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BIO–EFFICACY OF PLANT–EXTRACTS AGAINST THE MALARIAL VECTOR, *ANOPHELES STEPHENSI* (DIPTERA: CULICIDAE)

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ABSTRACT

Mosquito control is facing a threat due to mosquito resistance to synthetic insecticides. Insecticides of botanical origin may serve as suitable alternatives. During the present study the larvicidal and pupicidal activity of leaf extracts of *Sphaeranthus indicus, Sesbania grandiflora* and *Calotropis procera* against the first to fourth instar lavae and pupae of the laboratory–reared malarial vector, *A. stephensi* were evaluated. The dried plant materials were powdered by an electrical blender. From each sample, 500g powder was macerated with 1.5 litres of methanol for 8 hours, using Soxhlet apparatus, and filtered. The extracts were concentrated at reduced temperature on a rotary evaporator and stored at a temperature of 4°C. The plant extracts showed larvicidal and pupicidal effects against all larval instars and pupae. Larval mortality was observed after 24 hours of exposure. The maximum larvicidal activity was observed for methanolic extract of *C. procera* followed by *S. indicus* and *S. grandiflora*. No mortality was observed in the control. The observed mortality were statistically significant at $P<0.05$ level. The LC_{50} values for the 3 plant extracts against the different larval stages of *A. stephensi* were worked out. *C. procera* showed the highest mortality rate against the mosquito larvae in laboratory.

Key words: *Sphaeranthus indicus, Sesbania grandiflora*, *Calotropis procera, Anopheles stephensi*, larvicidal, pupicidal, malarial vector.

INTRODUCTION

Mosquito–borne diseases are endemic in more than over 100 countries, causing mortality of nearly two million people every year, and at least one million children die of such diseases each year, leaving as many as 2100 million people at risk around the world. Mosquitoes constitute a major public health problem as vectors of serious human diseases like malaria, filariasis, *Japanese encephalitis*, dengue fever, chikungunya, and yellow fever. Mosquitoes alone transmit disease to more than 700 million people annually (Jang *et al*., 2002; Taubes, 2000). Malaria is one of the most common vector–borne diseases widespread in tropical and subtropical regions, including parts of the America, Asia, and Africa (WHO 2007). Worldwide, there were about 247 million malaria cases with 0.881 million deaths reported in 2006 (WHO 2008). In India and other West Asian countries, *A. stephensi* L. is the primary vector of malaria and improved methods of control are urgently needed (Burfield and Reekie, 2005; Mittal *et al*., 2005). The highest number of malaria, *P. falciparum* cases, and malaria–related deaths are recorded from the state of Orissa located in the eastern part of India (Sharma *et al*., 2010).

Chemical control is an effective strategy used extensively in daily life and is presently at the forefront among mosquito–controlling agents. However, the environmental threat that these chemicals pose, effects on non–target organisms, and the resistance of mosquitoes to insecticides have all increased during the last five decades (Wattanachai and Tintanon, 1999; Amer and Mehlhorn, 2006). Biopesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans, and other advantages (Liu *et al*., 2000). Many herbal products have been used as natural insecticides before the discovery of synthetic organic insecticides (ICMR Bulletin, 2003).

Natural botanicals have provided numerous sources of phytochemicals utilized in the development of effective mosquito control agents. Plant material offer not only effective mosquito control agents, but also promise to be environmentally safe. Since these chemicals are taken from natural sources, they are expected to have low toxicity and a high degree of biodegradation (Choochote *et al*., 1999). The larvicidal activities of crude hexane, ethyl acetate, petroleum ether, acetone and methanol extracts of

Abutilon indicum, *Aegle marmelos*, *Euphorbia thymifolia*, *Jatropha gossypifolia*, and *Solanum torvum* were assayed (Rahuman *et al*., 2008), as well as the peel and leaf extracts of *Citrus sinensis*, *Ocimum canum*, *Ocimum sanctum*, and *Rhinacanthus nasutus* (Bagavan *et al*., 2009); and the water, hot water leaf, stem–bark and flower extracts of *Acacia arabica*, *Cedrus deodara*, *Hibiscus rosa–sinensis*, *Mangifera indica*, *Nerium indicum*, *Nicotiana tabacum*, *Pongamia pinnata*, and *Solanum nigrum* (Rahuman *et al*., 2009). Mullai *et al* (2008) have evaluated the leaf extract of *Citrullus vulgaris* with different solvents (e.g., benzene, petroleum ether, ethyl acetate, and methanol) for larvicidal, ovicidal, repellent, and insect growth regulatory activities against *A. stephensi*. The larvicidal efficacy of the crude leaf extracts of *Ficus benghalensis* in methanol, benzene and acetone was tested against the early second, third, fourth–instar larvae of *Cx. quinquefasciatus*, *Ae. aegypti*, and *An. stephensi* and the maximum efficacy was observed in methanol extract (Govindarajan, 2010).

