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LARVICIDAL ACTIVITY OF AQUEOUS EXTRACTS OF PLANTS

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ABSTRACT: In order to develop an environment-friendly botanical mosquito larvicide alternative to the chemical larvicides, extracts were made from the leaves of *Azadirachta indica, Piper nigrum, Calotropis gigantea, Vitex negundo, Myristica fragrans* and *Clerodendrum infortunatum* and *Annona muricata*. In the Present study *Azadirachta indica, Piper nigrum, Myristica fragrans* and *Annona muricata* showed 100% mortality in 24 hours. The mosquito larvae exposed to plant extracts showed significant behavioural changes. The most obvious sign of behavioural changes observed in *Aedes albopictus* was restlessness and loss of equilibrium which finally led to death. These good experimental results can further be explored. The present investigations suggest the possible use of this blend of botanical extracts as an ideal ecofriendly, larvicide against *Aedes albopictus.*

KEYWORDS: *Aedes albopictus*, Larvicidal Activity, *Azadirachta indica, Piper nigrum*.

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1.INTRODUCTION

Mosquitoes are the major vector for the transmission of malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis (JE) [9]. In India, malaria is one of the most important causes of direct or indirect infant, child and adult mortality with approximately two to three million new cases arising every year. *Anopheles subpictus* Grassi is distributed throughout

India, Afghanistan, Borneo, China, Malaysia, Philippines, Sri Lanka, Java and Indonesia. It is a dominant species in Haryana and Uttaranchal States [14]. Though it is a non-vector species, same infected specimens with malarial parasite have been reported from India, Indonesia and Java [11]. *An. culicifacies* is the main vector of malaria, and *An. subpictus* is a significant secondary vector in Sri Lanka [1]. *An. subpictus* is recognized as the secondary vector of malaria in South East Asia, with a large number of cases being reported from India. 2,400 million (about 40%) of the world's population [5, 6]. India contributes 77 per cent of the total malaria in Southeast Asia [12]. *Cx. tritaeniorhynchus* is a primary vector of Japanese encephalitis (JE) virus, with a distribution throughout Southeast Asia and South Asia. [10] have reported that global annual incidence and mortality estimates for JE are 30,000 to 50,000 and 10,000 respectively. Control of mosquito populations involves preventing wastewater that stands for longer than 4 days; keeping weeds down around ponds, in ditches, and in shallow wetlands and irrigating properly so that all surface water is gone within 4 days [13]. Mosquito larval control becomes important in cases where mosquito breeding sites cannot be eliminated such as the drinking ponds, watering troughs and hoof prints. Mosquito control at larval stage has the advantage of controlling the vector before it can acquire and transmit the disease. Mosquito larvae can be controlled by substances added directly to the water. These substances may be organisms that consume them such as larvivorous fish [3], biological compounds that poison them or cause fatal infections that are specific to them (*Bacillus thuringiensis israelensis*) [15], chemicals that disrupt their development or physiology such as the insect growth regulator, methoprene [13], [9] or oils and films that suffocate them [2]. Biological compounds tend to be more expensive than chemical controls but they affect fewer non-target organisms [7]. Chemical controls are typically very effective against mosquitoes but they are toxic to non-target organisms and inaccessible due to high cost and limited availability in nearby markets, particularly in resource-poor communities. In addition, in cases where the chemicals are continuously used mosquitoes develop resistance. Many plant extracts have been tested against various species of mosquitoes, focusing on larvicidal action (Shaalan et al., 2005). The use of plant extracts against noxious insects has the advantage that the closely related compounds within these complex mixtures often act synergistically [8]. The exposure of a target organism to a group of phytochemicals, rather than to a single active principle, lowers the probability for that organism to develop resistance or behavioural desensitization. In the Present study we have evaluated larvicidal activity of crude aqueous extracts of leaves of *Azadiracta indica, Piper nigrum, Calotropis gigantea, Vitex negundo*, *Myristica fragrans* and *Clerodendrum infortunatum* and *Annona muricata*.

2. MATERIALS AND METHODS

Fully developed leaves of *Azadirachta indica* of Meliaceae family, *Piper nigrum* of Piperaceae family, *Calotropis gigantea* of Apocynaceae family, *Vitex negundo* of Verbanaceae family, *Myristica fragrans* of Myristicaceae family and *Clerodendrum infortunatum* of Lamiaceae family and *Annona*

muricata of Annonaceae family were collected from Thiruvananthapuram District Kerala, India. It was Authenticated by a plant taxonomist from the Department of Botany, S. T. Hindu College, Nagercoil.

Surface Sterilization of Leaves: The collected leaves were thoroughly washed with running tap water and immersed in 0.3% Dettol solution for 20 min.

Extraction: About 10 gm of sample is macerated with 10ml of sterile water. Then the mixture was filtered.

Raring of Aedes albopictus (Test Organism): Coconut Shells containing 100ml of water (culture medium) were placed in different parts of the field. Larvae of mosquitoes seen after 8-10 days were collected and fed with biscuit (nutrient medium)

Larvicidal Bioassay: 1 ml of crude aqueous extract was added to 5 numbers of larvae in 5 ml of water. Corresponding control was maintained. The number of larvae surviving at the end of 12 hours and 24 hours were recorded and the percent mortality was calculated.

