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LARVICIDAL, PUPICIDAL AND SMOKE TOXICITY EFFECT OF *KAEMPFERIA GALANGA* TO THE MALARIAL VECTOR, *ANOPHELES STEPHENSI*

ABIRAMI DHANDAPANI*, SHOBANA KUMAR AND MURUGAN KADARKARAI,
Division of Entomology, Department of Zoology, School of Life Sciences,
Bharathiar University, Coimbatore - 641 046, INDIA
E-mail: sunabiphd@gmail.com

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***Corresponding author**

ABSTRACT
Laboratory investigation have been made to test the larval and pupal toxicity, smoke toxicity and repellent potential of methanolic extract of *Kempferia galanga* at different concentrations (0.25%, 0.5%, 1.0%, 2.0% and 4.0%) against the different instar (I, II, III and IV) larvae and pupae of *Anopheles stephensi.* Methanolic extract of *Kaempferia galanga* showed considerable toxicity effect against larvae and pupae of *Anopheles stephensi.* Lethal concentration (LC_{50} and LC_{90}) has been worked out on different larval stags of *Anopheles stephensi*. The LC_{50} and LC_{90} values of *K. galanga* for I instar larvae were 0.63 %,3.15 %, II instar 0.86 %, 3.66%, III instar 1.12%, 4.14%, IV instar 1.43%, 4.55%, respectively. The LC_{50} and LC_{90} values of pupae were 0.69%, 3.05%. Smoke toxicity effect also worked out on the adult mosquito of *Anopheles stephens,* the smoke emerged from *Kaempferia galanga* plant parts have considerably affected the adult mosquitoes and brought out considerable mortality and also treated adults laid minimum number of eggs.

