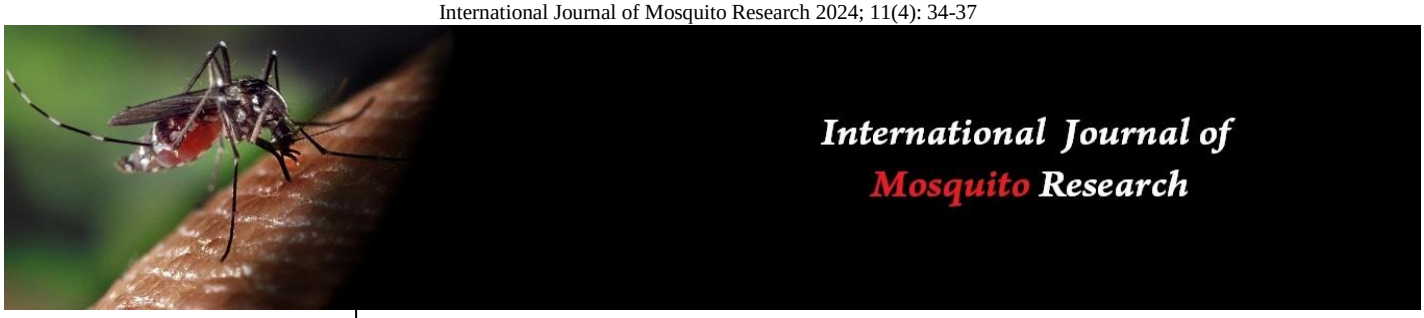

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Botanical insecticides: Assessment of *Vitex negundo* and *Acorus calamus* acetone leaf extracts against *Aedes aegypti* larvae and pupae (Diptera: Culicidae)

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Abstract
Aedes aegypti, the primary vector for diseases such as dengue fever, Zika virus and chikungunya, poses a significant threat to public health globally. The increasing resistance of mosquitoes to conventional insecticides has led to a search for alternative larvicidal and pupicidal agents derived from natural sources. This study aimed to evaluate the larvicidal and pupicidal potential of acetone leaf extracts from *Vitex negundo* and *Acorus calamus* against *Aedes aegypti*. The larvicidal and pupicidal activities of the extracts were assessed through bioassays following WHO guidelines. Various concentrations of the extracts were tested against different life stages of *Aedes aegypti* to determine their efficacy. The acetone leaf extracts from both *Vitex negundo* and *Acorus calamus* exhibited significant larvicidal and pupicidal activities against *Aedes aegypti*. The mortality rates of larvae and pupae increased with higher concentrations of the extracts. The results indicated that these plant extracts have the potential to be used as alternative larvicidal and pupicidal agents against *Aedes aegypti*. The findings of this study suggest that acetone leaf extracts from *Vitex negundo and Acorus calamus* have promising larvicidal and pupicidal properties against *Aedes aegypti*. Further research is warranted to elucidate the active compounds responsible for the observed insecticidal properties.

Keywords: Larvicidal, pupicidal, *Vitex negundo*, *Acorus calamus*, vector mosquito

Introduction
Mosquitoes transmit more diseases than any other group of arthropods and afflict millions of people throughout the world. The World Health Organization has identified mosquitoes as 'Human's first adversary' [1]. Vector-borne diseases have become a challenging public health concern due to their social and economic repercussions, especially in subtropical and tropical countries [2]. On an annual basis, millions of people die from mosquito-borne diseases include dengue fever, malaria, chikungunya, filariasis and Japanese encephalitis, which affect both human beings as well as domestic animals worldwide [3]. *Aedes aegypti* (Linnaeus, 1762) is a mosquito species that is known to spread arboviruses such as dengue, chikungunya, and zika virus over the world. It is a diurnal mosquito that can adapt quite well to domestic and urban settings [4,5]. The rapid increase in rates of urbanization in tropical regions, lack of basic infrastructure, and limited or non-existent sanitation, associated with favourable climatic conditions for mosquito development, have contributed to the expansion of the occurrence range of arboviruses transmitted by female *A. aegypti* [6]. A certain mosquito species, Aedes aegypti, plays a crucial role in transmitting viral diseases like Dengue, Dengue hemorrhagic fever, and yellow fever, among others, in tropical regions. This mosquito species is a primary culprit in spreading these infections, which pose a significant threat to public health in tropical countries[7]. Dengue viruses, which are the main cause of dengue fever and more dangerous dengue haemorrhagic fever/dengue shock syndrome, infect almost 100 million individuals each year [8]. Synthetic insecticides pose environmental risks, including the development of insecticide resistance in vector populations, the accumulation of non-biodegradable chemicals in the ecosystem, and their impact on biological magnification through food chains.

Additionally, their toxicity can harm human health and other organisms [9]. These problems have spurred scientists' efforts to discover remedies to these issues on a worldwide scale. Green chemistry, which use natural plant phytocompounds to control mosquitoes, has recently gained popularity. Natural compounds generated from plants, especially in the field of infectious diseases, are widely known to be efficient, safe, and often used [10]. More concentrated efforts need to be made into these studies in order to make these environmentally friendly compounds feasible for field application and large-scale vector control initiatives [11].

