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## Larvicidal potential of *Acorus calamus* L. essential oil against filarial vector mosquito *Culex quinquefasciatus* (Diptera: Culicidae)

A Senthilkumar, V Venkatesalu\*

Department of Botany, Annamalai University, Annamalai Nagar-608002, Tamil Nadu, India

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### ABSTRACT

**Objective:** To identify the chemical composition and larvicidal activity of *Acorus calamus* (*A. calamus*) rhizome essential oil against the filarial vector mosquito, *Culex quinquefasciatus* (*Cx. quinquefasciatus*). **Methods:** Essential oil was isolated by hydro-distillation and the chemical composition of the oil was analysed by gas chromatography and gas chromatography mass spectroscopy analysis. The larvicidal activity of the essential oil was analysed at different concentrations, viz., 12.5, 25.0, 50.0, 100.0 and 200.0 mL/L. Early 4th instar larvae were used for the larvicidal assay. The larval mortality was calculated after 24 h of the exposure. **Results:** The gas chromatography and gas chromatography mass spectroscopy analysis showed that the essential oil extracted from the rhizome of *A. calamus* contained 20 chemical compounds representing about 99.99% of the total oil. Beta-asarone (33.36%), *cis*-beta-terpineol (23.44%), limonene (13.08%), carvone (5.64%) and amyl isovalerate (4.92%) were identified as the major chemical compounds. The essential oil had promising larvicidal effect against the early 4th instar larvae of *Cx. quinquefasciatus* with LC<sub>50</sub> value of 63.43 mL/L and LC<sub>90</sub> value of 145.95 mL/L. **Conclusions:** The essential oil of *A. calamus* rhizome can be used as a natural larvicidal agent against the larvae of filarial vector mosquito, *Cx. quinquefasciatus*.

## 1. Introduction

Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases such as filariasis, malaria, dengue and Japanese encephalitis and also cause millions of deaths every year<sup>[1]</sup>. Lymphatic filariasis alone affects at least 120 million people in 73 countries in Africa, India, Southeast Asia and Pacific Islands. These diseases not only cause high levels of morbidity and mortality but also inflict great economic loss and social disruption on developing countries such as India and China<sup>[2]</sup>. India alone contributes around 40% of global filariasis burden and the estimated annual economic loss is about 720 crores<sup>[3]</sup>.

There are millions of rupees that are being spent on the purchase of personal prevention measures in the form of mosquito coils or vapourising mats or liquids to prevent mosquito bites. Repeated use of synthetic insecticides for mosquito control affects other beneficial organisms and

proves detrimental to animal life including man life. The development of resistance by vectors against plant derived bioactive molecules has not been reported so far. Hence, the use of natural larvicides is the most effective and alternative approach for mosquito control in their breeding sites itself. Recent studies showed that essential oils extracted from plants are those with the best results in this field and become potential candidates for the development of natural insecticides to control the mosquito borne diseases<sup>[4–6]</sup>.

*Acorus calamus* L. (*A. calamus*) (*Araceae*) is a semi-evergreen perennial with scented rhizomes, which originated in India, Central Asia, and Eastern Europe but now grows all over the world. It is a grass-like, rhizome forming perennial that can grow to 2 m high, resembling an iris. This species inhabits perpetually in wet areas like the edges of streams, around ponds and lakes, and in ditches and seeps. The thick, erect leaves are very similar in appearance, but with edges that are crimped. Plants very rarely flower or set fruit, but when they do, the flowers are 3–8 cm long, cylindrical in shape, greenish brown and covered in a multitude of rounded spikes. The fruits are small and berry-like and contains few seeds. Flowers from early to late summer depend on the latitude. The plants have long creeping roots that spread out just below the surface of the soil. These rhizomes spread horizontally and can grow to almost 2 m in length for old, well-established specimens. Rhizomes have a strong aroma and a pleasing

\*Corresponding author: Dr. V Venkatesalu, Department of Botany, Annamalai University, Annamalai Nagar-608002, Tamil Nadu, India.  
Tel: +91-414-4222265  
Mobile: +91-984-2998740  
E-mail: venkatesalu@yahoo.com

but bitter taste.

To best of our knowledge, the larvicidal activity of *A. calamus* rhizome essential oil has not been reported previously. The present study was focused on the chemical composition and larvicidal activity of the essential oil from *A. calamus* rhizome against the filarial vector mosquito, *Culex quinquefasciatus* (*Cx. quinquefasciatus*).

