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LARVICIDAL EFFICACY OF RHIZOME EXTRACTS OF ACORUS CALAMUS AND CURCUMA LONGA AGAINST THE DENGUE FEVER MOSQUITO VECTOR AEDES AEGYPTI

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Abstract: *Aedes aegypti* is a vector of dengue fever, which is a public health problem in the world. During epidemics, emphasis is laid on the use of insecticides for controlling mosquitoes. The application of easily degradable phytocompounds is considered to be one of the safest methods to control insect pests and vectors as an alternative to artificial insect killer. A study was made to monitor the effect of rhizome extract *Acorus calamus* and *Curcuma longa* on different larval instars and pupae of mosquito vector of *A. aegypti*. Bio-assay was performed using the solvent ethanol to find out the median lethal concentration. The study indicated that essential compounds were the only chemical used for the control of mosquito larvae while extract was used as the control of adult mosquitoes. The results suggest a potential utilization of the extracts of these two species for the control of *A. aegypti*.

Key words: *Aedes aegypti*, larvicidal activity, phyto-compounds, *Acorus calamus* and *Curcuma longa*

I. INTRODUCTION

Dengue fever is endemic over large areas of tropics and subtropics. Outbreaks of dengue have repeatedly occurred in world over the last 10 years. Despite of the fact that dengue outbreaks in the tropics have been worldwide for over 200 years, it is still the most important mosquito transmitted viral disease affecting man. The etiological agent is an arbovirus and the major vector is the *Aedes aegypti* mosquito, which is found in 3600 Brazilian municipalities. While most patients are asymptomatic, reinfection with different serotypes of dengue viruses may lead to hemorrhagic fever with high mortality. During outbreaks, public health authorities in Brazil have standardized the use of aerolized pyrethroid insecticides that can cause allergies. This measure only partially controls the mosquito population since it eliminates the adult flying insects but does not eliminate the breeding places. In these breeding sites, the larvicide used is usually the organophosphorate Temephos, although very slightly toxic may cause headaches, loss of memory and irritability[1].

Dengue is a viral disease that has major public health consequences in many parts of the world. The principal vector of dengue fever, including the haemorrhagic form, is the mosquito *Aedes aegypti* [2]. Presently, mosquito control primarily depends worldwide on continued applications of conventional toxic synthetic insecticides to which resistance has been reported in many areas where it is widely used [3-4]. This has stimulated the investigation of natural insecticides as an alternative control, focused on plant-derived compounds, including volatile chemical constituents (essential oils), as potentially bioactive substances against mosquito larvae.

Aedes aegypti (Diptera: Culicidae) is an arbovirus vector responsible for yellow fever in central and South America and in West Africa. It is also the vector for dengue hemorrhagic fever (DHF), endemic to south-east Asia, the Pacific Islands, Africa and the Americas. It is estimated that 2.5 billion people are currently at risk for dengue fever (DF), DHF, and dengue shock syndrome (DSS) [5]. The size and spread of the dengue pandemic, the unpredictability of the epidemic occurrences and the circulation of virulent and non-virulent strains make DHF/DSS a model for emerging infectious disease. Despite of this challenge, the development of dengue virus vaccines is still a long way to be of any use due to several obstacles [6].

Acorus calamus Linn. commonly known as Sweet Flag, belongs to the family Araceae (Adoraceae). It is also called as *Acorus odoratus*. The genus *Acorus* derived from *Acoron* (coreon = the pupil of the eye) and the

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species *calamus* is derived from the Greek word *Calamos* (a reed). The family *Araceae* comprises about 110 genera and more than 1,800 species. The members of the family are rhizomatous or tuberous herbs. *Acorus calamus* commercially occurs in both peeled and unpeeled forms. This perennial herb is common on the banks of streams and in damp marshy places. The sweet flag oil present in this plant is a unique source of oxygenated sesquiterpenes of great structural variety [7]. Apart from this terpenes a few commonly occurring steroids and xanthenes had also been reported. The rhizome of the plant has medicinal properties against bugs, moths, lice, emetic stomach in dyspepsia; etc [8]. Common names of *Acorus calamus* which are used in different parts in India are Bach (Hindi), Vashampu (Tamil), Baje (Kannada) and Vasa (Telugu).

