Biological activity of certain botanical extracts as larvicides against the yellow fever mosquito, *Aedes aegypti*.L

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ABSTRACT

As a part of a programme on possible utilization of indigenous plant extracts in pest management practices, acetone extracts of eight plant species collected in the state of Andhra Pradesh, India, were tested for their larvicidal activity against the yellow fever mosquito, *Aedes aegypti* L. The buds of Tail Pepper, *Piper cubeba* L, Capers *Capparis spinosa L* and Indian Black Berry, *Syzygium cumini* L. the florals of Indian Oleander, *Nerium indicum* (Mill.), Indian Cork tree, *Millingtonia hortensis L.* and Royal Poinciana, *Delonix regia* L., leaves of Wood Apple, *Limonia acidissima* L. and Physic Nut, *Jatropha curcas* L were collected locally, shade dried and extracted in the soxhelet apparatus. Six of the 8 plants studied exhibited toxicity against the 3rd instar larvae. The extracts of *D. regia* and *L. acidissima* were most active and showed toxicity up to 100 %. The dry bud extractions of *S. cumini* and *J. curcas* also showed significant larval mortality. Acetone extract of *P. cubeba* and *C. spinosa* were less active, and needed higher concentrations to obtain 50% toxicity. Hence, these active plant extracts may be used in control of the *A. aegypti* causing dengue fever and many other diseases.

Key words: Larvicidal, *Aedes aegypti*, botanicals, plant extracts.

INTRODUCTION

Vector control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting either countermeasures or development of newer insecticides (Chandre *et al.*, 1998). At the same time these pesticides may affect other beneficial organisms and prove detrimental to animal life including man. Botanical insecticides may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, degradable, and are readily available in many areas of the world. Though several plants from different families have been reported for their mosquitocidal activity, only a few botanicals have moved from the laboratory to field use, like neem based insecticides, which might be due to the light and heat instability of phytochemicals compared to synthetic insecticides (Green *et al.*, 1991)

Aedes aegypti L., a vector of dengue, is widely distributed in the tropical and subtropical zones. About two-thirds of the world's population lives in areas infested with dengue vectors, mainly *A. aegypti*. Dengue viruses, causative agents of dengue fever and more severe dengue hemorrhagic fever (DHF) /dengue shock syndrome infect over 100 million people every year (Hahn *et al.*, 2001). The first outbreak of DHF was recorded in 1963 in Kolkata. Since then dengue has spread to all parts of India. The serious outbreak to hit the capital city Delhi was in 1996 when 10,252 cases with 423 deaths were recorded (Kaul *et al.*, 1998). In Maharastra, dengue fever has spread to 209 villages in the state infecting 31,000 patients. There have been reports of large scale outbreak of this virus in Southern India. At least 80,000 people in Gulbarga, Tumkur, Bidar, Raichur, Bellary, Chitradurga, Davanagere, Kolar and Bijapur districts in Karnataka state and Andhra pradesh are known to have been affected since December 2005 (Ravi, 2006). However, recent reports of large scale outbreaks of fever caused by chikungunya virus infection in several parts of Southern India have confirmed the re-emergence of this virus (WHO, 2006; Enserink, 2006).

Considerable amount of work has been reported on effect of plant extracts against mosquito larvae. The crude hexane extracts obtained from flower heads of *Spilanthes acmella*, *Spilanthes calva* and *Spilanthes paniculata* (Pandey *et al.*, 2007), seed extract of *Sterculia guttata* (Katade *et al.*, 2006); the ethyl acetate extract of fruit mesocarp of *Balanites aegyptiaca* (Wiesmanr *et al.*, 2006); partially purified extracts of leaves of *Vitex negundo*, *Nerium oleander* and seeds of *Syzygium jambolanum* (Pushpalatha and Muthukrishnan, 1995), the petroleum ether root extract of *Solanum xanthocarpum* (Mohan *et al.*, 2007), leaves of *Artemisia annua* and *Azadirachta indica* (Tonk *et al.*, 2006), *A. annua* (Sharma *et al.*, 2006),

the acetone crude extract of *Fagonia indica* and *Arachis hypogaea* (Chaubal *et al.*, 2005), extracts of *Nerium indicum* and *Thuja orientelis* (Sharma *et al.*, 2005), *Murraya koenigii*, *Coriandrum sativum*, *Ferula asafoetida*, *Trigonella foenum* (Harve and Kamath, 2004) were tested against mosquito larvae and among these *D. regia and S. cumini* were proved to have excellent toxic effects on larval population of different mosquito species.

MATERIALS AND METHODS

Mosquito culture

Aedes aegypti colonies were maintained in our insectary in large enamel basins (45×45×40 cm) and rearing conditions were 28±2°C temperature, 65±5% relative humidity (RH) and photoperiod of 14:10 h light and dark period as per the procedure of Sharma and Saxena (1994). The egg strips were obtained from Osmania University mosquito control board, Hyderabad to start the colony. The strips were immersed in dechlorinated tap water for hatching. Larvae were fed with a diet of finely ground brewer yeast and dog biscuits (3:1). The emerged adults were fed with rabbit blood and with 10% glucose solution. Small porcelain dishes having 50 ml of tap water lined with filter paper was kept inside the cage for oviposition.