The Percentage of mortality was calculated by

$$\frac{\text{No. of Larva Dead}}{\text{Total no of Larva}} \times 100$$

3. RESULTS AND DISCUSSION

Aqueous extracts of *Azadirachta indica*, *Piper nigrum, Calotropis gigantea*, Vitex negundo, *Myristica fragrans* and *Clerodendrum infortunatum* and *Annona muricata* were screened for larvicidal activity. It was found that aqueous extracts of *Azadirachta indica* was highly toxic to *Aedes albopictus*. Among the extracts *Myristica fragrans, Annona muricata* and *Piper nigrum* showed 100% mortality in 24 hours. *Clerodendrum infortunatum* and *Vitex negundo* 80% mortality in 24 hours and *Calotropis gigantea* 60 % mortality in 24 hours. The mosquito larvae exposed to plant extracts showed significant behavioural changes. The most obvious sign of behavioural changes observed in *Aedes albopictus* was restlessness and loss of equilibrium which finally led to death. Results are shown in Table 1, Plates 1, 2 below

Table 1. Larvicidal Activity of Aqueous plant extracts at different time intervals

Sl. No.	Plants	Mortality Rate	
		12 hours	24 hours
1	*Azadirachta indica*	100%	80%
2	*Myristica fragrans*	100%	80%
3	*Piper nigrum*	100%	80%
4	*Annona muricata*	40%	100%
5	*Calotropis gigantea*	10%	60%
6	*Clerodendrum infortunatum*	Nil	80%
7	*Vitex negundo*	10%	80%

*

Plate 1

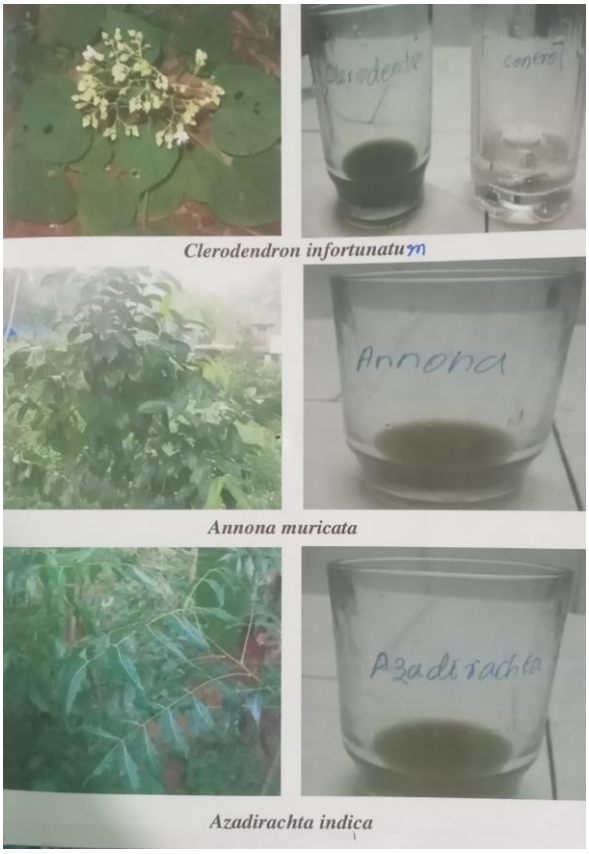
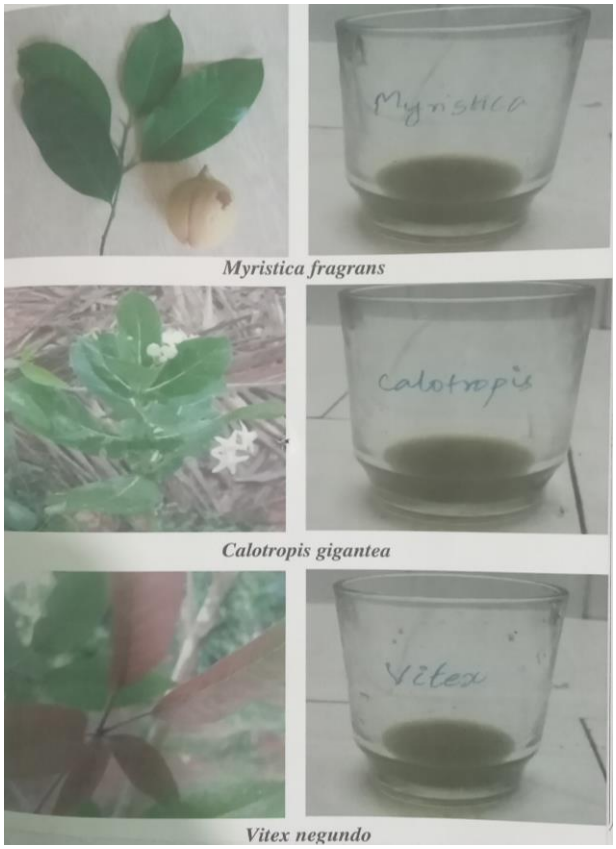


Plate 2



Several studies have focused in natural products for controlling *Aedes mosquitoes* as insecticides and larvicides but with varied results. [4], [1], [9]. In the present study *A. indica, P. nigrum, M. fragrans* and *A. muricata* showed 100% mortality in 24 hours. Moreover, behavioural changes were observed in the movement of the larvae. These effects may be due to the presence of neurotoxin compounds in plant extracts. No, behavioural changes were obtained in control group. Furthermore, the crude extracts may be more effective compared to the individual active compounds, due to natural synergism that discourages the development of resistance in vectors.

4. CONCLUSION

Crude extracts or isolated bioactive phytochemicals from the plants could be used in stagnant waterbodies which are known to be breeding grounds for mosquitoes. The pronounced taste of the extracts will probably be a problem regarding their use in drinking water. Use of these botanical derivatives in mosquito control instead of derivatives of synthetic insecticides could reduce the cost, free from harmful effects and environmental pollution. Further studies on their effects on non-target organisms and the environment and regarding formulations to improve the insecticide potency and stability are needed for these naturally occurring mosquito larval control agents to be used in practice.

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CONFLICT OF INTEREST

Authors have no any conflict of interest.

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