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Efficacy of *Melia azedarach* L. extract on the malarial vector *Anopheles stephensi* Liston (Diptera: Culicidae)

Sengottayan Senthil Nathan [a,b,*], G. Savitha [b], Dency K. George [b], Alagirisamy Narmadha [b], Laxmanan Suganya [b], Paul Gene Chung [a]

[a] *Department of Environmental Engineering, Chonbuk National University, Jeonju City, Chonbuk 561 756, South Korea*
[b] *Post Graduate and Research Department of Biotechnology, Vivekanandha College (W), Trichengode, Namakkal 637 205, Tamil Nadu, India*

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Abstract

Methanolic extracts of leaves and seeds from the chinaberry tree, *Melia azedarach* L. (Meliaceae) was tested against mature and immature mosquito vector *Anopheles stephensi* Liston (Diptera) under laboratory condition. The extract showed strong larvicidal, pupicidal, adulticidal, antiovipositional activity, repellency and biting deterrency. The *M. azedarach* seed and leaf extracts were used to determine their effect on *A. stephensi* adults and their corresponding oviposition and consequent adult emergence in comparison with the control. The seed extracts showed high bioactivity at all doses, while the leaf extracts proved to be active, only in the higher dose. Results obtained from the laboratory experiment showed that the seed extracts suppressed the pupal and adult activity of *A. stephensi* even at low dose. In general, first and second instar larvae were more susceptible to both leaves and seed extracts. Clear dose–response relationships were established with the highest dose of 2% plant extract evoking 96% mortality. Entire development of *A. stephensi* was inhibited by *M. azedarach* treatment. Less expensive (less than US$0.50 per 1 kg seed), naturally accruing biopesticide could be an alternative for chemical pesticides.

Abbreviations: LE, leaf extract; SE, seed extract; EC_{50}, effective concentration; ±SE, standard error

Keywords: Malarial vector; Chinaberry; Larvicidal; Biology; Mortality; Repellency

1. Introduction

The Meliaceae plant family is known to contain a variety of compounds, which show insecticidal, antifeedant, growth regulating and development modifying properties (Nugroho et al., 1999; Greger et al., 2001; D'Ambrosio and Guerriero, 2002; Nakatani et al., 2004). *Melia azedarach* L. (Sapindales: Meliaceae) known as Chinaberry or Persian lilac tree is a deciduous tree that is native to northwestern India and has long been recognized for its insecticidal properties but yet to be well analyzed. This tree typically grows in the tropical and subtropical parts of Asia, but nowadays it is also cultivated in other warm regions of the world because of its considerable climatic tolerance. Fruit extracts of *M. azedarach* elicit a variety of effects in insects, such as antifeedant, growth retardation, reduced fecundity, moulting disorders, morphogenetic defects, and changes of behavior (Schmidt et al., 1998; Hammad et al., 2001; Gajmer et al., 2002; Banchio et al., 2003; Wandscheer et al., 2004). The effects of compounds,

* Corresponding author. Address: Department of Environmental Engineering, Chonbuk National University, Jeonju City, Chonbuk 561 756, South Korea. Tel.: +82 63 270 2444; fax: +82 63 270 2449.
E-mail addresses: senthil@moak.chonbuk.ac.kr, senthilkalaidr@hotmail.com (S.S. Nathan).

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products and extracts obtainable from *M. azedarach* on insects have been reviewed by Ascher et al. (1995). Effects of *M. azedarach* extracts on many insects have been already reported (Saxena et al., 1984; Schmidt et al., 1998; Juan et al., 2000; Carpinella et al., 2003; Senthil Nathan and Saehoon, 2005).