Plant materials

The buds of Tail Pepper, *Piper cubeba* L, Capers *Capparis spinosa L* and Indian Black Berry, *Syzygium cumini* L. the floral extract of Indian Oleander *Nerium indicum* (Mill.), Indian Cork tree, *Millingtonia hortensis L.* and Royal Poinciana, *Delonix regia* L. leaves of Wood Apple, *Limonia acidissima* L. and Physic Nut, *Jatropha curcas* L were collected from campus of Indian Institute of Chemical Technology, Hyderabad, Andhra Pradesh, India in February 2008 and were authenticated by Dr. T. Pullaih, Department of Botany, Sri Krishnadeva University, Anantapur, Andhra Pradesh, India. The voucher specimens have been deposited in the Botany laboratory.

Preparation of plant extracts

The dried plant materials (800g) were powdered mechanically using commercial electrical stainless steel blender and extracted with acetone in a soxhlet apparatus (2,000 ml) until exhaustion. The extract was concentrated in rotary evaporator under reduced pressure at 45°C, and the residue obtained was stored at 4°C.

Biological tests

Screening procedure

Crude extracts were bioasssayed against third instar larvae of *Aedes aegypti* under controlled room temperature maintained at 28±2ºC and 65±5% relative humidity. The serial dilution of plant extract made in Dimethyl sulfoxide (DMSO), dilutions of 1 ml of extracts and 249 ml of water that gives final concentrations of 0.5, 1.0 and 2.0mg / ml. 250 ml of test solution was placed in to 500 ml glass beakers along with 20 third instar *A. aegypti* larvae. Controls received solvent (DMSO) only. All the experiments were replicated 3 times and each set of experiments have 5 replicates per samples. Mortality was recorded after 24 h and corrected using the formula of Abbot (1925).

Accurate tests

Twenty late-3rd or young-4th instar mosquito larvae were collected from the rearing basin and transferred into glass beakers (mean diameter, 6 cm; height, 9 cm) containing 99 mL of filtered tap water. The plant extracts selected in the

Table 1. Insecticidal activity of various botanical extracts (mg/ml) to the third instar larvae of *A. aegypti*.

Plants	Mortality (in %)		
	0.5	1.0	2.0
Syzygium cumini L.	20	50	60
Jatropha curcas L	0	30	70
Delonix regia L	15	55	100
Limonia acidissima L.	30	60	100
Millingtonia hortensis L.	0	15	38
Capparis spinosa L	0	0	40
Piper cubeba L,	0	20	80
Nerium indicum (Mill.)	0	0	20

Table 2. LC_{50} and LC_{95} with fiducial limits (95%) of tested botanical extracts against larvae of *A. aegypti*.

Plants	Activity (mg/ml) (95% FL)	
	LC_{50} (LCL–UCL)	LC_{95} (LCL–UCL)
Syzygium cumini L.	0.86(0.69-0.99)	1.95(1.63-2.68)
Jatropha curcas L	0.85(0.68-0.99)	2.03(1.67-2.87)
Delonix regia L	1.07(0.87-1.25)	3.02(2.29-5.42)
Limonia acidissima L.	1.24(0.98-1.64)	3.62(3.02-5.94)
Millingtonia hortensis L.	1.61(1.50-1.74)	2.35(2.08-2.98)
Capparis spinosa L	1.77(1.63-1.99)	2.77(2.33-4.12)
Piper cubeba L,	1.83(1.56-2.52)	5.01(3.24-8.13)
Nerium indicum (Mill.)	-	-

FL: Fiducial limits, UCL: upper confidence limit. LCL: lower confidence limit

preliminary screening were tested at 27±1ºC as solutions in DMSO. The total volume of DMSO added in each glass beakers was adjusted to 200ìl. Each concentration (or control) was performed using four glass beakers of 20 larvae. Mortality was recorded at 24 hours intervals. Mortality data was used to determine LC_{50} and LC_{95} values for each extract using probit analysis.

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating lethal concentrations LC_{50} and LC_{95} and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit were calculated (Raymond *et al.*, 1993).

RESULTS

The effect of various botanical extracts on the mortality of *A. aegypti* larvae in screening procedure are presented in Table I. From the results it appears that 5 of the 8 extracts tested possessed high larvicidal activities in various degrees. The floral extract of *D. regia* is the most lethal, followed by *S. cumini*, among the 8 plant extracts tested in the screening procedure. At dosage of 2 mg /ml two plant extracts caused 100% mortality in the treated larvae and almost all the extracts were ineffective in producing mortality at lower doses such as 0.5 mg/ ml against *A. aegypti* larvae. A gradient increase in mortality with increase in concentration was observed in all the treatments. The extracts of *P. cubeba, C. spinosa, S. cumini, J. curcas* and *L. acidissima* were moderately toxic and needed at least 2 mg/ ml to obtain 50% mortality. No significant difference in larval mortality was found in less than 0.5mg/ml concentrations compared to the control treatment.

