SCIENTIA

Peer Reviewed National Science Journal

Volume 14. No.1 ◆ Jan-Dec.2018 ◆ ISSN: 0976-8289

Published by:

MERCY COLLEGE

PALAKKAD 678006, KERALA, INDIA

Scientia ISSN: 0976-8289
Vol. 14, No.1, Jan.-Dec., 2018, pp.21-25

Potency of plant extracts in mosquitocidal activity

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Abstract

Mosquitoes have annoyed man and undermined his health for centuries. The present study tried to assess the mosquitocidal efficacy of leaf extracts of *Ocimum basilicum* and Simarouba glauca on the developmental stages of Culex species of mosquitoes. The present investigation showed that higher the concentration of the extract, higher was the percentage larval mortality and lower pupal emergence. Maximum larval mortality (93.3%) was observed in 120h (5th day) in 10,000ppm concentration of extracts of *Ocimum basilicum* , while minimum mortality of 3.3% in 2000ppm at 48h. Many abnormal developmental stages were also obtained in S. glauca leaf plant extracts.

Key Words: Mosquitocidal, Non-melanized pupae, hyper-melanized and pupal-adult intermediates

Introduction

Mosquitoes are the most important single group of insects in term of public health because of their ability to transmit a number of outrageous diseases like Japanese encephalitis, filariasis, malaria, chickungunya and dengue, causing millions of deaths every year throughout the world[1,2]. Mosquito species are abundant in the tropics and almost unbelievably large swarms of them occur in the Arctic. In India, these ubiquitous insects occur at elevation of 4,300 meters in Kashmir and 1,160meters below sea level in gold mines of south India[3].

Transmission of mosquito-borne diseases is governed by a variety of specific ecological, epidemiological, geographical and social factors. The north–eastern state of Assam is endemic for the mosquito borne diseases with uneven distribution pattern. Malaria is highly endemic in Barak valley and lower Assam areas[4].

Most of the present day research on mosquitoes is concerned directly with methods of killing them. The control of mosquitoes is complicated by the fact that breeding places of the larvae are often inaccessible or in public water supplies. Burning problem in mosquito control operation is development of resistance by the mosquito to several chemical insecticides and repellents. Extract of neem seed kernel[5], leaf extract of *Lucas aspera*, *Ocimum sanctum, Azadiracta indica* and *Allium sativum*, Rhizome extract of *Curcuma longa*[6] and fumigation by peel of different Citrus species[7] have been tried.

Larvicidal efficacy of ethanolic extracts of *Annona squamosa* (Annonaceae) over the filarial vector, *Culex quinquefasciatus* was tested. The results revealed that ethanolic leaf extracts of *A. squamosa* plant can be used effectively as a potential, ecofriendly, biodegradable and economic larvicide in integrated mosquito control programme[8]. Bioassays against larvae of *Ae. aegpti* with neem seed kernel extracts obtained by extraction with water and organic solvents. Permanent exposure of first instar larvae to treated water resulted in a conspicuous growth disrupting effect, mainly characterized by morphogenetic effects[9]. The effectiveness of the extracts increased with decreasing polarity of the solvents used for extraction. The seed kernel extract caused an extreme prolongation of the larval period when first instar larvae were continuously exposed to treated

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water until adult emergence. As Angamaly is a mosquito prone area, an investigation was undertaken to assess the potency of selected botanicals against Culex species of mosquito.

Materials and Methods

Collection of larvae

Hay infusion method was adopted for collecting the mosquito larvae[10]. Eggs were laid by mosquitoes after 1-2 days. The rafts were collected and maintained in the laboratory. The 2nd instar larvae emerging from the culture medium were collected and maintained separately. A pinch of dog biscuit was provided as food for these larvae. When the larvae moulted and entered into 3rd instar, they were segregated and used for the study.

Collection of test materials

Leaves of *Ocimum basilicum* (Fig. 1) and *Simarouba glauca* (Fig. 2) were collected from Morning Star Home Science College campus Angamaly, Ernakulam.

a) Preparation of leaf powder

Healthy green leaves were collected, washed once in water and shade dried. 100g of dried leaves were weighed and ground to powder.

b) Preparation of extracts

10g of leaf was weighed in an electronic balance. Leaf powder was mixed in 200ml of distilled water. The mixture was stirred for 16 h in an electrical shaker at120rpm and left to stand for 48 h at room temperature. The mixture was centrifuged at 3000rpm for 20 minutes twice. The supernatant was collected and used in this study.

Experimental Setup

The experimental set up consisted of six treatments ranged from 2000ppm to 10,000ppm each with three replications. For bioassay studies, the required quantity of different concentrations of leaf extracts were introduced into 250ml beaker containing 50ml of tap water and thoroughly mixed. 10 newly emerged 3rd instar larvae were introduced into each beaker containing the rearing medium for specific treatments. Dog biscuit was provided *ad libitum* as food. In control only tap water was used. Concentrations of 2000ppm, 4000ppm, 6000ppm, 8000ppm and 10,000ppm were taken as treatments.

Fig1:*Ocimum basilicum*

Fig 2: *Simarouba glauca*

Table 1 Effect of *O. basilicum* leaf extract on the developmental stages of Culex species.

