUT♂	6.66	93.3	3	200	98	02	96	__

15 each treated females and males were taken in each replicate.

Values are significantly different than the control (Duncan's multiple rang test, p<0.01)

OBSERVATIONS AND DISCUSSION

Effect of the crude extract was seen on the growth, development and metamorphosis of fourth and second instar larvae. Table (1) reported the developmental deffects due to chloroform extract of *Ageratum conyzoides* which was also inversely dependent to dosage. From the results it appear that due to larval and pupal mortality maximum fall in vector population was registered to be 92% against 2 % in the control group of insects. This was quite dose

dependent. The growth index was found to be significantly reduced 1.14 at 250 ppm concentration as compared to 10.31 growth index noticed in the control group which was quite significant.

Table (2) showed chloroform extract of *Ageratum conyzoides* fecundity and fertility effect on adults of *Culex quiquefasciatus.* It was observed when treated males and treated females cross together more than 40% female died during the course of experiment. It tooks 5 days for oviposition as compared to 3 days period taken by the untreated female. The average no. of eggs obtained were considerably less in number in group -I (82) as compared to the eggs laid by untreated female (200 eggs). Percentage of hatching was less (62%) than the untreated group 4th, sterility index was found to be maximum (74.06%) in group first when treated male cross with treated female. The results were quite significant than the control ($p<0.01$).

Toxic effect of plant derived compound on mosquito development was examined by Spilmen and Skaff (1967)[11]. The prolongation of the larval developmental period and decreased in growth in *Culex quinquefasciatus* by *Annona squamosa* extract was found to be quite similar as observed by previous worker like Supavan et al.(1974)[12]. Saxena et al. (1993)[9] had also noticed similar larvicidal and chemosterilant activity of *Annona squamosa* alkaloid against *Anopheles stephensi*. Kabir et al. (2011)[13] have also reported the potent larvicidal and growth disrupting activity of *Apium graveolense* seed extract on *Ades aegypti* mosquito.

CONCLUSION

The findings of present studies, therefor suggest the growth inhibitory activity in *Ageratum conyzoides* along with larvicidal activity. Our results may provide new tools for effective control approaches against *Culexquinquefasciatus.*

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