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Larvicidal Activity of Some Medicinal Plant Extracts Against Malaria Vector *Anopheles stephensi*

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Abstract: Mosquitoes are vectors of etiologic agents of malaria, filariasis and viral disease. *Anopheles stephensi* Liston (Diptera) is the primary vector of malaria in India and other West Asian countries and improved methods of control are urgently needed. In the present investigation the effect of certain plants on the larval mortality, biology and biochemical parameters of *Anopheles stephensi* were studied. Ten medicinal plants were collected, dried and extracted with methanol and used for the present study. Out of ten plants tested 100% mortality of *Anopheles stephensi* was observed in four plants namely *Albizia amara*, *Areca catechu*, *Leucas aspera* and *Ocimum sanctum* after 24 h treatment. The pupation and adult emergence was appeared to be dose dependent (1, 3 and 5% of extracts). The treated larvae showed reduction in the levels of carbohydrate, DNA and RNA when compared to the control which proved that the vector management is possible by using different medicinal plants. The medicinal plants were easily biodegradable than the synthetic insecticides, the plant products are less hazardous; they afford a rich storehouse of chemicals of diverse biological activities.

Key words: *Anopheles stephensi*, larvicidal activity, *Albizia amara*, *Areca catechu*, *Leucas aspera*, *Ocimum sanctum*

INTRODUCTION

Mosquitoes are responsible for the biological transmission of several dreaded diseases like filariasis, dengue fever, Japanese encephalitis etc. Despite an array of control measures taken to suppress the mosquito population, the latter flourish unabatedly to take heavy toll of human life every year, particularly in developing countries having poor socio-economic conditions (Sagar and Schgal, 1997).

Malaria exists in many parts of the world; but the incidence varies from place to place. As with other diseases, malaria is said to be endemic in an area when it occurs at a relatively constant incidence by natural transmission over successive years. Recognized categories of endemicity are based on the incidence and severity of symptoms (spleen enlargement) in adults and children, an epidemic occurs when the incidence in endemic area rises or a number of cases of the disease occur in a new area. Malaria is said to be in a stable state when there is little seasonal or annual variation in the disease incidence, predominantly transmitted by a strongly anthropophilic, *Anopheles* vector species.

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Anopheles mosquitoes occur almost worldwide, with the exception of cold temperate areas and there are over 400 known species. The four species of human pathogenic Plasmodium are transmitted significantly in nature by only some 30 species of *Anopheles.* Some of these have very local significance; others can be infected experimentally but have no natural role and perhaps 75% of Anopheles are quite refractory (intolerant) to malaria. Of the vectorial species, a handful is important in stable malaria, while others only become involved in epidemic spread of unstable malaria). Vectorial status can vary across the range of a taxon, an observation that may be due to the hidden presence of sibling species that lack morphological differentiation, but differ slightly in biology and have substantially different epidemiological significance, as in the *Anopheles gambiae* complex.

Mosquito control is a difficult task and is becoming even more so due to a variety of factors including the development of insecticide resistance and concern over environmental pollution and many of them are immuno suppressants (Srivastava and Sharma, 2000). Because of resistance in the vectors, conventionally insecticides, the chief weapon of the vector, are becoming ineffective (Vincent, 2000).

Plants are rich source of bioactive organic chemicals and synthesize a number of secondary metabolites to serve as defence chemicals against attack. These chemicals may serve as insecticides, antifeedants, oviposition deterrents, repellents, growth inhibitors, juvenile hormone mimics, moulting hormones, as well as attractants (Murugan *et al*., 1996). The botanicals offer an advantage over synthetic pesticides as they are less toxic, less prone to be development of resistance and easily biodegradable. Although only 10,000 secondary metabolites have been chemically identified, their total number may exceed 4, 00,000 (Ignacimuthu, 2000).

Botanical derivatives in mosquito control, especially for larvae, as an alternative to synthetic insecticides offer a more environmentally friendly method of insect control (Irungu and Mwangi, 1995). Various plants species have been exploited, throughout the world, to control the mosquito populations. (Muthukrishnan *et al*., 1997). Medicinal plants are very effective in mosquito control. A variety of plants are reported to contain insecticidal compounds (Grainge and Ahmed, 1998). Application of these plant extracts is eco-friendly and it is also beneficial to human being.

Hence, in the present investigation the effect of certain medicinal plants on the mortality, biology and biochemical parameters of *Anopheles stephensi* has been studied.

MATERIALS AND METHODS

Plant Collection

Medicinal plants were collected from Erode district, Tamilnadu, India. The plant species studied were given in the Table 1.