Treatments	Larval mortality (%)						Pupal emergence (%)	Pupal mortality	Adult emergence (%)
	24h	48h	72h	96h	120h	144h			
2000ppm	0.00	3.3	6.66	10	33.3	50	50	30.00	20.00
4000ppm	16.6	16.6	16.6	40	63.3	80.0	20	-	20.00
6000ppm	10	10	13.3	33.3	50	60	40	23.4	16.6
8000ppm	13.33	13.3	13.3	50.0	86.6	-	10.4	10.4	-
10,000ppm	36.6	40	43.3	53.3	93.3	-	3.3	-	3.3
Control	0.00	0.00	0.00	0.00	0.00		100.00	-	100.00

Observation was made at every 24h duration on larval and pupal mortality. Dead larvae and pupae were removed at 24h interval after the exposure. Various abnormalities noted during the period and moulting were also recorded. Pupae emerging out were counted, killed and preserved. A record of number of adults emerging also was maintained.

Results

Ocimum basilicum

The effect of *O. basilicum* leaf extracts on larval mortality was found to increase in proportion to the increase in the concentration of the leaf extract (Table 1). Maximum mortality of 93.3% was observed in 120h (5th day) in 10,000ppm concentration, while minimum mortality of 3.3% in 2000ppm at 48h. No mortality was recorded in the control as well as in 2000ppm at 24h. In control 100% pupal emergence was observed while in 10000ppm treatment minimum pupal emergence of 3.3% was observed. Adult emergence could be seen in all treatments except 8000ppm. Minimum adult emergence could be observed in 10000ppm and it was 3.3%.

Simarouba glauca

Effect of *S. glauca* leaf extract on larval mortality indicated 10,000ppm treatment as an effective one producing maximum mortality in 240h (10th day)(86.6.00%) (Table 2)

Larval extension could be observed and it was maximum in 2000 ppm (312h; 13th day). The pupal emergence was 100% in control and 10,000ppm recorded minimum pupal

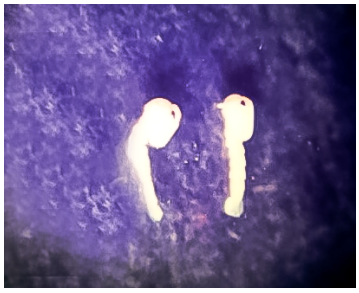

Fig 3. Non-melanized Pupae

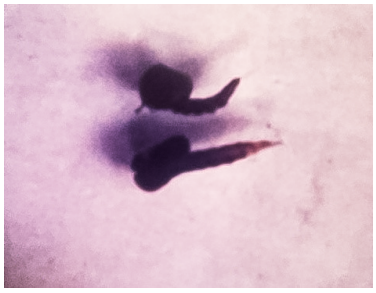

Fig 4. Hyper-melanized pupae

Fig 5. Pupal- Adult intermediates

Table 2 Effect of *S. glauca* leaf extract on the developmental stages of Culex species

Treatments	Larval mortality (%)				Pupal Emergence(%)	Pupal Mortality(%)	Adult emergence (%)
	24h	48h	72h	96h			
2000	0.00	0.00	0.00	30.00	20.00	3.3	16.7
4000	3.33	13.3	16.6	16.6	23.00	3.3	20.4
6000	0.00	0.00	0.00	6.6	20.00	3.3	16.7
8000	0.00	0.00	0.00	3.3	16.00	3.3	12.7
10,000	20.00	30.00	30.00	40.00	10.00	3.3	6.7
Control	0.00	0.00	0.00	0.00	100.00	0.00	100.0

emergence (3.3%). Pupal – adult intermediate was observed in 2000ppm (3.3%).Adult emergence was maximum in 4000ppm (20.4%) and minimum in 10000 ppm (6.7%).

Abnormalities observed

Abnormalities were observed in pupal stage. Few pupae were non-melanized and they were white in colour (Fig.3), many were hypermelanized (Fig.4) and pupal adult intermediates (Fig. 5) were also observed.

Discussion

In the present study aqueous leaf extracts of *O. basilicum* and *S. glauca* were found to be potent agents for the control of Culex spp. Both these leaf extracts had larvicidal properties and larval extension was observed in *S. glauca* and it was extented up to 13th day.

In the present investigation higher the concentration of the extract, higher was the percentage larval mortality and lower was the percentage of pupal emergence. The optimal dose which brought about maximum mortality and minimal emergence was found to be 10,000ppm in *O. basilicum* leaf extract and in *S. glauca.* In addition to this, no adult emergence was observed in 8000 ppm of *O. basilicum. In S. glauca,* the lower concentration showed larval extension showing its efficacy over other treatments.

Studies using acetone extracts of neem seed coat showed pupal mortality in *Cu. quinquefasciatus* even in lower concentrations, but not in *Ae. aegypti*. In the present work, in comparison with the leaf extract treatment of *O. basilicum* and *S. glauca* pupal emergence was minimum and pupal mortality was significantly high in higher concentrations[11].

Various defective stages such as unmelanized pupae, partially melanized pupae and hyper-melanized pupae and pupal-adult intermediate were observed in this bioassy study[9,12]. The induction of morphogenetic abnormalities is generally attributed to the interference of active ingredients of extracts with the endocrine system. The incidence of endocrine forms in different extracts of *O. basilicum* and *S. glauca* in the present study might be due to the juvenomimetic activity of substance present in the extract.

Conclusion

O. basilicum and *S. glauca* species are available in plenty in our locality and will be a cost-effective mosquitocidal material to be used by all. The present investigation is an attempt to identify some commonly

available effective botanicals that can be used by both rural and urban people in mosquito management. Further studies may be undertaken to study the active ingredients of the extracts and field trials can be undertaken.

Acknowledgement

We express our sincere thanks to Kerala State Council for Science Technology and Environment for the financial support given under SPYTiS-II 2017(138/SPYTiS-II/2017/KSCSTE).

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Scientia (Annual)

Volume 14. No.1 ◆ Jan-Dec.2018 ◆ ISSN: 0976-8289

ISSN 0976-8289
9 770976 828007