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Efficacy of *Melia azedarach* on the larvae of three mosquito species *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae)

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Abstract

Bioassays with an ethanolic extract of *Melia azedarach* L. were performed on the larval stages of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* [*Stegomyia aegypti* sensu Reinert *et al*., 2004]. The leaf and fruit extracts of *M*elia *azedarach* were prepared using 80% ethanol 1:10 (w/v) and soxhlet for 6 h. and evaporated to dryness at room temperature. The dried material was dissolved in an emulsifier. The present study revealed that both seed and leaf extracts produced significant larval mortality in all larval stages of the three mosquito species. The fruit extract (50 ppm) produces 81% larval mortality on the IV instar larvae of *An*. *stephensi* and 79% larval mortality in *Cx*. *quinquefasciatus* and *Ae*. *aegypti*. In the present study it was observed that the first and second instar larvae of *An*. *stephensi* and *Cx*. *quinquefasciatus* were more susceptible to all concentrations of leaf and fruit extracts. When compared to the first two instar stages, the third and fourth instar larvae of the three mosquito species exhibited lower mortality when exposed to all the concentrations (10, 20, 30, 40 and 50 ppm). Therefore, it can be inferred from the present study that the *M. azedarach* extracts may be an effective larvicidal agent which could be used to control populations of *Cx*. *quinquefasciatus*, *An*. *stephensi* and *Ae*. *aegypti*.

Key words: *Aedes aegypti* (*Stegomyia aegypti)*, *Anopheles stephensi*, *Culex quinquefasciatus*, larval mortality, larvicidal agent, *Melia azedarach,* mosquito.

Introduction

Worldwide, mosquitoes are a major public health problem. They are estimated to transmit diseases to more than 700 million people annually and are predicted to be currently responsible for the deaths of about one in 17 people (WHO, 2005). Insect vectors, especially mosquitoes, are responsible for spreading serious human diseases like malaria, Japanese encephalitis, yellow fever, dengue and filariasis. *Anopheles stephensi* has a wide distribution and is a major vector of malaria in the Indian subcontinent as well as in some of the west Asian countries. Of the 2.5 billion people at risk, more than 500 million become severely ill with malaria every year, and more than one million die from the effects of the disease (WHO, 2007). *Aedes aegypti* [*Stegomyia aegypti* sensu Reinert *et al*., 2004] is considered to be a vector of dengue fever, a disease endemic to South East Asia, Africa, and the Americas (Maillared *et al*., 1993). The incidence of dengue fever has increased fourfold since 1970, and nearly half the world's population is now at risk. In 1990, almost 30% of the world population (1.5 billion people) lived in regions where the estimated risk of dengue transmission was greater the 50% (Hales *et al*., 2002). The lymphatic filariasis caused by *Wuchereria bancrofti* and transmitted by the mosquito *Culex quinquefasciatus* is found to be endemic in the Indian subcontinent (Rajasekariah *et al*., 1991). For a number of the vector- borne infections, the most effective method of controlling their transmission is through control of their vectors. As chemical insecticides are often toxic to nontarget organisms (Srivastava & Sanjay, 1997), this has prompted a search for biologically active plant materials with larvicidal properties.

The flora of India has a rich aromatic plant diversity with potential for development of natural insecticides for the control of mosquito and other pests. The Meliaceae plant family is known to contain a variety of compounds, which show insecticidal, antifeedent, growth regulating and development modifying properties (Nugroho *et al*., 1999; Greger *et al*., 2001; D'Ambrosio & Guerriero, 2002; Nakatani *et al*., 2004). *Melia azedarach* L. (Sapindales: Meliaceae), know as Chinaberry or Persian lilac tree, is a deciduous tree native to northwestern India and has long been recognized for its insecticidal properties but yet to be properly 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. The effects of compounds, products and extracts obtained from *Melia azedarach* on insects have been reviewed by Ascher *et al*. (1995).

