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# TOXICITY AND SMOKE REPELLENCY EFFECT OF *MIMOSA PUDICA* L. AGAINST THE MALARIAL VECTOR *ANOPHELES STEPHENSI* (DIPTERA: CULICIDAE)

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**ABSTRACT**
*Mimosa Pudica* Linn is a commonly used herbal drug against many diseases. The antivectorial activity of ethanolic leaf extract of *Mimosa Pudica* Linn was investigated in the laboratory. Different concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 ppm) against different immature stages of *Anopheles stephensi* were tested and showed considerable toxicity effect against the immatures of *Anopheles stephensi*. Lethal concentration ($LC_{50}$ and $LC_{90}$) has been worked out on different larval stages of *Anopheles stephensi*. The $LC_{50}$ values of *M. pudica* for I instar larvae was 0.723%, II instar was 1.150%, III instar was 1.540%, IV instar was 2.073%, and pupa was 2.835%, respectively.The $LC_{90}$ values such as I instar was 3.578%, II instar was 4.079%, III instar was 4.833%, IV instar was 5.333 % and pupa was 6.717%, respectively. The smoke toxicity effect of *M. pudica* leaves exhibited a good knock down effect when compared with the commercial synthetic mosquito coil. The smoke affected gravid females and they lay only a fewer number of eggs and egg hatchability was also reduced. The percentage of population reduction was 79.5% in the plant exposed mosquitoes and in the positive control (Mortein coil), the percentage of reduction was 71.8%.

## INTRODUCTION

Mosquito-transmitted disease continues to be a major source of illness and death. In India, malaria is one of the most important causes of direct or indirect infant, child, and adult mortality. About 2 million confirmed malaria cases and 1,000 deaths are reported annually, although 15 million cases and 20,000 deaths are estimated by WHO South East Asia Regional Office. India contributes 77% of the total malaria in Southeast Asia. Mosquitoes are also becoming increasingly resistant to traditional chemical pesticides and there is growing concern about the potential health and environmental risks surrounding these products. Environmental protection agencies have banned or placed severe restrictions on the use of many pesticides which were formerly used in mosquito control programmes and there are now fewer adulticides available than there have been for the last 20 years (Carvalho *et al.*, 2008). Mosquitoes are well known group of insects, which transmit many dreadful diseases causing serious health problems to human beings.

Plant products have been used by traditionally human communities in many parts of the world against the vectors and species of insects. The phyto-chemicals derived from plant sources can act as larvicides, insect growth regulators, repellents and ovipositional attractants and have deterrent activities (Babu and Murugan, 1998). Repellents provide a mechanism for protecting man from the bites of arthropods. Murugan *et al*., (2003) studied the desired qualities in the design of an arthropod repellent includes having a long-lasting repellent activity, being of low toxicity to humans, and being non-irritating. *Mimosa pudica* L. is a creeping annual or perennial herb and it belongs to the Fabaceae family. *Mimosa pudica* is native to Brazil, but is now a pan tropical weed. The other names given to this plant are Humble plant, Shame plant, Touch me not (Germplasm Resources Information Network, 2008). The stem is erect in young plants, but becomes creeping or trailing with age. *Mimosa Pudica* Linn. is a commonly used herbal drug against many diseases. This plant has a history of use for the treatment of various ailments and the most commonly used plant part for this purpose is the root, but flowers, bark and fruit can also be utilized. Several research works have been carried out to study about the phytochemical components of *Mimosa pudica* (Arthur, 1954; Deininger, 1984; Ahmad and Beg, 2001) and also about the antimicrobial activity of the plant (Palacios and Reyes, 1991; Ojalaa *et al*., 1999).The aim of the present study was to evaluate the larvicidal, pupicidal and smoke repellent potential of *Mimosa pudica* plant against the malarial vector, *Anopheles stephensi*.