INTRODUCTION

Mosquitoes are important vectors of several tropical diseases, including malaria, filariasis, and numerous viral diseases, such as dengue, Japanese encephalitis and yellow fever. In countries with a temperate climate they are more important as nuisance pests than as vectors (Jaswanth *et al*., 2002). There are about 3000 species of mosquito, of which about 100 are vectors of human diseases. Control measures are generally directed against only one or a few of the most important species and can be aimed at the adults or the larvae. *Anopheles* is a genus of mosquito (Culicidae). There are approximately 460 recognized species: while over 100 can transmit human malaria, only 30-40 commonly transmit parasites of the genus *Plasmodium* that cause malaria which affects humans in endemic areas. The known vectors of *Anopheles* species, which are common in India include *An. stephensi*, *An. culicifacies, An. fluviattis, An. minimmus, An. sudanicus* and *An. philippinensis* malaria is caused by plasmodium, viz: *P.flaciparam, P.malaiae, P.ovale* and *P.vivax* .Presently, 420 species of *Anopheles mosquitoes* have been recorded through the world out of which 50 are known vectors of malaria. In India, 58 species of *Anopheles* are present and among them, 6 are primary vectors of malaria (Nagpal and Sharma, 1995). Among the *Anopheles* species, *Anopheles stephensi* is recognized as a major vector of urban malaria in India (Mittal *et al*., 2005). Many studies on plant extract against mosquito larvae have been conducted around the world. It has well known that plant may be an alternative source of mosquito repellent agents because they constitute a rich source of bioactive chemicals (Wink, 1993).The plant extract or phytochemicals act as potential source of commercial mosquito repellent agents. Managing mosquitoes, although extractives and essential oil of *foeniculum* fruits are active as insecticidal (Kim and Ahn, 2001) an acaricidal agents (Perruch, 1995). Many plant extracts and essential oils with high volatility, such as alkanes, terpenoids, alcohols and aldehydes act on mosquitoes in the vapor phase (Brown, 1977). These volatile compounds were effective against mosquitoes for a relatively short period, typically 15 min to 10 h (Barnard, 2000). The most promising botanical mosquito control agents are in the families Asteraceae, Cladophoraceae, Labiate, Meliaceae, Oocystaceae and Rutaceae (Sukumar *et al.,* 1991). The repellent constituents are mainly monoterpenoids such as geraniol, citronellol, linalool, terpineol and carvone (Vartak and Sharma, 1993). The leaf extract of *Datura metal* was reported to be toxic to *Spotoptera litura* (Murugan et al., 1999); The interactive effect of botanicals (Neem, Pongamia) and Leucas aspers, *Bacillus sphericus* against the larvae of *Culex quinquefasiatus* (Murugan *et al*., 2003) .The methanol extract of *Sphaeranthus indicus* showed macrofilaricidal activity by worm motility and subsequent mortality was observed (Nisha *et al*., 2007). The highest larval mortality was found in whole plant petroleum ether extract of *C. colocynthis* (Rahuman *et al*., 2008 a). The acetone crude extract of *Ocimum canum, Ocimum sanctum*, and *Rhinacanthus nasutus* (Kamaraj *et al*., 2008); *Nerium indicum* and *Thuja orientelis* (Sharma *et al*., 2005) were tested against mosquito larvae.The larvicidal efficacy of *Povonia zeylanica* L. and *Acacia ferruginea* D.C. against *Culex quinquefasiatus*. The ethyl acetate extract of leaf extract of *Acalypha indica* (Govindarajan *et al.*, 2008) extract of fruit mesocarp of *Balanites aegyptiaca* (Wiesman and Chapagain, 2006) the crude hexane extracts obtained from flower heads of *Spilanthes acmella, Spilanthes calva,* and *Spilanthes paniculata* (Pandey et *al.*, 2007) seeds extract of *Sterculia guttata* (Katade *et al.*, 2006) the methanol extracts of *Cryptomeria japonica* (Cheng *et al.*, 2008) *Abutilon indicum* (Rahuman *et al.*, 2008 b) were tested against the larvae of *A. aegypti, A. stephensi*, and *C. quinquefasciatus*. *Kaempferia galanga* Linn.is one of the plants in Zingiberaceae family. The rhizome extract has been potently active against bacterial infections. Indigenous medical protectionists use these rhizomes for treatment of bacterial infections, tumor and it is also applied externally for abdominal pain in women and used topically for treatment of rheumatism (Hirschhorn, 1983). Aromatic Ginger is found primarily in open areas in southern China, Taiwan, Cambodia and India, but is also widely cultivated throughout SE Asia. The plant has thick rounded leaves that lay flat on the ground. New leaves start growing in mid spring from the small dormant rhizomes. In summer, one or two flowers are produced successively from the centre of the growing tip. Flowering lasts over a two month period. The plant becomes dormant in winter, leaves die down in late autumn and rhizomes remain underground through winter. Dried or fresh rhizomes, which are very aromatic, are used in Asian cuisine as a spice.

The rhizomes of aromatic ginger have been reported to include cineol, borneol, 3-carene, camphene, kaempferol, kaempferide, cinnamaldehyde, p-methoxycinnaamic acid, ethyl cinnamate and ethyl p-methoxycinnamate (Kanjanapothi *et al*., 2004). Extracts of the plant using methanol have shown larvicidal activity against the second stage larva of dog roundworm (*Toxocara canis*). It was also found to be effective as an amebicide in vitro against three species of Acanthamoeba which cause granulomatous amebic encephalitis and amebic keratitis. The rhizome extract was found to inhibit activity of Epstein-Barr virus. Further research has demonstrated that the extract effectively kills larvae of the mosquito *Culex quinquefasciatus* and repels adult *Aedes aegypti* mosquitoes, both of which are serious disease vectors. As a result of these findings, research is underway to evaluate the plant extract's use as an insect repellent, with preliminary findings suggesting that it is a non-irritant to the skin of rats (Kanjanapothi *et al*., 2004).

The aim of this work was to evaluate the larvicidal, pupicidal and repellent potential of *K. galanga* against malarial vector, *A. stephensi*.

MATERIALS AND METHODS

Collection of eggs and mosquitoes

The eggs of *Anopheles stephensi,* were collected from local in and around Coimbatore districts drinking water bodies, water stored container with the help of 'O' type brush, for the laboratory bioassay. These eggs were brought to the laboratory and were transferred to 18 X 13 X 4 cm size enamel trays containing 500 mL of water and keep for larval hatching.

Maintenance of larvae

The mosquito larval culture was maintained in our laboratory at 27+2ºC, 75-85% RH, under14L: 10 D photoperiod cycles. The mosquito larvae were fed with dog biscuits and yeast at 3:1ratio. The feeding was continued till the larvae are transformed into the pupa stage.

