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Mosquito larvicidal activities of essential oils of *Alpinia* roxb. (Zingiberaceae)

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Abstract
The objective of the present study was to investigate the mosquito larvicidal activities of six *Alpinia* species on their toxic effects on *Aedes albopictus* (fourth instar larvae).From the study conducted, essential oils are rich sources of terpenoids mainly mono and sesquiterpenoids. All the tested species of *Alpinia* except *A. purpurata* exhibited potential larvicidal activity at higher concentrations of extracted essential oils. The result obtained in the present study indicates that the essential oils extracted from the rhizomes find wide application as eco-friendly larvicides.

Keywords: alpinia, terpenoids, *Aedes albopictus*, larvicidal activity

Introduction

Insect-transmitted diseases remain a major cause of illness and death worldwide (Pavela, 2009)[21]. Mosquitoes are the most important group of insects responsible for transmitting some of the most dreaded infectious diseases on human beings such as malaria, filariasis, dengue fever, Japanese encephalitis, etc. causing millions of deaths every year. No other organism influences the socioeconomic development of humans more than mosquitoes. Mosquito borne diseases are endemic over 100 countries, causing mortality of nearly two million people every year, and at least one million children die of such diseases each year, leaving as many as 2,100 million people at risk around the world (Kundsen and Slooff 1992; Weir and Stewart 1997; Klempner *et al*., 2007) [16, 32, 15]. Mosquitoes also cause allergic responses on humans that include local skin and systemic reactions such as angioedema (Peng *et al*., 1999) [22].

Dengue is a mosquito-borne infection that in recent decades has become a major international public health concern. It is found in tropical and sub-tropical regions around the world, predominantly in urban and semi-urban areas. Dengue haemorrhagic fever affects most Asian countries and has become a leading cause of hospitalization and death among children in the region (WHO, 1981) [33]. In India, *Aedes albopictus* which is currently the most invasive mosquito species in the world, has often been incriminated as a dengue vector in urban environment and also occasionally in rural settings (Reuben *et al*., 1988) [25]. It is a competent experimental vector of several other arboviruses, notably chikungunya, Ross fever, and Japanese encephalitis viruses (Shroyer 1986; Mitchell 1995; Moutailler *et al*., 2009) [27, 19, 20] and can support development of yellow fever virus (Johnson *et al*., 2002) [12].

The primary means for controlling mosquito larvae depends on the use of synthetic chemical insecticides. Frequent use of these chemical agents has created several environmental and health concerns such as disruption of natural biological control systems, undesirable effects on non-target organisms, outbreaks of other insect species and widespread development of resistance. These problems have initiated the search for new environmental friendly measures for mosquito larval control.

Natural products of plant origin especially essential oils constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and are found to be potential natural alternatives to the use of synthetic chemical insecticides. The effectiveness of several plant essential oils against mosquito larvae has been reported by many researchers (Chantraine *et al*., 1998; Cheng *et al*., 2003, 2004; Jantan *et al*., 2005; Thomas *et al*., 2004)[4,5,11,30]. Phytochemicals derived from plant sources can act as larvicides, insect growth regulators, repellents and ovipositor attractants, as observed by Venketachalam and Jebasan (2001) [31] and Thomas *et al*., (2004) [30]. Besides being target specific, plant essential oils are less toxic to humans and therefore can control a variety of insect pests and vectors in an environmentally safe way.

Materials and methods

In the present study, essential oils extracted from rhizomes of six *Alpinia* species have been selected for investigation on their toxic effects on *Aedes albopictus*, targeting the fourth instar larvae.

Plant materials

Six species of *Alpinia* Roxb. (Zingiberaceae) *viz*., *A. calcarata* Roscoe, *A. galanga* (L.) Sw., *A. malaccensis* (Burm. F.) Roscoe, *A. purpurata* (Vieill.) K. Schum., *A. smithiae* M. Sabu *et* Mangaly and *A. zerumbet* (Pers.) B.L. Burtt and R.M.Sm. collected from the wild as well as from collections maintained in the Calicut University Botanical Garden were used for the study. All the plants were authenticated and voucher specimens (CALI-37331, CALI-39164, CALI-95677, CALI-78811, CALI- 17563 and CALI- 39146) were deposited at the Herbarium (CALI) of Department of Botany, Calicut University.

