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Smoke Repellent Action of Eicosatrienoic Acid from Gliricidia Sepium against Filarial Vector Mosquitoes

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Abstract: *Mosquito-transmitted diseases continue to be a major cause of illness and death. Prolonged exposure to the synthetic insecticides may lead to irritation, severe allergic dermatitis, nausea, vomiting, headache and other central nervous system disturbances. In recent years interest in plant-based products has been revived because of the development of resistance, cross-resistance and possible toxicity hazards associated with synthetic insecticides and their rising cost. Due to these circumstances, the effectiveness of 8, 11, 14-eicosatrienoic acid from Gliricidia sepium, in controlling mosquito was studied and found very effective. Also, the effect of the newly found phytochemical on non target organisms was studied and found non-toxic.*

Keywords*:* *Mosquito, Culex quinquefasciatus, Gliricidia sepium, Phytochemical, Mosquito coil*

1. INTRODUCTION

Mosquitoes are still representing the world's number-one vector of human and domestic animal diseases. 40 million people in India suffer from mosquito borne diseases annually. These diseases transmitted by mosquitoes devastate Indian economy every year [Jaswanth et al., 2001]. Filariasis, a disease affecting the arms, legs and genitals, caused by Wuchereria bancrofti is transmitted by *Culex quinquefasciatus* mosquitoes which are widespread in the country now. There are 45 million cases of lymphatic filariasis, in India alone [Bowers et al., 1995]. Use of synthetic insecticides to control mosquitoes has caused physiological resistance and adverse environmental effects. Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects [Ghosh, 1991]. These factors have created the need for environmentally safe, degradable, and target specific insecticides against mosquitoes. Recent studies stimulated the investigation of insecticidal properties of botanicals and concluded that they are environmentally safe, degradable and target specific [Govindarajan et al., 2008].

2. MATERIALS AND METHODS

2.1 Selection of Plant Material and Extraction

An ethnobotanical survey revealed that leaves of *Gliricidia sepium*[Jacq] (Fabaceae) were commonly used for smouldering as a mosquito repellent in many areas of Kerala state. Fresh leaves of *G.sepium* were collected from Vagamon, located 1100 m above sea level at Idukki district, in Kerala state. Petroleum ether extract of dried powdered leaves were extracted again with acetonitrile and column chromatographic separation was done using acetone: methanol (9:1) after extraction with chloroform. The eluted compounds were tested for their larvicidal activity and the compound with high desired quality was undergone spectral analysis GCMS, FTIR, NMR and identified as 8,11,14-eicosatrienoic acid.

2.2 Rearing of Mosquitoes

Culex quinquefasciatus Mosquitoes collected from field were used for raising the colony. After oviposition, eggs were collected in filter paper and kept separately at 27±2 °C. The female mosquitoes were fed on blood meal. Both females and males were provided with 10% glucose solution. The larvae were fed on a diet of finely powdered biscuits and yeast in the ratio 3:1. The water in the tray was changed every day and dead larvae were removed (Gerber *et al*., 1994).

2.3 Preparation of Bio Coils

Coils were made manually by using 8, 11, 14-eicosatrienoic acid (0.1%), saw dust (52.9 %), coconut shell charcoal powder (35%),and starch powder (12%) w/w. These are thoroughly mixed with distilled water to form a semi solid paste. Mosquito coil of thickness 0.8 cm with weight approximately 27 g was prepared manually from the paste by shade drying. The procedures of Saini *et al*., 1986 and Pauluhn, 2006 were followed.

2.4 Smoke Toxicity Test

Smoke test was conducted according to the methods of Singha *et al*., 2011 and Zaridah *et al*., 2006. The smoke toxicity experiment was conducted in a glass chamber measuring 140 x120 x60 cm^3 with window at the mid-bottom on one side of the chamber. Group 1 mosquitoes were exposed to the smoke of a blank coil (without chemical). A test carried out without coil served as control group II. Group III were exposed to coil with 8, 11, 14-eicosatrienoic acid. The coil was kept on a stand in the middle of the chamber and allowed to burn for 30 min before 30 sucrose-fed mosquitoes were released into the chamber. Knock-down mosquitoes (i.e. those keeping abnormal posture or were unable to fly) were recorded at 10 min intervals up to 2 h. Knocked-down mosquitoes were placed in a clean container containing cotton wool soaked with 5% sucrose solution and the mortality of the mosquitoes was observed after 24 h. The above procedures were carried out in triplicates for each coil formulation.

