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LABORATORY EVALUATION ON THE REPELLENCY OF SELECTED PLANT PRODUCTS IMPREGNATED IN INCENSE STICKS AGAINST THE BITING POPULATION OF *AEDES AEGYPTI* LINN.(DIPTERA: CILICIDAE) MOSQUITOES

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ABSTRACT

The repellent effect of smoke of incense sticks impregnated with herbal products of Cinnamon -*Cinnamoum verum* (Family: Lauraceae), cloves *Syzygium aromaticum* (Family Myrtaceae), black pepper *Pepper nigrum* (Family: Piperaceae), black cumin *Cuminum cyminum* (Family: Apiaeceae) was studied against the biting population of *Aedes aegypti* under laboratory conditions. Starved adult females of *Aedes aegypti* were used in mosquito cages to assess their biting behavious in laboratory conditions. The percentage protection was calculated and comparison was made with controls. While comparing the protective effect of smoke of incense sticks in experimental and control, possible adverse effect was also observed. The percentage protection in the control chamber was 0.00 and in the experimental trails it ranged from 94.76 to 100.00. Ingredients caused by control incense sticks, which were devoid of herbal products did not exert protection effect for the starved females of *Aedes aegypti*. The study revealed the protective effect of smoke for a period of 1 hour. Results of the study indicates the repellent effect of smoke of the admixture of herbal plant products and ingredients of incense sticks However, long range effect of smoke of incense sticks needs to be explored.

INTRODUCTION

Botanical insecticides are considered as alternatives to hazardous chemical for the control of pest and vectors. Insect repellent formulations consisting of oil of citronella, spirits of camphor, oil of tar, oil of pennyroyal and castor oil have been shown to provide long-lasting protection against insects [1-3]. Penfold and Morrison [4] (1952) reported the effectiveness of forty essential oils against mosquitoes. The utility of these plants to repel mosquitoes needs special consideration due to the development of hazardous nature of synthetic insecticides and its concern over pollution. Repellency is a characteristic of personal protection tools such as mosquito coils, liquid vaporizers, vaporizer mats and ambient emenators [5] (WHO 2009) Spatial action of smoke, mosquito coils and vaporizer mats offer protection against mosquito bites through emanated vapour or airborne particles [6]. Using smoke inside and outside houses as a personal protection measure against mosquito bites had been in use in many countries for a long time and little work had been on the efficacy of smoke in reducing man-mosquito/vector contact [7]. Cheaper method of achieving personal protection in remote areas using herbal plant products is a felt need in rural communities. The present

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study is aimed to assess the possibility of repellency of incense sticks prepared with Cinnamon -*Cinnamoum verum* (Family: Lauraceae), cloves *Syzygium aromaticum* (Family Myrtaceae), black pepper *Pepper nigrum* (Family: Piperaceae) and black cumin *Cuminum cyminum* (Family: Apiaeceae) against the biting population of *Aedes aegypti* in laboratory conditions and there is a need for safe, effective nature or synthetic – product based repellents to reduce the nuisance of blood sucking arthropods such as mosquitoes [8].

MATERIALS AND METHODS

The mosquito species selected for the study was Aedes aegypti which is a known vector for dengue and chikunguinea. The trials was carried out in laboratory cages, net cage (45 X 30 X 45 cm2) and adult mosquitoes were collected from field and the emerged female mosquitoes was given sucrose solution for 3 days. These 3 days starved adult mosquitoes were used in the experiment.

Preparation of incense sticks:

The detailed account of ingredients in the incense sticks are presented below:

Camphor: Camphor is a waxy, white or transparent solid with a strong aromatic odour. It is found in the wood of camphor laurel (*Cinnamoumm camphora*).

Aromatic resin: This is locally called as jiggath This is obtained from the trees of the genus *Boswellia*. This resin is commonly used in agarbathis, dhoop etc.,

Cinnamon (*Cinnamoum verum* (Family: Lauraceae): Cinnamon bark is a spice obtained from the inner bark of several trees from the genus *Cinnamomum* that is used in both sweet and savoury foods. Cinnamaldehyde is the bioactive chemical component of this plant is known for its repellent and insecticidal activity [9].