Table 1: List of plants selected for larvicidal activity against *Anopheles stephensi*

Botanical name	Common name	Medicinal property	Parts used for mosquitocidal action
Adathoda vasica Nees.	Adothoda	Leaves and roots are used in cough, chronic bronchitis, asthma etc.	Leaves
Albizzia amara Roxb.	Albizia	Cooling effect, antidandruff, used in stomach ailments	Leaves
Areca catechu L.	Betal	Act as a stimulus upon the digestive organs and as preventive of dysentery	Leaves
Eucalyptus globulus Labill	Eucalyptus	Used as an antiseptic, used in respiratory system disorders	Leaves
Leucas aspera Spreng.	Tumbai	Antipyretic, leaves are used in chronic rheumatism	Leaves
Musa paradisiaca L.	Banana	Root and stem are used as tonic in blood and venereal diseases	Leaves

Table 1: Continued

Botanical name	Common name	Medicinal property	Parts used for mosquitocidal action
Ocimum sanctum L.	Tulsi	Used to treat digestive complaints	Leaves
Phyllantus amarus	Leucas	Antipyretic, leaves are	Leaves
Schum and Thonn		used in chronic rheumatism	Leaves
Piper nigrum L.	Pepper	Leaves and roots are used in cough, chronic bronchitis, asthma etc.	
Tamarindus indica L.	Tamarin	Used to treat digestive complaints	Leaves

Extraction

Plants leaves were air-dried under shade, powdered and extracted (crude) with methanol in the ratio of 1:10 (W/V) (Pushpalatha and Muthukrishnan, 1995).

Mosquito Collection

Third and fourth instar larvae of the mosquito, *Anopheles stephensi* were collected from the stagnant rainwater between rocks, near C.N.C College, Erode Tamilnadu, India.

Mortality Bioassay

The dried powdered leaves of each plant were dissolved in methanol to obtain 10% stock solution. Ten milliliter of the solution was delivered in a tray, mixed thoroughly with 100 mL of distilled water. In each tray, ten mosquito larvae were introduced. Larval food consisted of 0.4 g of ground dog biscuit and 100 mL of distilled water with 0.2 mL of methanol served as control. Mortality counts were taken 24 h after treatment.

Biology

The dried powdered leaves of each plant were dissolved in methanol to obtain a 10% stock solution. 1.0, 3.0 and 5.0% of each sample were prepared in different trays by mixing thoroughly with 100 mL of distilled water. In each tray, ten mosquito larvae were introduced. Larval food consisted of 0.4 g of ground dog biscuit. One hundred milliliter of distilled water with 0.2 mL of methanol served as control. Any abnormal behavior was recorded. Dead larvae were removed from each tray and recorded every 24 h.

Feeding

The feeding behavior of the treated mosquito larvae was observed in all the treatments.

Biochemical Parameters

After the treatment with various plant extracts the larvae was taken for further evaluations viz., carbohydrate (Dubios *et al*., 1958), isolation and estimation of DNA and RNA by Burton *et al*. (1956) and Ceriotti (1995), respectively.

RESULTS

Larval Mortality of III Instar Larvae of *Anopheles stephensi* after the Application of Plant

Figure 1 shows the percentage of mortality of III instar larvae after the treatment of plant extracts. The extracts exerted maximum insecticidal activity after 24 h exposure, out of the ten plants selected *Albizia amara*, *Areca catechu*, *Leucas aspera* and *Ocimum sanctum* were found to be very effective and showed 100% mortality after 24 h. The rest of the plants *Adathoda vasica*, *Eucalyptus globulus*, *Musa paradisiaca*, *Phyllanthus amarus*, *Piper nigrum* and *Tamarindus indica* showed 50, 80, 90, 85 and 90% mortality, respectively after 24 h. In the control more than 90% of the larvae developed into