## MATERIALS AND METHODS

### Collection and Maintenance of mosquitoes

The eggs of *A. stephensi* were collected from in and around Coimbatore districts (drinking water bodies, water stored container) with the help of 'O' type brush, for the laboratory bioassay. These eggs were brought to the laboratory and transferred to 18 X 13 X 4 cm size enamel trays containing

500 mL of water and keep for larval hatching. The mosquito larval and pupal culture was maintained in our laboratory by following the standard procedures. The plastic jars will be kept in 90 X 90X 90 cm size mosquito cage for adult emergence.

**Plant collection and preparation of phyto extracts**

*Mimosa pudica* leaves were collected together and shade dried. Since certain compounds get denatured in sunlight, it is dried under shade to avoid decomposition room temperature for 7-10 days, and then they were powdered using a blender. One hundred grams of plant material was used for extraction with 300 mL, ethanol for 8 hours in a Soxhlet apparatus (Vogel, 1978). The plant extracts was evaporated to dryness in a vacuum rotary evaporator. *Mimosa pudica yielded* 200 mg of residue. One gram of each plant residue was dissolved separately in a 1% stock solution of 100 mL of acetone from which various concentrations (0.2, 0.4, 0.6, 0.8 and 1.00 ppm.) were prepared.

**Preliminary Phytochemical Screening**

Phytochemical screening of the plant extract was carried out as per the methods and tests given by Dey and Raman (1957) to decipher the presence or absence of various phytocompounds.

**Test for larvicidal and pupicidal activity (WHO, 2005)**

The larvicidal bioassay was assessed by using standard WHO protocols (WHO, 2005). For experimental treatment, 1ml of whole plant ethanolic extract was added to 100ml of distilled water in a 250 mL of enamel bowl which was shaken lightly to ensure a homogenous test solution. Then 25 1$^{st}$, 2$^{nd}$, 3$^{rd}$ and 4$^{th}$ instars larvae of *Anopheles stephensi* were transferred by means of strainers to that bowl. Each experiment was performed in 4 replicates with a final total of 100 larvae for each concentration and the equal number of control were setup simultaneously with distilled water to which 1 mL of ethanol was added. Experiments were conducted at 27 ± 1ºC and 85% relative humidity (RH) with photoperiod of 12L: 12D. Symptoms of the treated larvae were observed and recorded immediately and at timed intervals and no food was offered to the larvae. Mortality and survival was registered after 24h and 48h of the exposure period. The moribund and dead larvae were combined in four replicates and expressed as a percentage of larval mortality of each concentration. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region, moribund larvae were those incapable of rising to the surface (with a reasonable period of time) or showing the characteristic diving reaction when the water position, tremors, un-coordination of rigor were also counted. In case of experiment for determining pupicidal activity, the mouth of each bowl containing pupae was covered with muslin cloth to prevent the escape of any emerged adult mosquitoes. Mortality in larvae and pupae was recorded at 24h and 48h.

**Smoke Toxicity Test**

*Mimosa pudica* leaves were used for smoke toxicity assay. The mosquito coils were prepared by following the method of Saini *et al.,* (1986) with minor modifications by using 4g of coconut shell, 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 three or four 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 coil and the synthetic coil. After the experiment was over, the fed, unfed (active and dead) mosquitoes were counted. The protection given by the smoke from plant coil and the commercial coil against the biting of *Anopheles stephensi* was calculated in terms of percentage of unfed mosquitoes due to treatment. Data were analyzed using analysis of variance (ANOVA) and means separated by Duncan's multiple range tests.

$$= \frac{\text{No. of unfed mosquitoes in treatment - No. of unfed mosquito in control 1}}{\text{No. of mosquitoes treated}} \times 100$$

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 raisons 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 2.5L 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 was calculated using the formula.

$$\text{Population reduction (\%)} = \frac{\text{No. of larvae hatched in control 1- No. of larvae hatched in treated}}{\text{No. of larvae hatched in control 1}} \times 100$$

**Statistical analysis**

All data were subjected to analysis of variance (ANOVA) and the means separated by Duncan's multiple range test (DMRT) (Alder and Rossler, 1977).

