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Rahana VK
Biopesticides and Toxicology Laboratory, Department of Zoology, University of Calicut, Kerala, India

Anju Viswan K
Biopesticides and Toxicology Laboratory, Department of Zoology, University of Calicut, Kerala, India

Pushpalatha E
Biopesticides and Toxicology Laboratory, Department of Zoology, University of Calicut, Kerala, India

Correspondence
Pushpalatha E
Biopesticides and Toxicology Laboratory, Department of Zoology, University of Calicut, Kerala, India

Response on growth regulatory activity of three indigenous plant extracts against dengue vector *Aedes albopictus* (Skuse)

Rahana VK, Anju Viswan K and Pushpalatha E

Abstract
Development of resistance in mosquitoes against synthetic insecticides led to alternative control strategies. In the present study, three plant products from viz., *Mimusops elengi* (L.), *Erythrina variegata* (Linn.) and *Pongamia pinnata* (L.) were screened for their mosquitocidal activities. Twenty four hours LC_{50} of the methanolic seed extracts of the selected plants against the 1st, 2nd, 3rd and 4th instar larvae of *Aedes albopictus* ranged from 11ppm to 232 ppm. Extension of larval duration was also observed as 13.00 and 17.33 days respectively for 1st and 2nd instars when compared to control, with 8.00days. It was found that at lower concentrations, there was an overall increase in the developmental periods (49.21days), however at higher treated concentrations an increased mortality rate was observed irrespective of the plants selected. The results obtained in the present study open the possibility for further investigations on the biochemical and physiological aspects of Insect Growth Regulatory (IGR) activities of these three plants against *Aedes albopictus*.

Keywords: *Mimusops elengi*, *Erythrina variegata*, *Pongamia pinnata*, *Aedes albopictus*, plant extracts, larvicidal activity, IGR.

1. Introduction
Aedes albopictus (Skuse), the 'Asian Tiger Mosquito', is a native to the tropical and sub-tropical areas of South Asia, and is a significant vector in many communities, which transmit many viral pathogens including the yellow fever virus, dengue virus and chikungunya virus [1], as well as several filarial nematodes such as *Dirofilaria immitis* [2]. Among them dengue is a serious arboviral disease and *Aedes albopictus* and *Aedes aegypti* are the vectors of dengue [3]. The spread of dengue throughout the world can be directly attributed to the proliferation and adaptation of these mosquitoes. Various kinds of mosquito control measures manage the population of mosquitoes to reduce their effects socio-economically. Depending on the situations, source reduction, chemical control, biocontrol, larviciding, adulticiding and Sterile Insect Technique (SIT) can be used to control mosquito population. Insect Growth Regulators (IGRs) are other safe and effective tool for the control of a variety of insect pests and disease vectors. IGRs show high level of activity against mosquitoes and have low toxicity for mammals, birds and other non- target organisms [10]. In mosquito control, most IGRs have delayed activity, induced mortality and morphogenetic anomalies in stages of development [11]. Among these control programmes, the use of synthetic insecticides are the fast acting and highly effective method, but their continuous use has resulted in the development of resistance in vectors and also leads the environmental contaminations [4].The *Aedes albopictus* mainly feeds during the day and rest during the night hours. Suppress or to control the population of *Aedes albopictus* is very difficult due to its remarkable ability to adapt to various environments as well as its complicated reproductive biology. Surveillance study of *Aedes albopictus* was initiated in 1986 and this species is continuous to be monitored by public health agencies [5]. Due to the development of resistance to Malathion, temephos and bendiocarb, management of adult population is more complicated than other species. Considerable efforts have been made to overcome such challenging situations and one of the strategy using botanical products have become more prominent alternative to this problem [6].
Plants are the storehouse of various phytochemicals such as terpenoides, alkaloids, steroids, resins, saponins and oils which act on insect's body in different ways [7]. Phytochemical based insecticides are considered to be more eco-friendly, biodegradable and safer than synthetic

Insecticides [8]. Botanicals with mosquitocidal properties such as general toxicant, repellents, growth and reproductive inhibitors and oviposition- deterrents have been projected as potent alternative natural insecticides in future mosquito control programmes [9].