Control of mosquito is required, because many species of mosquitoes are vectors of malaria filariasis, many arboviral diseases, and also simply because they constitute an intolerable biting nuisance (Youdeowei and Service, 1983; Curtis, 1994; Collins and Paskewitz, 1995). In a worldwide consideration, malaria has been said to be the most epidemic disease. Thus, the effect of vector borne diseases is a major threat to human survival on earth. *Anopheles stephensi* Liston (Diptera: Culicidae) predominantly breeds in wells, overhead or ground level water tanks, cisterns, coolers, roof gulters, and artificial containers (Collins and Paskewitz, 1995). Furthermore, it has a wide distribution and is a major vector in India as well as in some of the West Asian countries and has been shown to be directly responsible for about 40–50% of the annual malarial incidence (Curtis, 1994; Collins and Paskewitz, 1995). It transmits malaria in the plains of rural and urban areas of India. Biotechnologists and Entomologists agree that, mosquito control efficiency should be with selectivity for a specific target organism. New control methodologies aim at reducing mosquito breeding sites and biting activity by using a combination of chemical–biological control methods soothing several advocated biocontrol methods to reduce the population of mosquito and to reduce the man–vector contact (Service, 1983).

Recently, there has been a major concern for the promotion of botanicals as environmentally friendly pesticides, microbial sprays, and insect growth regulators amidst other control measures such as beneficial insects and all, necessitate an integration of supervised control (Ascher et al., 1995; Senthil Nathan et al., 2004, 2005b,c,d). The development of insects growth regulators (IGR) has received considerable attention for selective control of insect of medical and veterinary importance and has produced mortality due to their high neurotoxic effects (Wandscheer et al., 2004; Senthil Nathan et al., 2005a).

Although, biological control has an important role to play in modern vector control programs, it lacks in the provision of a complete solution by itself. Irrespective of the less harmful and ecofriendly methods suggested and used in the control programmes, there is still need to depend upon the chemical control methods in situations of epidemic out break and sudden increase of adult mosquitoes. Hence, insecticides are known for their speedy action and effective control during epidemics. Nonetheless, they are preferred as effective control agent to reduce the mosquito population irrespective of their side effects.

Recent studies stimulated the investigation of insecticidal properties of plant derived from materials or botanicals and concluded that they are environmentally safe, degradable, and target specific (Senthil Nathan and Kalaivani, 2005). Muthukrishnan and Puspalatha (2001) evaluated the larvicidal activity of extracts from *Calophyllum inophyllum* (Clusiaceae), *Rhinacanthus nasutus* (Acanthaceae), *Solanum suratense* (Solanaceae) and *Samadera indica* (Simaroubaceae), *Myriophyllum spicatum* (Haloragaceae) against *A. stephensi*. Several indigenous plants viz, *Ocimum basilicum*, *Ocimum santum*, *Azadirachta indica*, *Lantana camera*, *Vitex negundo* and *Cleome viscosa* were studied for their larvicidal action on the field which collected fourth instar larva of *Culex quinquefasciatus* (Kalyanasundaram and Dos, 1985). Murugan and Jeyabalan (1999) reported that *Leucas aspera*, *O. santum*, *A. indica*, *Allium sativum* and *Curcuma longa* had a strong larvicidal, antiemergence, adult repellency and antireproductive activity against *A. stephensi*. In addition *Pelargonium citrosa* (Jeyabalan et al., 2003), *Dalbergia sissoo* (Ansari et al., 2000a) and *Mentha piperita* (Ansari et al., 2000b) were shown to contain larvicidal and growth inhibitory activity against *A. stephensi*.

The Meliaceae plant family has been known as a potential source for insecticide properties. Extracts from the neem and other plants seeds and leaves have shown excellent insecticidal properties against mosquito vector and were at the same time very eco-friendly (Schmutterer, 1990; Senthil Nathan et al., 2005a). Recently, the efficacy of these neem products on mosquitoes were established (Chavan, 1984; Zebitz, 1984, 1986; Schmutterer, 1990; Murugan et al., 1996; Su and Mulla, 1999). The present investigation was undertaken to study the effect of *M. azedarach* against the larvae of *A. stephensi* (Liston) mosquito.