Maintenance of pupae and adult

The pupae were collected from the culture trays and were transferred to plastic containers (12 X12 cm) containing 500 mL of water with the help of a dipper. The plastic jars were kept in 90 X 90X 90 cm size mosquito cage for adult emergence. The cage is made up of wooden frames and covered with polythene sheets on four sides (two laterals, one back and other one upper) and the front part as covered with a muslin cloth bottom of the cage is fitted with 10% sugar solution for a period of three days before they will be provided with animal for blood feeding.

Blood feeding of adult *Anopheles stephensi*

The adult female mosquitoes were allowed to feed on the blood of a rabbit (exposed on the dorsal side) for two days, to ensure adequate blood feeding for 5 days. After blood feeding enamel trays with water from the culture trays was placed in the cage for the adults to lay eggs.

Collection of *Kaempferia galanga* and preparation of concentration

Collection of plant materials

Kaempferia galanga (Zingiberaceae) was collected from our Department garden, Bharathiar University, Coimbatore, India.

Preparation of Plant extract

Kaempferia galanga rhizomes and leaves were washed with tap water shade dried at room temperature. The dried plant and root materials were powdered by an electrical blender. From the powder l00g of the plant materials were extracted with 2.5 liter of organic solvents (methanol) for 8 hr in soxlet apparatus (Vogel, 1978). The crude plants extracts were evaporated to dryness in rotary Vacuum evaporator.

Preparations of required plant extract concentration

One gram of the plant residue was dissolved in 100 mL of acetone (stock solution) considered as 1% stock solution. From this stock solution different concentrations were prepared ranging from 2 to 10%, respectively.

Larval toxicity test of plant extract

A laboratory colony of *Anopheles stephensi* larvae were used for the larvicidal activity. Twenty five numbers of first, second, third and fourth instar larvae kept in 500 mL glass beaker containing 249 mL of dechlorinated water and 1 mL of desired concentration of plant extracts. Larval food was given for the test larvae. At each tested concentration 2 to 5 trials were made and each trial consisted of three replicates. The control was setup by mixing 1 mL of acetone with 249 mL of dechlorinated water.The larvae exposed to dechlorinated water without acetone served as control. The control mortalities were corrected by using Abbots formula (Abbott, 1925).

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

$$\text{Percentage mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larve introduced}} \times 100$$

LC_{50}, LC_{90} were calculated from toxicity data by using Probit analysis (Finney, 1971)

Papal Toxicity test

A laboratory colony of mosquito pupae was used for pupicidal activity. Twenty freshly emerged pupae were kept in 500 mL glass beaker containing 249 mL of de-chlorinated water and 1mL of desired concentration of plant extracts. Five replicates were set up for each concentration and control was setup by mixing 1 mL of de-chlorinated water. The control mortality was corrected by Abbott formula (Abbott, 1925).

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

$$\text{Percentage mortality} = \frac{\text{Number of dead pupae}}{\text{Number of Pupae introduced}} \times 100$$

LC_{50}, LC_{90} were calculated from toxicity data by using profit analysis (Finney, 1971).

Adult repellency

The cotton pads were soaked in different concentrations of *Kaempferia galanga* extracts mixed with goat blood and kept in glass containers. Twenty adult mosquitoes were recovered into each container with one treated pad and one control pad placed in opposite direction. The number of mosquitoes landing on the treated and control pads were recovered. The repellency of extract *Kaempferia galanga* and control was calculated by the following formula.

$$\text{Repellency rate} = \frac{C - T}{C + T} \times 100$$

Where, C is the number of mosquito on control pad, and T is the number of mosquitoes on treated pad.