2.5 Studies of the Effect of Bio Coil on Rat Embryo

The experiment involved Wistar albino variety of pregnant rats. They were kept inside a partially ventilated closed room (4×3.5×3.5 m^3), with free access to food and drinking water. They were divided into two groups of six each, group I served as control while group II were fumed with bio coil. Experimental period was only for 21 days till the end of their gestation. The experiment was carried out by continuously igniting mosquito coils every day for 8 h. The control animals were kept exactly in similar conditions for the same period of time except mosquito coil smoke. Weanlings produced were tested for toxicity studies after one week.

2.6 Specimen Collection

At the end of the experimental period, blood was collected in sterilised bottles containing anticoagulant heparin for biochemical analysis, by excising the jugular vein. Blood collected in centrifuge tubes, was allowed to clot, and spun at 3000 rpm for 5 min to get the serum. The organs (liver, kidney, lungs, spleen and heart) were collected and transferred into ice-cold containers for histopathological evaluations.

3. RESULTS

Results of smoke toxicity test is given in Table 1

Table 1. *Knock-down assessment values (KD_{50} and KD_{90}) of coils against C.quinquefasciatus mosquitoes*

Groups	KD_{50}(min) (95% CL)	KD_{90}(min) (95% CL)	Mortality in mosquitoes
I	-	-	4±0.06
II	-	-	0±0.0
III	41.80 (37.99-45.11)	94.67 (84.64-110.69)	10±0.23

Group I-Blank coil, Group II-no coil, group III- coil with 0.1% 8,11,14-eicosatrienoic acid.

The phytochemical from *G.sepium* exhibited strong knock-down (KD_{50}) value at 41.80 min (37.99 - 45.11 with 95% C.L) and KD_{90} at 94.67min (84.64-110.69 with 95% C.L).The mortality was reported as 10 in number which is highly significant from control values.

4. BIOCOIL FUMING STUDY ON RAT EMBRYO

4.1 Haematological Studies

Effect of biomosquito coil smoke on haematological parameters are given in Table 2

Table 2.

Parameters	Group I	Group II
Hemoglobin (gm%)	17.35± 0.83	17.53± 0.45^{ns}
RBC (million/cumm)	8.45± 0.40	8.37± 0.23 ^{ns}
PCV %	48.83± 3.17	48.80± 2.61 ^{ns}
MCV F1	57.78	58.30 ^{ns}
MCH pg	20.53	20.94 ^{ns}
MCHC %	35.53	35.92 ^{ns}
Neutrophil%	15.68± 1.51	15.33± 1.63 ^{ns}
Basophil%	00	00
Eosinophil %	2.08± 1.10	2.85± 0.75 ^{ns}
Lymphocyte%	78.57±3.96	78.82±3.14^{ns}
Monocyte%	3.67±1.5	3.0±1.27^{ns}

- Group I-control, group II-fumed with biocoils
- $^{ns-}$ non-significant
- Average of six values in each group ± SD

4.2 Biochemical Studies

The effect of biocoil on biochemical parameters are given in Table 3

Table 3.

Parameters	Group I	Group II
cholesterol mg%	164 ± 1.41	163 ± 0.63 ^{ns}
HDL mg%	27.8±1.01	27.9±0.91 ^{ns}

AST IU/L	35.8± 3.78	35.3± 2.14 ^{ns}
ALT IU/L	34.6 ± 2.07	33.9 ± 1.97 ^{ns}
ALP IU/L	121.2± 1.41	121.1± 1.17 ^{ns}
Urea mg%	29.5± 1.87	30± 1.37 ^{ns}
Creatinine mg%	0.75± 0.10	0.78± 0.04 ^{ns}
Bilirubin mg%	0.50± 0.01	0.50± 0.01 ^{ns}
Albumin gm%	2.58± 0.20	2.58 ±0.12 ^{ns}
Protein gm%	5.7± 0.14	5.65 ±0.23 ^{ns}