2. Methods

2.1. Mosquito culture

A. stephensi eggs were collected from in around the Vivekananda College Campus, Namakkal District, Tamil Nadu and larvae were reared in plastic and enamel trays in tap water. They were maintained at 27 ± 2 °C, 75–85% relative humidity under 14:10 light and dark photo period cycle. Larvae were fed with diet Brewers Yeast, dog biscuits and algae collected from ponds in the ratio of 3:1. Pupae were transferred from the trays to a cup containing tap water and placed in screened cages ($23 \times 23 \times 32$ cm) where the adult emerged. The adults of *A. stephensi* were reared in the glass cages of $30 \times 30 \times 30$ cm dimension. Adults were continuously provided with 10% sucrose solution in a jar with cotton wick. On day 5 postemergence the adult females were

deprived of sugar from 12 h then provided with a mice placed in resting cages overnight for blood feeding. Seven days after emergence they were maintained at the physical condition as that of larva.

2.2. Methanolic extracts of leaves and seeds of *M. azedarach*

Methanolic extracts of leaves and seeds of *M. azedarach* were collected from trees of natural forests of Kolli hills, Namakkal District, Tamil Nadu. Extracts of seed and leaves were obtained according to the following methodology. First, the plant seeds and leaves were crushed to fine particle size and dried in an oven at 35 °C for 20 h. Extraction was carried out according to the procedure of Warthen et al. (1984). In a 1000 ml flask, 100 g of crushed and dried plant materials in 1000 ml of methanol were stirred for 3 h. After leaving the methanolic solution to rest overnight, it was filtered through Whatman no. 40 filter paper (Whatman International Ltd., Maidstone, England). The solid filtration residue was extracted again following an identical procedure, and the two filtrates were combined. The solvent was removed by vacuum evaporation in a rotary evaporator, and an oily dark red residue from seeds, dark green color extract from leaves was obtained. That crude extract was used to prepare stock solution.

2.3. Preparation of stock solution

The known amount (100 mg/ml) of filtered crude extract obtained from the above process was serially diluted to obtained the desired concentration. The stock solution was serially diluted to prepare the test solutions of 0.25%, 0.50%, 1.0% and 2.0%. One drop of emulsifier (Tween 20, Sigma Chemical Company, St. Louis, MO, USA) was added with the seed and leaf extracts to ensure complete solubility of the material in water.

2.4. Bioassays and larval mortality

Bioassays were performed with first to fifth instars of *A. stephensi* using concentration from 0.25%, 0.5%, 1.0% and 2.0% of the *M. azedarach* seed and leaf extracts. A minimum of 20 larvae/concentration were used for all the experiments and this experiments were replicated five times. The effective concentration (EC_{50}) was calculated using Probit analysis (Finney, 1971).

For mortality studies, 20 larvae each of first to fourth instars and pupae were introduced in 250 ml glass beaker containing various concentrations (0.25%, 0.5%, 1.0% and 2.0%) of the *M. azedarach* seed and leaf extracts supplemented with 50 mg/l of yeast extract. A control was maintained. The treatments were replicated five times and each replicate set contained one control. The percentage mortality was calculated by using the formula (1) and corrections for mortality when necessary were done using Abbot's (1925) formula (2)

$$\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100 \quad (1)$$

$$\text{Corrected percentage of mortality} = \left(1 - \frac{n \text{ in T after treatment}}{n \text{ in C after treatment}}\right) \times 100 \quad (2)$$

where n = number of larvae, T = treated, C = control.

2.4.1. Adulticidal assay

A. stephensi fresh adults were exposed to filter paper treated with 0.25–2% *M. azedarach* leaf extract. The paper was kept inside the beaker. Muslin cloth covering the beaker was also treated. Control insects were exposed only to methanol treated paper and muslin cloth. Mortality count was taken after 24 h (Sharma et al., 1992).

