Effect of certain plant extracts against the mosquito, *Anopheles stephensi* Liston

Use of biologically-active plant materials with antilarval properties has attracted considerable interest[1,2]. Because of their biodegradable nature and being relatively safer for human beings and nontarget organisms in the environment, extensive survey of the flora was undertaken to search for potential plant extracts, which could be used in the management of agricultural and household pests[3]. Moreover, investigations on the insecticidal properties of plant extracts have been given an impetus because of imposition of restrictions on the use of chemicals for insect control. Larvicidal efficacy of the oils of *Ocimum basilicum* on *Culex fatigans* has been reported[4]. Dhillion *et al.*[5] have reported that the extracts of *Rhizocnium kiercolyphicum* and *Chlorella ellipsoides* have toxic properties against the larvae of *Aedes aegypti*, *Culex quinquefasciatus* and *Caliseta incidens*. Previously, Murugan *et al.*[6] had reported the antipupational effect of neem oil and neem seed kernel extract against mosquito larvae of *Anopheles stephensi*. The neem latex was shown to have adult repellent activity against *Musca domestica*[7]. Recently, Babu and Murugan[8] have reported that neem gum extracts potentiate the neem seed kernel extract activity against *C. quinquefasciatus*. As a follow up, we screened for effects of extracts of *Leucas aspera*, *Ocimum sanctum*, *Azadirachta indica*, *Allium sativum* and *Curcuma longa* on the larval mortality, pupation, adult emergence, repellency, and ovipositional deterrency of *Anopheles stephensi* at 1% to 4% concentrations.

A. stephensi, predominantly breeds in wells, overhead or ground level water tanks, cisterns, coolers, roof gutters and artificial containers. It has a wide distribution and is a major vector in India as well as in some of the West Asian countries. It has been shown to be directly responsible for about 40–50% of the annual malarial incidence. It transmits malaria in the plains of rural and urban areas of India.

For our studies we used laboratory colonies of *A. stephensi* which were maintained at $27 \pm 2°C$, under a 10 : 14 light/dark photoperiod cycle and 75% relative humidity. Plant extracts were prepared as previously described[6].

Leaves of *L. aspera*, *O. sanctum*, *A. indica*; pulp of *A. sativum*; and rhizome of *C. longa* were collected from in and around Bharathiar University Campus, Coimbatore, and dried to constant weight. The dried leaves, pulp and rhizomes were separately ground to powders. The powders were stirred for 16 h with 20 times their weight of distilled water and left to stand for 48 h at room temperature. The solution was filtered twice and diluted serially to obtain the desired concentrations.

The required quantity of different concentrations (1, 2, 4%) were mixed thoroughly with 200 ml of rearing water in 500 ml plastic troughs. One hundred early fourth-instar mosquito larvae were released into each trough. Larval food consisted of 1 g of finely ground dog biscuit per day per trough. Dried coconut midribs were placed over water as the substratum for pupation. The plastic trough containing only 200 ml of rearing water served as the control. Dead larvae and pupae were removed and counted at 24 h intervals. Observations on larval mortality, percentage of pupation, and adult emergence were recorded. The experiments were replicated 5 times. The percentage of corrected mortality from the observed mortality was calculated by using Abbott's formula[9].

Different quantities of the stock solution of plant extracts were individually mixed thoroughly with 200 ml of rearing water in 250 ml glass jars to obtain the concentrations desired for the tests with *A. stephensi*. Ten gravid females were given a choice between treated and control jars. During the tests, the groups of females were kept separately for 48 h in cages measuring $25 \times 25 \times 30$ cm. After the eggs were counted, the oviposition activity index (OAI) was calculated using the formula:

$$OAI = (N_c - N_t)/(N_c + N_t) \times 100,$$

where N_c is the number of eggs in the control, and N_t is that in the treatment.

Different concentrations of plant extracts were mixed thoroughly with 10 ml of goat blood in glass plates. The untreated blood served as the control. Ten adult females were released into each cage. The number of females landing on the treated blood and untreated blood was recorded. The repellent index of plant extracts was calculated as previously described[7]. All data was subjected to analysis of variance (ANOVA) and means were separated by using Duncan's multiple range test[10].

The leaf, pulp and rhizome extracts of *L. aspera*, *O. sanctum*, *A. indica*, *A. sativum* and *C. longa* significantly reduced larval survival compared to the control (Table 1). Among the various treatments administered, the leafs extracts of *L. aspera*, *O. sanctum* were most effective. The per cent larval mortality at 4% concentration of *L. aspera* and *O. sanctum* leaves was 90 and 84%, respectively, whereas in *A. indica*, *A. sativum*, and *C. longa* it was 71, 68 and 54%, respectively.

The significant reduction in pupation and adult emergence was observed in all the treatments when compared to the control (Table 1). The leaf extract of *L. aspera* and *O. sanctum* at 1% concentration, significantly reduced percentage of pupation and adult emergence than other treatments.

The leaf extracts of *L. aspera* and *O. sanctum* at 4% concentration, significantly increased the adult repellency, to 89% and 78% respectively, whereas in *A. indica*, *A. sativum* and *C. longa*, it was 60, 48 and 41%, respectively. However the ovipositional deterrency was significantly greater in *A. indica*, *L. aspera* and *O. sanctum* than in *A. sativum* and *C. longa* (Table 2).

