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Smoke toxicity effect of *Artemisia Parviflora* against dengue vector *Aedes Aegypti*

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ABSTRACT:

Background and Objectives: This paper reports the smoke toxicity effect of Asteraceae plant species against the dengue vector, adult *Aedes aegypti.* These plants are used traditionally as source of medicine.

Methods: The mosquito coils were prepared by using leaves, root, and the stem of *Artemisia Parviflora* by mixing with coconut shell and charcoal powder as burning material. Test mosquito coil were compared with commercially available coils. The percentage of unfed mosquitoes and % of population reduction was calculated.

Results: Smoke emerged from the coil made up of leaves showed maximum protection 51% and population reduction was 83.8%.Contol II showed highest toxic effect and more population. The smoke from root and stem coils showed moderate amount of protection.

Conclusion: The result suggested that the smoke toxic effect of *Artemisia Parviflora* affects the central nervous system and hence affects the neuroendocrine system to inhibit the hatchability of eggs and reduces the egg laying capacity as well the egg hatchability of the mosquitoes.

Keywords:
Aedes aegypti, *Artemisia Parviflora*, smoke toxicity, knock down effect.

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INTRODUCTION

Blood-feeding arthropods are responsible for the transmission of a large number of diseases world-wide. Insect-transmitted disease remains a major cause of illness and death worldwide (Pavela,2009).Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filaria, dengue, japanese encephalitis, etc. causing million of deaths every year (Rahuman *et al*., 2008). There are approximately 3,500 species of mosquitoes grouped into 41 genera ((NCID, 2004). *Aedes aegypti*, a vector of Dengue, Dengue hemorrhagic fever and chikungunya which is a widely distributed tropical and subtropical disease, is now endemic in more than 100 countries and threatens the health of approximately 2.5 billion people. Worldwide, around 80 million people are infected annually at an attack rate of 4% (Monath, 1994). In recent years, *Aedes aegypti* (Diptera:Culicidae) spread the virus chikungunya which affected the southwest Indian ocean islands in 2005, spread out to india, and resulted in an ongoing outbreak that has involved >1.5 million patients (Taubitz *et al*.,2007).

Today, the environmental safety of an insecticide is considered to be of paramount importance. Insecticide applications are although highly efficacious against the target species, vector control is facing a threat due to the development of resistance to chemical insecticides resulting in rebounding Victoria capacity (Liu *et al*., 2006). Furthermore, they are responsible for substantial hazards to a variety of non-target organisms and environment in the form of biomagnifications (Gold *et al*., 2001). In recent years, many researchers have been looking for new botanical insecticides. Plants contain many chemicals, which are important in their defense against insects. They fall into several categories including repellents, feeding deterrents, toxins and growth regulators. There are thousands of chemical compounds that act in one or more of these ways. Sukumar *et al*., (1991) reviewed the bioactivity of 344 plant species against mosquitoes. They showed that some photochemical acts as general toxicants to all life stages of mosquitoes, whereas others interfere with growth and reproduction or act on the olfactory receptors eliciting responses of attractancy or repellency. Uma Devi *et al*., 2010 reported the larvicidal activity of ethanolic leaf extract of *Artemisia parviflora* tested against *A.stephens.*

Smoke is the most widely used means of repelling mosquitoes utilized in the rural tropics. Waste plant materials are frequently burned in Sri Lanka as a mosquito repellent, even though indoor residual spraying has been carried out by the government for many years (Silva, 1991). In rural Guinea – Bissau, 86% of residents used an unimpregnated bed net in conjunction with mosquito coils or plant-based smoke (Palsson and Jaenson, 1999a). In the Solomon Islands a recent survey revealed that fire with coconut husks and papaya leaves was more prevalent among the other personal protections from mosquitoes, being used by 52% of residents (Dulhunty *et al*., 2000). In the Gambia *L. cheraliera* leaves are traditionally used as mosquito repellents. *L. javanica* is commonly found in Southern Africa and is frequently used as a repellent (Lukwa, 1994). Hot leaf infusion of the leaves of these plants are traditionally used as remedies for a variety of ailments, including malaria. The leaves have a strong lemon smell (Van Wyk *et al*., 1997) to which belief in its healing abilities can probably be attributed. *L. cheraliera* is also burned in the Gambia as a mosquito repellent smoke (Aikins *et al*., 1994). A thorough study carried out in Zimbabwe by Lukwa *et al.* (1999) revealed that 29% of the population used plants to protect themselves from mosquitoes, mainly by burning the leaves of *L. javanica.*