## RESULTS AND DISCUSSION

Table 1 illustrates the larval and pupal mortality of *Anopheles stephensi* after the treatment of ethanolic extract of *Mimosa*

**Table 1: Toxicity effect of *Mimosa pudica* ethanol extract against the immature stages of malarial vector *Anopheles stephensi***

| Larval instar And pupa | Larval mortality (%) Concentration of M.P (ppm) | | | | | $LC_{50}$ and ($LC_{90}$) | 95% Confidence limit $LCLLC_{50}(LC_{90})$ | $UCLLC_{50}(LC_{90})$ |
|---|---|---|---|---|---|---|---|---|
| | 0.5 | 1.0 | 1.5 | 2.0 | 2.5 | | | |
| I | 47±0.2 | 55±0.5 | 62±0.5 | 71±0.9 | 80±1.0 | 0.723(3.578) | 0.202(2.965) | 1.020(4.851) |
| II | 39±0.5 | 48±0.8 | 55±0.5 | 64±0.7 | 73±0.6 | 1.150(4.079) | 0.791(3.345) | 1.408(5.625) |
| III | 35±0.9 | 41±0.9 | 49±1.1 | 57±0.8 | 65±0.7 | 1.540(4.833) | 1.233(3.837) | 1.862(7.165) |
| IV | 28±1.5 | 33±0.6 | 40±0.7 | 48±1.0 | 58±0.3 | 2.073(5.333) | 1.771(4.204) | 2.593(7.983) |
| pupa | 22±0.8 | 27±0.3 | 33±0.8 | 40±0.8 | 45±0.6 | 2.835(6.717) | 2.319(4.980) | 4.188(11.829) |

Chi square value Significant at $p < 0.05$ level; Within a column ± stands for the (SEM)

*pudica* leaf extract respectively. The considerable mortality was evident after the treatment of MPLE for the immature stages of *A. stephensi* such as I, II, III, IV instars and pupa. Mortality was increased as the concentration was increased. Among the different larval stages, the I instar larvae was more susceptible than the other instar larvae. The plant extract also showed considerable pupal mortality. It was recognized that the fourth stage larvae and pupae of mosquitoes were more tolerant to toxicant than early instar (Mulla, 1961; Thomas, 1965; Rettich, 1976). Larval mortality may be due to effect of the chemicals like mimosine, norepinephrine, crocetin-dimethyl-ether and sitosterol. Similar trend has been noted for all the larval instars and pupae of *A.stephensi* at different concentrations of MPLE treatment. The $LC_{50}$ and $LC_{90}$ values were represented as follows: $LC_{50}$ value of I instar was 0.723%, II instar was 1.150%, III instar was 1.540%, IV instar was 2.073%, and pupa was 2.835%, respectively. $LC_{90}$ value of I instar was 3.578%, II instar was 4.079%, III instar was 4.833%, IV instar was 5.333 % and pupa was 6.717%, respectively.

Table 2 provides the qualitative photochemical analyses of MPLE extract. The analysis reports the presence of Alkaloids, Flavonoids, Tannins, Terpenoids, Saponins and Steroids. The mortality resulted by the immature stages of the mosquito may due the presence of essential phytochemicals also. We observed a functional response by all immature life stages of *A. stephensi* to the acetone extract of leaves of these plant species. This biological activity is attributed to the compounds in the leaves, including alkaloids, flavonoids, phenols and steroids that together or independently produce morbidity and mortality effects in *A. stephensi*.