In case of *A. indica* leaf extract, the highest concentration (4%) tested, resulted in 87% ovipositional deterrency, whereas in *L. aspera* and *O. sanctum* it was 83% and 79% only. However it was statistically not significant according to Duncan's multiple range test. All the extracts significantly increased ovipositional deterrency index. Among the various treatments administered, leaf extracts of *L. aspera*, *O. sanctum* and *A. indica* were superior to those of *A. sativum* and *C. longa* extracts.

Biologically active plants show great promise for their potential efficiency as

Table 1. Effect of plant extracts on the mortality, pupation and adult emergence of *A. stephensi*

Treatment	Larval mortality	Percentage of pupation	Adult emergence
Control	00^{g}	98^{a}	96^{a}
Leucas aspera (leaf)			
1.0%	49^{d}	36^{ef}	27^{ef}
2.0%	64^{bc}	20^{g}	14^{fg}
4.0%	90^{a}	7^{h}	2^{g}
Ocimum sanctum (leaf)			
1.0%	40^{de}	39^{ef}	35^{e}
2.0%	58^{c}	25^{fg}	18^{f}
4.0%	84^{ab}	12^{gh}	6^{g}
Azadirachta indica (leaf)			
1.0%	35^{e}	51^{d}	46^{d}
2.0%	52^{cd}	34^{ef}	29^{ef}
4.0%	71^{b}	20^{g}	14^{fg}
Allium sativum (pulp)			
1.0%	27^{ef}	60^{c}	55^{c}
2.0%	44^{d}	42^{e}	38^{de}
4.0%	68^{b}	32^{f}	25^{f}
Curcuma longa (rhizome)			
1.0%	18^{f}	73^{b}	64^{b}
2.0%	32^{e}	56^{cd}	50^{cd}
4.0%	54^{cd}	40^{ef}	34^{e}

Within a column means followed by the same letter are not significantly different at 5% level by Duncan's multiple range test.

Table 2. Adult repellency and ovipositional deterrency of *A. stephensi* after the treatment of plant extracts

Treatment	Adult repellency (%)	Ovipositional deterrency (%)
Control	00^{g}	00^{g}
Leucas aspera (leaf)		
1.0%	48^{d}	45^{d}
2.0%	62^{c}	60^{bc}
4.0%	89^{a}	83^{a}
Ocimum sanctum (leaf)		
1.0%	40^{de}	42^{de}
2.0%	57^{c}	55^{c}
4.0%	78^{b}	79^{a}
Azadirachta indica (leaf)		
1.0%	34^{e}	52^{c}
2.0%	48^{d}	69^{ef}
4.0%	60^{c}	87^{a}
Allium sativum (pulp)		
1.0%	22^{f}	35^{e}
2.0%	35^{e}	46^{cd}
4.0%	48^{d}	60^{bc}
Curcuma longa (rhizome)		
1.0%	15^{f}	18^{f}
2.0%	28^{ef}	32^{e}
4.0%	41^{de}	45^{d}

Within a column means followed by the same letter are not significantly different at 5% level by Duncan's multiple range test.

larvicides. The leaf extracts of *L. aspera*, *O. sanctum*, *A. indica*; pulp extract of *A. sativum*; and rhizome extracts of *C. longa* showed their potential in controlling *A. stephensi*. However, *L. aspera* and *O. sanctum* leaf extracts were found to be more effective than the rest, specially in the case of larval mortality, pupation, and adult emergence. During the bioassay studies it was found that the percentage of pupation and adult emergence progressed in dose response manner. All the plant extracts tested showed larvicidal activity against *A. stephensi*. Sharma and Saxena[11] observed the larvicidal activity of crude extract of *Sphaeranthus indicus* against *C. quinquefasciatus*. Sujatha *et al.*[12] have also observed differential susceptibility with petroleum ether extracts of *Acorus calamus*, *Ageratum conyzoids*, *Annona squamosa*, *Bambusa arundanasus*, against *C. quinquefasciatus* and *A. stephensi*.

Supavarn *et al.*[13], using methanol extracts of plant species from 17 families, reported that in addition to acute toxicity, compounds from these plants significantly lengthened the larval period in *A. aegypti*, and suggested that this was due to interference in normal moulting hormonal activity.

Treatment with leaf extracts of *L. aspera*, *O. sanctum* and *A. indica* on larvae exhibited high mortality, especially during the moulting process or the subsequent processes of malanization and tanning. Such processes are under the influence of the ventral nerve cord neurosecretory cells, which release the tanning hormone[14]. These extracts may have an inhibiting influence on such cells, or may act directly on epidermal cells which are responsible for the production of enzymes for the tanning or cuticular oxidation process. This is further evidenced by the fact that many of the larvae treated with higher concentration do not successfully moult to pupae but rather moult to larval–pupal intermediates.

The leaf extracts of *A. indica*, *L. aspera*, *O. sanctum*; pulp extracts of *A. sativum*; and rhizome extract of *C. longa*, acted as potent repellents against *A. stephensi*. These extracts on being mixed with blood meal resulted in remarkable suppression of feeding activity of *A. stephensi*. The potent repellent activity may be due to the presence of biologically active components in the plant extracts.

Thus, our observations suggest that the leaf extracts of *L. aspera* and *O. sanctum* are highly toxic to the mosquito larvae. In addition, these extracts have exhibited a high deterrent effect on the adult mosquitoes. Further, *O. sanctum* extracts are easy to prepare and handle, inexpensive, and safe natural products for mosquito control. The extracts of tulsi, wheat and neem can also be used for disinfecting water. Tulsi leaf extracts can be used in water tanks to suppress *A. stephensi* population as well act as water disinfectant.

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