Essential oil extraction

Rhizomes of the selected *Alpinia* species (50 g each, in triplicate) were air-dried, powdered, and subjected to hydrodistillation using a modified Clevenger-type apparatus for 4 h (Cheng *et al*., 2005) [7]. The yield of essential oils obtained were averaged over three experiments and

calculated according to dry weight of the plant materials. Essential oils were stored in airtight glass vials in the refrigerator at 4°C until used for the various analyses.

Analysis of essential oils

The GC-MS analyses of the oil was performed on a Hewlett Packard (HP) 6890 GC interfaced with a Hewlett Packard 5973 Mass Selective Detector (MSD) system operating at 75 eV and 250°C. The GC column used was: HP-5(DB5), fused silica capillary-0.32mm x 30m with film thickness 0.25µ. Helium was used as the carrier gas with a flow rate of 1.4 mL/min. The temperature program for the HP-5 column was set initially at 60°C for 1 min and then heated at the rate of 3°C/ min to 246°C. Runtime was 62 min. The components were analysed and various components were ascertained with the help of Wiley Library 275 combined with the analyser.

Mosquito larvicidal activity

The experiment was conducted using laboratory reared larvae of *Aedes albopictus* Skuse which was grown in a dish that was kept opened. Decaying leaves and yeast granules were added to water and allowed to remain stagnant till larvae appears. Excess of food (yeast granules) was avoided to prevent excessive microbial growth which may lead to the non-specific death of test organism. The larvae of different mosquitoes developed after 8-10 days in dish were covered using a mosquito net to prevent their escape. Among the different adult mosquitoes that developed after 15-20 days, *Aedes albopictus* mosquitoes were identified and selected by its distinguishing silvery patch on the back of its mesotonum.

All other types of mosquitoes were killed selectively to rear *A. albopictus* mosquitoes alone in a dish. The fourth instar larvae of *A. albopictus* were found to develop within 10-15 days depending on the climatic conditions.

The larvicidal activity of the oil was evaluated using fourth instar larvae of *A. albopictus* as per the method recommended by WHO (1981)[31].The essential oil extracted using Clevenger apparatus was used to prepare stock solution of 10mg/ml in acetone. The stock solutions were diluted with distilled water to obtain the test solutions of 200, 100, 50, 25, 20, 10 and 5 ppm. Concentrations of the test solutions were determined based on the literature survey (Sakthivadivel and Thilagavathy 2003; Cetin *et al*., 2010; Kaushik and Saini 2008) [26,3,14]. Test organisms in acetone and distilled water alone were kept as control.

The fourth instar larvae (25 each) were picked from the rearing dish with a rubber teat aided wide mouth pipette provided with a flame blunted orifice to prevent injury to the larvae from sharp glass and these were introduced to each of the test solutions as well as control. For each dose four replicas were kept at a time. To prevent non-standard dilution of test solution by the suspending water of picked larvae, the required larvae were taken in a 100ml beaker and the suspending water sucked out and discarded with a flame blunted pasteur pipette taking care not to bruise the larvae in the process. The larval mortality was recorded after twenty-four hours. The bioassay was repeated with three different batches of mosquito larvae. The larvae were considered as dead, if they were not responsive to a gentle prodding with a fine needle. Toxicity and effect were reported as LC_{50} and LC_{90}, representing the concentrations in ppm with 50% and 90% larvae mortality rate in 24 h, respectively.

Results and discussion

Chemical composition analysis of essential oils

The major essential oil components identified in the present investigation broadly belongs to monoterpenoids, sesquiterpenoids and few phenols. Various aromatic species of *Alpinia* possess oils that are rich in odoriferous monoterpenoids, *viz*., camphene, 1,8- cineole, β-pinene, myrcene, p-cymene, limonene, γ-terpinene, α-thujone, L-camphor, terpinene-4-ol, α-fenchyl alcohol, α-pinene, β-fenchyl acetate, methyl cinnamate, sabinene, α- terpinene, geranyl acetate, β-fenchyl alcohol, α-phyllandrene, fenchol, α-terpineol, α-terpinolene, fenchane, borneol, 1,4-terpineol, citral etc. Phenols such as methyl eugenol and acetyl eugenol also contribute towards the peculiar aroma and flavour of various taxa studied. Fragrant monoterpenes, oxygenated monoterpenes, monoterpene derivatives and phenols are probably responsible for the characteristic odour of the essential oils. However some taxa are poor in these odoriferous compounds. Instead they contain high amounts of sesquiterpene hydrocarbons like β-caryophyllene, α-humulene, epizonaren, valencene, azulenol, α-selinene, α-farnesene, germacrene-D, β-bisabolene, β-sesquiphellandrene, nerolidol, trans-caryophyllene, zerumbone, caryophyllene oxide, γ-cadinene, β-elemene, alloaromadendrene, germacrene-A, eudesmol, neo-intermediol, azulene and alliodorin. A wide range of chemical compounds were detected in the GC/MS analysis of essential oils of six species of *Alpinia* in the present study.