## 2. Materials and methods

### 2.1. Plant materials and essential oil extraction

The rhizomes of *A. calamus* were collected from Moolai Palli Patti (11°29'47 N, 78°18'10 E), Namakkal District, Tamil Nadu, India in April 2008. The rhizomes were shade dried, milled in an electrical blender and subjected to hydrodistillation using a Clevenger apparatus for 4 h. The obtained essential oil was dried over anhydrous sodium sulphate and the purified essential oil was stored at 4 °C for further analysis and larvicidal assay.

### 2.2. Gas chromatography (GC) and gas chromatography–mass spectroscopy (GC–MS) analysis

GC analysis was conducted using Varian 3800 gas chromatography equipped with mass selective detector coupled to front injector type 1079. The chromatograph was fitted with DB–5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The injector temperature was set at 280 °C, and the oven temperature was initially at 45 °C, then programmed to 300 °C at the rate of 10 °C/min and finally held at 200 °C for 5 min. Helium was used as a carrier gas with the flow rate of 1.0 mL/min. One microlitre of the sample (1:10 diluted in acetone) was injected in the split mode at a ratio of 1:100. The percentage of the essential oil was calculated by the GC peak areas.

GC–MS of essential oil was performed using Varian 3800 gas chromatography equipped with Varian 1200 L single quadrupole mass spectrometer. GC conditions were the same as reported for GC analysis and the same column was used. The mass spectrometer was operated in the electron impact mode at 70 eV. Ion source and transfer line temperature was kept at 250 °C. The mass spectra were obtained by centroid scan of the mass range from 40 to 1 000. The compounds were identified based on the comparison of their retention indices (RI), retention time (RT) and mass spectra of WILEY, NIST library data of the GC–MS system.

### 2.3. Larvicidal assay

The egggrants of *Cx. quinquefasciatus* were collected from the drainage of local residential area of Annamalainagar and reared at (29±3) °C with relative humidity of 75%–85% in laboratory. The larvae were fed with Brewer's yeast:dog biscuit (1:3, w/w). The larvicidal activity was analysed as per the standard procedures recommended by the World Health Organisation[7]. The essential oil was dissolved in 1 mL of acetone and prepared into different concentrations with distilled water, viz., 12.5, 25.0, 50.0, 100.0 and 200.0 mL/L. Twenty larvae of the early 4th instar stage were used for the larvicidal assay and five replicates were maintained for each concentration. The larval mortality was calculated after 24 h of the exposure. The lethal concentrations LC<sub>50</sub> and LC<sub>90</sub> and their 95% confidence limits of upper and lower confidence levels were calculated by profit analysis (SPSS version 11.5).

## 3. Results

The dried rhizomes of *A. calamus* yielded 0.13% (v/w) of essential oil. Table 1 shows the chemical compounds identified in the rhizome essential oil and listed in the order of elution from DB–5MS column. A total of 20 compounds were detected representing about 99.99% of total oil. Beta-asarone, *cis*-beta-terpineol, limonene, carvone and amyl isovalerate were identified as the major chemical compounds. The larvicidal activity of *A. calamus* rhizome essential oil was investigated. The oil had promising larvicidal activity against the early 4th instar larvae of *Cx. quinquefasciatus* with LC<sub>50</sub> value of 63.43 mL/L (95% confidence limit of 33.02–101.84 mL/L) and LC<sub>90</sub> value of 145.95 mL/L (95% confidence limit of 105.82–283.11 mL/L). The mortality of *Cx. quinquefasciatus* was (11±2.9)%, (31±3.0)%, (52±3.6)%, (71±3.1)% and (97±2.3)% when the essential oil of *A. calamus* rhizome was at a concentration of 12.5, 25.0, 50.0, 100.0, 200.0 mL/L respectively. No mortality was recorded in the control.

**Table 1**

Chemical composition of the essential oil of *A. calamus* rhizome.

Chemical compounds <sup>a</sup>	Retention time (min)	Percentage
Alpha-thujene	33.304	0.77
Alpha-pinene	34.849	0.20
2-Methylbutyl acetate	35.585	1.50
Limonene	36.189	13.08
Amyl isovalerate	36.690	4.92
Perillen	37.130	0.89
Alpha-cubebene	37.677	1.30
Octyl acetate	38.416	2.30
Linalool	39.541	2.48
Beta-copaene	39.985	2.53
Beta-asarone	40.520	33.36
<i>Cis</i> -beta-terpineol	41.256	23.44
Alpha-humulene	42.158	0.49
Gamma-murolene	42.551	0.92
Carvone	43.668	5.64
<i>Cis</i> -calamenene	45.051	0.83
Globulol	46.287	3.28
Gamma-eudesmol	47.686	0.68
Terreyol	48.602	0.72
<i>Trans</i> -alpha-bergamotol	49.828	0.66

<sup>a</sup>Compounds listed in order of elution from DB–5MS column.