From this literature survey, we could be certained that there were no information was available on the smoke toxicity activities with respect to the experimental plant species given here. Therefore present study aimed to evaluate the smoke toxicity effect of

Artemisia parviflora plant parts against the adult *Aedes aegypti*. *Artemisia parviflora* come under the family Asteraceae. The family Asteraceae comprises of many aromatic and medicinal plants. *Artemisia parviflora* (Asteraceae) is an important medicinal plant found in Western Ghats, northern Himalayas, Coimbatore hills, Nilgiris and hills of Travancore above 3000 feet. *Artemisia parviflora* is an important medicinal plant belonging to family Asteraceae, commonly used for skin diseases, cuts and wounds (Kimothi and Shah, 1989). It is also considered for the treatment of high blood pressure, diabetes, and anthelmintic (Ahmad *et al*., 2006). These activities are because of complex mix of phytochemicals present in *A.parviflora.* It also possesses anti-viral properties (Ambasta, 1986; Anonymous, 1985).

MATERIALS AND METHODS

Colonization of *Aedes aegypti*

Collection of eggs

The eggs of *A.aegypti* were collected from National Institute for Communicable Diseases (NICD), Mettupalayam, Coimbatore,Tamil Nadu, India without expose to any insecticide and in and around Coimbatore,India at different breeding habitats with the help of a 'O' type brush. The eggs were then brought to the laboratory and transferred to 18 x 13 x 4 cm size enamel trays containing 500 ml water and kept for larval hatching. They were hatched and reared have been still maintained from many generations in the laboratory. The eggs and larvae obtained from this stock were used for different experiments.

Maintenance of larvae

The freshly hatched larvae were fed with dog biscuits and yeast at 3:1 ratio. The feeding was continued till the larvae transformed into the pupae stage. The larvae reared in plastic cups. Water was changed alternate days. The breeding medium was regularly checked and dead larvae were removed at sight. The normal cultures as well as breeding cups used for any experimental purpose during the present study were kept closed with muslin cloth for preventing contamination through foreign mosquitoes.

Maintenance of pupae and adult

The pupae were collected from culture trays and were transferred to glass beakers containing 500 ml of water with the help of a sucker. The pupae containing glass beaker were kept in 90 x 90 x 90 cm size mosquito cage for adult emergence. The cage was made up of wooden frames and covered with polythene sheets on four sides (two laterals, one back and other one upper) and the front part was covered with a muslin cloth. The bottom of the cage was fitted with strong cardboard. The freshly emerged adults were maintained 27.2°C, 75 - 85% RH, under 14L: 10D photoperiod cycles. The adults were fed with 10% sugar solution for a period of three days before they were provided an animal for blood feeding.

Blood feeding of adult *A.aegypti* and egg laying

The females were fed by hand every alternate days at 6.00 a.m. Feeding mosquitoes on human arm for experimental purposes was suggested by Judson (1967) and Briegel (1985). Both females and males were provided with 10% glucose solution as described by Villani *et al*., (1983) on cotton wicks. The cotton was always kept moist with the solution and changed every day. Theoder and Parsons (1945) noticed that glucose as well as ordinary sugar appeared equally attractive to the mosquitoes. An egg trap (cup) lined with filter paper containing pure water was always placed at a corner of the cage. This arranged made collection of eggs easier.

Smoke toxicity test

Artemisia parviflora (leaves stem and roots) used for smoke toxicity assay. The mosquito coils were prepared by following the method of Saini *et al.,* (1986) with minor modifications by using 2.5 gram of plant ingredients, 4 grams of coconut shell and charcoal powder as burning material. These ingredients were thoroughly mixed with distilled water to form a semisolid paste. A Mosquito coil (0.6 cm thickness) was prepared manually and shade dried. The control coils will be prepared by without the plant ingredient. The experiments were conducted in glass chamber measuring 140 X 120 X 60 cm. A window measuring 60 X 30 cm was situated at mid bottom of one side of the chamber.