The essential oil of *A. calcarata* is dominated by monoterpenes, 1, 8- cineole (36.94%), β- fenchyl acetate (17.56%), limonene (4.14%), camphene (6.62%),β- pinene (7.06%), myrcene (0.64%), β -fenchyl alcohol (4.02%).

The essential oil obtained from *A. galanga* is rich in a monoterpenoid, 1, 8-cineole (63.31%). The other monoterpenoids identified were sabinene (0.39%), β-pinene (1.12%), myrcene (0.87%), limonene (2.37%), camphene (0.96%) and β- fenchyl acetate (0.70%).

The essential oil of *A. malaccensis* is dominated by a sesquiterpenoid, zerumbone (54.77%). Monoterpenoids present are fenchol (12.19%), α- phellandrene (12.9%), limonene (4.21%), camphene (0.96%), β-pinene (3.34%), myrcene (1.2%), β-fenchyl acetate (6.17%) and p-cymene (1.33%).

The major components of *A. purpurata* are α-selinene, a sesquiterpenoid (58.3%) and β-pinene, a monoterpenoid (41.7%).

The chemical components in *A. smithiae* are monoterpenoids, 1,8-cineole (30.94%), β-fenchyl acetate (17.60%), α-terpineol (9.84%), α-pinene (2.61%), camphene (2.15%), β-pinene (6.79%), myrcene (0.75%), α-fenchyl alcohol (1.06%) and 1,4- terpineol (1.70%).

The major constituents of essential oil of *A. zerumbet* are monoterpenoids and sesquiterpenoids. Monoterpenoids observed were β-pinene (0.97%), myrcene (0.17%), α-terpinene (1.70%), limonene (0.51%), 1,8-cineole (3.65%), γ-terpinene (3.69%), camphene (2.57%) and terpinene-4-ol (4.87%).The sesquiterpenoids detected were α-humulene (4.89%) and zerumbone (72.76%).

Effect of essential oils on mosquito larvae

All the tested species of *Alpinia* except *A. purpurata* showed potential larvicidal activity against fourth instar larvae of *Aedes albopictus* at higher concentrations of

extracted essential oils. The essential oils of *A. zerumbet, A. malaccensis, A. galanga and A. smithiae* induced 100% larval mortality against *A. albopictus* with in 24 h at 200 ppm. However *A. zerumbet* (LC_{50} =5.02 ppm; LC_{90} = 25 ppm) and *A. malaccensis* (LC_{50} =5.9 ppm; LC_{90}= 33.0 ppm) were found to be promising among these plants. The lowest activity at this concentration was shown by *A. purpurata* (62%) followed by *A. calcarata* (97.3%).

A. zerumbet and *A. malaccensis* showed promising activity even at the concentration at 5ppm showing 51.33% and 46.67% mortality respectively. No significant differences were noted among the others at this concentration. At 10 ppm, the highest activity was shown by *A. malaccensis* (78%) followed by *A. zerumbet* (58%). No mortality was observed in *A. smithiae* and *A. purpurata* at this concentration. *A. galanga* showed activity but was at par with *A. calcarata*.

At concentrations 25ppm and 50ppm, the essential oils showed significant difference in their activity. *A. zerumbet* (90 and 99.3%) and *A. malaccensis* (87.3 and 96%*)* showed higher mortality percentage at these concentrations followed by *A. smithiae* (39.33 and 82.66%). Considerable activity was showed by *A. galanga* at 25ppm (54%) which is at par with *A. calcarata* (47.3%). But the activities of these two were significantly different at 50ppm where *A. galanga* (64%) showed higher activity than A. calcarata (49.3%). *A. purpurata* showed no activity at these concentrations.

At 100ppm, *A. zerumbet*, *A. malaccensis* and *A. smithiae* induced 100% mortality followed by *A. galanga* (88%) and *A. calcarata* (78.67%) whereas *A. purpurata* induced only 9.3% mortality. At 200 ppm, *A. galanga* also showed 100% larvicidal activity after 24 hrs. No mortality was observed in controls. The overall results suggest that the essential oils of *A. zerumbet*, *A. malaccensis*, *A. smithiae* and *A. galanga* have the potential to use as alternative source for developing larvicides against larvae of *Aedes albopictus* and thereby control diseases borne by this mosquito.