Effects of *Melia 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 & Saehoon, 2005; Defago *et al*., 2006). The efficacy of *M. azedarach* against *Anopheles stephensi* (Senthil Nathan *et al*., 2006), and *Aedes aegypti* (Wandscheer *et al*., 2004; Prophiro *et al*., 2008) have also been reported. Although biological control has an important role to play in modern vector control programmes, it is not a complete solution by itself. The present investigation was undertaken to study the effect of *Melia azedarach* against the larvae of *Ae. aegypti*, *Cx.* q*uinquefasciatus* and *An. stephensi*.

Materials and Methods

Mosquito culture

The eggs of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* were obtained from the National Institute of Communicable Diseases (NICD), Mettupalayam of Coimbatore, Tamil Nadu. Individual larvae were reared in plastic trays in tap water and were maintained at 23± 4^{o}C, 60- 80% relative humidity under 10:14 light and dark photoperiod cycle. The larvae were fed on a diet of brewers yeast and dog biscuits in the ratio of 3:1. Newly formed pupae were transferred from the trays to a cup containing water and placed in screened cages (60×30×45 cm) where the adult emerged. The adults were continuously provided with 5% sucrose solution mixed with zincovit vitamin drops in a jar with a cotton wick. On day four post emergence the adult females were deprived of sugar for 12 hours then provided with a shaved chicken placed in resting cages overnight for blood feeding. Wet filter paper was placed on the corners for egg laying.

Preparation of *Melia azedarach* extract

The seeds were collected from the local area and air dried at room temperature. The dried material was powdered using an electronic grinder. Extraction was carried out according to the procedure of Warthen *et al*. (1984). 20g. of powder was mixed with 250 ml. of methanol and extracted with soxhlet apparatus for 6 h. The obtained extract was air dried at room temperature and the crude extract was used to prepare stock solution.

Preparation of stock solution

A known amount (100 mg./ ml.) of filtered crude extract obtained from the above process was serially diluted to obtain the desired concentration. The stock solution was serially diluted to prepare the test solutions of 10, 20, 30, 40, & 50 ppm. One drop of emulsifier (Tween 20, Sigma Chemical Company, St. Louis, MO, USA) was added with the extract to ensure complete solubility of material in water.

Bioassays and larval mortality

Bioassays were performed with first to fourth instars of *An. stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* using concentration of 10, 20, 30, 40 and 50 ppm of *M. azedarach* extracts. A minimum of 10 larvae/ concentration was used for all the experiments and replicated five times. The percentage mortality was calculated using formula (I) and the results corrected using Abbot's (1925) formula (II)

$$\textbf{(I)}\quad \textbf{Percentage of mortality} = \frac{\textbf{Number of dead larvae}}{\textbf{Number of larvae introduced}} \times \mathbf{100}$$

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

Results

The percentage mortality of fourth instar larvae of *An. stephensi* increased significantly with concentration of the extract (Table 1). Continued survival of larvae of all instars tested indicates that Tween 20 does not adversely affect larval development. It was also observed that first and second instar larvae were more susceptible to the extracts (Figure 1). When compared to second stage larvae, the third stage larvae exhibited lower larval mortality 43.9% at 10 ppm increased to 86.1%

at 50 ppm. Likewise a similar pattern of larval mortality was observed in fourth stage larvae from 40.7% to 81.9% when treated with 10 ppm and 50 ppm.

Table 1. Effect of *Melia azedarach* on the larvae of three mosquito species

Mosquito species	Concentration of plant extract (ppm)	Percentage larval mortality			
		Larval stage			
		I instar	II instar	III instar	IV instar
Anopheles stephensi	10	48.2	47.8	43.9	40.7
	20	54.5	51.1	48.3	46.4
	30	75.9	69.1	56.2	53.5
	40	86.2	81.9	77.5	76.1
	50	95.1	93.0	86.1	81.9
Culex quinquefasciatus	10	48.5	47.2	43.5	40.5
	20	53.9	51.5	48.2	46.1
	30	76.5	68.1	56.8	53.6
	40	85.9	81.7	77.2	76.9
	50	94.6	92.9	86.5	81.3
Aedes aegypti	10	41.0	38.5	36.4	32.4
	20	54.2	49.2	48.2	39.2
	30	75.3	54.8	50.2	50.0
	40	86.4	78.2	71.0	75.4
	50	88.6	82.5	80.2	78.8

Values are the mean of five replications,
Computed as C- T / C 100,
Where T= Percentage damage in treatment,
C= Percentage damage in control.

