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Research Article

MOSQUITO OVICIDAL ACTIVITY OF *ARISTOLOCHIAINDICA* LINN. (ARISTOLOCHIACEAE) EXTRACTS AGAINST *AEDESAEGYPTI* (LINN.), *ANOPHELES STEPHENSI* (LISTON) AND *CULEXQUINQUEFASCIATUS* (SAY) (DIPTERA :CULICIDAE)

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A B S T R A C T

Plants may be a source of alternative agents for control of mosquitoes because they are rich in bioactive chemicals, are active against a limited number of species including specific target insects, and are biodegradable. They are potentially suitable for use in integrated pest management programs. Therefore, the present study was aimed to investigatethe ovicidal efficacy of different extract of *Ariitolochiaindica* (Aristolochiaceae) against *Aedesaegypti, Anopheles stephensi*and *Culexquinquefasciatus*. Ovicidal activity if selected mosquitoes eggs/eggeraft were exposed to different concentrations ranging from 100 – 500ppm and were assayed in the laboratory condition by using the standard protocol. The oval mortality was observed after 24 h of treatment. Among five solvent extracts tested the maximum efficacy was observed in the methanol extract and followed by ethyl acetate, dichloromethane, diethyl ether and hexane. The extract of methanol exerted 100% mortality at 300, 400 and 500ppm selected mosquitoes. From the results it can be concluded the crude extract of *A. indica* was a potential for controlling vector mosquitoes.

INTRODUCTION

Mosquitoes are the major vector for the transmission of several communicable diseases like malaria, dengue fever, yellow fever, filariasis, schistosomiasis, Japanese encephalitis, etc., causing millions of deaths every and also cause allergic responses in humans that include local skin and systemic reactions such as angioedema (WHO, 2009; 2010 ;WHO, 1999; Pancharoen *et al*., 2002).The disease remains endemic in more than 100 developing tropical countries, and its control is a major goal for improved worldwide health. Mosquito control has been becoming increasingly difficult because of the indiscriminate uses of synthetic chemical insecticides which have an adverse impact on the environment and disturb ecological balance. Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and spreading toxic effects. The increased use of these insecticides may enter into the food chain, and thereby, the liver, kidney, etc., may be irreversibly damaged. They even result in mutation of genes and these changes become prominent only after a few generations (Gosh, 1991). Mosquito control is very costly. In larval mosquito control, application of insecticides in ponds, wells, and other water bodies may cause health hazards to human and larvivorus fishes.

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Nowadays, mosquito coils containing synthetic pyrethroids and other organophosphorus compounds cause so many side effects, such as breathing problem, eye irritation, headache, asthma, itching, and sneezing to the users. These problems have highlighted the need for the development of new strategies for selective mosquito control. Phytochemicals are advantageous due to their eco-safety, target-specificity, non-development of resistance, reduced number of applications, higher acceptability, and suitability for rural areas. Botanicals can be used as alternative to synthetic insecticides or along with other insecticides under integrated vector control programs. The plant product of phytochemical, which is used as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites. Phytochemicals obtained from the whole plant or specific part of the plant by the extraction with different types of solvent such as aqueous, methanol, chloroform, benzene, acetone, etc., depending on the polarity of the phytochemical. Some phytochemicals act as toxicant (insecticide) both against adult as well as larval stages of mosquitoes, while others interfere with growth and growth inhibitor or with reproduction or produce an olfactory stimulus, thus acting as repellent or attractant (Markouk *et al*., 2001). Plants may be a source of alternative agents for control of mosquitoes because they are rich in bioactive chemicals, are active against a limited number of species including specific target insects, and are biodegradable. They are potentially suitable for use in integrated pest management programs (Alkofahi, 1989; Dharmshaktu *et al*., 1987; Green *et al*., 1991). In view of the recently increased interest in developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal activity of different extracts of *Aristolochiaindica* against vector mosquitoes.

MATERIALS AND METHODS

Plant collection and processing

Several hundred medicinal plant species from the Indian sub-continent have been identified and their usage documented in the ethno botanical literature. These literature reports guided the selection of plant for the present study. Plant sampling (*Aristolochiaindica*) was collected from in and around Yercaud hills, Salem district, Tamil Nadu, India. At the time of collection, two pressed voucher herbarium specimens were prepared per species. Bulk samples were air-dried in the shade and after drying each sample was ground to a fine powder using an electric blender.

Extraction

The leaves were washed with tap water, shade-dried, and finely ground with the help of electrical blender. The finely ground plant leaf powder (1.0 kg) was loaded in Soxhlet apparatus and was extracted with different solvents by adapting a standard protocol (Vogel, 1978). The solvents from the extracts were removed using a rotary vacuum evaporator (Rota vapour, Systronics India Ltd., Chennai, India) to collect the crude extract. Standard stock solutions were prepared to 100 and 500ppm concentrations.