2.4.2. Oviposition assay

Different quantities of plant extract from a stock solution were mixed thoroughly with 200 ml of rearing food in 250 ml glass jars to obtain the concentration desired for the tests with *A. stephensi*. The gravid females were given a choice between treated and control jars. During the tests, the groups of females were kept separate for 48 h in cages measuring $25 \times 25 \times 30$ cm. After the eggs were counted, the oviposition deterrence index (ODI) (Hwang et al., 1982) was calculated by using the formula (3)

$$\text{ODI} = \frac{\text{Nt} - \text{Ns}}{\text{Nt} + \text{Ns}} \times 100 \quad (3)$$

2.4.3. Ovicidal assay

A. stephensi eggs were released in water. The test extracts were added in desired quantities (0.25–2.0%) and hatching was observed for 1 week. The eggs were then exposed to deoxygenated water and the number of hatching eggs was recorded. Percentage hatching was compared with the control in which only methanol was used (Sharma et al., 1992).

2.4.4. Repellency activity

Different concentrations of plant extract were mixed thoroughly with 10 ml of goat blood in glass plates. The untreated blood served as the control. 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 the plant extracts was calculated as previously described (WHO, 1996).

2.4.5. Total larval and pupal duration

To assay the growth factors of *A. stephensi*, test solutions of sublethal concentrations like 0.1%, 0.25%, and

0.5% were prepared in an enamel tray of $30 \times 25 \times 5$ cm dimension. A known number of eggs were made to hatch and the total larval duration (days) was calculated from hatching to pupation period. The pupa was placed in a small container closed with a transparent mesh, so that the adults were kept trapped. The pupal duration (days) was calculated from the pupal molt to the emergence of imago.

2.5. Fecundity

The fecundity experiments were conducted by taking equal number of male and female *A. stephensi*, which had emerged from the control and treated sets of each concentration. They were mated in the cages of $30 \times 30 \times 30$ cm dimension individually to each concentration. Three days after the blood meal, eggs were collected daily from the small plastic bowls containing water kept in ovitraps in the cages. The fecundity was calculated by the number of the eggs laid in the ovitraps divided by the number of female let to mate. (The death of the adult in the experiment was also considered.)

2.6. Hatchability

The eggs collected for the fecundity tests were used for the hatchability tests. The eggs were placed in the enamel tray for hatching. The hatchability percentage was calculated by the number of eggs hatched.

2.7. Effect of plant products on mosquito longevity

The adult longevity of male and female *A. stephensi* was also recorded. This was calculated by the number of days lived by the imago. The emergence days and mortal days of the adult were recorded and the means were calculated to give the mean longevity in days.

2.8. Statistical analysis

The analysis program Probit (Finney, 1971) was used for the determination of EC_{50}. Data from biology, mortality, oviposition deterrence and effective concentrations were subjected to analysis of variance (ANOVA of arcsine square root transformed percentages). Differences between the treatments were determined by Tukey's multiple range test ($P \leqslant 0.05$) (SAS Institute, 1988; Snedecor and Cochran, 1989).

3. Results

Results on the mortality, reproduction, egg hatchability, ovipositional deterrency and repellency effects of 2% of these extracts on the *A. stephensi* reported in the present study, confirm their potential for control

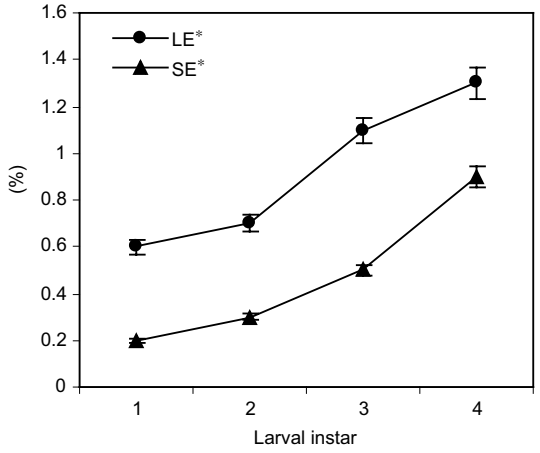

Fig. 1. Effective concentrations (EC_{50}) of *Melia azedarach* against first to fourth instar larvae of *A. stephensi*. *Values are mean of five replicates, LE—leaf extract, SE—seed extract, ± standard error.

