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Studies on efficacy of two botanical repellents, *Zanthoxylum rhetsa* and *Amorphophalus sylvaticus* against *Culex quinquefasciatus*

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Abstract
A laboratory study was conducted to evaluate the repellent activity of *Zanthoxylum rhetsa* and *Amorphophalus sylvaticus* against the adult mosquitoes of *culex quinquefasciatus*. The ethanolic fruit extract of *Zanthoxylum rhetsa* at 100, 200, 300, 400 and 500 ppm offered 26, 46.8, 77, 90.9 and 100 percentage protection against mosquito bite during the time 5-6 p.m. The percentage protection against the mosquito bite was 29, 48, 74, 91 and 100 at 100, 200, 300, 400 and 500 ppm when treated with corm extract of *Amorphophalus sylvaticus*. Both of the results showed maximum percentage of protection in higher concentration (500 ppm) and minimum percentage of protection in lower concentration (100 ppm) during the time 5-6 pm, 6-7 pm, 7-8 pm and 8-9 pm respectively. After the smoke exposure of *Zanthoxylum rhetsa* coil the adult mortality of *Culex mosquitoes* was 54.4% where as in negative control (without plant ingredients) it was 0% and in the case of positive control (synthetic coil) it was 50.80%. In the case of *Amorphophalus sylvaticus* coil the adult mortality noticed after smoke exposure was 57.6% where as in negative control (without plant ingredients) it is 0% and in the case of positive control (synthetic coil) it was 50.8%. From the above results it is clear that both *Zanthoxylum rhetsa* and *Amorphophalus sylvaticus* possess good smoke toxic effect and they can be used as effective repellents against mosquitoes.

Keywords: *Zanthoxylum rhetsa*, *Amorphophalus sylvaticus*, *Culex quinquefasciatus*, percentage of protection, repellent activity

Introduction
Mosquitoes are of great importance due to the transmission of various diseases like pathogens, species of virus, nematodes and protozoa; they pose a great health threat till today. Mosquitoes act as a vector for-fatal human diseases like Malaria, filariasis, dengue, yellow fever, encephalitis and various viral infections in all around the world [6]. *Culex quinquefasciatus* is one of the most annoying vectors which transmit lymphatic filariasis and Japanese encephalitis in India [5]. It is the potential vector of Bancroftian filariasis is widely distributed in southeast regions such as Kerala, Mysore, Tamil Nadu, Andhra Pradesh and Maharashtra with around 120 million people infected worldwide and 44 million people having common chronic manifestation [3]. *Culex pipiens* are common house mosquito which transmits several infections for example West Nile fever, Sindbis fever, lymphatic filariasis, and Japanese encephalitis [4]. Female *Cx. pipiens* normally feed on the blood of vertebrae including human beings and birds and hence transmits infectious diseases from birds to humans and from humans to humans. Eggs are laid in clumps on the surface of stagnant water bodies, such as pools, ditches, water tanks, and vases [7].
The use of insecticides and repellents represent major strategies for preventing mosquito-borne diseases [29]. Plants like *O. gratissimum, Clausena dentate, Eclipta prostrate, Tagetes erecta* have been reported to possess strong repellent activity against mosquitoes [11, 22].
The use of repellents to protect people from bites of mosquitoes has been acknowledged as part of an overall integrated insect-borne disease control programme [19]. Most commercial repellents are produced by using chemical components such as N, N-diethyl-metatoluamide (DEET), Allethrin, N, N-diethyl mendelic acid amide, and Dimethyl phthalate [20]. It has been reported that these chemical repellents are not safe for public health and should be used with caution.

Prolonged and frequent use of synthetic repellents in mosquito control has resulted to development of resistance to insecticides, resurgence in mosquito populations, and adverse impact on non-target organisms [29, 13]. Accordingly, the idea of using natural mosquito repellent products as an alternative to develop new eco-friendly repellents could be an amicable solution to reverse the undesirable effects on environment and human health [12]. By contrast, plant-derived repellents and insecticides, especially plant essential oils, have shown significant advantages such as rapid biodegradability, eco-friendliness, and safety for insect and mosquito control [1, 23, 30]. Therefore, it is essential to identify new plant-derived repellents and insecticides for mosquito control. So the present study was conducted to evaluate the efficacy of repellency of *Zanthoxylum rhetsa* and *Amorphophalus sylvaticus* against the adults of *culex quinquefasciatus* to avoid the adverse effect of synthetic repellents.

