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REPELLENT ACTIVITY AND SMOKE TOXIC EFFECT OF ZANTHOXYLUM RHETSA AND AMORPHOPHALUS SYLVATICUS AGAINST YELLOW FEVER MOSQUITO AEDES AEGYPTI

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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 *Aedes aegypti*. The ethanolic fruit extract of *Zanthoxylum rhetsa* at 100, 200, 300, 400 and 500 ppm offered 27.42, 48, 78.57, 91.42 and 100 percentage protection against mosquito bite during the time 5-6 p.m. The percentage protection against the mosquito bite was 25, 42, 71, 91.6 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 *Aedes mosquitoes* was 57.6 % where as in negative control (without plant ingredients) it was 0 % and in the case of positive control (synthetic coil) it was 50.4 %. In the case of *Amorphophalus sylvaticus* coil the adult mortality noticed after smoke exposure was 49.6 % where as in negative control (without plant ingredients) it is 0 % and in the case of positive control (synthetic coil) it was 46.0 %. 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 *Aedes aegypti*.

KEYWORDS: *Zanthoxylum rhetsa, Amorphophalus sylvaticus, Aedes aegypti*, Percentage of protection, Repellent activity.

1. INTRODUCTION

Mosquitoes are the most important in public health importance, which transmit a number of diseases like malaria, filariasis, dengue, Japanese encephalitis, etc causing millions of deaths every year (Shanmugapriyan *et. al.,* 2017). Emerging infections, including dengue, zika, and chikungunya, caused by arboviruses that are transmitted by *Aedes aegypti* L. are of great concern to the World Health Organization (Leta *et. al*., 2018). Globally, 2.5 billion people live in high-risk areas, especially in the tropical and subtropical regions of the world, where temperature and humidity promote the proliferation of such vectors (Kraemer *et. al*., 2015.) According to the World Health Organization, 390 million people are infected annually with the dengue virus, 96 million of which have clinical manifestations (Bhatt *et. al.,* 2013). There are various symptoms, the first is usually high fever (39–40 ◦C) with headache, prostration, arthralgia, anorexia, asthenia, nausea and others. Some clinical aspects often depend on patient age. There is no specific treatment for dengue and the more complicated cases of the disease can cause hemorrhage, shock and even death (WHO 2019). Dengue fever has become an important public health problem as the number of reported cases continues to increase, especially with more severe forms of the disease, dengue hemorrhagic fever and dengue shock syndrome, or with unusual manifestations such as central nervous system involvement (Pancharoen *et. al*., 2002). The yellow fever mosquitoes, *Aedes aegypti*, are responsible for dengue fever in India where the number of dengue fever cases has increased significantly in recent years. Dengue viruses occur as four antigenically related but distinct serotypes, which cause abroad range of disease, including clinically asymptomatic forms, classic dengue fever and the more severe forms such as dengue hemorrhagic fever–dengue shock syndrome (Fundacaõ Nacional de Saú de 2002).

Mosquito control is an effective alternative for preventing the spread of these diseases, and management programs focus as much on the control of the immature vectors (egg, larvae, and pupae) as on adult mosquitoes (Chellappandian *et. al*., 2018; Wang *et. al*., 2018). Synthetic insecticides and repellents continue to be the first line of defense owing to their fast action and easy application (Murugan *et. al*., 2012). However, prolonged use of these compounds leads to ecological imbalance and consequent harmful effects on non-target organisms in addition to the development of resistance. Therefore, the search for natural alternatives that are safe, selective, economically viable, and biodegradable is important (Ravindran *et. al*., 2012; Vivekanandhan *et. al*., 2018). 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 (Laura *et. al.*, 2020). In view of an increasing interest in developing plant origin insecticides as an alternative to chemical insecticides, this study was undertaken to assess the repellent activitiy and smoke toxic effect *Zanthoxylum rhetsa* and *Amorphophalus sylvaticus* against dengue vector, *Aedes aegypti* as target species.

2. MATERIALS AND METHODS

2.1 Culture of *aedes aegypti*

To satisfy the number of mosquitoes needed for the day to day bioassays a colony was essential. The eggs of *Aedes aegypti* 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. 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 eggs of *Aedes aegypti* 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 (Medhi *et. al.*, 2013).