Figure 1. The effect of *Melia azedarach* on the larvae of three mosquito species

The larvicidal activity of *M. azedarach* extracts on *Cx. quinquefasciatus* larvae is also presented in Table 1. It is evident that all concentration of extracts showed moderate and high larvicidal effect (Figure 1). First instar larval mortality was 48.5% at 10 ppm and 94.6% at 50 ppm concentration after 24 h. However, the second instar larvae of *Cx. quinquefasciatus* exhibited 47.2% mortality at 10 ppm and rose to 92.9% at 50 ppm. Compared to the first two instars, the third stage showed lower larval mortality at all the concentrations: 43.5%, 48.2%, 56.8%, 77.2% and 86.5% larval mortality at 10, 20, 30, 40, and 50 ppm respectively. The fourth stage larva was exhibited low mortality (40.5%) at 10 ppm and 81.3% at 50 ppm concentration.

*M*elia *azedarach* extract was an effective tool on controlling *Ae. aegypti* (Table 1). The highest larval mortality of 88.6% was noted at 50 ppm concentration on first stage larvae, and a lower mortality of 41.0% was recorded at 10 ppm. Treatment with 10 ppm to 50 ppm extracts on second stage larvae gave 38.5% and 82.5% mortality. The corresponding figures for third stage larvae were 36.4% and 80.2% and for fourth stages, 32.4% and 78.8%.

Discussion

The present study confirmed that *M. azedarach* seed extracts were potential agents for the control of mosquito population.

The intensive use of pesticides produces side effects on many beneficial insects and also poses both an acute and a chronic threat to the environment (Abudulai *et al*., 2001). The Meliaceae plant family is used as a growth regulator against many insect pests (Saxena *et al*., 1984; Jacobson, 1987; Schmatterer, 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 the Meliaceae has emerged as a potent source of insecticide. Furthermore; the crude extracts of plants may be more effective compared to the individual active compounds, due to natural synergism that discourages the development of resistance in the vectors (Maurya *et al*., 2007). Al-Sharook *et al*. (1991) reported the *M. azedarach* extracts to be eco-friendly and not toxic to vertebrates. It is clearly proved that crude or partially purified plant

extracts are less expensive and highly efficacious in the control of mosquitoes compared to purified compounds or extracts (Jang *et al*., 2002; Cavalcanti *et al*., 2004).

All of the ethanol extracts of *M. azedarach* tested caused mortality among the larvae of vector mosquito species to some extent. However, in the present study higher mortality was observed in all the larval stages of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*. The larval mortality slowly decreased when the larval ages increased. Similar results were also obtained by Nirmal Sharma (1998); Rossi *et al*. (2007) and Prophira *et al*. (2008). The direct and indirect contribution (reduced larval feeding) need to be properly understood in order to improve the use of *M. azedarach* for management of mosquitoes.

The phytochemical azadirachtin, and other limonoids relating to *Azadirachta indica*, exhibit feeding inhibitory and growth regulatory activities. These compounds are highly biodegradable and pose only weak toxicity to non-target organisms and have low persistence of systemic action (Schaaf *et al*., 2000). The leaves of *M. azedarach* also contain sufficient quantity of the limonoids with insecticidal activity, for them to act synergistically.

Thus it is concluded from the above study that extract seeds of *M. azedarach* is a potent larvicide against vector mosquito species, and its toxic potentiality depends on concentration of extract, larval stage and exposure time. An extract of *M. azedarach* is easy to prepare and it is a safe and cheap natural product to be used to suppress mosquito population. Further studies such as mode of action, synergism with the biocides under field condition are required.

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