of the mosquito populations. An EC_{50} value of the malarial vector was shown in Fig. 1. Seed extract was most potent in all experiments with least EC_{50} (0.2%, 0.3%, 0.5% and 0.9% first to fourth instars respectively). It is clearly pointed out that the high concentration (2%) of the seed extracts produced high mortality in the initial larval stages (96%). At 2% concentrations the seed extracts killed more than 92% of the fifth instar larvae, pupae and more than 91% of adult respectively. Larval mortality was higher in 2% concentration of seed extract (more than 96%) in first instar when compared with all other instars. Larval mortality was considerably less in leaves extract when compared with seeds extract (82%) in first instar. The second to fourth instar larvae showed highest mortality in 2% concentration (94%, 94%, 93% and 92% respectively) of seeds extract than that of leaves (Table 1). The pupal (Table 1) and adult mortality was higher (92.3% and 90.9%) in 2% concentration of seed extract.

The effect of *M. azedarach* extract on larval, pupal and adult of *A. stephensi* is shown in Fig. 2(a–d). It was also observed that first and second instar larvae were more susceptible to both leaves and seed extract. The same trend was also noticed in adult fecundity and longevity. Adult longevity and fecundity also markedly decreased by the *M. azedarach* extract treatment (Fig. 2c and d). An adverse sub-lethal effect in pupa exposed to *M. azedarach* was evident. In addition to significantly lower survivorship and protracted development, larval duration was reduced markedly (Fig. 2a). The plant extracts drastically reduced the fecundity of the females and only few adults survived. Plant extracts reduced adult longevity (Fig. 2c). The duration of larval instars and the total developmental time was prolonged even when larvae were fed on lower (0.25% and 0.5%) concentrations of *M. azedarach* leaf and seed extract (Fig. 2a–d). In the present study, application of *M. azed-*

Table 1
Percentage mortality of *A. stephensi* after the treatment of *M. azedarach*

M. azedarach treatment concentration (%)	Larval instar				Pupal mortality (%)	Adult mortality (%)
	I	II	III	IV		
Control	02.1 ± 0.01^{g}	01.7 ± 0.001^{h}	01.2 ± 0.01^{i}	09.00 ± 0.01^{h}	08.0 ± 0.01^{g}	01.2 ± 0.01^{g}
LE						
0.25	15.2 ± 1.0^{f}	13.4 ± 0.9^{g}	11.2 ± 1.0^{h}	10.6 ± 0.9^{fg}	11.1 ± 0.8^{f}	8.9 ± 0.5^{f}
0.50	32.6 ± 2.6^{e}	29.4 ± 2.5^{e}	20.6 ± 1.8^{f}	17.4 ± 1.2^{f}	15.3 ± 1.1^{f}	12.7 ± 0.8^{f}
1.0	58.4 ± 6.3^{d}	54.9 ± 6.3^{d}	47.4 ± 4.9^{d}	39.4 ± 3.5^{d}	37.2 ± 3.6^{d}	32.9 ± 2.9^{d}
2.0	82.3 ± 7.7^{b}	79.5 ± 6.7^{b}	76.2 ± 7.1^{b}	74.5 ± 6.9^{b}	72.7 ± 6.9^{b}	74.6 ± 6.2^{b}
SE						
0.25	19.5 ± 0.7^{g}	16.3 ± 1.4^{g}	15.8 ± 1.0^{g}	13.4 ± 0.9^{f}	13.1 ± 0.8^{f}	11.2 ± 0.7^{f}
0.50	38.4 ± 4.2^{e}	35.9 ± 2.9^{e}	31.6 ± 2.7^{e}	28.1 ± 1.5^{e}	27.4 ± 2.5^{e}	25.3 ± 1.9^{e}
1.0	71.6 ± 7.2^{c}	68.5 ± 8.2^{bc}	65.4 ± 7.3^{bc}	62.7 ± 7.2^{c}	60.2 ± 6.9^{c}	61.5 ± 5.9^{c}
2.0	96.4 ± 4.6^{a}	93.7 ± 6.3^{a}	94.1 ± 6.0^{a}	92.6 ± 7.0^{a}	92.3 ± 7.2^{a}	90.9 ± 7.0^{a}

Means (±SE) followed by the same letters within column of indicate no significant difference ($P \leqslant 0.05$) in a Tukey test.
Values are mean of five replicates, LE—leaf extract, SE—seed extract, ± standard error.