Vitex negundo is the plant of family Verbenaceae found throughout Southern India, Ceylon- Afghanistan, tropical Africa, Madagascar, China, Philippines, Burma and Bengal. It is prevalent in damp areas, riverbanks, waste areas near villages, and deciduous forests [12, 13, 14]. Various reports claim that the plant products of *V. negundo* have insecticidal efficacy against tobacco leaf-eating larvae, house flies, mosquito larvae, and pests of stored products.

Research also indicates that the plant's oil has the ability to deter pests found in stored goods [14]. Herbal remedies are made from almost every component of the plant. It is well known that the plant has anticancer, antibacterial, antifeedant, anti-inflammatory, antihyperpigmentation, hepatoprotective, antihistaminic, analgesic, and related properties [14].

Acorus calamus Linn belongs to the Acoraceae plant family and is a semiaquatic perennial known as "sweet flag" or "calamus" [15]. Records indicate that the essential oil extracted from A. calamus possesses qualities that deter insects, prevent them from feeding, and chemically sterilize. The rhizomes are thought to possess sedative, nervine, stimulant, carminative, anthelmintic, aromatic, nauseous, and expectorant properties. Additionally, they are used to treat glandular problems, dysentery, recurrent fevers, bronchial carcinoma, long-term diarrhoea, psychiatric disorders, and abdominal malignancies [16, 17]. In recent times, plant extracts and their unique bioactive ingredients have emerged as a viable substitute for synthetic insecticides. Hence, in the present study, an attempt was made to explore the larvicidal and pupicidal activity of *A. aegypti* through acetone leaf extracts of *V. negundo* and *A. calamus*.

Materials and Methods

Acquisition of plant material

The plant, *Vitex negundo* (Verbenaceae) and *Acorus calamus* (Acoraceae), leaves were collected from various parts of Thoothukudi district, Tamil Nadu, India. The plants were identified and authenticated by the botanist.

Preparation of crude solvent extracts

The leaves of selected plants were cleaned with running tap water and rinsed with double-distilled water to remove dust particles. Following a thorough cleaning, the plant leaves were left to dry naturally at room temperature (27 ºC to 37 ºC) for around 20 days. The dried plant leaves were ground into a fine powder using an electric blender and sieved. Both the plant powder extract was derived with the help of Soxhlet apparatus using acetone as an only solvent for 8 hours. The resulting extracts were filtered via a Buchner funnel using Whatman Number 1 filter paper. The residue was concentrated and kept at 5 ºC for future use.

Rearing of Aedes aegypti larvae

The *Aedes aegypti* eggs were purchased from ICMR- Vector Control Research Centre, Madurai, Tamil Nadu, India. Then the purchased eggs were transported to the laboratory in small petri dishes covered with cotton. The hatched larvae were reared in enamel pans containing water as culture medium at a laboratory temperature of 29º C. The water in the rearing trays was changed daily to prevent the mortality of the larvae. The larvae were fed with sterilized yeast powder and dog biscuits in the ratio of 3:1 (Rajkumar and Jebanesan, 2005) [18]. The adults were provided with 5% glucose solution and honey was given to male and female. These laboratory-colonized mosquito larvae were utilized in the bioassays. In this study, all the four larval instars and pupae were used.

Larvicidal bioassay

The World Health Organization (2005) suggested methodology was used to assess the larvicidal properties of the chosen plant extracts. One gram of raw plant extract was diluted in 100ml of acetone (1% stock solution). To achieve the necessary quantities, 1ml of plant extract was added to 249ml of dechlorinated water in a 500ml glass beaker holding 100 of each of the four stages of larval instars and pupae. Larvae were treated with varying concentrations from the stock solution according to their developmental phases with the prepared dechlorinated water from stock solution. With 249ml dechlorinated water, 1ml of acetone was kept as control. The number of mortalities of all the four instars and pupae of *A. aegypti* was observed at the end of 24hrs. The percentage of mortality was calculated by

$$\text{Percentage of mortality} = \frac{\text{No. of. Larva/ pupa dead}}{\text{No. of. Larvae/ pupae introduced}} \times 100$$

$$\text{Corrected mortality} = \frac{\text{observed mortality in treatment} - \text{observed mortality in control}}{\text{100- control mortality}}$$

Statistical analysis

Statistical analysis is a valuable tool for comprehending and analysing data. Probit analysis is a popular statistical tool for studying mortality data. In the present investigation, the larval mortality data were subjected to Probit analysis in order to calculate LC_{50}, LC_{90}, 95% confidential of upper confidential limit and lower confidential limit by using the software SPSS 20 to ensure the accuracy of results.