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 (Raaman 2006).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 2002 with modification. Three to four days old blood starved 50 adult females of *Aedes aegypti* 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.,* 1986 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 *Aedes aegypti* was calculated in terms of percentage of unfed mosquitoes due to treatment (Thangam. and Kathiresan1992).

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

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 *Aedes aegypti* 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 *Aedes aegypti* the percentage of protection during 100 ppm is 25 %, 200 ppm it is 42 %, 300 ppm it is 71 % , 400 ppm it is 91.6 % 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 *aedes aegypti.*

Repellent activity observed time (Time)	**% of protection**					
	Control	**100 ppm**	**200 ppm**	**300 ppm**	**400 ppm**	**500 ppm**
5.00-6.00 pm	0	27.42	48	78.57	91.42	100
6.00-7.00 pm	0	33.46	50	69.54	89.62	98.46
7.00-8.00 pm	0	29.23	44	65	87	97
8.00-9.00 pm	0	29	49	78	92	99

*The mean difference is significant at 0.001

Table 2: Repellent potential of ethanolic corm extract of *amorphophalus sylvaticus* on *aedes aegypti.*

Repellent activity observed time (Time)	**% of protection**					
	Control	**100 ppm**	**200 ppm**	**300 ppm**	**400 ppm**	**500 ppm**
5.00-6.00 pm	0	25	42	71.	91.6	100
6.00-7.00 pm	0	32.4	44	66	81	99
7.00-8.00 pm	0	36	47	68	86	100
8.00-9.00 pm	0	29	47	72	88	100

*The mean difference is significant at 0.001

From the present study it is clear that both *Zanthoxylum rhetsa* and *Amorphophalus sylvaticus* possess high repellent activity on *Aedes aegypti*. 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 *Aedes aegypti* 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.

Plant products can be used, either as an insecticide for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites, depending on the type of activity they processes (ICMR, 2003). Repellents have an important place in protecting man from the bites of insect pests. An effective repellent will be useful in reducing man vector contact and in the interruption of disease transmission. Mosquito repellents may be one of the most effective tools for protecting human from vector-borne diseases and nuisance caused by mosquitoes (Barnard, 2000). Repellents are substances that act locally or at a distance, deterring an arthropod (insect/mosquito) from flying to, landing on or biting human or animal skin (Choochote *et. al.*, 2007). Usually, insect repellents work by providing a vapour barrier deterring the arthropod (insect/mosquito) from coming into contact with the surface (Brown and Hebert, 1997) and sometimes, applied on to the skin for protection.

Amer and Mehlhorn (2006 b) evaluated 41 plant extracts and11 oil mixtures against the *A. aegypti, A. stephensi,* and *C.quinquefasciatus* using the skin of human volunteers to findout the protection time and repellency. The five most effective oils were those of Litsea *(Litsea cubeba),* Cajeput*(Melaleuca leucadendron),* Niaouli *(Melaleuca quinquenervia),* Violet *(Viola odorata), and* Catnip *(Nepeta cataria),*which induced a protection time of 8 h at the maximum anda 100 % repellency against all three species. Govindarajan (2010a) evaluate the larvicidal activity of crude extract of *Sida acuta* against three important mosquitoes with LC50 values ranging between 38 and48 mg/L; the crude extract had strong repellent action against three species of mosquitoes, as it provided 100 % protection against *A. stephensi* for 180 min followed by *A.aegypti* (150 min).

Neem products are good mosquito repellents showing 90–100 % protection against malaria vectors and about 70 % against *C. quinquefasciatus* (Sharma and Ansari 1994). One controlled study evaluated the efficacy of a cream formulation containing 5% neem oil against *C.quinguefasciatus* and *A. culicifacies*. About 4–5 g of the cream was applied to the exposed skin areas of human volunteers in Ghaziabad, India in the summer months of May/June and the monsoon months of August/September. Neem cream was found to offer 82 % protection against *Culex* bites and 100 % protection against Anopheles bites, as compared to untreated controls (Nagpal *et al.* 2001). The ethanolic extracts of the orange peel *C. sinensis* was tested for the toxicity effect on the larvae of the yellow fever mosquito *A. aegypti* (Amusan *et. al.*, 2005; Murugan *et. al*., 2012). All these results obtained in these studies has supported for the present study.