Curcuma domestica, or Turmeric, is a robust, rhizomatous, perennial herb native to Tropical Asia. The rhizomes are short and tuberous with a yellow flesh. It is from these rhizomes that we get the spice Turmeric. The rhizomes are dried and ground to yield the spice. Plants will reach up to 3 feet (0.9 m) tall. The dark to medium green leaves reach about 1.5 feet (0.45 m) long by 8 inches (20.3 cm) wide. They are attractive plants that do very well in containers. The plants are hardy in the landscape in USDA zones 8-11. *Curcuma domestica* was featured as Plant of the Week August 10-16, 2007. Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger family, *Zingiberaceae*. It is native to tropical Indian Subcontinent and needs temperatures between 20 °C and 30 °C (68 °F and 86 °F) and a considerable amount of annual rainfall to thrive. Plants are gathered annually for their rhizomes, and propagated from some of those rhizomes in the following season.

When not used fresh, the rhizomes are boiled for about 30–45 minutes and then dried in hot ovens, after which they are ground into a deep orange-yellow powder commonly used as a spice in Indian cuisine and even curries, for dyeing, and to impart color to mustard condiments. Its active ingredient is curcumin and it has a distinctly earthy, slightly bitter, slightly hot peppery flavor and a mustardy smell. Curcumin has been a centre of attraction for potential treatment of an array of diseases, including cancer, Alzheimer's disease, diabetes, allergies, arthritis and other chronic illnesses. In the present work rhizome extract of *Acorus calamus* and *Curcuma longa* were investigated for potential larvicidal activity. To identify and characterized the compounds of therapeutic value extracted from *Acorus calamus* and *Curcuma longa*.

II. MATERIALS AND METHODS

Plant material and oil distillation: The medicinal herbs of *Acorus calamus* and *Curcuma longa* rhizomes were purchased from herbal store at Pattukkottai, Thanjavur district, Tamil Nadu, South India. The rhizomes were identified with the help of flora of presidency, Tamil Nadu and Karnatic flora [9-10] and standard references [11].

The fresh rhizomes of *Acorus calamus* and *Curcuma longa* were collected and a part of the rhizomes material was washed under running tap water. Small hairs of *A. calamus* and *C. longa* were removed and could be chopped and dried at low temperature and then homogenized to fine powder and stored in airtight bottles. Powder of rhizome extracted with 90% w/w ethanol using a soxhlet apparatus. The ethanol was removed under pressure using a rotary evaporator. The dried residue of the crude extract was stored in a dark bottle at 4°C in airtight bottles for further studies. Approximately, 5 g of extract was obtained from 100 g of dried powder material. The extracts were dried in an air conditioned room at 25°C, milled and submitted to hydro distillation in a Clevenger-type apparatus for 4 hours. The extracts were dried in anhydrous sodium sulphate, filtered, stored in amber glass bottles in a refrigerator (4°C) for investigation of chemical constituents and larvicidal activity.

Collection and storage of experimental animals: Larvae of *A. aegypti* were obtained from a permanent colony. The larvae were cultured and maintained in the laboratory at 27 ± 2°C and 50 - 75% relative humidity. Larval forms were maintained in tray by providing dog biscuit and yeast powder in the ratio 3:1.

Test for Larvicidal activity [12]: The laboratory colonies of *A. aegypti* were used for the larvicidal activity. The instar II and instar IV larvae and pupae of the selected mosquito species were kept in 1 litre glass beaker and different concentrations of selected plant extract were added to find out LC₅₀.