One hundred 3-4 day's old blood starved adult female mosquitoes were released into the chamber and were provided with 10% sucrose solution. 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 separate days including control groups using mosquitoes of same age. The data were pooled and average values were subsequently used for calculations. Control was maintained in two sets. One set was run with coil lacking the active ingredient of plant powder (control I) another one was a commercial coil (control 2), which was used for positive control to compare the effectiveness of plant coils. After the experiment was over, the fed, unfed (active and dead) mosquitoes were counted. The protection given by the smoke from plant samples against the biting of *A.aegypti* was calculated in terms of percentage of unfed mosquitoes due to treatment

$$= \frac{\text{Number of unfied mosquitoes in treatment - Number of unfed mosquito in control 1}}{\text{Number of mosquitoes treated}} \times 100$$

The live blood fed mosquitoes were reared in a mosquito cage, measuring 30 x 30 x 15 cm. The top and bottom of the cage were fit with glass and all other sides were covered with muslin cloth. Water soaked raisins and a 5% sucrose solution soaked in cotton balls were provided as a food source. Water containing powdered yeast and dog biscuits were also kept inside the cage in a glass bowl for oviposition. The eggs from the cage were collected daily till all the mosquitoes died. A total 50-100 eggs were allowed to hatch in plastic trays measuring 30 x 25 x 6 cm, containing about 2.5 liters of unchlorinated tap water. Hatched larvae's were fed with a mixture of dog biscuits and yeast powder in the ratio of 2:1 and water in the tray was changed daily. Survival and dead instars were counted and reduction in the population from the smoke treated

mosquitoes were calculated using the formula.

$$\text{Population reduction (\%)} = \frac{\text{Number of larvae hatched in control 1 - Number of larvae hatched in treated}}{\text{Number of larvae hatched in control 1}} \times 100$$

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) and means separated by Duncan's multiple range tests (DMRT).

RESULTS AND DISCUSSION

Table 1 provides the results of smoke toxicity effect of *A. parviflora* leaf on *A.aegypti*. The control was maintained without plant ingredients. It acts as negative control. The commercially available (Mortein) mosquito coil was used as positive control. One hundred, 4-3 days starved *A.*aegypti were used. After the treatment of the plant, the fed and unfed mosquitoes were counted. There were 19 fed and 81unfed mosquitoes counted after the treatment of leaf smoke exposure,21fed and 79 unfed from roots and 24 fed and 76 unfed mosquitoes were counted after the treatment of stem smoke exposure of *A. parviflora.* The comparisons of these plant parts the smoke from leaves showed very high efficacy. The effect of plant leaves showed good smoke toxicity effect on *A.aegypti*. This may be due to the presence of active chemical compounds in the leaves. Plant derived smoke contains an array of chemicals with different mode of action, which kill mosquitoes. The smoke from the blank coil also showed considerable toxic effect and control II showed higher toxic effect against *A.aegypti*.

Table 2 shows the smoke toxicity effect of *Artemisia parviflora* from ((leaves, stem and root)

Table 1: Smoke toxicity effect of *Artemisia parviflora* parts against biting activity of *Aedes aegypti.*

Artemisia parviflora parts used	No. of mosquitoes tested	Fed mosquitoes	Unfed mosquitoes		Total	% unfed over control I
			Alive	Dead		
Leaf	100	19[c]	61[a]	20[a]	81[ab]	51[a]
Root	100	21[bc]	49[b]	30[c]	79[ab]	49[ab]
Stem	100	24[a]	40[c]	36[b]	76[b]	46[b]
Control I*	100	70[a]	30[d]	0[e]	30[c]	0[c]
Control II*	100	18[cd]	40[c]	42[a]	82[a]	-

Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT.Control I* -Negative control - blank without plant material Control II*-Positive control - mortein coil

different parts ensured population of the *Aedes aegypti*. The numbers of eggs laid by the alive, fed females were shown. Number of eggs laid and the hatchability were greatly reduced or affected by the exposure of smoke. Only 5 of 25 mosquitoes oviposited a total of 1357 eggs of which only 950 eggs had hatched. The percentage of reduction was 83.8% in the plant exposed mosquitoes in the leaf smoke, 82.6% from root and the 53.7% from stem. The leaves showed a significant effect on the fecundity and hatchability. The smoke exposure affects the central nervous system and hence affects the neuroendocrine system to inhibit the hatchability of eggs and reduces the egg laying capacity as well as the egg hatchability of the mosquitoes.

The control of adult mosquitoes has been considered too, either by adulticiding or preventing method such as repellency or mosquito coil burning. There are four major types of insecticidal products used by general people in their residence like aerosols, mosquito coil, liquid vaporizers and vaporizing mats, out of which mosquito coils are preferred as anti-mosquito product in low communities in India. The common active ingredients in coil are various pyrethroids and different toxic chemicals and frequently contain octa chlorodiprophyl ether include undefined geno toxicagent (Pauluhn and Mohr, 2000). Plant derived smoke contains an array of chemicals which has been used since early time to deter mosquitoes and it is cheap target specific and highly toxic to adult mosquitoes at low dose. Smoke toxicity from *Moringa oleifera* (Prabhu *et al*., 2011) *Mesua ferra* (Someshwar *et al*., 2011) *Spathodea campanulata* (Aarthi and K Murugan.,2010), *C.occidentalis* Abirami Dhandapani and Murugan Kadarkarai (2011) *Albizzia amara* and *Ocimum basilicum* (Murugan *et al*., 2007) considerably affect the mosquito survival and pronounced high repellent potential. In the present smoke toxicity test also the plant showed a good smoke repellency activity. The smoke exposed females also laid only limited number of eggs, in that also only a limited hatched out. This reduces the mosquito population also. The results of this study indicated that *A.parviflora* leaves enhances in the smoke

Table 2. Smoke toxicity effect of *Artemisia parviflora* parts ensured population of *Aedes aegypti.*

Artemisia parviflora parts used	No. of mosquitoes tested	No of egg rafts laid by fed mosquitoes	Total No of eggs	Total No. of Larvae hatched from the eggs rafts	% of reduction in population over control I
Leaf	25	5[bc]	1357[a]	950[a]	83.8[a]
Root	25	8[b]	955[c]	440[c]	53.7[b]
Stem	25	7[b]	1357[a]	950[a]	82.6 [ab]
Control I*	25	12[ab]	1048[b]	675[b]	28.9[c]
Control II*	25	4[c]	380[e]	165[d]	-

Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT. Control I* -Negative control - blank without plant material Control II* Positive control - mortein coil

toxicity test and it may be an effective alternative to conventional synthetic insecticides for the control of *A.aegypti* smoke emitted from the *A.parviflora* also showed a good knock down effect. The smoke exposure affects the central nervous system and hence affects the neuroendocrine system to inhibit the hatchability of eggs and reduces the egg laying capacity as well as the egg hatchability of the mosquitoes. According to Thangam and Kathiresan (1992) stated that smoke from burning various dry materials has been used since early times to determine insects, especially mosquitoes. Pandian *et al*., (1995) studied powdered preparations of the leaves of *Adhatoda vasica*, *Azadirachta indica* and Ocimum sanctum, which on burning with charcoal produced smoke that repelled *Armigeres subalbatus* and *Culex quinquefasciatus* and prevented their biting activity for 6 –8 h. However, compared with the previous study, our study showed greater smoke toxicity. Traditional repellents not only provide protection against mosquito bites but also curtail disease transmission. Hence, these plant parts can be preferably employed for the development of mosquito coil in future.

CONCLUSION

The finding of the present investigation revealed that *A.parviflora* has good toxic and repellent effect against *Aedes aegypti*. Smoke from medicinal plants are tremendous to the human beings, also they are biodegradable and easily available**.**

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