Test organisms

The larvae of selected mosquitoes, *Ae.aegypti, An. stephensi* and *C. quinquefasciatus* were collected from the agricultural gardens and field, reared in the laboratory. The larvae were fed on dog biscuits and yeast powder in the 3:1 ratio. Adults were provided with 10% sucrose solution and 1-week-old chick for blood meal. Mosquitoes were held at 28 ± 2°C, 70–85% RH, with a photo period of 12L: 12D.

Ovicidal activity

For ovicidal activity, slightly modified method of Su and Mulla (1998) was performed. The eggs/egg rafts of selected mosquitoes were collected individually and exposed to selected concentrations. The different leaf extracts diluted in the appropriate solvent to achieve various concentrations ranging from 100 to 500 ppm. Eggs of selected mosquito species (100) were exposed to each concentration of leaf extracts. After treatment, the eggs from each concentration were individually transferred to distilled water cups for hatching assessment after counting the eggs under microscope. Each experiment was replicated five times along with appropriate control. The hatch rates were assessed 48 h post treatment by following formula.

RESULTS

Mosquitoes are the major vector for the transmission of several communicable diseases like malaria, dengue fever, yellow fever, filariasis, schistosomiasis, Japanese encephalitis, etc., causing millions of deaths every and also cause allergic responses in humans that include local skin and systemic reactions such as angioedema.The disease remains endemic in more than 100 developing tropical countries, and its control is a major goal for improved worldwide health.Ovicidal activity of selected mosquitoes eggs/ egge raft were exposed to different concentrations ranging from 100 – 500ppm and were assayed in the laboratory condition by using the standard protocol. The oval mortality was observed after 24 h of treatment. Among five solvent extracts tested the maximum efficacy was observed in the methanol extract and followed by ethyl acetate, dichloromethane, diethyl ether and hexane. The extract of methanol exerted 100% mortality at 300, 400 and 500ppm selected mosquitoes (table 1). From the results it can be concluded the crude extract of *A. indica* was a potential for controlling vector mosquitoes.

Table 1 Ovicidal activity of *A. indica*extracts against the eggs of the selected mosquitoes

Solvent tested	Name of the species	Ovicidal activity				
		Concentrations tested (ppm)				
		100	200	300	400	500
Hexane	*Ae. aegypti*	24.55±1.62[b]	35.81±1.22[b]	63.47±1.68[b]	78.53±2.14[b]	84.31±2.41[b]
	An. stephensi	27.76±1.73[c]	38.72±1.63[c]	65.12±1.99[c]	81.81 ±2.35[c]	86.45 ±2.39[c]
	Cx. quinquefasciatus	29.84±1.66[d]	40.85±1.71[d]	68.84±1.62[d]	84.25±2.89[d]	88.62±2.92[d]
	Control	1.65±0.20[a]	1.65±0.20[a]	1.65±0.20[a]	1.65±0.20[a]	1.65±0.20[a]
Diethyl ether	*Ae. aegypti*	27.38± 1.81[b]	47.80±2.58[b]	64.26±2.79[b]	77.58±2.89[b]	100.0±0.0[b]
	An. stephensi	29.90±1.20[c]	49.42±2.39[c]	67.76±2.17[c]	79.77±2.25[c]	100.0±0.0[b]
	Cx. quinquefasciatus	31.46±1.79[d]	51.85±2.48[d]	69.59±2.33[d]	81.21±2.54[d]	100.0±0.0[b]
	Control	1.88±0.75[a]	1.88±0.75[a]	1.88±0.75[a]	1.88±0.75[a]	1.88±0.75[a]
Dichloromethane	*Ae. aegypti*	32.15±1.28[b]	55.13±1.66[b]	76.94±1.68[b]	100.0±0.0[b]	100.0±0.0[b]
	An. stephensi	36.80±2.96[c]	59.74 ±1.17[c]	79.26±1.22[c]	100.0±0.0[b]	100.0±0.0[b]
	Cx. quinquefasciatus	29.43±2.18[d]	65.62±2.42[d]	84.73±2.77[d]	100.0±0.0[b]	100.0±0.0[b]
	Control	1.72±0.63[a]	1.72±0.63[a]	1.72±0.63[a]	1.72±0.63[a]	1.72±0.63[a]
Ethyl acetate	*Ae. aegypti*	48.51±1.33[b]	78.32±2.15[b]	100.0±0.0[b]	100.0±0.0[b]	100.0±0.0[b]
	An. stephensi	51.68±1.22[c]	86.76±2.96[c]	100.0±0.0[b]	100.0±0.0[b]	100.0±0.0[b]
	Cx. quinquefasciatus	56.40±1.48[d]	89.44±2.84[d]	100.0±0.0[b]	100.0±0.0[b]	100.0±0.0[b]
	Control	1.83±0.29[a]	1.83±0.29[a]	1.83±0.29[a]	1.83±0.29[a]	1.83±0.29[a]
Methanol	*Ae. aegypti*	54.13±1.42[b]	79.84±2.61[b]	100.0±0.0[b]	100.0±0.0[b]	100.0±0.0[b]
	An. stephensi	58.22±1.49[c]	82.62±1.70[c]	100.0±0.0[b]	100.0±0.0[b]	100.0±0.0[b]
	Cx. quinquefasciatus	65.76±1.13[d]	86.83±1.59[d]	100.0±0.0[b]	100.0±0.0[b]	100.0±0.0[b]
	Control	2.61±0.42[a]	2.61±0.42[a]	2.61±0.42[a]	2.61±0.42[a]	2.61±0.42[a]