Table 2: Biological evaluation of *Anopheles stephensi* after the treatment

	Days after treatment								
	1			2			3		
Treatments	LM	P	M	LM	P	M	LM	P	M
Control	-	-	A	+	+	A	-	+	A
Adathoda vasica									
1%	+	-	LA	+	+	LA	+	+	IA
3%	+	-	LA	+	+	IA	+	-	IA
5%	+	-	LA	+	-	IA	+	-	IA
Albizzia amra									
1%	+	-	LA	+	+	LA	+	-	LA
3%	+	-	LA	+	+	IA	+	-	LA
5%	+	-	LA	+	-	IA	+	-	IA
Areca catechu									
1%	+	-	LA	-	-	LA	+	-	IA
3%	+	-	IA	-	-	IA	+	-	IA
5%	+	-	IA	-	-	IA			
Eucalyptus globulus									
1%	-	+	LA	+	+	LA	+	-	IA
3%	+	-	LA	+	-	IA	+	-	
5%	+	-	IA	+	-	IA	+	+	
Leucas aspera									
1%	-	+	LA	+	-	LA	+	-	IA
3%	+	+	LA	+	-	IA	+	-	IA
5%	-	-	LA	+	-	IA	+	-	IA
Musa paradisiaca									
1%	-	-	A	+	+	LA	+	+	LA
3%	+	+	LA	+	-	LA	+	-	IA
5%	+	+	LA	+	-	LA	+	-	IA
Ocimum sanctum									
1%	-	+	LA	+	+	LA			
3%	+	-	IA	+	-	IA			
5%	+	-	IA						
Phyllantus amarus									
1%	-	+	LA	+	+	LA			
3%	+	-	IA	+	-	IA			
5%	+	-	IA						
Piper nigrum									
1%	+	-	LA	+	+	LA	+	-	LA
3%	+	-	LA	+	+	IA	+	-	IA
5%	+	-	IA	+	-	IA			
Tamarindus indica									
1%	-	-	A	+	+	LA	+	+	
3%	+	+	LA	+	-	LA	+	-	IA
5%	+	-	LA	+	-	IA	+	-	IA

LM: Larval mortality, LA: Less active, IA: Inactive, P: Pupation, M: Movement, A: Active, +: sign indicates the positive effect (dose and hours dependent manner). -: Non effect of plant extracts on *Anopheles stephensi*

normal adults. This study indicates that the continuous exposure of the larvae to the plant extracts effectively inhibited the pupal and adult emergence.

Effect of Plant Extracts on the Biology of III Instar Larvae of *Anopheles stephensi*

Table 2 showed the biological evaluations of III instar larvae after the treatment of plants extracts. The larval biology in 1 and 3% was less affected when compared to 5% treatment. The pupation and adult emergence appeared to be affected in a dose dependent manner. Pupation in 5% treatments was very less when compared to 1 and 3%. No mortality occurred among control larvae, 98% of them pupated and successfully developed into normal adults. Pupae arising from the treated larvae were also affected following treatment. Some of the pupae were straight and could not emerge from the pupal case.

After the treatment the larvae showed some abnormality in their movement. Some of the larvae were found to be less active and some were found to be inactive. The abnormality in their movement was dose dependent. In 5% treatments the larvae showed abnormal movement in the first day itself.

Biochemical Profiles of *Anopheles stephensi* after the Treatment

In the control the carbohydrate concentration was 1.7 mg/0.1 g. After the treatment with *Musa paradisiaca*, *Areca catechu*, *Ocimum sanctum* and *Piper nigrum* the carbohydrate concentration was significantly reduced to 0.3 mg/0.1 g as whereas when the larvae were treated with *Albizia amara* the carbohydrate concentration was reduced to 1.2 mg/0.1 g (Fig. 2).

The DNA and RNA profiles of the III instar larvae after the treatment were shown in Fig. 3 and 4, respectively. The DNA concentration of the control larvae was 3.5 mg/0.1 g. The treatment with *Musa paradisiaca* and *Tamarindus indica* reduced the concentration to 2.5 mg/0.1 g and 2.9 mg/0.1 g respectively, where as after the treatment with *Eucalyptus globulus* the concentration was 3.3 mg/0.1 g in the control larvae, the RNA concentration was 3.0 mg/0.1 g. The treatment

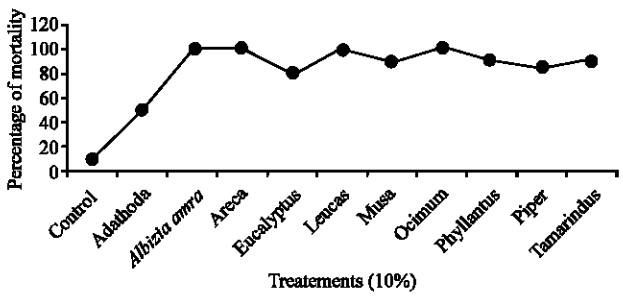

Fig. 1: Larval mortality of III instar larvae of *Anopheles stephensi* after one day of treatment by plant extracts