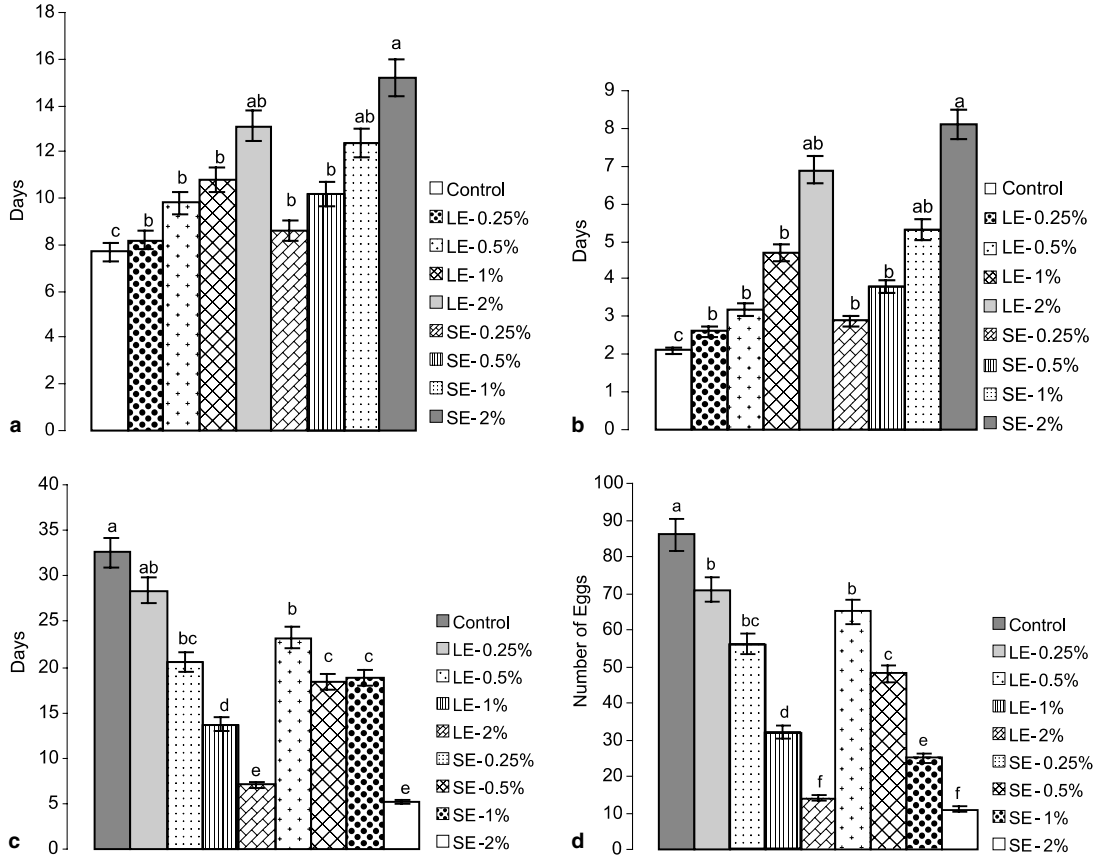

Fig. 2. (a) Total larval duration of *A. stephensi* after treatment with *M. azedarach*. Means (±SE) followed by the same letters within bars of indicate no significant difference ($P \leqslant 0.05$) in a Tukey test. (b) Total pupal duration of *A. stephensi* after treatment with *M. azedarach*. Means (±SE) followed by the same letters within bars of indicate no significant difference ($P \leqslant 0.05$) in a Tukey test. (c) Total adult duration of female *A. stephensi* after treatment with *M. azedarach*. Means (±SE) followed by the same letters within bars of indicate no significant difference ($P \leqslant 0.05$) in a Tukey test. (d) Number of eggs (fecundity) laid by the female after treatment with *M. azedarach*. Means (±SE) followed by the same letters within bars of indicate no significant difference ($P \leqslant 0.05$) in a Tukey test. LE—leaf extract, SE—seed extract, ± standard error.

arach extract greatly affected the growth of *A. stephensi*. The lower dose treatments affected their growth inhibition, malformation and mortality in a dose dependent manner. After *M. azedarach* treatment at a higher dose, the larvae die immediately before their pupal stage. The larvae become abnormal and irregular in movement.