2. Materials and Methods

2.1 Insect rearing

Aedes albopictus larvae were collected from the nearby areas of Calicut University campus and identification was done at the Department of Zoology, University of Calicut, Kerala, India. The larvae were kept in plastic trays containing water at 27± 2° C and 75–85% relative humidity under 14:10 light and dark cycles. Larvae were fed a diet of Brewer's yeast and dog biscuits in a ratio of 1:3 respectively. Pupae were transferred to a bowl and were kept in the standard cage (45 x 45 x 40 cm) for emergence. Adults emerged were maintained in cages and were provided with 10% sucrose solution as food.

2. 2 Plant collection and preparation of extracts

The seeds of the plants *Mimusops elengi*, *Erythrina variegata* and *Pongamia pinnata* were collected in and around the Calicut University campus and the taxonomic identification was made, from the Department of Botany, University of Calicut, Kerala, India. The seeds were powdered separately using commercial electrical blender and extracted with Methanol in a Soxhlet apparatus. The extracts were concentrated at 40 °C and the residue obtained was stored at 4 °C.

2.3 Larvicidal Bioassay

Bioassay for larvicidal activity was carried out using WHO protocol (1996) Desired concentrations of the methanol extracts of the selected plants (*Mimusops elengi*, *Erythrina variegate and Pongamia pinnata*) were prepared. Twenty numbers of freshly hatched 1st, 2nd, 3rd and 4th instar larvae were introduced in plastic cups containing 200 mL of water with desired concentrations of the extracts. Methanol and water controls were kept and similar numbers of larvae were introduced as that of the experiment set. Triplicates were maintained for each of the experiment and controls (both methanol and water control). Mortality was recorded after 24 hours and the control mortality was corrected using Abbott's Formula [12].

$$\text{Percent mortality} = \frac{\%\text{ mortality in treated} - \%\text{ mortality in control}}{100 - \%\text{ mortality in control}} \times 100$$

2.4 Developmental indices

I, II, III and IV instars of *Aedes albopictus* were exposed to the crude methanolic seed extracts of *Mimusops elengi*, *Erythrina variegate* and *Pongamia pinnata* at different concentrations such as 5ppm – 200ppm for 1st instars, 10ppm – 200ppm for 2nd instars, 10ppm- 400ppm for 3rd in stars and 50 ppm, –600ppm for 4th instars respectively for *Mimusops elengi*. Concentrations of *Erythrina variegate* varies from 50ppm – 600ppm for 1st and 2nd instars, 50ppm – 800ppm for 3rd and 4th instars respectively. Furthermore, *Pongamia pinnata* showed the lowest concentrations as 2ppm – 70ppm for 1st and 2nd instars, 5ppm – 100ppm for 3rd in stars and 10ppm – 200ppm for 4th instars respectively. Thereafter, the larvae were transferred to saline solution containing larval food, in which they were kept till emergence. Observations on larval extension duration, morphogenetic variation and adult emergence were recorded.

2.5 Statistical analysis

Probit analysis [13] was used for determination of LC_{50} and LC_{90} data from mortality and Graphpad quickcalcs were subjected to analyzed larval extension duration.

3. Results & Discussions

3.1 Larvicidal Activity

The larvicidal activities of the methanolic seed extracts of *Mimusops elengi*, *Erythrina variegate* and *Pongamia pinnata* were summarized in the Table 1, 2 & 3 respectively. Twenty four hours LC_{50} of the methanolic extracts of seeds of selected plants against the different larval instars of *Aedes albopictus* ranged from 49.963(127.131) - 207.063(430.141), 201.830(426.635) - 231.817(54.636) and 11.222(46.752) - 42.664(103.326) respectively. No mortality was observed in control.