## 4. Discussion

Many papers have been published on the biological activity of essential oils, but the data show much discordance between the same essence[8]. Therefore, the biological activity of the essential oils greatly depends upon its chemical composition.

The larvicidal activity of *A. calamus* rhizome essential oil may be due to the presence of the major chemical compounds beta-asarone and limonene. Beta-asarone is –the most discussed compound due to its biological activity. Growth inhibition and antifeedant activity of asarones isolated from *A. calamus* were tested against variegated cutworm, *Peridroma saucia*[9]. Beta-asarone showed

antigonadal activity in insects. The vapors of the rhizome of *A. calamus* oil have exhibited complete inhibition of ovarian development when given to a number of stored grain insects<sup>[10]</sup>. Eleni *et al.* reported the larvicidal activity of limonene rich essential oil from *Citrus aurantium* subsp. bergamia against West Nile virus vector, *Culex pipiens*. The oil had the LC<sub>50</sub> values of 58.73 mg/L<sup>[11]</sup>. The essential oil of *Mentha longifolia* has been reported for larvicidal activity against *Culex pipiens*. The oil contained 20% of limonene and had the LC<sub>50</sub> value of 78.28 mg/L after 48 h of exposure<sup>[12]</sup>. Zoubiri and Baaliouamer identified limonene as one of the major chemical compounds in *Verbena officinalis* and the oil showed 100% larval mortality against *Culex pipiens* at 500 mg/L<sup>[13]</sup>. It is also possible that the larvicidal effect might be due to the synergism between the chemical constituents present in the essential oil<sup>[14–20]</sup>. The result of the present study is also similar to that of the earlier reports. Govindarajan reported the larvicidal activity of the essential oil of *Clausea anisata* against *Cx. quinquefasciatus*<sup>[21]</sup>. Pavela studied mosquito larvicidal activity of essential oils from 22 aromatic plant species against *Cx. quinquefasciatus* and found that essential oils obtained from *Thymus vulgaris*, *Satureja hortensis* and *Thymus satureioides* plants showed the highest larvicidal effect, with LC<sub>50</sub> values of 33, 36 and 44 µg/mL, respectively<sup>[22]</sup>. Khandagle *et al.* evaluated the essential oil of *Zingiber officinalis* (rhizome), *Achyranthes aspera* (stem) and *Achyranthes aspera* (leaf) for larvicidal activity. *Zingiber officinalis* showed the highest larvicidal activity against *Aedes aegypti* and *Cx. quinquefasciatus*, respectively<sup>[23]</sup>.

The results of the present study suggest that the essential oil of *A. calamus* rhizome may be used as a local resource in controlling *Cx. quinquefasciatus* larvae because *A. calamus* is cultivated as a major aromatic plant in Tamil Nadu. The essential oil may be used directly as a larvicidal agent in places where water is stagnant to control the filarial vector mosquito.