Larvicidal bioassay: Different concentrations of extract (0.600, 0.500, 0.400, 0.300, 0.200, 0.150, 100 and 0.50 mg/ml) were prepared using solvent. The mosquito larvae were treated with extract by using the method of WHO [13]. Ten larvae of *A. aegypti* were introduced in different test concentration of both plant extracts along with a set of control containing distilled water without any test solution. After adding the larvae, the glass dishes were kept in laboratory at room temperature. By counting the number of dead larvae at 24hrs of exposure, the mortality rate and the median lethal concentration were obtained. Three replications were maintained for each treatment. Dead larvae were removed as soon as possible in order to prevent decomposition which may cause rapid death of the remaining larvae. The water used for

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the study was analyzed by using the method of APHA [14]. Mortality was recorded after 24 h of exposure during which no nutritional supplement was added. The experiments were carried out $27 \pm 2^{\circ}\text{C}$. Each test comprised of three replicates with four concentrations (600, 500, 200, 100 and 50 $\mu\text{g/ml}$).

Statistical Analysis: Data were evaluated through regression analysis. From the regression line, the LC_{50} values were read representing the lethal concentration for 50% larval mortality of *A. aegypti*.

III. RESULTS AND DISCUSSION

Physical and chemical characteristics of water used for the study, like temperature $28 \pm 0.2^{\circ}\text{C}$, pH 7.1 ± 0.2 , dissolved oxygen 3.9 ± 0.2 mg/l, dissolved carbon dioxide 1.5 ± 0.2 mg/l, salinity 2.1 ± 0.2 ppt and alkalinity 132 ± 0.2 mg/l were within the permissible limits throughout the study period.

The 24h bioassay is a major tool for evaluating the toxicity of phytochemicals and a number of researchers have been applying this method to assess the toxic effect of different plant extracts against mosquitoes [15]. The mosquito larvae exposed under plant extracts showed significant behavioral changes. The changes were observed within 30 minutes of exposure. The most obvious sign of behavioral changes observed in *A. aegypti* was inability to come on the surface. The larvae also showed restlessness, loss of equilibrium and finally death. Remia and Logaswamy [16] reported that these behavioral effects were more pronounced in case of *Catharanthus roseus* than *Lantana camara* extracts after exposure. These effects may be due the presence of neurotoxic compounds in both the plants. In the present study the behavioral effects were more pronounced in case of *Acorus calamus* than *Curcuma longa* extracts after exposure. No such behavioral changes were obtained in control groups.

Results of the experiment conducted for evaluating the larvicidal efficacy of both plants showed that they are toxic to *A. aegypti* larvae. Three replicates of each extract and control were performed in order to ascertain the consistency of the results (Tables 1).

Table 1. Larvicidal effects of ethanolic extracts of *Acorus calamus* and *Curcuma longa* on larvae of *A. Aegypti* after a 24 h treatment at room temperature

S. No	Concentration of the extract (mg/ml)	No. of larvae Dead/No. exposed (<i>A. calamus</i>)	No. of larvae Dead/No. exposed (<i>C. longa</i>)	Mortality
1	Control	0/30	0/30	0
2	0.50	3/30	3/30	10
3	0.100	6/30	6/30	20
4	0.150	9/30	9/30	30
5	0.200	12/30	12/30	40
6	0.250	15/30	15/30	50
7	0.300	18/30	18/30	60
8	0.400	24/30	24/30	70
9	0.500	30/30	30/30	80
10	0.600	30/30	30/30	100

The crude extract of *C. longa* was found to be active against the IV instar larvae of *A. aegypti*. The larvicidal activity varied with the concentration and exposure. The larvicidal activity of *C. longa* was comparable to that of *A. calamus*. *A. calamus* has been reported to contain sufficient amount of tetranortriterpenoids [17-18] responsible for the larvicidal activity. The observed mosquito larvicidal effects could possibly be due to these compounds.

