

REPELLENCY OF LANTANA CAMARA LEAVES SMOKE AGAINST FEMALE ANOPHELES MOSQUITOES

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Abstract: The study was done to investigate the Mosquito repellency properties of the smoke of *Lantana camara* in different solvents viz; methanol, ethyl acetate, ethanol and diethyl ether. Plant samples were dried, burned and extracted using various solvents. The extracts were concentrated using a Rota vapor and various concentrations ranging from 50ppm-1000ppm of the repellents were prepared and evaluated. Methanol extract at 400ppm concentration significantly gave the highest protection against the bites of female Anopheles mosquito (P> 0.01) for over 300 minutes. This extract yielded significantly the better protection time (P> 0.05). The methanol extract seemed to have a steady repellency over a longer period than the rest of the extracts. The results from this study shows that the plant *Lantana camara* can be used effectively to repel Anopheles mosquitoes, hence a great achievement in the development of safe organic repellants to control transmission of malaria by female anopheles mosquitoes. However, more research needs to be done to identify the specific compounds which are repelling the mosquitoes their structures and also mode of action.

Keywords: Anopheles mosquito, Repellants, Lantana camara, Smoke, Leaves.

INTRODUCTION

Lantana camara is a weed which is low erected or subsequent, vigorous shrub with scout recurved prickles and a strong black currents odour ^[1]. The plant grows to height of 1-2 m and also occur luxuriously in elevation of 2000 m in both sub-tropical and tropical regions. The plant has been named as one of the most important medicinal plants all over the world ^[2].

Female Anopheles mosquitoes are a key concern in sub-Saharan Africa. As a vector, Anopheles mosquitoes are responsible for the transmission of malaria from one individual to the other. It is, therefore, very important to limit the vector-man contact, if the battle against malaria has to be won. An important aspect of malaria prevention is mosquito repellency. Although synthetic chemicals and insecticides have been used to control these vectors, they have triggered degenerate irremediable damage to the eco-system since many of them are non-degradable ^[3].

Malaria remains a problem all over the world despite adverse struggle to eliminate it from the globe. Malaria is one of the six killer diseases in the world today with an estimation of about two million children and pregnant women dying from the disease every year^[4]. Malaria is mainly transmitted by female *Anopheles* mosquitoes. Several measures have been put forward to combat the vectors but with little success due to the mosquito resistance to the drugs used in fighting them^[5]. According to Odetola^[6], it has been evident that some plant compounds can be used

as mosquito repellents against variety of Anopheles and Culex species. Mosquitoes are insects which men would wish to live without especially when they carry fatal microbes in them such as *Plasmodium falciparum* which cause malaria.

A lot of data has been provided on the harmful effects of repellents used against mosquitoes. A few of these include; Synthetic pyrethroids e.g. allethrin whose main means of action is the sodium channel, cause sub-normal or super-normal excitability by affecting the sodium channel opening time. As reported by Liu^[7], an exposure of rats to the mosquito coil smoke for 60 days results in focal deciliation of the tracheal epithelium, metaplasia of epithelial cells and morphological alterations of the alveolar macrophages. Ahlbom et al.,^[8] reported changes in the density of muscarinic acetylcholine receptors (MAChRs) in cerebral cortex of mice treated neonatally with DDT, who later as adults received bioallethrin, causing the irreversible MAChR changes and behavioral disturbances.

Some of the effects of synthetic repellents include skin and eye irritation, unpleasant smell which may lead to suffocation and induction of some kinds of allergy. Others remain lethal to other life form for a very long period of time. Chemicals like N, N-diethyl mendelic acid amide (DEM), N-N-diethyl-m-toluamide (DEET) and dimethyl phthalate (DMP) among others have been used as vector repellents and have shown remarkable

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Faculty of Chemistry, University of Eastern Africa, Baraton, P.O Box. 2500 – 30100 Eldoret, Kenya. toxicity and hence considered not safe for use^[9]. Despite much efforts to prevent mosquito bits on human and also transmission of diseases by the organisms, mosquitoes still transmit diseases to over 700 million people annually and are responsible for the death of approximately one person out of the seventeen people currently alive^[10].

The first plant based mosquito repellent was invented in 1990 in a research done at the University of Florida^[11]. Natural products are considered most appropriate for use by man as they are renewable, highly biodegradable and pose no toxicity to life. It is, therefore, highly necessary to unveil an extensive exploration to uncover the much vital eco-friendly biological repellents for control of mosquitoes^[11]. Plant derived phytochemicals act as insect repellents antibiotics, larvicides, insect growth regulators, and ovipositional attractants among other activities. Traditionally phytochemicals have been used to repel and kill the mosquitoes in many parts of the world. One such plant which has shown remarkable mosquito repellency is Lantana camara. The flowers of Lantana camara (Vervanaceae) repellency against Aedes mosquitoes was reported by Dua *et al.*,^[12]. According to Dua et al., Lantana camara flowers extract in coconut oil has shown repellency effect against Aedes mosquitoes. This extract provided 94.5% protection from Aedes albopictus and Aedes. Aegypti with a mean protection time of 1.9 hours. In the current report, the repellency of Lantana camara leaves' smoke extract in the form of cream was evaluated against female Anopheles mosquitoes and reported.

Lantana camara has been used as tonic and stimulant in folk medicine ^[13 & 14]. The plant has also been investigated for anti- insect properties against various insects ^[15]. *Lantana camara* insecticidal activity, antioviposition and growth regulating effects against various insects have been reported ^[16 & 17].