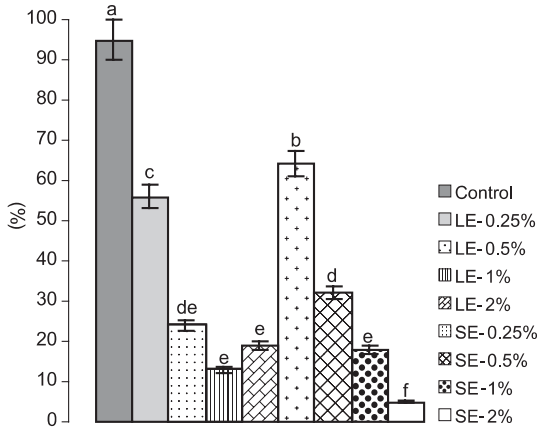

Fig. 3. Percentage egg hatchability of *A. stephensi* after treatment with *M. azedarach*. Means (±SE) followed by the same letters within bars of indicate no significant difference ($P \leqslant 0.05$) in a Tukey test. LE—leaf extract, SE—seed extract, ± standard error.

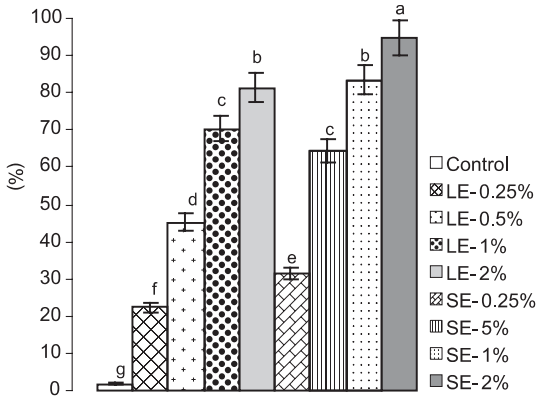

Fig. 5. Percentage repellency of *A. stephensi* after the treatment of *M. azedarach*. LE—leaf extract, SE—seed extract, ± standard error.

The present study clearly indicates that application of *M. azedarach* extract can disrupt the normal process of feeding and physiological response. In addition the percentage of egg hatchability drastically declined in 2% treatment of both leaf and seed extract (Fig. 3) also ovipositional deterrency significantly increased in highest concentration tested (Fig. 4). All the concentrations of leaf and seed extract used in these studies exhibited repellency activity against the adult mosquito of *A. stephensi* (Fig. 5). At 2%, *M. azedarach* leaf extract exerted a detrimental effect on *A. stephensi*, which was manifested by their very slow movements. These results suggest that at concentrations exceeding a certain threshold, repellents can act as insecticides.

M. azedarach extract treatment exhibited a detrimental effect upon larval growth and development of *A. stephensi*. The concentration-dependent reduction in weight relative to controls is directly related to the reduction in food consumption. Larval development was delayed and mortality rate increased in the *M. azedarach* treatment in 1% and above concentrations. These toxic effects may be independent of each other due to the differences in the way the larvae were exposed to the plant extracts.