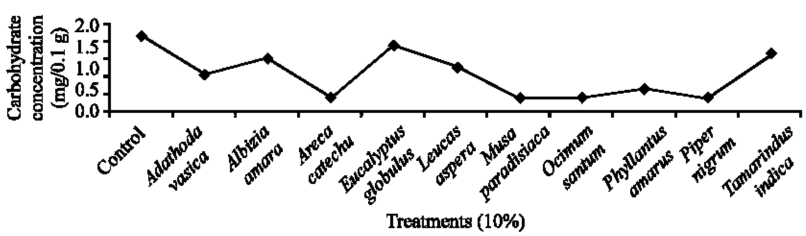

Fig. 2: Effect of Medicinal plants on the carbohydrate profiles of *Anopheles stephensi*

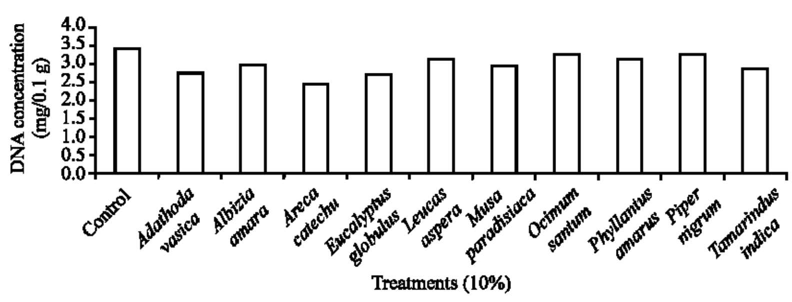

Fig. 3: Effect of Medicinal plants on the DNA profiles of *Anopheles stephensi*

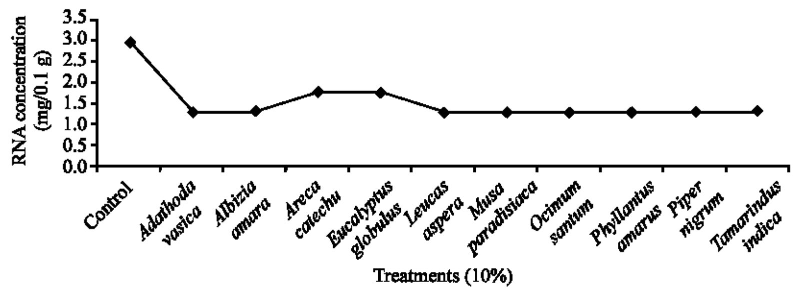

Fig. 4: Effect of Medicinal plants on the RNA profiles of *Anopheles stephensi*

with *Albizia amara*, *Musa paradisiaca* and *Leucas aspera* significantly reduced, the concentration to 1.2 mg/0.1 g, whereas in the case of *Area catechu* and *Eucalyptus globulus*, the concentration was 1.8 mg/0.1 g.

DISCUSSION

Mosquito problem has become acute in recent years and the death of millions of people every year due to mosquito borne diseases has resulted in the loss of socio-economic wealth in many countries (Venkatachalam and Jebanesan, 2001). Chemical control of mosquito was not favored at present because of insecticide resistance among vectors and environmental imbalance created. Therefore alternative control methods are needed.

Understanding the ecology of mosquitoes and the mechanism of disease management was a prerequisite before adopting any type of control. In general, the population of the vector species must be of sufficient size in order to continue the transmission of vector borne diseases. If the population of the vector fell below a critical density, the transmission of these diseases was not very effective.

In view of growing concern of mosquito nuisance and vector borne diseases in urban and tribal populations, the use of plant extracts should be promoted to reduce the toxic load of insecticides on the environment (Raja and Ignacimuthu, 2001). Synthetic chemicals, which were harmful in the long term, did not provide absolute protection. Application of chemical insecticides to control vector mosquitoes resulting, in problems like residual effects, vector resistance and toxicity to the components of the ecosystem alternatively, plant extracts could be effectively employed in mosquito control programs.

Plant sources possess a wide range of pharmaceutical and insecticidal properties. The insecticides of plant origin did not disturb the environment. Besides reducing the cost-factor plants exhibit different, degrees of toxicity to the different stages and conditions of mosquitoes such as larvicidal, pupicidal, adulticidal, growth and reproduction inhibition, repellent and ovipositional deterrents. The very high activity of the extracts especially *Albizia amara*, *Areca catechu*, *Leucas aspera* and *Ocimum sanctum* against the larvae of *Anopheles stephensi* suggested that the methanol extracts might be used directly as larvicidal agents in small volume aquatic habitats or breeding sites of around human dwellings.