3.2 Developmental Indices

Growth inhibiting effects on the various developmental stages occurred at lower dose treatments. Larval duration significantly increased in treated in stars and total larval periods extended as 39.2±0.73 (*Mimusops elengi*), 49.21± 1.11 (*Erythrina variegata*) and 36.98± 1.33 (*Pongamia pinnata*) compared with control 21± 2.00 days (Figure-1 & Table- 4). IGR effects of the selected plants were also observed with several morphogenetic variations like larval-pupal intermediates, pupal- adult intermediates, pupa with straight abdomen and partly emerged pupae with attached head capsule (Plates 1 & 2).

Table 1: Twenty fours hrs LC_{50} and LC_{90} (ppm) and associated statistics of the methanol extract of *Mimusops elengi* tested against different larval instars of *Aedes albopictus.*

Larval instars	LC_{50} (LC_{90})	95% confidence level		Chi square
		LFL	UFL	
I	49.963 (127.131)	17.883 (88.025)	89.965 (280.630)	13.690*
II	86.960 (162.415)	63.910 (128.338)	117.598 (242.736)	7.373*
III	131.666 (265.819)	117.315 (238.075)	147.832 (304.140)	3.067*
IV	207.063 (430.141)	110.221 (320.571)	314.308 (773.319)	14.487*

*Significant at P<0.05

Table 2: Twenty four hrs LC $_{50}$ and LC$_{90}$ (ppm) and associated statistics of the methanol extract of *Erythrina variegata* tested against different larval instars of *Aedes albopictus*

Larval instars	LC_{50} (LC_{90})	95% confidence Level		Chi square
		LFL	UFL	
I	201.830 (426.635)	178.340 (387.083)	225.847 (479.106)	3.646*
II	207.100 (398.746)	186.190 (363.396)	229.160 (445.310)	4.855*
III	258.581 (533.196)	179.914 (424.839)	345.597 (771.064)	8.471*
IV	231.817 (541.636)	110.343 (434.574)	319.088 (777.427)	7.871*

*Significant at $P<0.05$

Table 3: Twenty fours hours LC_{50} and LC_{90} (ppm) and associated statistics of the methanol extract of *Pongamia pinnata* tested against different larval instars of *Aedes albopictus*

Larval instars	LC_{50} (LC_{90})	95% confidence level		Chi square
		LFL	UFL	
I	11.222 (46.752)	14.787 (29.028)	28.882 (139.475)	18.004*
II	12.709 (46.637)	-25.368 (27.043)	39.778 (241.127)	25.930*
III	23.587 (70.277)	7.470 (54.747)	35.959 (102.344)	18.187*
IV	42.664 (103.326)	18.990 (81.990)	58.847 (159.809)	7.082*

*Significant at $P<0.05$

Table 4: Effect of the methanolic seed extracts of *Mimusops elengi*, *Erythrina variegate* and *Pongamia pinnat*a on different larval instars of *Aedes albopictus.*

SI No.	Name of Plant/ parts	Extract	Conc. (ppm)	Extension of larval duration (Days)			
				I instars	II instars	III instars	IV instars
1.	*Mimusops elengi* / seed	MeOH	5	9.33±o.58	____	____	____
			10	12.00±0.00	12.33±0.58	7.33±0.58	7.33±1.53
			50	11.00±1.00	12.67±0.58	8.33±0.58	7.33±1.53
			100	____	13.00±0.00	9.00±1.00	8.00±1.00
	Control	Saline/MeOH	____	4.00±2.00	4.00±2.00	4.00±2.00	4.00±2.00
2.	*Erythrina variegata* / seed	MeOH	50	12.67±1.15	15.67±1.15	9.67±1.15	7.00±0.00
			100	13.00±1.00	17.33±2.31	11.00±1.73	8.00±1.00
			200	15.00+1.00	17.33±1.15	11.67±1.15	9.33±0.58
	Control	Saline/MeOH	____	4.00±2.00	4.00±2.00	4.00±2.00	4.00±2.00
3.	*Pongamia pinnata* / seed	MeOH	2	8.67±0.58	9.33±0.58	____	____
			5	9.33±0.58	10.33±0.58	6.67±0.58	7.00±1.00
			10	10.67±1.15	12.33±0.58	8.00±0.00	9.67±1.15
			20	____	____	8.33±1.15	____
			50	____	____	____	10.67±0.58
	Control	Saline/MeOH	____	4.00±2.00	4.00±2.00	4.00±2.00	4.00±2.00