The results from *A. aegypti* larvicidal assay using two different extract are shown in Table 2. The most active essential compounds against third instar larvae of *A. aegypti* were those of *Acorus calamus* and *Curcuma longa*. Abbott [19] reported that *C. citratus* causes significant growth inhibition and mortality in later developmental stages of *A. aegypti*. The analysis of the essential oil of this plant from the state of Ceará, showed that its major components are geranial (60.3%) and neral (39.7%). *Lippia sidoides* essential oil and its main constituent thymol were shown to be very active against *A. aegypti* larvae [20]. Abbott [19] studied *Ocimum americanum* and showed that solvent extracts from the

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whole plant have ovipositional deterrence against *A. aegypti*. Matos [21] reported that *O. gratissimum* essential oil displays antifungal (*Aspergillus* and *Trichoderma*) and antibacterial (*Staphylococcus*) activities. *O. gratissimum* oil presented antihelminthic activity against *Haemonchus contortus*, the main nematode of ovines and caprines in Northeastern Brazil [22]. The citrus oils, although they have insecticidal activities and *Hyptis suaveolens* that is used as mosquito repellent [28] were not effective in the larvicidal test. Supavarn [23] tested 36 vegetable extracts on *A. aegypti* and found that 11.1% were capable of producing mortality at a concentration of 500 ppm but only 2.8% produced the same effect at a concentration of 100 ppm.

The use of vegetable oil presents a better option in comparison to chemical pesticides for the larval mosquito control, as chemicals may cause environmental hazards proving troublesome in the long run [24]. Extensive research has been carried out on the effect of botanical derivatives of the neem tree and its derivatives [25]. Methanolic extract of the leaves of *Atalantia monophylla* were evaluated for mosquitocidal activity against the immature stages of mosquitoes, *Culex quinquefasciatus*, *Anopheles stephensis* and *A. aegypti* in the laboratory [26]. A survey of literature on control of different species of mosquito revealed that assessment of the efficacy of different phytochemicals obtained from various plants has been carried out by a number of researches on the field of vector control *Ageratina adenophora* (Spreng.) showed toxic effects on the mosquito species of *A. aegypti* and *C. quinquefasciatus* [27]. *Albizia amara* and *Ocimum sanctum* showed larvicidal and repellent properties against *A. aegypti* and neem seed kernel extracts showed higher larvicidal activity of *A. aegypti* [28,15]. A detailed laboratory study on extracts of fruit of *Piper nigrum* against larvae of *C. pipines*, *A. aegypti* and *A. togoi* was carried out [29]. The authors determined the LC₅₀ and observed the behavioural changes and mortality in the larvae. Similar observations were noticed in the present study supporting the potential application of these herbs in mosquito control measures.

Table 2. Percentage larval and pupal mortality of *A. aegypti* for different concentrations of extract of *Acorus calamus* and *Curcuma longa* following 24 h exposure

Plants used	Stages of exposure	Parameters	Effective concentration in µg/ml					
			Control	100	200	300	400	500
<i>A. calamus</i>	II instar	Larval mortality (%)	Control	100	200	300	400	500
			0	10	22	34	45	60
	IV instar	Larval mortality (%)	Control	320	360	400	440	480
			0	15	23	34	46	51
	Pupae	Pupal mortality (%)	Control	400	450	500	550	600
			0	15	21	28	48	55
<i>C. longa</i>	II instar	Larval mortality (%)	Control	50	100	150	200	250
			0	13	25	36	49	63
	IV instar	Larval mortality (%)	Control	100	200	300	400	500
			0	19	28	36	48	57
	Pupae	Pupal mortality (%)	Control	400	450	500	550	600
			0	18	25	33	51	60

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Molluscicidal and mosquito larvicidal efficacy of *Heliotropium indicum* and mosquito larvicidal property of *Momordica charantia* have already been reported [7, 30-31] as safe for human health. In conclusion the rhizomes extract of *Acorus calamus* and *Curcuma longa* are highly toxic even at low doses thus proving to be effective larvicide. Further analysis is required to isolate the active principles and optimum dosages, responsible for larvicidal and adult emergence inhibition activity in *A. aegypti*. The product of these plants can be well utilized for preparing phytochemicals from which all the non-target organisms can be rescued from harmful vectors. These plants may serve as eco-friendly and suitable alternative to synthetic insecticides as they are relatively safe, inexpensive and readily available in many areas of the world.

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