Among these extracts, the most promising ones are the floral extract of *D. regia* and leaf extract of the *L. acidissima* in the screening procedure. It was observed that larvae became slowly inactive within 12 h and began to fall towards the bottom of the beaker. Microscopic examination of dead larvae revealed that the extract has penetrated into larval digestive system. The treated larvae showed curling up, agitation, vigorous body movements. *J. curcas* extract extended the duration of the various larval instars and of pupation at very low concentration and showed toxicity at higher concentrations.

More accurate data on the toxicity of the plant extracts were obtained by calculation of their LC_{50} and LC_{95}. In contrast to the results obtained from screening procedure it was observed that *C. spinosa, S. cumini, M. hortensis and J. curcas* showed increased mortality rate while *D. regia* and *P. cubeba* showed reduced activity in the accurate test (Table 2). *S. cumini* showed high toxicity with a LC_{50} of 0.85 mg/ml and LC_{95} of 1.95 mg/ml. In the similar way *J. curcas* showed the second highest mortality with a LC_{50} at 0.85 mg/ ml and LC_{95} at 2.03 mg/ml. All the plant extracts tested showed LC_{50} Less than 2 mg/ ml while LC_{95} values ranges from 1.95 to 5.0 mg/ ml as shown in the Table II .

DISCUSSION

Today, the environmental safety of an insecticide is considered to be of paramount importance. An insecticide does not have to cause high mortality on target organisms in order to be acceptable (Kabaru and Gichia, 2001) but it should prevent the breeding. Phytochemicals may serve as suitable alternatives to synthetic insecticides in future as they are relatively safe, inexpensive, and are readily available throughout of the world. According to Bowers *et al.*, (1995) the screening of locally available medicinal plants for mosquito control would generate local

employment, reduce dependence on expensive imported products and stimulate local efforts to enhance public health. The biological activity of these plant extracts might be due to the various compounds, including phenolics, terpenoids, and alkaloids, existing in plants(Park *et al.*, 2000). These compounds may jointly or independently contribute to produce larvicidal activity against *A. aegypti*.

This work demonstrates the potency of *S. cumini* extract in the control of *A. aegypti* mosquito larvae with LC_{50} value 0.86mg/ml. Previous studies have shown that *S. cumini* seed extracts possessed significant effect on central nervous system (CNS) due to the presence of saponins (Kumar *et al.*, 2007). It is proposed that the saponin molecules itself played an important role in the larvicidal activity of the *S. cumini* extract.

Jatropha curcas extract extended the duration of the various larval instars and of pupation at very low concentration tested. Our results coroboate with the results obtained by Pushpalatha and Muthukrishnan, (1995) who stated that the fractions of *Vitex negundo* and *Syzygium jambolanum* prolonged the larval and pupal duration of *Culex quinquefasciatus* and *Anopheles stephensi*. However, the mechanism by which molecule kills the larvae is the subject of research currently under way by our team. *Nerium indicum* showed less activity among the tested plant extracts with 20% morality 2mg/ml. Sharma *et al.* (2005) examined crude alcoholic and acetone extracts of *N. indicum* leaf against *An. stephensi* and obtained results of LC_{50} at 185.99 ppm after 24 h of exposure. Though the required quantity of the plant extract in large breeding sites is more, presently the preparation of suitable formulation using additives might enhance their efficiency.

Till now, there has been no promising solution for sustainable control of dengue vectors. The trend for dengue vector *A. aegypti* control in this region has shifted from relying solely on insecticides to biological control, source reduction and environmental management through community participation (Gubler and Kuno, 1997). These findings have re-emphasised the need to explore the possibility of using herbal-based larvicides as supplementary and complimentary measures for malaria control. This will reduce the chemical burden on the environment. It is noteworthy that foliar extract of *S. cumini* and floral extracts of *D. regia* were very promising. Furthermore, these plants grow wild in uncultivated dry zones in the southern India hence the plant materials could be easily collected without any additional cost. Therefore, plant materials could be used as a larvicidal agent in an integrated vector control programme. Research into their mode of action, effect on non target organisms and field evaluation are presently under investigation. Further investigations are needed to elucidate this activity against a wide range of mosquito species and also the separation of active ingredient(s) of the extract responsible for larvicidal activity in *A. aegypti* should be identified and utilized. The two plant extracts show promising activity in mosquito control and commercial utilization seems to be very much feasible.

ACKNOWLEDGEMENTS

The authors thank Dr. J. S. Yadav, Director IICT, Hyderabad, India for his constant support and encouragement.

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