Cloves (*Syzygium aromaticum* (Family Myrtaceae) : Cloves are the aromatic flower buds of a tree *Syzygium aromaticu* and a major component of clove bud is imparted by the chemical eugenol and this plant is also known for its insecticidal property [10].

Pepper: Black pepper (*Piper nigrum* (Family: Piperaceae) is a flowering vine in the family Piperaceae, cultivated for its fruit, which is usually dried and used as a spice and seasoning. The phytochemicals in the seeds contain amides, piperidines, pyrrolidines and trace amounts of safrole which are known or their insecticidal effects [11]. The black pepper corns were used for study.

Black cumin (*Nigella sativa* (Family: Apiaeceae): Locally known as *karunjeeragam*, the seeds of this plant contain thymoquinone and is utilized locally for medicinal purposes. The seeds of black seeds were procured from a local market and the seeds of this plant is also known for its repellency against anopheline mosquitoes [12].

600 gm of the above materials in equal amount are taken in mortar and mixed thoroughly with a pestle and made into a fine powder. For incense stick making charcoal (200 gm), jiggath – a resin binding material (200gms) and sawdust (200gm) were used. All the material were dried under shade mixed and ground and incense sticks were made using mould which are used traditionally.

Assessment of Bite protection/ repellency:

Tests were carried out in mosquito cages where 50 nos of the starved unfed female mosquitoes were kept for 15 minutes initially towards acclimatization. The hands of person were cleaned with ethanol and then air dried prior to introduction into mosquito net cages. Incense sticks smoke was kept in the mosquito net cage in which 50 starved adult females *Aedes aegypt* were also kept. A control cage was also maintained where incense sticks made with charcoal sawdust and jiggath without any plant products were used. Incense blocks were lit and kept in the cages and visually smoke emission was verified as they get filled inside the cage. Bare hand was introduced into the control and experimental cages simultaneously. The dorsal side of the hand was used to exposure to landing/probing mosquitoes. Mosquitoes probing to bite/land were observed and recorded. The trials was carried out in three times and data were on mosquito landing on human hand were recorded. Total duration of smoke emission from incense sticks was observed for a period of 1 hour. It was assured that there was no skin irritation due to smoke exposure and also nasal and eye irritation.

The percentage protection as described by Karunamoorthy et al (2010) [13] was calculated using the following formula:

% of protection =
Number of bites/landing observed in the control arm - Number of bites/landing observed in the experimental arm ÷ Number of bites/landing observed in the control arm* 100

The guidelines recommended by WHO to assess the efficacy of repllency og mosquito repllents or human skin was adopted[5]. As the starved mosquitoes in the control chamber started feeding and the readiness of mosquitoes for feeding was observed, hands were shaken off and to rid of mosquitoes and the number of mosquitoes landing on skin surface was recorded. In the control chamber however, the bare hand was kept continuously allowing exposure to both smoke and starved mosquitoes. The number of mosquitoes landed on human skin at the interval of 30 minutes are recorded and the experiment was continued for 1.5 hours and the data was collected

RESULTS AND DISCUSSION

The tested proposition of incense sticks with herbal ingredients and incense sticks with ingredients of incense sticks alone are presented below (Table.1):

Data collected are presented in Table 2, & 3 for the each replicate. Number of mosquitoes probing to bite

or land on human hand were so within few minutes after introduction and the control cage when hand was introduced, all the starved females mosquitoes landed on the skin immediately and a quick count was made and recorded. Since keeping the hand in the control cage might allow feeding of starved mosquitoes, hand was taken out of the cage and then introduced at 30 minutes interval time. In the experimental cage after introduction of bare hand, though mosquitoes land on skin and did not attempt to bite, the hand was exposed in the presence of smoke for one half an hour period and the observations at 0.5 hour segement was repeated. Percentage protection by the smoke of incense sticks having plant products is shown in Fig 2.