Results

The results showed that larvicidal activity of acetone extracts of *Vitex negundo* and *Acorus calamus* against all the four larval instars and pupal stage of Aedes aegypti are shown in the (Tables 1 and 2). The study found that mosquito larvae mortality increases with concentration. These findings suggest that *Vitex negundo* acetonic leaf extracts are more toxic

Table 1: The larvicidal and pupicidal activity of *Vitex negundo* acetone leaf extracts against various larval stages and pupal stage of *A. aegypti*

Larval & pupal stages	Concentration (ppm)	Mortality %	LC_{50} (LCL- UCL) (ppm)	LC_{90} (LCL- UCL) (ppm)	Regression equation
First larval instar	control	0	1.563 (1.164- 2.038)	20.728 (12.699- 43.422)	Y= -2.21+ 1.142X
	0.5	24			
	1	48			
	5	70			
	10	82			
Second larval instar	control	0	4.620 (2.591- 5.997)	26.514 (18.704- 60.333)	Y= -1.12+ 1.68X
	5	56			
	10	64			
	12	72			
	15	88			
Third larval instar	control	0	11.021 (9.213- 12.392)	27.703 (23.741- 35.812)	Y= -3.33+3.20X
	10	46			
	15	64			
	20	80			
	25	88			
Fourth larval instar	control	0	20.937 (17.843- 23.151)	43.038 (38.430- 51.874)	Y= -5.40+4.09X
	20	48			
	30	72			
	35	80			
	40	90			
Pupal stage	control	0	28.866 (23.317- 32.528)	65.178(56.839- 83.994)	Y= 1.015+ 0.62X
	30	56			
	40	64			
	50	80			
	60	90			

to larvae and pupae than *Acorus calamus* extracts, as indicated by the lower LC_{50} and LC_{90} values. This implies that *Vitex negundo* will be more effective larvicidal agent than *Acorus calamus*. The data show statistically significant differences (p<0.01) in larval mortality between the treatment groups and control. Notably, zero mortality was observed in the control group following 24 hours of treatment.

Table 2: The larvicidal and pupicidal activity of *Acorus calamus* acetone leaf extracts against various larval stages and pupal stage of *A. aegypti*

Larval & pupal stages	Concentration (ppm)	Mortality %	LC_{50} (LCL- UCL) (ppm)	LC_{90} (LCL- UCL) (ppm)	Regression equation
First larval instar	control	0	2.829 (2.075- 3.971)	61.015 (29.350- 205.016)	Y= -0.43+0.96X
	0.5	22			
	1	38			
	5	50			
	10	76			
Second larval instar	control	0	11.190 (9.892- 12.964)	38.753 (27.558- 73.367)	Y= -2.49+2.37X
	5	24			
	10	36			
	12	50			
	15	70			
Third larval instar	control	0	13.379 (11.793- 14.727)	33.534 (27.986- 45.476)	Y= 0.55+0.45X
	10	40			
	15	50			
	20	62			
	25	90			
Fourth larval instar	control	0	25.855 (22.743- 28.290)	62.767 (51.214- 93.486)	Y= -4.70+3.32X
	20	42			
	30	46			
	35	60			
	40	86			
Pupal stage	control	0	37.370 (34.751- 39.646)	64.820 (58.882- 74.889)	Y= 1.02+0.63X
	30	32			
	40	56			
	50	70			
	60	90			

*Death rate of larvae and pupae recorded after 24-hour treatment duration. LC_{50} = Lethal Concentration corresponds to 50% fatal dose, LC_{90} =Lethal Concentration corresponds to 90% fatal dose, LCL = Lower Confidence Limit, UCL= Upper Confidence Limit, statistically significant at P<0.01.

Discussion

Mosquito control is critical public health strategy for preventing the spread of vector- borne diseases like malaria, dengue and Zika. While synthetic pesticides have been widely used for mosquito control, their limitations and drawbacks have led to a growing interest in alternative approaches. Plant extracts offer a promising solution, with several advantages over synthetic pesticides. Firstly, plant extracts are biodegradable and non-toxic, reducing risk of environmental contamination and human exposure. In contrast, synthetic pesticides have been linked to various health problems, including cancer, neurological damage and reproductive issues [19, 22]. The current study investigates the larvicidal properties of two plant species, *Vitex negundo* and *Acorus calamus*, as a biological control agent against *Aedes aegypti* mosquito larvae and pupae. The highest mortality was observed in *Vitex negundo* extracts against various larval instars and pupae. Similar findings were observed in a study by (Vasanth Raj *et al*., (2009), which demonstrated that both aqueous and methanolic extracts of V.*negundo* exhibited significant larvicidal efficacy against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* [20]. The findings are consistent with those reported by Vaikkunnel House, S. T. (2023), which showed that the highest mortality rate (100%) was achieved with both acetone and ethanol extracts after 48 and 72 hours of exposure, respectively [21].

Conclusion

The acetone leaf extracts of V. *negundo* show promise for mosquito control and further research is to investigate the optimal dosages, formulation and field efficacy of these plant extracts as mosquito control agents, as well as their potential impact on non- target organisms and the environment.

Appendix

ºC= Degree Celsius.
PPM = Parts Per Million.
LC_{50} = Lethal Concentration for 50% mortality.
LC_{90}= Lethal Concentration for 90% mortality.
LCL= Lower Confidence Limit.
UCL= Upper Confidence Limit.

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