MATERIALS AND METHODS

Mosquito rearing:

The eggs of the mosquito were obtained from International Centre for Insect Physiology and Ecology (ICIPE). The eggs were then put in trough containing distilled and deionized water free from exposure of insecticides and pathogens and incubated at a temperature range of 37 - 41°C until they hatched in to the first instar. They were allowed to grow to maturity in a cage. The larvae were fed with larvae food which is powdered dog biscuits and yeast in the ratio 3:1 and adult mosquito fed on 10% glucose solution. The adult mosquitoes were separated into female and male mosquitoes based on their physical and behavioral characteristics. The female *Anopheles* mosquitoes were then used for the study.

Sample Collection and Preparation:

The herb was randomly collected in the natural forest around University of Eastern Africa, Baraton. The plant samples were collected and identified by a taxonomist in the Biology Department, Baraton University. The samples were thoroughly mixed and spread to dry at room temperature for about three weeks. They were then ground into fine powder and put in transparent polyethene bags.

Extracts preparation:

The smoke used as mosquito repellents were obtained by burning dried leaves of *Lantana camara* (*Verbenaceae*). This smoke was dissolved in various solvents viz; methanol, ethyl acetate, ethanol and diethyl ether by allowing it to slowly bubble through. The extracts were later concentrated and several concentrations ranging from 50ppm -1000ppm made from stock solution of 1000ppm in pure petroleum jelly.

Plants extract repellency analysis:

Repellency trials were conducted in a test chamber $(30 \times 30 \times 62.5 \text{ cm})$ *in vitro*. Three concentrations of 50ppm, 100ppm and 500ppm of the test jelly were serially evaluated on the hands of a human subject. The hands were thoroughly cleaned by soapy water, rinsed, wiped by methylated spirit, dried before applying the test jelly each time before exposure. The jelly was applied on dorsal side of one hand between the wrist and finger tips.

The hand was inserted in a cage containing 100 unfed female anopheles mosquitoes (3-days old) for 10 minutes in dark. The hole of the cage was carefully plugged with a cloth to prevent escape of mosquitoes. Female Anopheles mosquito progenies were obtained from laboratory colony maintained on honey solution in a cloth cage (50 x 50 x 50 cm) under controlled temperature ($28 \pm 2^{\circ}$ C) and relative humidity range (78 $\pm 2^{\circ}$). The tests were repeated at every 30 min interval using a different set of unfed female Anopheles mosquitoes in a different cage. The tests were replicated eight times for each concentration of the test jelly.

Control was set by placing a hand inside the test cage. Only pure petroleum jelly was applied to this hand. The test jelly which had the highest repellency was subjected to further test by serially exposing a given concentration to several test cages in order to determine the protection of the said test jelly. After each test, the number of fed and unfed female *Anopheles* mosquitoes were counted and recorded. This data was later subjected to Randomized Block Design test (RBD).

RESULTS AND DISCUSSION

It was observed that out of the four herbal repellents, the methanol extract exhibited better protection against the bites of female *Anopheles* mosquitoes.



Fig. 1: Effect of different extracts on the feeding habit of female *Anopheles* mosquito.

On analyses, methanol, ethanol and acetone extracts of *L. camara* were found more effective and afforded significantly the better protection time than the diethyl ether extract at 400ppm concentration petroleum jelly. Methanol extract at 400ppm concentration significantly gave the highest protection against the bites of female *Anopheles* mosquito (> 0.01) for over 300 minutes. This extract yielded significantly the better protection time (> 0.05) (fig.2).



Fig. 2: Comparative minimum protection time of various extracts against the unfed female *Anopheles* mosquito.

The fact that *Lantana camara* leaves' smoke extract obtained from different solvents showed different level of repellency against female anopheles mosquitoes is evidence that the chemical compositions and essential oils obtained from *Lantana camara* were quite different. This is an advocate for the fact that a number of different plant volatiles are robust insect repellents. Aforementioned studies have proved that many plant secondary metabolites, especially those that are volatile under steam distillation conditions possess remarkable repellent effects against different insects, mosquitoes included^[21].

The results obtained in this research are inconformity with previously obtained data in which according to Ogendo^[18], the plant showed significance repellency potential against Sitophillus zeamais (Coleoptera: Corculionidae) in stored maize grains with 87.5% of the insects being repelled within a period of 21days and insect mortality of 82.7 - 90% observed. The repellency activity of the plant could be attributed to the important phytochemicals found in the plant, which the plant uses them for protection against its enemies. According to Sharma et al.,^[3], viz. steroids, saponins, tannins, triterpenoids, alkaloids and flavonoids were found to be present in the plant, this is also in conformity with results obtained by Patel et al.,^[19] and Naz et al.,^[20].

CONCLUSION

This study reveals that high temperature does not completely render *Lantana camara* ineffective. *Lantana camara* methanol smoke extract can either be used singly or in combinations with other mosquito repellents for the preparation of another potent mosquito repellent product. These could be in form of spray, cream, candle and sticks or any other applicable form, prepared using suitable carriers to get better protection from mosquito bites. Such formulations could reduce the harmful effects on human health ranging from breathing problems, eye irritation, headache, bronchial irritation and so on of synthetic mosquito repellents.

Further studies on isolation and characterization of active compounds, their toxicity and field trials are necessary in order to recommend the active fraction(s) of *Lantana camara* leaves' smoke extract for development into a green, eco-friendly repellent against haematophagous insects like mosquitoes.

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