Table 2 and 3 clearly indicates the repellent effect of smoke of the admixture of herbal plant products and ingredients of incense sticks. The number of mosquitoes probing to bite or land on human bare hands is an indication of feeding habits of starved adult female mosquitoes of *Aedes aegypt* despite presence of smoke in mosquito cages. The probable reason for this seems to be in lesser denser smoke areas undernourished/starved mosquitoes seem to exert an effort to feeding. In the experimental cages, however the smokes of plants products and ingredients of incense sticks caused a negative effect for feeding. Repellent efficacy and % of protection ranged from 96.74% to 100.00 among the different trials. In the control setup, Ingredients of incense sticks alone did not offer protection and it can be inferred that despite the release of plain smoke cannot be expected for repellency or bite protection. However, in the experimental trials a considerable protection against the bite of *Aedes aegypti* was notices during the onset of experiment, at the time of 30 minutes and 60 minutes interval (Fig.2).

However in the present study protection at different concentrations was not attempted. The incense stics in the experimental trial had 50% of total mass with remaining portion by ingredients of incense sticks. It can be expected that a continued observation for more than 6 hours may also yiled the same protection. Repellent efficacy and % of protection ranged from 96.74% to 100.00 among the different trials. In the control setup, Ingredients of incense sticks alone did not offer protection and it can be inferred that despite the release of plain smoke cannot be expected for repellency or bite protection. However, in the experimental trials a considerable protection against the bite of *Aedes aegypti* was noticed during the onset of experiment, at the time of 30 minutes and 60 minutes interval (Fig.2). However in the present study protection at different concentrations was not attempted. The incense stics in the experimental trial had 50% of total mass with remaining portion by ingredients of incense sticks. It can be expected that a continued observation for more than 6 hours may also yiled the same protection. Even though no attempts were made in the present study to measure the density of smoke, the mere presence of smoke of plants of herbal natures confirms its repellency effect.

Figure 1. No. of mosquitoes recorded in different times of observation

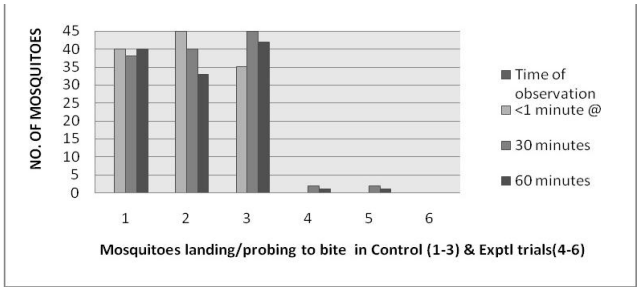

Figure 2. % bite protection observed during different time intervals in exptl trials

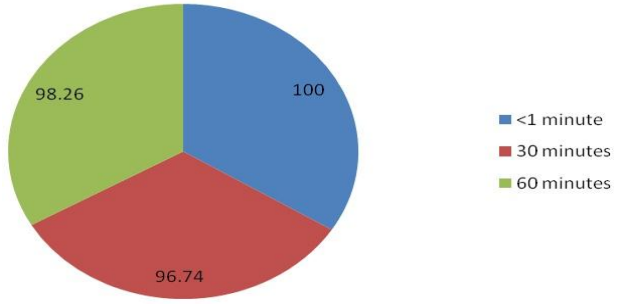

Table 1. Composition of incense sticks

Incense sticks	Camphor	Aromatic resin	cinnamon	Cloves	pepper	Black cumin	Charcoal	jiggath	Saw dust
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.33	0.33
Test	0.083	0.083	0.083	0.083	0.083	0.083	0.167	0.167	0.167

Table 2. Number of mosquitoes probed for biting/landing in the control and experimental chamber

Time of observation	Control	Test	% protection
<1 minute	40	0	100.00
30 minutes	38	2	94.74
60 minutes	40	1	97.5

Table 3. Number of mosquitoes probed for biting/landing, in the control and experimental chamber

Time of observation	Control	Test	% protection
<1 minute [@]	45	0	100.00
30 minutes	40	2	95.00
60 minutes	33	1	96.96

CONCLUSION

The study highlight repellence effects of herbal products against day time (host seeking and biting) behavior of *Aedes aegypti* the vector mosquitoes of dengue and chikungunya. This study is a first hand approach which can be utilized to formulate guidelines for specifying the timing of adulticidal measures such as thermal fogging in open spaces and outdoor habitations using herbal products. Newer formulation with indepth studies can enable to devise new products with low manufacturing costs.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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