In the present study, GC-MS data (Table 1) revealed that the species of *Alpinia* studied are rich in non-polar compounds namely terpenoids. Monoterpenoids were found to be the major class of compounds in the rhizome essential oils of *A. calcarata*, *A. galanga* and *A. smithiae*. Whereas in *A. malaccensis*, *A. zerumbet* and *A. purpurata*, the rhizome oils seem to be dominated by sesquiterpenoids. So it can be stated that the non- polar compounds, terpenoids present in these species of *Alpinia* may be responsible for their potential larvicidal activities.

The major constituent of *A. zerumbet* essential oil, the most effective essential oil in the present study, contains sesquiterpenoids namely zerumbone (72.76%) and α-humulene (4.89%) as major components. These may be responsible for its larvicidal activity as reported by Sutthanont *et al*. (2010)[29] who reported the activity of these compounds from *Zingiber zerumbet* against the larvae of *Aedes aegypti*. The activity of *A. malaccensis* with 54.77% activity is also supported by the above work. The essential oil obtained from *A. galanga* is rich in a monoterpenoid, 1,8-cineole (63.31%). The activity of *A. galanga* may be due to the presence of this compound as reported by Lucia *et al*., (2007) [18] who reported the larvicidal activity of *Eucalyptus grandis* essential oil containing 1, 8-cineole against the larvae of *Aedis aegypti.*

All the five species of *Alpinia* showed potential mosquito larvicidal effects against fourth instar larvae of *Aedes albopictus*. The essential oils of *A. zerumbet, A. malaccensis, A. galanga and A. smithiae* induced 100% larval mortality against *A. albopictus* in 24 h with a dosage of 200 ppm, whereas *A. calcarata* essential oil induced 97.3% larval mortality and *A. purpurata* essential oil induced 62% larval mortality. At 100ppm, *A. zerumbet, A. malaccensis* and *A. smithiae* induced 100% mortality. *A. galanga* and *A. calcarata,* induced 88% and 78.67% mortality respectively whereas *A. purpurata* induced only 9.3%. At medium concentrations of 50 and 25%, *A. zerumbet* and *A. malaccensis* exhibited very high mortality, whereas *A. smithiae*, *A. galanga* and *A. calcarata* showed considerable activity. Even at lower concentrations, *A. zerumbet* and *A. malaccensis* oil induced larval mortality. *A. purpurata* showed no activity at concentrations lower than 100ppm. No mortality was observed in controls.

Table 1: Chemical constituents of rhizome essential oils of the six Alpinia species

		Concentration (%)					
Constituents	**RT**	***A. calcarata***	***A. galanga***	***A.malaccensis***	***A. purpurata***	***A. smithiae***	***A. zerumbet***
Camphene	3.19	6.62	0.96	0.96		2.15	2.57
Sabinene	3.61		0.39				
β-pinene	3.66	7.06	1.12	3.34	41.7	6.79	0.97
Myrcene	3.93	0.64	0.87	1.2		0.75	0.17
α-phellandrene	4.19			12.9			0.18
α-pinene	4.32	0.64				2.61	
α-terpinene	4.45		0.43			0.99	1.7
p-cymene	4.64	0.68		1.33			0.84
Limonene	4.71	4.14.	2.37	4.21			0.51
1,8-cineole	4.81	36.94	63.31			30.94	3.65
γ-terpinene	5.46	0.58	0.9			0.57	3.69
α-thujone	6.23	0.96		0.86			
α-terpinolene	6.24					2.83	0.72
Fenchol	6.99			12.19			
L-camphor	7.89	3.75					
Terpinene-4-ol	9.05	0.94	2.14				4.87
α-fenchyl alcohol	9.33					1.06	
β-fenchyl alcohol	9.53	4.02					
β-fenchyl acetate	10.59	17.56	0.7	6.17		17.6	0.38
L-Borneol	11.19					2.56	
1,4-terpeneol	11.67					1.7	