Kranti Sharma reported that he mosquito coils available in market creates heavy smoke that can causes severe respiratory problems especially for patients of Asthma. Though the 'Fast card mosquito papers' are available in market, they contain of TFT molecules which has adverse effect on respiratory system. By repeated use of mosquito coils and fast card paper, mosquitoes may acquire resistance against smoke and TFT molecule. 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* (Muhammed *et. al*., 2013). The findings of the present fruit and corm extracts of the experimental plants exhibited excellent repelling action against *Aedes aegypti* 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 *Aedes aegypti*. 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 *Aedes aegypti* the adult mortality noticed after smoke exposure of *Zanthoxylum rhetsa* coil was 57.6 % where as in negative control (without plant ingredients) it was 0 % and in the case of positive control (synthetic coil) it was 50.4 % (Table.3). In the case of *Amorphophalus sylvaticus* coil the adult mortality noticed after smoke exposure was 49.6 % where as in negative control (without plant ingredients) it is 0 % and in the case of positive control (synthetic coil) it was 46.0 % (Table.4). From the present study it is clear that both

Zanthoxylum rhetsa and *Amorphophalus sylvaticus* possess good smoke toxic effect on *Aedes aegypti*. The findings agree with some of the previous reports.

Table 3: Smoke toxicity of ethanolic fruit extract of *zanthoxylum rhetsa* on *aedes aegypti*.

Coil	No. of mosquitoes tested	Fed mosquitoes	Mean % of alive mosquitoes	Mean % of dead mosquitoes
Zanthoxylum rhetsa (Herbal Coil)	50	19±1.08	29±1.4	57.6±1.6
Negative control (Without plant extract)	50	60±0.1	91.99±0.0	0.0±0.0
Positive control (Synthetic Coil)	50	16±0.9	36.8±0.7	50.4±0.8

*The mean difference is significant at 0.001

Table 4: Smoke toxicity of ethanolic corm extract of *amorphophalus sylvaticus* on *aedes aegypti*.

Coil	No. of mosquitoes tested	Fed mosquitoes	Mean % of alive mosquitos	Mean % of dead mosquitoes
Amorphophalus sylvaticus (Herbal Coil)	50	20±0.06	32.01±1.4	49.6±1.6
Negative control (Without plant extract)	50	72.5±0.9	90.03±0.02	0.0
Positive control (Synthetic Coil)	50	18.8±0.09	29.1±1.04	46.0±1.4

*The mean difference is significant at 0.001

Smoke is still the most widely used common method of repelling biting insects that is used throughout the world. Fresh and dried plants are frequently added to fires to enhance the repellent properties of the smoke. Several field evaluations, where plants were burned to repel mosquitoes have shown good reduction in mosquito landings ICMR Bulletin 2003. 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 (Vineetha &Murugan 2009).Plant products can be used, either as an insecticide for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites, depending on the type of activity they possess (Kweka *et. al*., 2011). Volatile extracts from the smoke of burning dried leaves were found to be more repellent than those from fresh leaves, which in turn were more repellent to mosquitoes than volatiles from dried leaves. The study also found that, no adverse effects were reported from the use of the prepared sample (Jibrin and Mohammed 2020). Murugan *et. al*., 2007 reported the smoke toxicity of leaves of *Albizzia amara* and *Ocimum basilicum* against the dengue vector, *Aedes aegypti*. Kamalakannan *et al* 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*. The remarkable repellent properties of *M. piperita* essential oil were established against adult's of *Aedes aegypti*. The application of oil resulted in 100% protection till 150 min. After next 30 min, only 1-2 bites were recorded as compared with 8-9 bites on the control arm (Sarita *et. al.,* 2011). 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, the present study proved remarkable mosquitocidal activity in both repellent bioassay and smoke toxicity studies against *Aedes aegypyi*. Hence both the experimental plants can be exploited as a potential source for the control of dengue vector mosquito, *Aedes aegypti* in mosquito control programmes.

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