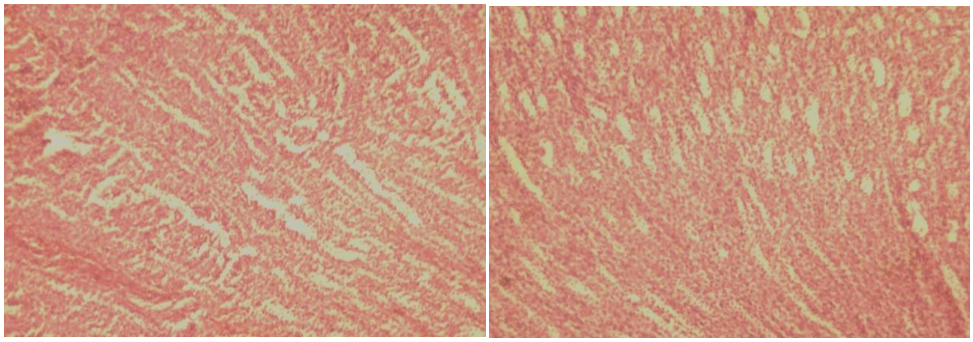
- Group I-control, group II- fumed with biocoils
- Average of six values in each group ± SD
- $^{ns-}$ non-significant

4.3 Histopathological studies

The mosquito coil made using eicosatrienoic acid extracted from *G.sepium* was fumed for the entire pregnancy period of rat and, effects on kidney, liver, heart, spleen and lungs embryo were analysed.

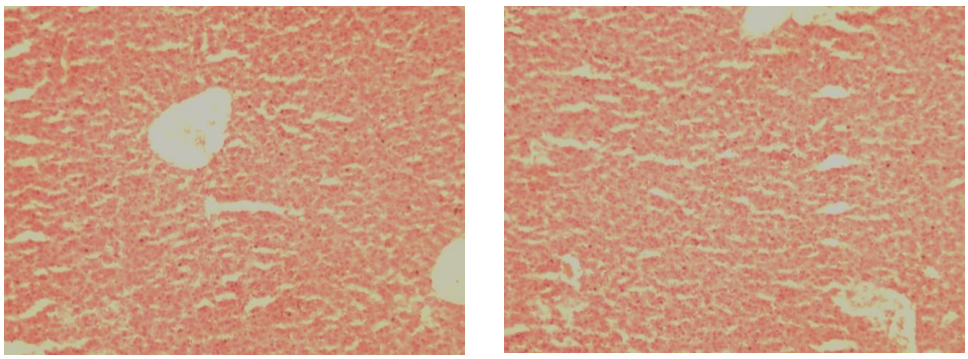
Effect of biocoil fuming on kidney of embryo

I II



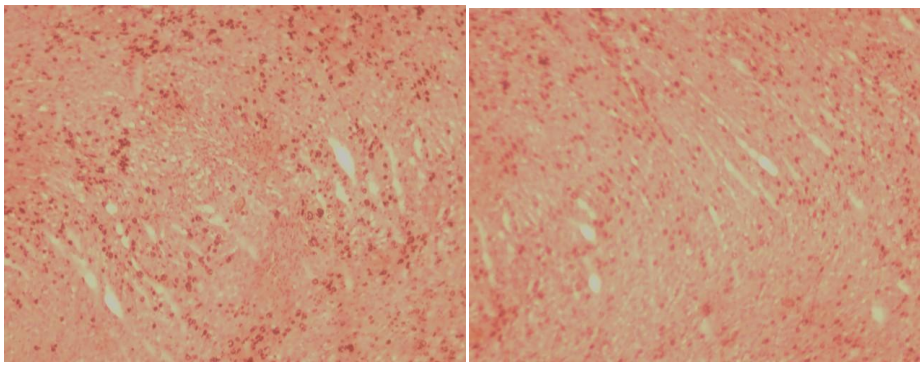
Effect of biocoil fuming on liver of embryo

I II



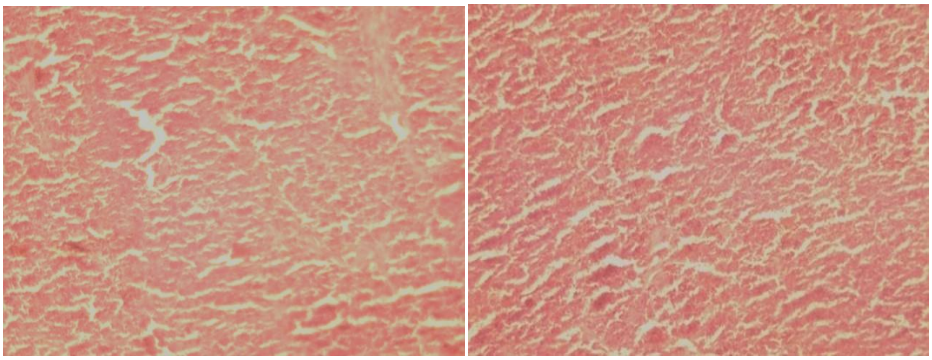
Effect of biocoil fuming on heart of embryo