In the present investigation, an attempt has been made to explore the effect of *S. indicus*, *S.grandiflora* and *C.procera* as a mosquito control agent under laboratory conditions. These plant extracts are reported to have larvicidal properties and to control the malarial vector, *A. stephensi*.

MATERIAL AND METHODS

Collection of eggs and maintenance of larvae The eggs of *A. stephensi* were collected from the National Centre for Disease Control (NCDC) field station of Mettupalayam, Tamil Nadu, India, using an 'O' type brush. These eggs were brought to the laboratory and transferred to 18×13×4 cm enamel trays containing 500 ml of water for hatching. The mosquito larvae were fed pedigree dog biscuits and yeast at 3:1 ratio. The feeding was continued until the larvae transformed into the pupal stage.

Maintenance of pupae and adults: The pupae were collected from the culture trays and transferred to plastic containers (12×12 cm) containing 500 ml of water with the help of a dipper. The plastic jars were kept in a 90×90×90 cm mosquito cage for adult emergence. Mosquito larvae were maintained at 27+2°C, 75–85% RH, under a photoperiod of 14L/10D. A 10% sugar solution was provided for a period of 3 days before blood feeding.

Blood feeding of adult mosquito *A. stephensi:* The adult female mosquitoes were allowed to feed on the blood of a rabbit (a rabbit per day, exposed on the dorsal side) for 2 days, to ensure adequate blood feeding for 5 days. After blood feeding, enamel trays with water from the culture trays were placed in the cage as oviposition substrates.

Plant collection and preparation of extract: The plants, *S. indicus*, *S. grandiflora*, and *C. procera*, were collected from Maruthamalai hills and Bharathiar University Coimbatore, Tamil Nadu, India. The four medicinal plants were washed with tap water and shade–dried at room temperature (27±2°C). The dried plant materials (500 kg) were powdered mechanically using commercial electrical stainless steel blender and extracted (1.5 L) with methanol in a Soxhlet apparatus (Vogel, 1978) boiling point range 60–80°C for 8 h. The extracts were concentrated at reduced temperature on a rotary evaporator and stored at a temperature of 4°C. The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. The yields of crude extract were 13.0, 12.0 and 13.5 g in *S. indicus*, *S. grandiflora*, and *C. procera*, respectively. One gram of the plant residue was dissolved in 100 ml of acetone (stock solution) considered as 1 per cent stock solution. From the stock solution, 50–250 ppm concentrations were prepared using de–cholorinated tap water.

Larval and pupal toxicity test: A laboratory colony of *An. stephensi* larvae/pupicidal pupae was used for the larvicidal activity. Twenty–five third instar larvae/pupae were kept in 500–ml glass beaker containing 249 ml of dechlorinated water, and 1 ml of desired concentration of plant extracts were added. Larval food was given for the test larvae. At each tested concentration, two to five trials were made, and each trial consists of five replicates. The control was setup by mixing 1 ml of acetone with 249 ml of dechlorinated water. The larvae exposed to de–chlorinated water without acetone served as control. The control mortalities were corrected using Abbott's formula (Abbott, 1925). LC_{50} and LC_{90} were calculated from toxicity data using Probit analysis (Finney, 1971).