2. Materials and Methods

2.1 Culture of *Culex quinquefasciatus*

To satisfy the number of mosquitoes needed for the day to day bioassays a colony was essential. The eggs of *Culex quinquefasciatus* were collected from standardized colony at National Institute of Communicable Disease (NICD) Mettupalayam in Tamil Nadu, India. The eggs were placed separately in sterilized glass containers with unchlorinated tap water, (Size 18cm diameter and 9cm height) in laboratory conditions. After 24 hrs freshly hatched larvae were collected and maintained in separate containers with tap water (capacity 2 litres) glucose biscuit and yeast (2:1) were given as the source of food. Water was changed on alternate days. The culture medium was regularly checked and the culture troughs were kept closed with mosquito net for preventing interference of foreign mosquitoes. The pupae were isolated from the culture troughs and were allowed to emerge into adults in the mosquito net cage (42 cm x 30cm x 30 cm).The freshly emerged adults were maintained at 27±2˚C, 75-85% RH, under 14 L: 10 D photo period cycles. Emerged adult males were fed with 10% sucrose solution soacked in cotton wick and female mosquitoes were fed with pigeon blood as a source of food as detailed by Meola and Readio [15]. Different batches of adults were maintained in the cage by introducing sufficient number of pupae. An oviposition trap was kept in cage to facilitate the female to lay the eggs. The egg rafts of *Culex quinquefasciatus* in the container were removed carefully and allowed to hatch. The incubation period of the normal eggs were 48 hours.

2.2 Collection and identification of experimental plants.

Two plants, a tree and a herb were collected from the Southern Western Ghats spanning Tamilnadu and Kerala (India) for the present study as the source plants for larvicidal and mosquitocidal compounds. The plants are identified as *Zanthoxylum rhetsa* (Roxb.) DC. and *Amorphophalus sylvaticus* (Roxb.) Kunth. By the competent authority at Botanical Survey of India, Southern circle, Coimbatore, with a voucher specimen and a certificate is issued for the same. The plants are selected based on the available literature of medicinal and insecticidal properties and abundant availability [14].

2.3 Preparation of solvent extract

The dried fruit with seed of *Zanthoxylum rhetsa* (Roxb.) DC. and corm of *Amorphophalus sylvaticus* are the parts selected in the respective extract preparation for the bioassays. Plant parts collected from southern Western Ghats are brought to the laboratory, washed with dechlorinated water, shade dried under room temperature (27±2˚C) for about 15 days. The completely dried fruits and tubers were blended separately with electrical blender and sieved to get fine powder. The powders were stored separately in air tight containers for further analysis. One kilogram of each powdered plant material was extracted with ethyl alcohol for a period of 72 hrs each and then filtered. (Cold perculation method[21]). The filtered content was then subjected to rotary vacuum evaporator until solvents completely evaporated to get the solidified crude extracts. The crude extracts thus obtained was stored in sterilized amber coloured bottles and maintained at 4 ˚C in a refrigerator for further experiments. Standard 1% stock solution (1000 ppm) was prepared by dissolving 100 mg of crude extract in 100 ml of distilled water.

2.4 Repellent bioassay

The repellent activity of the *Zanthoxylum rhetsa* and *Amorphophalus sylvaticus* were determined by using the techniques of Fradin and Day [8] with modification. Three to four days old blood starved 50 adult females of *Culex quinquefasciatus* were kept in different net cage (42 x 30 x 30 cm^3). The arms of the test person were cleaned with isopropanol. After air drying the arm of the test person, only 25 cm^2 dorsal side of the skin on each arm was exposed and the remaining area was covered with rubber gloves. The plant extracts were dissolved in ethanol which served as control. The exposed area was treated with plant extracts of varying concentrations
i.e. 100,200,300,400 and 500 ppm. The treated and controlled arms were interchanged regularly to eliminate bites and each test concentration was repeated five times. Volunteers were made to involve in the test of each concentration by inserting the treated and control arms alternatively into the same cage for one full minute for every fifteen minutes, until the first bite occurred or until the landing of two mosquitoes. It was observed that there was no skin irritation from the plant extracts. The percentage repellency was calculated by using the following formula.

$$\text{Percentage of repellency} = \frac{C - T}{C} \times 100$$

Where,
C is the number of bites received in control arm
T is the number of bites received in treated arm

2.5 Smoke toxicity test

Ethanolic fruit and corm extracts of *Zanthoxylum rhetsa* and *Amorphophalus sylvaticus* respectively *were* used for smoke toxicity assay. The mosquito coil was prepared by following the method of Saini *et al.* [24] with suitable modifications.
One gram of active ingredient, two grams of saw-dust as binding material and two grams of coconut shell charcoal powder a burning material was used in the preparation of the coil. All the three sample sources were thoroughly mixed with distilled water and a semisolid paste was prepared. Mosquito coils (0.6 cm thickness) were prepared manually from the semisolid paste and were shade dried. The negative control

(without plant ingredient) and positive control (Synthetic coil -Bramos mosquito coil) was used to compare the effectiveness of plant coils.