**Table 2: Qualitative phytochemical analysis of *Mimosa pudica* leaf extract**

| Name of the constituents | Name of the plants *Mimosa pudica* |
|---|---|
| Alkaloids | + |
| Flavonoids | + |
| Saponins | + |
| Steroids | + |
| Tannins | + |
| Terpenoids | + |

(+) Shows the Presence of phytochemical constituent; (-) Shows the Absence of phytochemical constituent

Table 3 provides the results of smoke toxicity effect of *Mimosa pudica* on biting activity of *A.stephensi*. Two gram of plant ingredients from *Mimosa pudica* leaves were used for smoke toxicity. The control was maintained without plant ingredients. It acts as negative control. The commercially available (Mortein) mosquito coil used as positive control. One hundred 4-3 days starved *A.stephensi* were used. After the treatment of the plant, the fed and unfed mosquitoes were counted. There were 20 fed and 80unfed mosquitoes counted after the treatment of *Mimosa pudica* leaf smoke exposure. The comparisons of positive control with the plant product showed very high efficacy. The effect of plant showed good smoke toxicity effect on *A.stephensi*. 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. *M.pudica* contains an alkaloid called mimosine, which has been found to have potent antiproliferative and apoptotic effects. The main chemicals in *M.pudica* include: Ascorbic-acid, crocetin, crocetin-dimethyl-ether, D-glucuronic-acid, D-xylose, linoleic-acid, linolenic-acid, mimosine, mucilage, norepinephrine, oleic-acid, palmitic-acid, sitosterol, stearic-acid etc., According to Thangam, and Kathiresan (1992) stated that smoke from burning various dry materials has been used since early times to deter insects, especially mosquitoes. Similarly Murugan *et al.,* (2007) studied the smoke toxicity after exposure of smoke from *Albizzia amara* and *Ocimum bascilicum* and found that smoke toxicity of *A.amara* was more effective against *A.aegypti* than the *O.bascilicum*. 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.

Table 4 provides the Smoke toxicity effect of *Mimosa pudica* leaf on reproduction and survival of *Anopheles stephensi*. After the treatment of Mimosa pudica leaf smoke, only 11 of 25 mosquitoes oviposited a total of 780 eggs of which only

**Table 3: Smoke toxicity effect of leaves of *Mimosa pudica* against biting activity of *Anopheles stephensi***

| *Coil Used* | No. of mosquito tested | | Fed mosquito Alive | Unfed mosquito Dead | Total | % unfed over control I |
|---|---|---|---|---|---|---|
| Leaf | 100 | $20^{d}$ | $33^{c}$ | $47^{b}$ | $80^{b}$ | $81^{ab}$ |
| Control I * | 100 | $85^{a}$ | $15^{e}$ | $0^{e}$ | $15^{d}$ | 0 |
| Control II * | 100 | $8^{e}$ | $34^{d}$ | $58^{a}$ | $90^{a}$ | $83^{cd}$ |

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

**Table 4: Smoke toxicity effect of different parts of *Mimosa pudica* on reproduction and survival of *Anopheles stephensi***

| No. of mosquito used | Total No. of eggs | Total No of larva hatched from the eggs | % of reduction in population over control 1 |
|---|---|---|---|
| 25 | 780 [e] | 275 [e] | 79.58 [bc] |
| 25 | 2112 [a] | 1347 [a] | *0 |
| 25 | 873 [d] | 379 [c] | **71.86 [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.

275 eggs had hatched. The percentage of reduction was 79.5% in the plant exposed mosquitoes and in the positive control (Mortein coil), the percentage of reduction was 71.8%. Among the comparison between the plant and the commercial coil, the *M.pudica* leaf smoke exhibited higher efficacy of percentage reduction on ensured population of *Anopheles stephensi*. In the light of literature many researchers have evaluated the effect of insecticides in mosquito coils and in the study of (Aarthi and Murugan, 2010) also the smoke emitted from the *Spathodea campanulata* also showed a good knock down effect. The smoke exposure affects the central nervous system and hence affects the neuroendocrine system to inhibit the hatachability of eggs and reduces the egg laying capacity as well the egg hatachability of the mosquitoes.

Globally, numerous studies including the present investigation results evidently suggest that the traditionally used plant-based insect repellents are promising and could potentially contain vectors of disease. The result of this study indicates that *M.pudica* leaf extracts enhances the larvicidal, pupicidal and smoke repellency activity and hence it may act as an effective alternative to conventional synthetic insecticides for the control of *A. stephensi*.

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