The 100% mortality might be due to the chemical constituents present in the leaf extracts that arrest the metabolic activities of the larvae, which caused higher percentage of mortality. The increase in turbidity at higher concentration might block the oxygen depletion to the larvae. The active fractions of *Leucas aspera* were found to be significant toxic to fourth instar larvae (Muthukrishnan *et al*., 1997). The active fractions of *Ocimum sanctum* were also found to be toxic (Kalyanasundram and Das, 1985). Out of the ten plants tested *Adathoda vasica* and *Musa paradisiaca* was found to be less toxic when compared to the other plants. The varying results were probably due to the differences in levels of toxicity among the insecticidal ingredients of each plant (Monzon *et al*., 1994).

Treatment with leaf extracts of *Leucas aspera* and *Ocimum sanctum* on larvae exhibited high mortality, especially during the moulting process. Such processes were under the influence of the ventral nerve cord neurosecretory cells, which release the tanning hormone. These extracts might have an inhibiting influence on such cells or might act directly on epidermal cells which were responsible for the production of enzymes for the tanning or cuticular oxidation process. This was further evidenced by the fact that many of the larvae treated with higher concentration did not successfully moult to pupae.

Various neem extracts were also known to act on various insects. The neem products work by intervening at several stages of an insect life (Murugan *et al*., 1996). The ingredients from this tree approximate the shape and structure of hormones vital to the lives of insects. The bodies of these insects absorb the neem compounds as if they were the real hormones, but these only block their endocrine systems. The resulting deep-seated behavioral and physiological abeuations leave the insects so confused in brain and body that they cannot reproduce and their populations plummet (Vincent, 2000). Some members of the family Labiatae such as *Ocimum basilicum* were reported to contain juvocimenes which exert juvenilising effect on insects (Jacobson, 1989).

Therefore, the larvicidal activity of *Leucas aspera* recorded in the present study might be due to juvocimene like substances present in it. Prolongation of development period and induction of morphogenic abnormalities of mosquito larvae treated with plant extracts were generally attributed to interference of the active ingredients of the extracts with the endocrine system (Zebitz, 1986).

In the present study, the treatment with medicinal plants rendered the *Anopheles stephensi* larvae inactive and motionless. Higher degree of disturbance in normal behavior of the larvae was observed in treatments of *Adathoda*, *Albizia*, Banana, *Eucalyptus*, *Phyllanthus*, *Leucas* and Pepper suggesting that these botanicals affect the normal behavioural physiology and biology in a significant manner.

The present study had shown that applications of the plant extracts disrupted the normal process of feeding of *Anopheles stephensi*, which reduced the duration of feeding and quantity of food digested. Similar, decrease in feeding was recorded in *Anopheles*, *Culex* and *Aedes* after treatment of neem (Vincent, 2000). Feeding in the mosquito larvae might have decreased after the medicinal plant treatments because of feeding deterrency/repellency, inhibition of normal digestive processes leading to indigestion (post ingestive toxic effects) and disruption of normal physiological and metabolic processes. Chemical from the botanicals might have insect repellent, anti-feedent, oviposition deterrent and IGR (Insect Growth Regulator) inhibitor effect (Schmutterer and Ascher, 1984).

In the present study a significant reduction of carbohydrates was observed when the larvae were treated with high dose. The carbohydrates were found to be reduced in the treated larvae. The present results had shown that the treatment caused a decrease in the DNA and RNA content when compared to the control. The decrease in nucleic acids in the treated larvae suggested that the plant extracts inhibits metabolic process like nucleic acid synthesis.

In the present investigations the plant extracts significantly increased the larval mortality and caused less food consumption. These results indicated that a certain finite amount of the plant extracts would be sufficient for the enhancing effect. Chemical analysis of the insects indicated that the carbohydrate was significantly affected due to the treatments resulting in overall collapse of the metabolism and growth.

The present findings have important implications in the practical control of mosquito larvae in the polluted aquatic ecosystem. The plants studied are available in large quantities. These extracts are easy to handle, inexpensive and safe natural products for mosquito control (Kalyanasundram and Babu, 1982). The extracts of tulsi can also be used for disinfecting water (Jeyabalan and Murugan, 1999). In view of residue problems in the environment and the development of insect resistance to synthetic insecticides like DDT and other chlorinated hydrocarbons, the recent trend is to explore plants to obtain extracts that are safe for non target animals and do not pose any residue problem but are still able to suppress pest populations. Though several compounds of plant origin have been reported as insecticides (Nayar, 1995), larvicides (Patterson *et al.*, 1975), there is a wide scope for the discovery of more effective plant products (Saxena and Yadav, 1986). Further research undoubtedly will lead to improved formulations with enhanced activity which may eventually become environmentally acceptable and replace objectionable conventional insecticides for mosquito control. It may be concluded that the nature possesses numerous medicinal plants, which may be useful for control of vector borne diseases.

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