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment - Observed mortality in control}}{\text{100 - Control mortality}} \times 100$$

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae/pupae}}{\text{Number of larvae/pupae introduced}} \times 100$$

Statistical analysis: The average larval mortality data were subjected to Probit analysis for calculating LC_{50} and LC_{90}, and other statistics at 95 per cent confidence limits of upper confidence limit (UCL) and lower confidence limit (LCL) and chi–square values were calculated using the SPSS 9.0 version (Statistical Software Package) to find the regression equation values. Results with $P<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Larval and pupal mortality of *An. stephensi* after the treatment with methanolic extract of *S. indicus* leaf was observed. Table (1) shows the larval and pupal mortality of *An. stephensi* (I to IV Instars) after treatment of *An. stephensi* at different concentrations (50–250 ppm). The mortality of 1st instar larvae was recorded to be 48 per cent by the treatment of *S. indicus* at 50 ppm; whereas, it was 94 per cent at 250 ppm. A similar trend was noted for all the instars of *A. stephensi* at different concentrations of *S. indicus*. The LC_{50} and LC_{90} values calculated have been tabulated. Likewise, larval and pupal mortality of *A. stephensi* (I to IV instars) after treatment with *S. grandiflora* at different concentrations (50 ppm to 250 ppm) has been presented in Table 2. Mortality recorded was 41 per cent for I instar larvae at 20 ppm; whereas, it was 89 per cent at 250 ppm. The mortality of pupae recorded was 28 per cent when treated with *S. grandiflora* at 50 ppm and was 65 per cent at 250 ppm. A similar trend was noted for all the instars of *An. stephensi* at different concentrations of *S. grandiflora*. The LC_{50} and LC_{90} values for the larvae and pupae have been presented in Table: 2. However, larval and pupal mortality of *An. stephensi* (I to IV instars) after the treatment with methanolic extract of *C. procera* leaves (Table 3) showed that at 40–200 ppm larval mortality was 96 per cent for 1st instar larvae. The LC_{50} and LC_{90} values worked out for the larvae and pupae have been presented in the Tables.

During the present study, methanolic extracts of three plants were evaluated for their effects against the malarial vector, *An. Stephensi*, of which, *C. procera* showed the maximum larvicidal activity followed by *S. indicus* and *S. grandiflora*. Various botanicals have been evaluated for their toxicity to mosquitoes by many workers; our findings conform to the work done by some of the investigators and have been elucidated here. The larvae of *An. stephensi* after treatment with methanolic extract of *Clarodendrum inerme* leaf extract showed 22 per cent mortality for I instar larvae at 20 ppm and 81 per cent at the dose of 100 ppm. Treatment of *An. stephensi* (I to IV instars) with methanolic extract of *Acanthus ilicifolius* at different concentrations (20 to 100 ppm) showed 23 per cent mortality for I instar at 20 ppm and 89 per cent mortality at 100 ppm. The sundried leaf powders of *C. inerme* and *An. ilicifolius* had mosquitocidal activity against *An. stephensi, Ae. aegypti*, and *C. quinquefasciatus* (Kovendan and Murugan, 2011). The methanol extract of *Sphaeranthus indicus* showed macrofilaricidal activity (Nisha *et al*., 2007); whereas, the petroleum ether extract showed ovicidal and growth disrupting activities against *C. quinquefasciatus* (Sharma and Saxena, 1996).

Phytochemical investigations on *Sphaeranthus indicus* revealed the presence of two eudesmanolides, along with a known eudesmanolide and two sesquiterpenoids, cryptomeridiol and 4–epicryptomeridiol have been isolated from flower heads (Supada *et al*., 1992). Similarly, 3 closely related new hydroxy lactones have been isolated from the chloroform extract of *S. indicus* (Gogte *et al*., 1986). Chughtai *et al.* (1992) isolated alkaloids from flowers. Compounds in the bioactive fraction showed reaction mainly with flavone reagent indicating the flavonoid type of constituents. Yadava and Kumar (1998) have isolated 7–hydroxy–3,4,5,6–tetramethoxy–flavone 7–O–D– (1'!4)–di glucoside, a new flavone glycoside from the stem of *S. indicus.* Some of these compounds and theirs derivatives have been previously reported for their insecticidal properties against stored–product pests.

Table 1. Bio–efficacy of *Sphaeranthus indicus* methanolic leaf extract against the malarial vector, *An. stephensi*