Values represent mean±S.D. of five replications. Different alphabets in the column are statistically significant at $p<0.05$. (mANOVA; LSD -Turkey's Test). Eggs in control groups were sprayed with no phytochemicals (Su and Mulla, 1998 and Abbott *et al.*, 1925).

DISCUSSIONS

Due to indiscriminate use of synthetic chemicals to control the mosquitoes in the natural habitats, they have developed strong resistance to almost all the chemicals that are available today. Moreover, chemical pesticides gradually altered the behaviour of non-target organisms. Thus, in this context, the world scientific community intensively searching for the alternative mosquitocidal agent preferably from plants available in nature. Today, the environmental safety of an insecticide is considered to be of important milestone in the field of pest control in general and vector control programme in particular. An insecticide must not cause high mortality in target organisms in order to be acceptable (Kabaru and Gichia, 2001). The extract treated eggs exhibited an allayed hatchability and this may be due to the action of phytochemicals present in the extract. The extract may inhibit the hatchability of the eggs by interfering with their chorion. It is evident from the present study that exposure of eggs of selected mosquitoes. Similar kind of observation was also noted earlier by several workers (Rajkumar *et al*., 2011; Aarthi and Murugan, 2011). The ovicidal activity indicated an important finding that the larvae which hatched out of the treated eggs were succumbed to death within an hour or two. In the present study, our aim was to determine whether *E. pedunculatum* could be used for mosquito control. We observed a functional response of the ovicidal activity exhibited by the ethanol extract. In the case of ovicidal activity, exposure to the freshly laid eggs was more effective than that to the older eggs. Similarly, ovicidal and gravid mortality effects of ethanolic extract of *Andrographispaniculata* was assessed by Kuppusamy *et al* (2008) against *An. stephensi*. Larvicidal and oviposition activity of *Cassia obtusifolia*leaf extract against *An. Stephensi* Liston was also evaluated by Rajkumar and Jebanesan (2009). Similarly, the aqueous and hydro-alcoholic extracts of *Meliaazedarach* leaves and seeds were tested to explore the in vitro ovicidal and larvicidal activity against *Haemonchuscontortus* (Kamaraj *et al*., 2010) and the results were comparable with our results. Additionally, through screening several plants for their larvicidal activity, Sharma *et al* (2006) found that *Artimisiaannua* was the most toxic against anopheles with an LC_{50} of 16.85 ppm and 11.45 ppm after 24 and 48 h of exposure, respectively. In addition, the larvicidal effects of *Momordicacharantia* fruit on *An. Stephensi* (LC_{50} of 66.05 ppm) were also investigated by Singh *et al* (2006). The biological activity of the plant extract might be due to a variety of compounds in *E. pedunculatum* may jointly or independently contribute to cause larvicdal and ovicidal activity against *An. stephensi*. The main chemical compounds present in the *E. pedunculatum* might responsible for the activities recorded in the present experiments. It would have been suggested that the direct and indirect contributions of such compounds to treatment efficacy while on the use of botanical insecticides for the control of *An. stephensi*. These and other naturally occurring insecticides may play a crucial role in vector control programs in the near future (Wandscheer *et al*., 2004). Since *An. Stephensi* breeds in drinking water tank, many of the plant extracts are subject to risk factors in mosquito control (Ahmed *et al*., 2011). The plant extracts which are highly toxic against *An. stephensi*are also toxic to human beings. In the present study, *Aristolochiaindica* extract showed promising effect on selected mosquitoesand it has no deleterious effects against human beings since it has been used in Indian auyurvedhic medicine for several ailments.

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