α-terpineol	12.20			0.6		9.84	
Citral	15.42					2.29	
Epizonaren	16.36	0.85					
Valencene	16.60	2.68					
Methyl cinnamate	16.89	0.88					
Geranyl acetate	17.12		2.23			1.23	
Methyl eugenol	17.93		0.5				
β-caryophyllene	18.05		0.62	1.47		4.28	0.52
α-humulene	19.39		1.27			0.93	4.89
β-elemene	19.91		11.49				
Germacrene D	20.48		0.69				
β-bisaboline	21.77		0.5				
α-farnesene	21.89		0.7				
α-selinene	22.17	3.03			58.3		
β-sesquiphellandrene	22.32		0.59				
Acetyl eugenol	22.69		5.68				
Caryophyllene oxide	24.29					4.38	0.97
Azulenol	24.83	8.03					
γ-cadinene	24.98					2.56	
Nerolidol	27.03		2.54			3.94	
Zerumbone	29.91			54.77			72.76

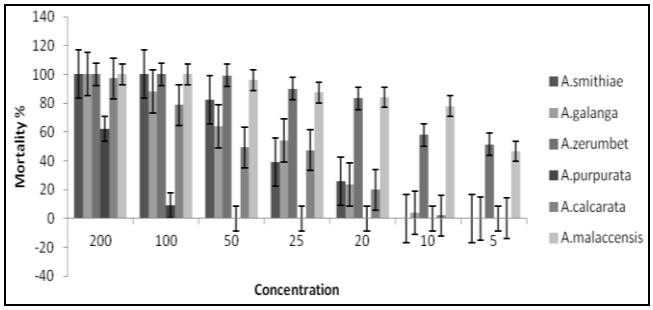

Fig 1: Mosquito larvicidal effects of essential oils from six species of Alpinia Roxb. Against fourth instar larvae of A. albopictus in 24 h

Statistical analyses

Mortality data obtained were analyzed by probit analysis to obtain regression equation, LC_{50} and LC_{90}. Standard errors with means of larval mortalities were also calculated. The analysis of variance (ANOVA) was conducted to analyze the significant difference among the tested essential oil's larvicidal activity. The LC_{50} and LC_{90} values were calculated by probit analysis and recorded in Table 2. The standard errors with means of larval mortalities were recorded in Table 3.

The LC_{50} and LC_{90} values indicated that the essential oil from *A.zerumbet* is the most effective, among the six *Alpinia* species studied, in larvicidal activity against *A. albopictus* larvae in 24 h with an LC_{50} of 5.02 ppm and LC_{90} of 25.0 ppm). This is followed by *A.malaccensis* (LC_{50} = 5.90 ppm; LC_{90} = 33.0 ppm), *A.galanga* (LC_{50} = 24.55 ppm; LC_{90}=112.0 ppm), and *A.smithiae* (LC_{50} = 29.51 ppm; LC_{90} = 70.0 ppm). The LC_{50} and LC_{90} values of *A.calcarata* were 55.08 and 161.50 respectively. The LC_{50} of *A.purpurata* was found to be 173.80 ppm but LC_{90} was too high to be considered as significant (Table 2). The control treatments (acetone and distilled water) had no larvicidal effect.

In the present study, GC-MS data (Table 1) revealed that all the species of *Alpinia* studied are rich in non-polar compounds namely terpenoids. Monoterpenoids were found to be the major class of compounds in the rhizome essential oils of *A. calcarata*, *A. galanga* and *A. smithiae*, whereas in *A.malaccensis*, *A.zerumbet* and *A.purpurata*, the rhizome oils seem to be dominated by sesquiterpenoids. So it can be stated that the non-polar compounds, terpenoids present in these species of *Alpinia* are responsible for their potential larvicidal activities.

The secondary compounds of plants make up a vast repository of compounds with a wide range of biological activities (Chowdhury *et al*., 2008)[8].The literature survey showed that the essential oil components detected in the present investigation possess reputed insecticidal properties. Camphene, α-terpineol, α-pinene, β-myrcene and limonene have anti-silverfish activity (Kuo *et al*., 2007) [17]. 1,8-cineole in *Xylopia aetiopica* essential oil showed significant level of toxicity to insects (Asawalam *et al*., 2006) [1].

