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Studies on effects of *Andrographis paniculata* (Burm.f.) and *Andrographis lineata* nees (Family: Acanthaceae) extracts against two mosquitoes *Culex quinquefasciatus* (Say.) and *Aedes aegypti* (Linn.)

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ABSTRACT

Objective: To investigate the studies on effects of *Andrographis paniculata* (*A. paniculata*) (Burm.f.) and *Andrographis lineata* (*A. lineata*) nees (Family: Acanthaceae) extracts against two mosquitoes *Culex quinquefasciatus* (*Cx. quinquefasciatus*) (Say.) and *Aedes aegypti* (*Ae. aegypti*) (Linn.). **Methods:** The aqueous and petroleum ether extracts of two plant species, *A. paniculata* and *A. lineate* were examined against the larvae of *A. aegypti* (L.) and *Cx. quinquefasciatus* with gradually increasing concentration *ie*. from 50 to 200 ppm of solvent extracts and to test their activity in combination with each other. **Results:** In a 24 h bioassay experiment with plant extracts, highest mortalities were recorded at 200 ppm of concentrations for leaves of *A. lineta* and *A. paniculata* individually. For combination effect, only 150 ppm of the mixture of solvent extracts of petroleum ether: aqueous (1:1) extracts showed 100% mortality after 24 h of exposure. **Conclusions:** The results show that, insecticides of plant combination is ecofriend and has better larvicidal activity compared to individual extracts.

1. Introduction

The mosquito is the principal vector of many of the vector-borne diseases affecting human beings and other animals. Mosquitoes constitute a major public health problem as vectors of serious human diseases[1] several mosquito species belonging to genera *Anopheles*, *Culex* and *Aedes* are vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis, dengue fever, dengue haemorrhagic fever and yellow fever[2]. *Aedes aegypti* (*Ae. aegypti*) (L.) is generally known as a vector for an arbovirus responsible for dengue and Chikungunya fever, which is endemic to Southeast Asia, the Pacific island area, Africa, and the Americas. *Culex quinquefasciatus* (*Cx. quinquefasciatus*) is important vector of *Brancraftian filariasis* in tropical and subtropical regions. Mosquito control represents an important strategy for prevention of disease transmission and epidemic outbreaks. The continuous and indiscriminate use of insecticides over the years has resulted in the development of resistance to certain molecules belonging to different classes of insecticides in different parts of the world[3]. To overcome these problems, it is necessary to search for alternative methods of vector control. Bio-pesticides provide an alternative to synthetic pesticides because of their generally low environmental pollution, low toxicity to humans, and other advantages[4]. Even today, several plant based products are used to control a wide variety of insects.

Andrographis paniculata (*A. paniculata*) Nees is a traditional medicinal plant that has been used for centuries to successfully treat malaria[5] and filarial as well as being used for pest control[6] and as an insect repellent[7,8] and in a variety of infectious diseases. Since ancient times, The methanol and aqueous extracts of *Andrographis lineata* (*A. lineata*) established pharmacological evidence to support the folklore claim that it is used traditionally as a

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antipyretic, anti-inflammatory[9] anti-diabetic, jaundice, diabetes, snake bite, skin diseases and also have been attributed to this plant in the traditional system of Indian medicine[10-13] hepatoprotective agent[14]. Therefore, it is necessary to establish the scientific basis for therapeutic action of this plant. The present paper is to investigate the combination effect of leaves of *A. paniculata* and *A. lineata* extracts, against the larva of *Ae. aegypti* and *Cx. quinquefasciatus*

2. Materials and methods

2.1. Plant material

The leaves of *A. paniculata* and *A. lineata* were collected from different regions of Cuddalore District, Tamilnadu, India, and were botanically authenticated by Herbal garden maintained in Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India. The voucher specimen was numbered and kept in our research laboratory for further reference.