Smoke Toxicity Test

Kaempferia galanga parts (leaves, stem and roots) were used for smoke toxicity assay. The mosquito coil was prepared by following method of (Saini *et al.,* 1986) with minor modifications by using 4g from each plant powered sample considered as active ingredient two grams of saw- dust as binding material and two gram of coconut shell charcoal powder as burning material. All the three were thoroughly mixed with distilled water to form a semisolid past and were shade dried. The control coils were prepared without plant ingredient. After the experiment was over the fed, unfed (active and dead) mosquitoes were counted. The protection given by the smoke from plant samples against the biting of *Anopheles Stephensi* was calculated in terms of percentage of unfed mosquitoes due to treatment.

$$= \frac{\text{Number of unfed mosquitoes in treatment - Number of unfed mosquito in control 1}}{\text{Number of mosquito treated}} \times 100$$

The reduction in the population from the smoke treated mosquitoes was calculated using the formula

$$\text{Population reduction} = \frac{\text{No. of larvae hatched in control 1 - No. of larvae hatched in treated}}{\text{No. of larvae hatched in control 1}} \times 100$$

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) and the means separated by Duncan's multiple range test (DMRT) (Alder and Rossler, 1977).

RESULTS AND DISCUSSION

The results of larvicidal and pupicidal activity of *Kaempheria galanga* are presented in the Table 1. The plant extract exhibited larvicidal activity to different instars (I,II,III,IV) and pupae of *Anopheles stephensi.* Among the different larval stages, the I instar larvae was more susceptible than the other instar larvae. The plant extract also showed considerable pupal mortality. Larval mortality may be due to effect of the chemicals like cineol, borneol, 3-carene, camphene, kaempferol, kaempferide, cinnamaldehyde, p-methoxycinnaamic acid, ethyl cinnamate and ethyl p-methoxycinnamate present in the methanolic extract of *Kaempferia galanga* (Kanjanapothi *et al.*, 2004). The higher mortality of mosquito larvae was due to the combined action of plant compounds that might be acting on the midgut epithelium cells and exerted their toxic effects on mosquito. The differential susceptibilities of larvae of three mosquito species to petroleum ether extracts of *Acorus calamus*, *Citrus madica* (Sujatha *et al*., 1988).The crude extract of the fruit pods from *Swartzia madagascariensis* Desvaux produced higher mortality in larvae of *Anopheles gambiae* than larvae of *A.aegypti* but was ineffective against larvae of *Culex quinquefasiatus* (Minijas and Sarda, 1986).The effect of some indigenous properties in *Anopheles stephensi* (Murugan and Jeyabalan, 1999).

With regards to the present findings, the repellent activity of different concentrations of *K. galanga* (0.25, 0.5, 1.0, 2.0 and 4.0 %) on the malarial vector, *Anopheles stephensi* was shown in Table 2. Among the different concentrations, the 4% of concentration showed higher repellent activity in *Kaempferia galanga*. The percentage of protection at 0.25% concentration showed 68% and at 4.0% concentration showed 90%. The percentage of production was increased as increasing concentration of plant extracts. This may be due to presence of active compounds in the leaves and root of *K. galanga.* The reduction is presumably caused by chemosensory effects of *K. galanga* either olfactory or gustatory. The highest concentration of 0.02 and 0.015% provided over 100 minutes protection against mosquito bites. Lower concentration provided 70 to 90 minutes of protection. The control provided only 2.2 minutes of protection. The results clearly shows that repellent activity was does dependent Rajkumar and Jebanesan (2005). The repellents have an important place in protecting man from bites of insect pests. An effective repellent will be useful in reducing man –vector contact and in the interruption of disease transmission. Repellent compounds should be non-toxic, irritating and long lasting (Kalyanasundaram and Das, 1985).

Smoke is the most widely used means of repelling mosquitoes utilized in the rural tropics.Waste plant materials are frequently burned in Sri Lanka as a mosquito repellent, even though indoor residual spraying has been carried out buy the government for many years (Silva, 1991). In the present study the smoke emerged from the *K. galanga* considerably affected the adult mosquito survival, pronounced high mortality and

Table 1: Toxicity evaluation of methanolic extracts *Kaempheria galanga* on the larval instars and pupae of *Anopheles stephensi* Liston