### Conflict of interest statement

We declare that we have no conflict of interest

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### References

- [1] Das NG, Goswami D, Rabha B. Preliminary evaluation of mosquito larvicidal efficacy of plant extracts. *J Vect Borne Dis* 2007; **44**: 145–148.
- [2] Madhumathy AP, Aivazi AA, Vijayan VA. Larvicidal efficacy of *Capsicum annum* against *Anopheles stephensi* and *Culex quinquefasciatus*. *J Vect Borne Dis* 2007; **44**(3): 223–226.
- [3] Hotez PJ, Remme HJF, Buss P, Alleyne G, Morel C, Breman JG. Combating tropical infectious diseases: Report of the disease control priorities in developing countries project. *Clin Infect Dis* 2004; **38**(6): 871–878.
- [4] Senthilkumar A, Kannathasan K, Venkatesalu V. Chemical constituents and larvicidal property of the essential oil of *Blumea mollis* (D. Don) Merr. against *Culex quinquefasciatus*. *Parasitol Res* 2008; **103**(4): 959–962.
- [5] Cheng SS, Chang HT, Lin CY, Chen PS, Huang CG, Chen WJ, et al. Insecticidal activities of leaf and twig essential oils from *Clausea excavata* against *Aedes aegypti* and *Aedes albopictus* larvae. *Pest Manag Sci* 2009; **65**(3): 339–343.
- [6] Conti B, Canale A, Bertoli A, Gozzini F, Pistelli L. Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito *Aedes albopictus* (Diptera: Culicidae). *Parasitol Res* 2010; **107**(6): 1455–1461.
- [7] World Health Organisation. Instruction for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO–VBC 1981; 81.807, pp. 1–6.
- [8] Lahlou M. Methods to study the phytochemistry and bioactivity of essential oils. *Phytother Res* 2004; **18**: 435–448.
- [9] Koul O, Smirle MJ, Isman MB. Asarones from *Acorus calamus* L. oil: Their effect on feeding behaviour and dietary utilisation in *Peridroma saucia*. *J Chem Ecol* 1990; **16**(6): 1911–1920.
- [10] Varma J, Dubey NK. Perspective of botanical and microbial products as pesticides of tomorrow. *Curr Sci* 1999; **76**: 172–179.
- [11] Eleni M, Antonies M, George K, Alexios–Leandos S, Prokopios M. High quality bergamot oil from Greece: Chemical analysis using chiral gas chromatography and larvicidal activity against the West Nile virus vector. *Molecules* 2009; **14**(2): 839–849.
- [12] Koliopoulos G, Pitarokili D, Kioulos E, Michaelakis A, Tzako O. Chemical composition and larvicidal evaluation of *Mentha*, *Salvia*, and *Melissa* essential oils against the West Nile virus mosquito *Culex pipiens*. *Parasitol Res* 2010; **107**(2): 327–333.
- [13] Zoubiri S, Baaliouamer A. Larvicidal activity of two Algerian *verbenaceae* essential oils against *Culex pipiens*. *Vet Parasitol* 2011; **181**(2–4): 370–373.
- [14] Cheng SS, Huang CG, Chen YJ, Yu JJ, Chen WJ, Chang ST. Chemical compositions and larvicidal activities of leaf essential oils from two eucalyptus species. *Bioresour Technol* 2009; **100**(1): 452–456.
- [15] Govindarajan M. Larvicidal and repellent activities of *Sida acuta* Burm. F. (Family: Malvaceae) against three important vector mosquitoes. *Asian Pac J Trop Med* 2010; **3**(9): 691–695.
- [16] Mandal S. Exploration of larvicidal and adult emergence inhibition activities of *Ricinus communis* seed extract against three potential mosquito vectors in Kolkata, India. *Asian Pac J Trop Med* 2010; **3**(9): 605–609.
- [17] Kovendan K, Murugan K, Vincent S, Barnard DR. Mosquito larvicidal properties of *Orthosiphon thymiflorus* (Roth) Sleesen. (Family: Labiatae) against mosquito vectors, *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *Asian Pac J Trop Med* 2010; **3**(9): 299–305.
- [18] Dipanwita Das, Goutam Chandra. Mosquito larvicidal activity of *Rauwolfia serpentina* L. seeds against *Culex quinquefasciatus* Say. *Asian Pac J Trop Med* 2010; **3**(1): 42–45.
- [19] Adhikari U, Singha S, Chandra G. *In vitro* repellent and larvicidal efficacy of *Swietenia mahagoni* against the larval forms of *Culex quinquefasciatus* Say. *Asian Pac J Trop Biomed* 2012; **2**(Suppl 1): S260–S264.
- [20] Prabhu K, Murugan K, Nareshkumar A, Ramasubramanian N, Bragadeeswaran S. Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Asian Pac J Trop Bioed* 2011; **1**(2): 124–129.
- [21] Govindarajan M. Chemical composition and larvicidal activity of leaf essential oil from *Clausea anisata* (Willd.) Hook. f. ex Benth (Rutaceae) against three mosquito species. *Asian Pac J Trop Med* 2010; **3**(11): 874–878.
- [22] Pavela R. Larvicidal property of essential oils against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Ind Crops Prod* 2009; **30**(2): 311–315.
- [23] Khandagle AJ, Tare VS, Raut KD, More RA. Bioactivity of essential oils of *Zingiber officinalis* and *Achyranthes aspera* against mosquitoes. *Parasitol Res* 2011; **109**(2): 339–343.