2.2. Preparation of plant extract

The leaves were washed with distilled water and were shade dried at room temperature. The dried parts are powdered with the help of an electric blender. The dried powder was subjected to extracts with Aqueous, petroleum ether and chloroform for 8 h in a Soxhlet apparatus (Sigma, Mumbai). The plant extracts were evaporated to dryness in rotary vacuum evaporator. The residue was then made into a 1% stock solution with acetone. The stock solutions for various test concentrations were prepared.

2.3. Test mosquitoes

The present study was conducted at Parangipettai (23°16′ N, 87°54′ E), Tamil nadu, India during April – June 2011. Larvae of *Cx. quinquefasciatus* were collected from cemented drains surrounding the university campus and kept in the plastic bucket with the addition of artificial foods (powdered mixture of dog biscuits and dried yeast powder in the ratio of 3:1). Larvae of *Ae. aegypti* were collected from municipal water tank and automobile old tyres and kept in the plastic bucket with the addition of artificial foods (powdered mixture of dog biscuits and dried yeast powder in the ratio of (3:1).

2.4. Larvicidal bioassay

The larvicidal bioassay was assessed by using standard[15]. The different solvent leaf extracts prepared from *A. paniculata* and *A. lineata* were subjected to dose-response bioassay for larvicidal activity against the larvae of *Ae. aegypti* and *Cx. quinquefasciatus*. In addition to the larvicidal activity of the plants *A. paniculata* and *A. lineata* extracts individually, both the extracts are mixed in an equal proportion (1:1), and mixed well for both the extracts to interact well, and the larvicidal assay was performed. The stock solution of the aqueous extracts are mixed in 1:1 ratio to give 100% concentration stock, which is diluted to required concentrations for combination extract. Batches of 30 fourth instar larvae were transferred to a small disposable test cups, each containing 200 mL, of water in the cups to obtain the desired target dosage (concentrations ranging from 50 to 200 ppm), starting with the lowest concentrations. Six replicates were set up for each concentration and an equal number of controls were set up simultaneously using tap water to which 1 mL of appropriate solvent was added. Symptoms of the treated larvae were observed and recorded immediately and at timed intervals and no food was offered to the larvae. Mortality and survival was registered after 24 h of the exposure period. Dead larvae were identified when they failed to move after probing with a needle in the siphon or cervical region or showing the characteristic diving reaction when the water was disturbed.

Table 1
Mean mortality of *Ae. aegypti* and *Cx.quinquefasciatus* larva exposed to different concentration of *A. paniculata* and *A. lineata* solvents extract (petroleum ether and aqueous each solvent extract of both plant were applied individually and 1:1 combination at different concentration).

Plants	Species	Solvents	Concentration (μg/mL)			
			50	100	150	200
A. paniculata	*Ae. aegypti*	Petroleum ether	34.00±1.31	53.00±0.21	77.00±0.11	100.00±0.00
		Aqueous	27.00±1.23	41.00±1.31	64.00±0.21	79.00±0.38
	Cx. quinquefasciatus	Petroleum ether	29.00±0.23	46.00±0.33	69.00±1.11	87.00±0.11
		Aqueous	25.00±1.21	37.00±0.21	54.00±1.11	79.00±0.31
A. lineata	*Ae. aegypti*	Petroleum ether	51.00±1.21	65.00±0.11	79.00±1.31	100.00±1.00
		Aqueous	47.00±0.11	57.00±0.31	71.00±0.21	86.00±1.21
	Cx. quinquefasciatus	Petroleum ether	45.00±1.00	59.00±1.11	64.00±0.33	83.00±1.00
		Aqueous	41.00±0.21	55.00±0.11	62.00±1.11	79.00±0.33
A. paniculata + *A. Lineata*	*Ae. aegypti*	Petroleum ether	55.00±1.21	76.00±0.33	95.00±1.11	100.00±0.00
		Aqueous	51.00±0.21	83.00±0.13	100.00±0.00	100.00±0.00
	Cx. quinquefasciatus	Petroleum ether	53.00±1.11	69.00±0.21	76.00±1.31	100.00±1.00
		Aqueous	49.00±0.21	77.00±0.11	100.00±0.00	100.00±0.00

Table 2

Log probit analysis of larvicidal activity of *A. paniculata* and *A. lineate* extract and combined extract on different larva of *Ae. aegypti* and *Cx. quinquefasciatus*.

