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

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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. culicicacies, An. fluviattis, An. minimus, An. sudanicus and An. philippinensis malaria is caused by plasmodium, viz: P.falciparam, 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 (Naggal 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, Ooctystaceae and Rutaceae (Sukumar et al., 1991). The repellent constituents are mainly monoterpoids such as geraniol, citronellol, linalool, terpineol and carvone (Vartak and Sharma, 1993). The leaf extract of Datura metal was reported to be toxic to Sporoptera litura (Murugan et al., 1999); The interactive effect of botanicals (Neeem, Pongamia) and Leucas aspers, Bacillus asper, 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. colocythis (Rahuman et al., 2008 a). The acetone crude extract of Ocimum canum, Ocimum sanctum, and Rhinacanthus nasutus (Kamaraj et al., 2008); Nerium indicum and Thuya 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

ABSTRACT
Laboratory investigation have been made to test the larval and pupal toxicity, smoke toxicity and repellent potential of methanolic extract of Kaempferia 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 (LC90 and LC90) has been worked out on different larval stags of Anopheles stephensi. The LC90 and LC90 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 %, 4.53 %, respectively. The LC90 and LC90 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.

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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-methoxy cinnamic acid, ethyl cinnamate and ethyl p-methoxycinnamate (Kanjanojith 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 (Kanjanojith 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+2°C, 75-85% RH, under 14L: 10 D photoperiod cycles. The mosquito larvae were fed with dog biscuits and yeast at 3:1 ratio. 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 100g 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).

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\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{control mortality}} \times 100
\]
was corrected by Abbott formula (Abbott, 1925).

mixing 1 mL of de-chlorinated water. The control mortality were set up for each concentration and control was setup by glass beaker containing 249 mL of de-chlorinated water and activity. Twenty freshly emerged pupae were kept in 500 mL 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 number of mosquitoes on treated pad.

Where, C is the number of mosquito on control pad, and T is observed mortality in treatment - observed mortality in control X 100. Corrected mortality =)

Percentage mortality = number of dead larvae / number of larvae introduced X 100.

LC = LC = were calculated from toxicity data by using Probit analysis (Finney, 1971).

Pupal 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 1 mL 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).

Corrected mortality = observed mortality in treatment - observed mortality in control / 100 - control mortality X 100.

Percentage mortality = number of dead larvae / number of larvae introduced X 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 Kaempferia 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-methoxycinnamic acid, ethyl cinnamate and ethyl p-methoxycinnaminate present in the methanolic extract of Kaempferia galanga (Kanjaniapothi 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 susceptibility 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 quinquefasciatus (Minijas and Sarda, 1986). The effect of some indigenous properties in Anopheles stephensi (Murungan 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
also treated individual laid minimum number of eggs. Hence, these plant extract can be employed for the control of Anopheles stephensi. 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).

References
EFFECT OF KAEMPFERIA GALANGA TO THE MALARIAL VECTOR