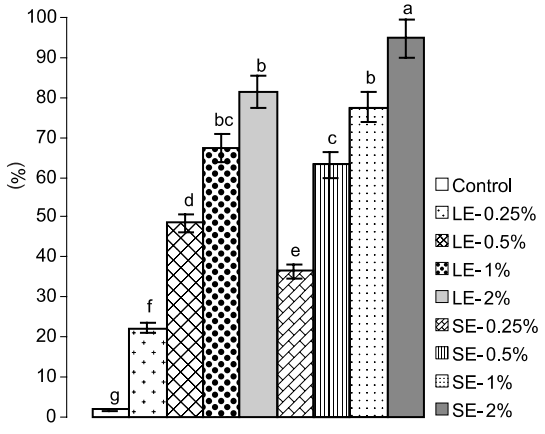

Fig. 4. Ovipositional deterrency (%) of *A. stephensi* after the treatment with *M. azedarach*. LE—leaf extract, SE—seed extract, ± standard error.

4. Discussion

The plants tested in the present study are reported to be eco-friendly and are not toxic to vertebrates (Al-Sharook et al., 1991). It is clearly proved that crude or partially purified plant extracts are less expensive and highly efficacious for the control of mosquitoes rather than the purified compounds or extracts (Jang et al., 2002; Cavalcanti et al., 2004).

The effects of these extracts on the biology, reproduction and adult emergence of the mosquitoes are remarkably greater than those reported for other plant extracts in the literature. For example 50% inhibition of the emergence of the adult mosquitoes was observed by the use of *C. inophyllum*, *S. suratense*, *S. indica* and *Rhinocanthus nasutus* leaf extracts (Muthukrishnan and Puspalatha, 2001). Similarly 88% of the adult mortality was observed by the use of *P. citrosa* leaf extracts at 2% concentration (Jeyabalan et al., 2003).

Meliaceae plant family is an insect growth regulator against many insect pests (Saxena et al., 1984; Jacobson, 1987; Schmutterer, 1990; Hammad et al., 2001; Gajmer et al., 2002; Banchio et al., 2003; Wandscheer et al., 2004). The growth regulatory effect is the most important physiological effect of *M. azedarach* on insects. It is because of this property that family Meliaceae has emerged as a potent source of insecticides.

Exposure of *A. stephensi* larvae to sub-lethal doses of neem leaves extract in the laboratory prolonged larval development, reduced pupal weight and ovipostion (Murugan et al., 1996; Su and Mulla, 1999). In the field, delayed phenology of surviving larvae and reduced

pupal weight are common occurrence after treatment with neem (Zebitz, 1984, 1986). The direct and indirect contribution of such effects to treatment efficacy through reduced larval feeding and fitness need to be properly understood in order to improve the use of *M. azedarach* for management of *A. stephensi*. The results of this study indicate the plant-based compounds such as limonoids (compounds present in the Meliaceae plant family seed) may be effective alternative to conventional synthetic insecticides for the control of *A. stephensi*.

Undoubtedly, plant derived toxicants are a valuable source of potential insecticides. These and other naturally occurring insecticides may play a more prominent role in mosquito control programs in the future (Mordue and Blackwell, 1993). The results of this study will contribute to a great reduction in the application of synthetic insecticides, which in turn increase the opportunity for natural control of various medicinally important pests by botanical pesticides. Since these are often active against a limited number of species including specific target insects, less expensive, easily biodegradable to non-toxic products, and potentially suitable for use in mosquito control programme (Alkofahi et al., 1989), they could lead to development of new classes of possible safer insect control agents. Plant allelochemicals may be quite useful in increasing the efficacy of biological control agents because plants produce a large variety of compounds that increase their resistance to insect attack (Berenbaum, 1988; Murugan et al., 1996; Senthil Nathan et al., 2005a).

The intensive use of pesticides produces side effects on many beneficial insects and also pose both acute and chronic effects to the milieu (Abudulai et al., 2001). Recently, bio-pesticides with plant origins are given for use against several insect species especially disease-transmitted vectors, based on the fact that compounds of plant origin are safer in usage, without phytotoxic properties; also leave no scum in the environment (Schmutterer, 1990; Senthil Nathan et al., 2004, 2005a,d). The present study clearly proved the efficacy of *M. azedarach* extract on larvae, pupae and adult of *A. stephensi*. Further studies such as mode of action, synergism with the biocides under field condition are needed.

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