Plants	Species	Solvents	LC_{50}	LC_{90}	χ^2
A. paniculata	*Ae. aegypti*	Petroleum ether	143.24	257.51	11.237
		Aqueous	129.31	235.19	9.970
	Cx. quinquefasciatus	Petroleum ether	117.73	239.33	10.313
		Aqueous	109.21	197.53	9.871
A. lineata	*Ae. aegypti*	Petroleum ether	193.10	270.37	15.214
		Aqueous	152.23	241.31	13.013
	Cx. quinquefasciatus	Petroleum ether	179.03	253.37	10.024
		Aqueous	147.45	239.16	9.943
A. paniculata + *A. lineata*	*Ae. aegypti*	Petroleum ether	101.31	175.01	9.112
		Aqueous	96.01	167.15	7.941
	Cx. quinquefasciatus	Petroleum ether	93.31	173.09	9.051
		Aqueous	89.54	146.36	7.027

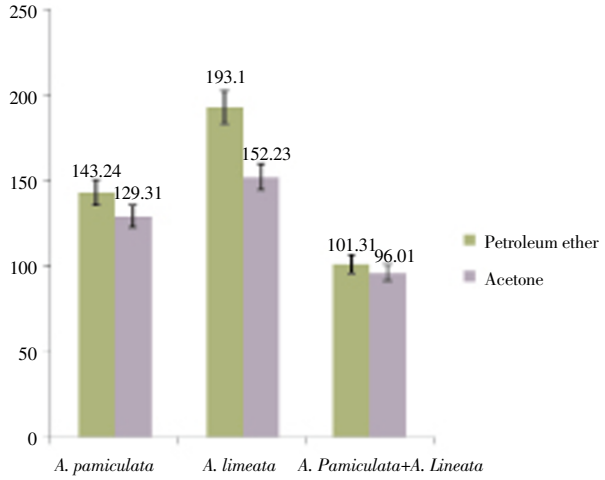
2.5. Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50}, LC_{90}, and other statistics at 95% confidence limits of upper confidence limit and lower confidence limit, and *Chi*-square values were calculated using the software. Results with $P<0.05$ were considered to be statistically significant.