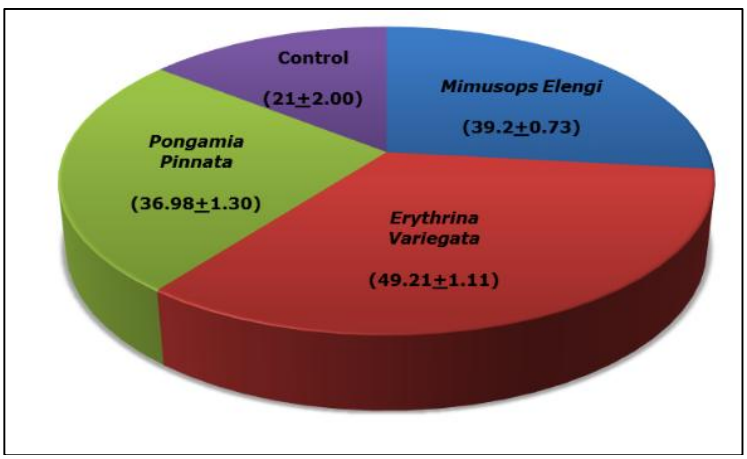

Fig 1: Total Extension of Larval Duration of different larval instars of *Aedes albopictus* exhibited by the methanolic seed extracts of *Mimusops elengi*, *Erythrina variegata* and *Pongamia pinnata*

Plate 1: Pupal – adult intermediate obtained at 300ppm concentration of the leaf extract of *Erythrina variegate.*

Plate 2: Larval – pupal intermediate observed at 50 ppm concentration of the leaf extract of *Pongamia pinnata*

4. Discussion

Integrated Vector Management (IVM) is defined as "rational decision-making process for the optimal use of resources for vector control". The approach seeks to improve the efficacy, cost-effectiveness, ecological soundness and sustainability of disease-vector control. The ultimate goal is to prevent the transmission of vector-borne diseases such as malaria, dengue, Japanese encephalitis, leishmaniasis, schistosomiasis and filariasis. Many existing vector control interventions are known to be effective against multiple diseases, so combining vector control programmes to simultaneously tackle several diseases could offer more cost-effective and therefore sustainable disease reductions.

Various vector management programmes, biopesticides are often considered to be important components of IVM strategies, and have received much practical attention as an alternative to synthetic insecticides [15]. Biopesticides may include alkaloids, terpenoids, phenolics like natural plant-derived products and other secondary chemicals. Such phytochemicals can be used as feeding deterrents, repellents, Insect Growth Regulators, confusants etc.