The experiments were conducted in a room (10×10m). A mosquito cage containing three days old blood starved hundred adult mosquitoes fed with sucrose solution was kept in to the condition chamber. A belly shaven pigeon was kept tied inside the cage in immobilized condition. The experimental chamber was tightly closed.

The experiment was repeated five times on five separate days including control using mosquitoes of same age groups.

After the experiment was over, the fed and unfed (alive and dead) mosquitoes were counted. The protection given by the smoke from plant samples against the biting of *Culex quinquefasciatus* was calculated in terms of percentage of unfed mosquitoes due to treatment [26].

$$\frac{\text{No. of unfed mosquitoes in treatment} - \text{No. of unfed mosquitoes in control}}{\text{Number of mosquitoes treated}} \times 100$$

3. Results and Discussion

3.1 Studies on repellency

The repellent activity of ethanolic fruit extract of *Zanthoxylum rhetsa* and *Amorphophalus sylvaticus* were tested against *Culex quinquefasciatus* and is represented in table 1 and 2. In the case of *Zanthoxylum rhetsa* the percentage of protection increases with the increase in concentration. The maximum percentage of protection was seen in higher concentration (500 ppm) and minimum percentage of protection was observed in lower concentration (100 ppm) in 5-6 pm, 6-7 pm, 7-8 pm and 8-9 pm respectively. Similarly in the case of ethanolic corm extract of *Amorphophalus sylvaticus* tested against *Culex quinquefasciatus* the percentage of protection during 100 ppm is 29%, 200 ppm it is 48%, 300 ppm it is 74%, 400 ppm it is 91% and at 500 ppm 100% protection attained during the time of 5-6 pm. Similar results are obtained in all the experiments. Here also the percentage of protection increased when the concentration of the corm extract increased (Table.2).

Table 1: Repellent potential of ethanolic fruit extract of *Zanthoxylum rhetsa* on *Culex quinquefasciatus*

Repellent activity observed time (Time)	% of protection					
	Control	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm
5.00-6.00 pm	0	26	46.8	77	90.9	100
6.00-7.00 pm	0	34.6	48	62	84	99
7.00-8.00 pm	0	33	49	64	85	100
8.00-9.00 pm	0	27	42	74	89	100

*The mean difference is significant at 0.001

Table 2: Repellent potential of ethanolic corm extract of *Amorphophalus sylvaticus* on *Culex quinquefasciatus*

Repellent activity observed time (Time)	% of protection					
	Control	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm
5.00-6.00 pm	0	29	48	74	91	100
6.00-7.00 pm	0	33	47	61	83	99
7.00-8.00 pm	0	31	43	66	82	99
8.00-9.00 pm	0	29	39	71	87	100

*The mean difference is significant at 0.001

From the present study it is clear that both *Zanthoxylum rhetsa* and *Amorphophalus sylvaticus* possess good smoke toxic effect on *Aedes aegypti* and *Culex quinquefasciatus.* The findings agree with some of the previous reports.The results of repellent activities in the present study showed that percentage protection is dose dependent. In *Culex quinquefasicatus* the maximum percentage of protection (100%) attained during 500 ppm in both *Zanthoxylum rhetsa* and *Amorphophalus sylvaticus* and the minimum percentage of protection attained in lower concentration in all the time intervals. So there noticed an increase in the mean number of bites at that time. From this it is clear that percentage of protection was dose dependent. When the percentage of extract increases the percentage of protection also increases.

Mullai and Jebanesan [17] observed the repellency activity of methanol, hexane, petroleum ether and ethyl acetate leaf extract of *Citrullus colocynthis* and *Cucurbiata maxima* against adult mosquitoes of *Culex quinquefasciatus.* In *Citrullus colocynthis* the maximum protection was observed in methanolic extract (271 min) and minimum protection was observed in ethyl acetate extract (214 min) at 5mg/cm^2. Ahmad *et al.* [2] also reported that *Anopheles Culicifacies* and *Culex quinquefasciatus* mosquitoes were unable to bite the protected person within 4 hour after his or her application of neem products which were safe and better than any other repellents without adverse reactions. The repellency percent at different observation periods (0, 1, 2, 4 and 6 hours) ranged from 80% to 100% at different concentrations of *Cyperus rotundus* extract against *Anopheles culicifacies*, *Anopheles stephensi* and *Culex quinquefasciatus*, suggesting that the extract might be applied as an effective personal protective measure against mosquito bites [9].

The results obtained in these studies has supported for the present study.