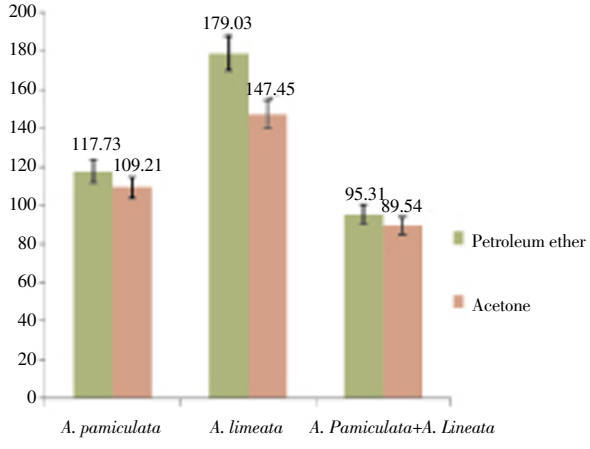
3. Results

The toxicity of crude leaf extract of *A. paniculata* and was tested against the larvae of *Ae. aegypti* and *Cx. quinquefasciatus* (Table 1). The data were recorded and statistical data regarding LC_{50}, LC_{90}, LCL, UCL and chi square value values were calculated (Table 2). The LC_{50} and LC_{90} values of Petroleum ether and Aqueous extract of *A. paniculata* and *A. lineata* against fourth instar larvae of *Ae. aegypti* were (LC_{50}=143.24, LC_{90}=257.51; LC_{50}=129.31, LC_{90}=235.19) and(LC_{50}=193.10, LC_{90}=270.37; LC_{50}=152.23, LC_{90}=241.31)and against the larvae of *Cx. quinquefasciatus*(LC_{50}=117.73 , LC_{90}=239.33; LC_{50}=109.21, LC_{90}=197.53) and (LC_{50}=179.03, LC_{90}=253.37; LC_{50}=147.45, LC_{90}=239.16) respectively. Maximum larvicidal activity was observed in the aqueous extract of *A. paniculata* and *A. lineata* followed by Petroleum ether extracts.

The combined activity of both the extracts is more effective than the individual activity in all the two types, the mortality rate increased considerably high when the extracts were combined together in equal proportion *i.e.*, 200 ppm concentration of the combined extract is capable of killing almost 100% in 24 h. The mortality rate was steadily increasing along with the time of exposure and concentration. No mortality was observed in control. *Chi*-square value was significant at $P<0.05$ level (Table 2 and Figure 1). The petroleum ether extracts 100% mortality (zero hatchability) at 200 ppm. In control experiments 100% hatchability.



(a) *Ae. aegypti*



(b) *Cx. quinquefasciatus*

Figure 1. Probit analysis of larvicidal activity of *A. paniculata* and *A. lineate* extract and combined extract on different larva of (A) *Ae. aegypti* and (B) *Cx. quinquefa.*

4. Discussion

The vector control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting either counter measures or development of newer insecticides. It is evident from our results that arise in the concentration of plant extracts was the main cause of mortality in *Cx. quinquefasciatus* and *Ae. aegypti* larvae. The highest larval mortality was found in petroleum ether and Aqueous, leaf extracts of *A. paniculata* (LC_{50}=143.24, LC_{90}=257.51; LC_{50}=129.31, LC_{90}=235.19) against the larvae of *Ae. aegypti* and against the larvae of *Cx. quinquefasciatus* (LC_{50}=117.73, LC_{90}=239.33; LC_{50}=109.21, LC_{90}=197.53) respectively. 100% mortality was recorded when 200 ppm mixed extract of *A. paniculata* and *A. lineata* at 1:1 combination was applied and it was found to the best combination when compared to other combinations. Similar results are found in the maximum repellent activity was observed at 500 ppm in methanol extracts of *Aegle marmelos*, *A. lineata*, and ethyl acetate extract of *Chamaecytisus hirsutus*, and the mean complete protection time ranged from 90 to 120 min against *Anopheles subpictus*[16-22]. was found in crude extracts of *Croton caudatus (C. caudatus)* fruits and *Tiliacora acuminata* (*T. acuminata)* flowers showed at 0.5% concentration when applied separately. 100% mortality was recorded when 0.2% crude mixed extract of *C. caudatus* fruits and *T. acuminata* flowers at 1:1 combination was applied and it was found to be the best combination when compared to other combinations and Second and third instars larvae of *Cx. quinquefasciatus* are more susceptible to crude extract mixture (1:1) of *C. caudatus* fruits and *T. acuminata* flowers. In our observation, the combination effects of leaf extract of *A. lineata* and *A. paniculata* petroleum ether and aqueous, possessed much effective than the individual extracts. So use of this combination in mosquito control can be of greater use[23].

In conclusion, with the above results obtained, we propose that these medically valuable plants, contains active compounds that are able to kill the mosquito larvae effectively, there by controlling the multiplication of the mosquitoes and also to eliminate the mosquitoes from an environment. The results reported here open the possibility of further investigations of efficacy on their larvicidal properties of natural product extracts for the control of mosquitoes and thereby prevent the environmental contamination

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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