As a pest, much more serious though, many species of mosquitoes play the roles as vectors of several dreadful diseases [17]. Larvicidal efficacy of *Mimusops elengi* against *Aedes aegypti* and *Culex quinquefasciatus* have been reported [18]. The crude extract of *Mimusops elengi* is found to be effective against the larvae of the chikungunya vector and the filarial vector. The anti-microbial potency of the petroleum ether, chloroform, ethyl acetate and methanolic flower extracts of *Mimusops elengi* was also reported [19]. Larvicidal activities of different solvent extracts of the fruits of *Mimusops elengi* against different species of vector mosquitoes have been also reported [20]. The present study therefore evaluated and envisaged the larvicidal and growth regulatory responses of the methanolic seed extract of *Mimusops elengi* against the dengue vector, *Aedes albopictus*. The experimental results pointed out the potent larvicidal efficacy of *Mimusops elengi* with the Lc_{50} values of 49. 963 (127.131) and 207.063 (430.141) for I and IV instars respectively. Among the three plants tested, *Pongamia pinnata* showed higher larvicidal activity with LC_{50} value 11.222 ppm, 42.664 ppm respectively for I and IV instars of *Aedes albopictus* at 24 hrs. The higher dose treatments caused mortality in a dose-dependent manner, which indicated that the rate of mortality were directly proportional to concentration.

It also evaluated the growth regulatory responses like the extended larval durations with 39.2± 0.73 days, when compared with the control as 21± 2.00 days. Several other deformities, including demelanized larvae, pupa with straight abdomen and partly emerged pupae with attached head capsule was also observed. Various chemical insecticides and phytochemicals are examined for their insect growth regulatory activities [21].

The leaf extract of *Abutilon indicum* (Linn.) possessed adult emergence inhibition and larvicidal activity against *Culex quinquefasciatus* due to the presence of various phytocompounds, including phenols, alkaloids and terpenoides [22]. The protection effect of the seeds of the plant *Erythrina variegate* on high fat induced, by hyperlipidemia may be attributed to a decrease in cholesterol synthesis, an increase in cholesterol excretion and expression of LDL receptor and subsequent catabolism was reported [23]. The results from the experiments reveals the fact that, the phytochemicals present in the seeds of the *Erythrina variegate* capable to control the dengue vector population, with the Lc_{50} of 201.830 (426.635) and 231.8817 (541.636) respectively for I and IV instars. Observations on the growth regulatory responses were also recorded the significant changes in the larval extension duration with a total larval period extended up to 49.21± 1.11 days, compared to control as 21.0± 2.00 days.

Crude extracts of the leaves, barks and seeds of *Pongamia pinnata* were investigated. Larvicidal activity was found in both methanol and hydroalcohol extracts of the bark of *P. pinnata* against the fourth instars larvae of *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* with Lc_{50} values of 84.8, 118.2 and 151.7 ppm [24]. The repellent and larvicidal activity of the ethanolic extracts of leaves of *Pongamia pinnata* against *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* were also evaluated [25]. The methanolic extracts of crude seed oil of *Pongamia pinnata* against the larvae of *Spodoptera litura, Trogoderma granarium* and *Tribolium castaneum* exhibited the antifeedant and growth reduction activities [26]. The crude methanolic extracts of the leaves and barks of *Pongamia pinnata* carry huge potential as mosquitocidal properties against *Culex quinquefasciatus* [27]. Oviposition deterrent activities of *Pongamia pinnata* were recorded against *Helicoverpa armigera* [28]. The data presented in the table-3 indicated the Lc_{50} and Lc_{90} values of the crude methanolic seed extracts of *Pongamia pinnata* against the dengue vector as 11.222 (46.752) and 42.664 (103.326) respectively for I and IV instars. Growth regulatory responses were also noticed with extended larval duration with 36.98± 1.33 days and control with 21± 2.00 days.

5. Conclusion

The experimental results of the present investigation reveal the toxic properties of the tested plant extracts against the different larval stages of the dengue vector, *Aedes albopictus*.

This study clearly demonstrated the growth regulator efficacy and the mosquitocidal properties of the tested materials. The screening results suggested that the methanolic extracts of *Mimusops elengi*, *Erythrina variegata* and *Pongamia pinnata* can be used as natural, biodegradable and cost effective materials to control the 'tiger mosquitoes' in its natural environment without causing any environmental pollution. Further studies need to elucidate the Inhibition of Growth Regulatory (IGR) activities of these three plants for their biochemical analysis, toxicity and their beneficial effects for field application of mosquito control.

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