In the present study the ethanolic fruit extract of *Zanthoxylum rhetsa* and corm extract of *Amorphophalus sylvaticus* did not cause any such discomfort or skin irritation to the volunteers. Moreover this kind of plant derived product does not cause any ill effect to other beneficial organisms. Similar results have been recorded in essential oils and extracts of six plants namely *Melissa officinalis, Rosmarinus officinalis, Lavandula officinalis, Citrus limonum, Eucalyptus globules and Ocimum basilicum* against *Anopheles stephensi* [16]. The findings of the present fruit and corm extracts of the experimental plants exhibited excellent repelling action against *Culex quinquefasciatus* and can be used it over the awe of mosquito borne diseases.

3.2 Studies on smoke toxicity

In the present study, the smoke toxic effect of fruit extract of *Zanthoxylum rhetsa* and *Amorphophalus sylvaticus* was evaluated against the adult mosquitoes of *Culex quinquefasciatus*. The smoke toxic effect of negative control (without plant ingredients) and positive control (synthetic coil) was also performed along with the test samples (Table.3 and 4). In *Culex quinquefasciatus* the adult mortality noticed after smoke exposure of *Zanthoxylum rhetsa* coil was 54.4% where as in negative control (without plant ingredients) it was 0% and in the case of positive control (synthetic coil) it was 50.80% (Table.3). In the case of *Amorphophalus sylvaticus* coil the adult mortality noticed after smoke exposure was 57.6% where as in negative control (without plant ingredients) it is 0% and in the case of positive control (synthetic coil) it was 50.8% (Table.4). From the present study it is clear that both *Zanthoxylum rhetsa* and *Amorphophalus sylvaticus* possess good smoke toxic effect on *Culex quinquefasciatus*. The findings agree with some of the previous reports.

Table 3: Smoke toxicity of ethanolic fruit extract of *Zanthoxylum rhetsa* on *Culex quinquefasciatus*

Coil	No. of mosquitoes tested	Fed mosqitoes	Mean% of alive mosquitoes	Mean% of dead mosquitoes
Zanthoxylum rhetsa (Herbal Coil)	50	23.02±0.07	32.03±1.06	54.4±1.7
Negative control (Without plant extract)	50	59±0.9	91.05±0.05	0.0
Positive control (Synthetic Coil)	50	17±1.5	32±0.9	50.8±1.0

*The mean difference is significant at 0.001

Table 4: Smoke toxicity of ethanolic corm extract of *Amorphophalus sylvaticus* on *Culex quinquefasciatus*

Coil	No. of mosquitoes tested	Fed mosquitoes	Mean% of alive mosquitoes	Mean% of dead mosquitoes
Amorphophalus sylvaticus (Herbal Coil)	50	18.6±0.07	30.7±1.3	57.6±1.6
Negative control (Without plant extract)	50	67.08±0.8	92±0.03	0.0
Positive control (Synthetic Coil)	50	15.09±0.9	39.8±0.9	50.8±1.7

*The mean difference is significant at 0.001

Plant derived smoke contains an array of chemicals with different mode of action which kills mosquitoes. Smoke toxicity may be due to the volatile compounds which affects the body functioning of mosquito. The smokes from plants are cheap, target specific, self-sustained and highly toxic to the adult mosquitoes even at very low doses [27]. Murugan *et al.* [18] reported the smoke toxicity of leaves of *Albizzia amara* and *Ocimum basilicum* against the dengue vector, *Aedes aegypti*. Kamalakannan *et al.* [10] have reported that the smoke toxicity effect of leaves of *Tridax procumbans* with 80% unfed and 20% fed adult mosquitoes after treatment in *Anopheles stephensi*. Smoke toxicity effect of *Aegle marmelos* and *Toddalia asiatica* were reported by Vineetha and Murugan [27]. The combination of two powders increased the toxicity of the smoke compared to individual plant powders. Smoke production lowers humidity by reducing the moisture carrying capacity of air. This makes mosquito susceptible to desiccation and reduces sensory input because mosquito chemoreceptors are more responsible in the presence of moisture. Singha *et al.* [25] also observed the similar reports on smoke repellency and mosquito larvicidal potential of *Mesua ferra* leaf extract against *Culex quinquefasciatus*. So finally we can conclude that these two plants contain smoke toxic effect, they may be used as repellants by burning plant material, either on a fire or in a mosquito coil to create an insecticidal smoke, which repels the mosquitoes.

4. Conclusion

In conclusion, these two botanicals showed promising mosquitocidal activity in both repellent bioassay and smoke toxicity studies against *Culex quinquefasciatus*. Hence both the experimental plants can be exploited as a potential source for the control of filarial vector mosquito, *Culex quinquefasciatus* in mosquito control programmes.

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