Larval Instar	Concentration (%)	Number of Larvae	Observed Mortality	Expected Mortality	Residual	Probit Value	Probit analysis	Chi Square
	0.25	100	34[e]	42.24	-8.23	0.42	LC50	
	0.5	100	46[d]	47.28	-1.28	0.47	0.63	
I	1	100	69[c]	57.41	11.59	0.57		9.053
	2	100	76[b]	75.72	0.28	0.76	LC 90	
	4	100	94[a]	95.71	-1.71	0.96	3.15	
	0.25	100	31[e]	38.99	-7.99	0.39	LC50	
II	0.5	100	43[d]	43.44	-0.44	0.43	0.86	
	1	100	61[c]	52.54	8.46	0.53		6.891
	2	100	73[b]	69.91	3.09	0.7	LC 90	
	4	100	90[a]	92.47	-2.47	0.92	3.66	
III	0.25	100	28[e]	35.52	-7.52	0.36	LC50	
	0.5	100	38[d]	39.55	-1.55	0.4	1.12	
	1	100	59[c]	47.91	11.09	0.48		
	2	100	65[b]	64.54	0.46	0.65	LC 90	7.889
	4	100	87[a]	88.94	-1.94	0.89	4.14	
IV	0.25	100	23[e]	31.47	-8.47	0.31	LC50	
	0.5	100	35[d]	35.19	-0.19	0.35	1.43	
	1	100	53[c]	43.05	9.95	0.43		
	2	100	61[b]	59.28	1.72	0.59	LC 90	7.96
	4	100	83[a]	85.43	-2.43	0.85	4.55	
Pupae	0.25	100	37[e]	40.63	-3.63	0.41	LC50	
	0.5	100	48[d]	45.95	2.05	0.46	0.69	
	1	100	59[c]	56.72	2.28	0.57		
	2	100	76[b]	76.15	-0.15	0.76	LC 90	0.966
	4	100	96[a]	96.36	-0.37	0.96	3.05	

Within the Column means followed by the same letter(s) are not significantly different at 5% level by DMRT

Table 2: Repellent potential of methanolic extract of *Kaempheria galanga* on the malarial vector, *Anopheles stephensi*

Concentration of extract (%)	Number of Adult Mosquito	No.of Mosquito landing(hrs)						% of repellency (After 6 hours)
		1/2	1	11/2	2	21/2	3	
0.25	50	6	8	10	12	14	16	68
0.5	50	4	6	10	13	14	15	70
1.0	50	0	0	5	6	10	12	76
2.0	50	0	0	4	4	6	8	84
4.0	50	0	0	0	0	3	5	90
control	50	12	17	22	29	35	47	6

Table 3: Smoke toxicity effect of *Kaempheria galanga* parts ensured population of *Anopheles stephensi*

Kaempheria galangal parts used	No.of mosquitoes used	No.of egg rafts laid by fed mosquitoes	Total No. of Eggs	Total No.of larva hatched from the egg refts	% of reduction in population over Control I
Root	25	3[d]	386[e]	153[e]	82.4[a]
Stem	25	6[b]	847[c]	356[c]	58.9[b]
Leaf	25	9[ab]	935[b]	597[b]	34.6[d]
Control I*	25	14[a]	1075[a]	867[a]	73.5[c]
Control II**	25	4[cd]	456[d]	230[d]	-

Within the Column means followed by the same letter(s) are not significantly different at 5% level by DMRT; Control I* = Negative control; Control II** = Positive control

also treated individual laid minimum number of eggs. Hence, these plant extract can be employed for the control of *Anopheles stephens*. Similarly, a powdered preparation of leaves of *Vitex negunda* and *Leucas aspera* were found more toxic to the filarial vector mosquito *C.quinquefasciatus* than the synthetic mosquito mats which contained 4 percent d-allethrin (Pandian *et al*., 1994). *Anophalas karwari* was repelled by coconut husks, ginger and betel nut leaves (Vernede and Marnix, 1994). In Soloman Islands, a recent survey revealed that fire with coconut husks and papaya leaves was the most prevalent form of personal protection from mosquitoes, being used by 52% of residents (Dulhunty *et al*., 2000). In the present study the earlier larval stages were most affected after the treatment of *K. galanga,* which could be due to the age and physiological status of larvae. The active substances of *K. galanga* were toxic to the younger instar larvae of *A. stephensi*. Thus, these products can be used as economically viable form of personal protection against mosquito vector. Moreover, this kind of plant derived product does not cause any ill-effect to other beneficial organism (Murugan, 2004).

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