Table 2: Efficacy of essential oils from six *Alpinia* species against fourth instar larvae of *A. albopictus* in 24 h treatment

Species	***Regression equation***	***LC_{50} (ppm)***	***LC_{90} (ppm)***
A. smithiae	0.13X + 4.89	29.51	70.0
A. galanga	0.12X+ 4.82	24.55	112.0
A. zerumbet	0.05X+ 17.98	5.02	25.0
A. purpurata	0.08X- 1.98	173.80	
A. calcarata	0.12X +3.55	55.08	161.5
A. malaccensis	0.04X+ 18.67	5.90	33.0

Table 3: Standard errors with means of larval mortalities induced by the six *Alpinia* species

Plant	***200 ppm***	***100 ppm***	***50 ppm***	***25 ppm***	***20 ppm***	***10 ppm***	***5 ppm***
A. smithiae	25 ±2.04	25±2.04	21 ±2.10	10 ±1.43	7 ±1.26	0	0
A. galanga	25 ±2.04	22 ±1.94	16 ±1.99	14± 1.69	6 ±1.10	1±0.62	0
A. zerumbet	25 ± 2.04	25 ±2.04	25 ±2.04	21 ± 2.02	15±1.91	15±1.60	13 ±1.54
A. purpurata	16 ±1.74	2.3±1.19	0	0	0	0	0
A. calcarata	24 ± 2.04	20 ±1.91	12 ±1.65	12 ±1.61	5 ±1.55	1±0.42	0
A.malaccensis	25 ± 2.04	25 ±2.04	24 ±2.03	22 ±1.96	21±1.93	20±1.86	12±1.68

Conclusion

According to GC-MS analyses, the major constituents of the essential oils are as follows: Zerumbone, α-humulene, β-pinene, myrcene, α- terpinene, limonene, 1,8-cineole, γ-terpinene, camphene and terpinene-4-ol in *A. zerumbet*; zerumbone, fenchol, α-phellandrene, limonene, camphene, β-pinene, myrcene, β-fenchyl acetate and p-cymene in *A. malaccensis*; 1,8-cineole, sabinene, β-pinene, myrcene, limonene, camphene and β-fenchyl acetate in *A. galanga*; 1,8-cineole, β- fenchyl acetate, α- terpineol, α- pinene, camphene, β- pinene, myrcene, α-fenchyl alcohol and 1,4-terpineol in *A. smithiae*; 1,8 cineole, β- fenchyl acetate, limonene, camphene, β- pinene, myrcene and β- fenchyl alcohol in *A. calcarata* and α- selinene and β- pinene in *A. purpurata*.

Results obtained from the larvicidal tests, using the essential oils from *A. zerumbet* and *A. malaccensis* showed that they had excellent inhibitory larvicidal effects against *A. albopictus* larvae. The results indicate that the essential oils of these species might be considered as potent sources for the production of fine natural mosquito larvicides. However, further investigations for the constituent's actions, mode of action, effects on nontarget organisms, and field evaluation are necessary. These results obtained are useful in search of more selective, biodegradable, and naturally produced larvicidal compounds.

Many promising, economical and environmental friendly botanical larvicides have also been reported from the families *viz*. Apiaceae, Araceae, Magnoliaceae, Piperaceae, Rutaceae (Sivagnaname and Kalyanasundaram 2004)[28], Annonaceae and Zingiberaceae. Several phytochemicals like alkaloids; phenolics and terpenoids exist in plants (Wink 1993) [34] which may jointly or independently contribute to the generation of mosquito larvicidal activities (Hostettmann and Potterat, 1997) [10].

The results of the present study suggest that chemical composition of extracts from different parts of the same or different plants may be different and require thorough understanding of the active ingredients present in these plants. For successful application of these phytochemical ingredients in insect bio-control, it is obligatory to understand the mechanisms of their action in the target insects as well as the spectrum of insects affected by them. Further work on these plant-derived derivatives is needed for developing them into effective formulations to be utilized in integrated vector control and in exploration of their multiple medicinal properties inherited by these plants. Further research is in progress to identify the biologically active constituents present in the rhizomes of these plant species.

The present study revealed that the rhizomes of *Alpinia* species contain active anti-larvicidal compounds. This makes it a more suitable candidate for the development of new potential eco-friendly larvicides.

Botanical larvicides can contribute remarkably to lowering the vector population of mosquitoes (Ascher and Meisner, 1989; Pizzarro *et al*., 1999; De Omena *et al*., 2007; Kamaraj *et al*., 2008; Rahuman *et al*., 2008; Pavela, 2009) [2, 23, 9, 13, 24, 21].

The biological activity of these plant extracts might be due to the various compounds, including phenolics, terpenoids, and alkaloids that exist in plants. These compounds may contribute jointly or independently to larvicidal and adult emergence inhibition activity.

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