Mosquito life stages	Larval and pupal mortality (%) Concentration of *S. indicus* (ppm)					LC_{50} and (LC_{90})	Regression equation (LC_{90})	Confidence Limit (95%)		Chi–square value
	50	100	150	200	250			LFL LC_{50} (LC_{90})	UFL LC_{50} (LC_{90})	
I instar	48	57	66	79	94	71.72962 (252.48069)	Y=0.50857 +0.00709=X	42.61925 (92.00851)	224.23324 (296.91634)	4.523
II instar	43	54	61	72	85	86.87218 (311.65966)	Y=–0.49527 +0.00570=X	53.80096 (109.36100)	269.15082 (386.40246)	1.210
III instar	38	49	57	68	80	108.58609 (340.33201)	Y=–0.60048 +0.00553=X	80.30636 (129.70162)	292.00784 (426.67642)	0.485
IV instar	33	46	52	61	74	133.07984 (386.12755)	Y=–0.67398 +0.00506=X	107.24802 (155.41821)	325.29140 (501.25089)	0.752
Pupa	30	41	50	58	68	153.33167 (419.28041)	Y=–0.73887 +0.00482=X	128.98186 (178.43718)	348.91303 (557.36806)	0.144

Control–Nil mortality, *LFL* = Lower Fiducidal Limit, *UFL* = Upper Fiducidal Limit, x^2 – *Chi*–square value, *df* – degrees of freedom, Significant at $P < 0.05$ level. Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT.

Table 2. Bio–effiacy of *Sesbania grandiflora* leaf extract against the malarial vector, *An. stephensi*

Mosquito life stages	Larval and pupal mortality (%) Conc. of *S. grandiflora* (ppm)					LC_{50} and (LC_{90})	Regression equation (LC_{90})	Confidence Limit (95%)		Chi–square value
	50	100	150	200	250			LFL LC_{50} (LC_{90})	UFL LC_{50} (LC_{90})	
I instar	41	53	64	76	89	89.12296 (274.41402)	Y=–0.61641 +0.00692=X	63.52067 (243.24580)	107.92111 (323.80939)	1.008
II instar	39	48	59	73	81	103.48771 (319.14339)	Y=–0.61498 +0.00594=X	76.42154 (277.13644)	123.63684 (391.00520)	0.385
III instar	35	42	50	66	74	132.75852 (372.74030)	Y=–0.70896 +0.00534=X	108.42591 (317.10285)	153.97054 (474.27778)	0.967
IV instar	30	37	44	60	69	160.08152 (403.56941)	Y=–0.84256 +0.00526=X	138.33301 (341.14788)	183.78225 (518.97804)	0.941
Pupa	28	34	41	56	65	177.58638 (432.84665)	Y=–0.89158 +0.00502=X	155.11123 (361.67329)	205.94045 (569.16821)	0.836

Control–Nil mortality, *LFL* = Lower Fiducidal Limit, *UFL* = Upper Fiducidal Limit, x^2 – *Chi*–square value, *df* – degrees of freedom, Significant at $P < 0.05$ level. Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT.

Table 3. Bio–efficacy of *Calotropis procera* leaf extract against the malarial vector, *An. stephensi*

Mosquito life stages	Larval and pupal mortality (%) Concentration of *C. procera* (ppm)					LC_{50} and (LC_{90})	Regression equation (LC_{90})	Confidence Limit (95%)		Chi–square value
	40	80	120	160	200			LFL LC_{50} (LC_{90})	UFL LC_{50} (LC_{90})	
I instar	46	58	71	83	96	57.51586 (181.89240)	Y=–0.59263 +0.01030=X	37.95599 (163.80107)	71.76643 (208.57238)	2.879
II instar	42	56	68	79	90	64.30137 (207.87838)	Y=–0.57395 +0.00893=X	42.96013 (184.96708)	79.65298 (243.62372)	0.354
III instar	40	52	61	72	83	76.12006 (251.37355)	Y=–0.55663 +0.00731=X	52.34248 (217.87934)	93.03713 (309.26676)	0.302
IV instar	37	48	56	64	74	93.82584 (311.56499)	Y=–0.55223 +0.00589=X	68.19681 (259.38463)	113.01818 (415.46012)	0.154
Pupa	35	46	53	61	69	106.29264 (345.78923)	Y=0.56877 +0.00535=X	80.99590 (281.57341)	127.41122 (483.65147)	0.165

Control–Nil mortality, *LFL* = Lower Fiducidal Limit, *UFL* = Upper Fiducidal Limit, x^2 – *Chi*–square value, *df* – degrees of freedom, Significant at $P < 0.05$ level. Within a column means followed by the same letter(s) are not significantly different at 5% level by DMRT.

Flavonoids (flavone, rutin, quercetin, myricetin, fisetin, quercitrin) from *Calotropis procera* (Ait.) R. Br. were toxic to adult *Callosobruchus chinensis* (L.) *via* contact on the filter paper bioassay (Salunke *et al*., 2005).

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