I II



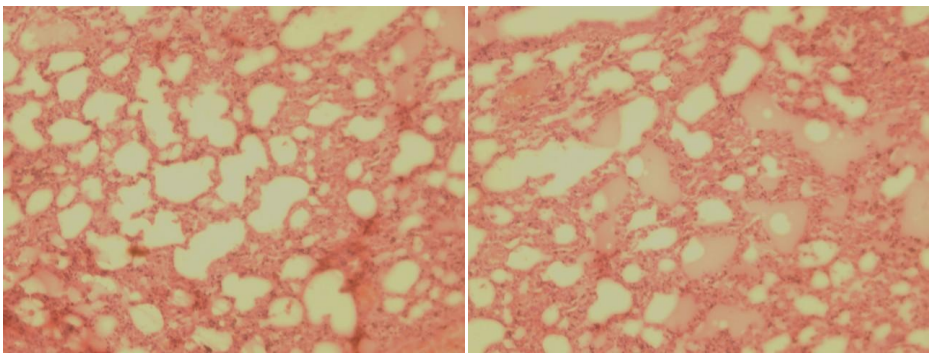
Effect of biocoil fuming on spleen of embryo

I II



I-Control group, II-Fumed with biocoil

Effect of biocoil fuming on lungs of embryo



I-Control group, II-Fumed with biocoil

No toxicity was induced to any organs by the biomosquito coils made from 8,11,14-eicosatrienoic acid which is extracted from *G.Sepium.*

5. DISCUSSION

In the fuming study against adult mosquitoes 8,11,14-eicosatrienoic acid exhibited high knock-down values KD_{50} at 41.80 min (37.99 - 45.11 with 95% C.L) and KD_{90} at 94.67min (84.64-110.69 with 95% C.L). Murugan *et al*., 2007 reported that smoke from *Albizza amara* is an effective repellent against *Aedes aegypti*. Singha *et al*., 2011, reported that 94% of *C.quinquefasciatus* mosquitoes were dropped down after 40 min coil fuming and there was 91% death, when *Mesua ferra* crude extract was used. Zaridha *et al*., 2006 assessed knock down values of plants after 2 min fuming and *Azadirachta* came first with KD_{50} value as 40.25 min and KD_{90} as 67.57min. Herbal products with proven potential as insecticide or repellent can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level (ICMR, 2003).

Today, the environmental safety of an insecticide is considered to be of paramount importance. Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily available throughout the world. In addition, the use of medicinal plants for mosquito control is likely to generate local employment, reduce dependence on expensive imported products, and stimulate efforts to enhance public health.

In the field of toxicological studies, unheeded inadequacy in ascertaining the physiology of side effects like thrust on the target tissues should be immediately reckoned and redeemed. This point towards the urgent need of programming strategies to avert side effects and to ensure protection against the hazards on health and environment, prior to its launching to the open market for the use of common man. The hazards caused by such synthetic materials though not explicit in adults are really untold in infants and babies.

Studies conducted on rats in the prenatal period revealed that there was no significant variation in any parameters tested, due to fuming of biocoils. According to Whyatt, *et al*., 2003 all women tested had detectable levels of at least three insecticides in personal air samples collected over 48 hr during the third trimester and also the insecticides were detected in blood samples collected from the mothers and newborns at delivery. Perera *et al*., 2003 reported an inverse association between chlorpyrifos levels in umbilical cord plasma and, birth weight and length. But in this study, using natural phytochemical, rat pups did not show any symptoms of toxicity as evidenced by haematological, biochemical and histopathological parameters. Since any report of this type of experiment is not available, no comparison could be made.

6. CONCLUSION

The approaches to isolate commercially phytochemicals provide important evidence for the potentiality of botanicals in public health integrated management and hence the plant can be used to control mosquito borne diseases like Malaria, Filariasis, and Chikungunea etc.

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