LARVICIDAL, ADULTICIDAL AND REPELLENT EFFECTS OF *KAEMPFERIA GALANGA*

W Choochote[1], D Kanjanapothi[2], A Panthong[2], T Taesotikul[2], A Jitpakdi[1], U Chaithong[1] and B Pitasawat[1]

[1]Department of Parasitology, [2]Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

Abstract. Four fractions of *Kaempferia galanga* (hexane fraction, dichloromethane fraction 1, dichloromethane fraction 2 and methanolic fraction) were tested for larvicidal activity toward fourth instar *Culex quinquefasciatus*. The hexane fraction was found to exhibit the highest larvicidal effect with the $LC_{50}$of 42.33 ppm. Testing for adulticidal activity, the hexane fraction did not show any promising adulticidal effect. However, it caused a knockdown effect which might be useful as a repellent. It was then tested for repellent activity in human volunteers both in laboratory and field studies. In a laboratory study, the hexane fraction possessed repellency against *Aedes aegypti* (ED_{50} value of 30.73 µg/cm^2), and provided biting protection for 3 hours. In a field study, it could protect against certain mosquitos, *ie, Armigeres subalbatus, Anopheles barbirostris, An. aconitus, Mansonia uniformis, Cx. quinquefasciatus, Cx. gelidus, Cx. tritaeniorhynchus* and *Ae. aegypti*. The hexane fraction did not cause dermal irritation when applied on human skin.

INTRODUCTION

Nowadays, the growing use of phytochemicals for control of the insects, may be attributed to the fact that populations throughout the world are coming to see the dangers inherent in conventional insecticides. Synthetic organic insecticides, although highly efficacious against target species such as mosquitos, pose a substantial hazard to the environment in the form of biomagnification and toxicity to a variety of animal life. Moreover, the use of alternative structural types is often rendered ineffective by cross or multiple resistance. Restriction on the use of chemical agents has stimulated investigation of other alternative controls. The use of plants might be an alternative approach. In addition to pharmaceutical uses, certain chemicals derived from various botanical sources have been reported for potential use as insecticides effective against mosquitos. Natural botanicals have provided numerous sources of phytochemicals utilized in the development of effective mosquito control agents. Plant materials offer not only effective mosquito control agents, but also promise to be environmentally safe. Since these chemicals are taken from natural sources, they are expected to have low toxicity and high degree of biodegradation. Although numerous plant-derived constituents have shown tendencies to exhibit slower actions and weaker effects on mosquitos compared with conventional synthetic insecticides, increasing awareness of quality of life and environment is stimulating the further investigation of plants. If their effects are high enough and they are harmless on nontarget organisms, plant-derived natural products have immense potential for the control of vector-borne diseases.

Plant families with several representative species which appear to have the greatest potential for providing future mosquito control agents include Asteraceae, Canellaceae, Cladophoraceae, Labiatae, Malvaceae, Meliaceae, Oocystaceae and Rutaceae (Arnason *et al*, 1988; Sukumar *et al*, 1991). Previous studies (Pitasawat *et al*, 1998) have demonstrated significant mosquito larvicidal activity in 3 of 10 plant extracts, including those from *Kaempferia galanga*, *Illicium vernum* and *Spilanthes acmella* having LC_{50} values of 50.54, 54.11 and 61.43 ppm, respectively. The most effective, *K. galanga* extract, was therefore chosen for the present study.

Kaempferia galanga (Zingiberaceae) is an acaulescent perennial, growing in Southern China, Indochina, Malaysia and India. The rhizomes of the plant have been used in decoction or powder for indigestion, cold, pectoral and abdominal pains, headache and toothache. Its alcoholic maceration is applied as liniment for rheumatism (Keys, 1976). The constituents of this rhizome hitherto reported include cineol, borneol, 3-carene, camphene, kaempferol, kaempferide, cinnamaldehyde, *p*-methoxycinnaamic acid, ethyl cinnamate, and ethyl p-methoxycinnamate. Ethyl *p*-methoxycinnamate was reported to inhibit monoamine oxidase.

MATERIALS AND METHODS

The larvae of *Culex quinquefasciatus* were collected from polluted street canals and *Aedes aegypti* larvae from artificial containers in Chiang Mai Province. The specimens were transported to the insectary of Department of Parasitology, Faculty of Medicine, Chiang Mai University. They were identified and reared separatedly in the insectary at the temperature of 25-30°C and 80-90% relative humidity. The mosquito larvae of at least F_8 progeny were used for investigations of larvicidal, adulticidal and repellent activities.

The rhizomes of *Kaempferia galanga* were purchased in Chiang Mai Province. The extraction was performed by macerating 1.5 kg of dried and powdered sample with 5 liters of hexane at room temperature for 2 days. After suction filtering through a Buchner funnel, the hexane filtrate was evaporated by rotary evaporator at 40°C, and then lyophilized to yield a hexane fraction. The residue after extraction with hexane was further extracted with 5 liters of dichloromethane. The dichloromethane solution was drained and then acidified to pH 2-3 with 5% HCl in order to partition between water and dichloromethane. The dichloromethane lower layer was separated, evaporated at 40°C and lyophilized. The acidulated solution was basified to pH 10-12 with Na_2CO_3 at 0°C, and then reextracted with dichloromethane. Separately extracted solution was evaporated at 40°C and lyophilized. The aqueous layer was discarded. The material obtained after dichloromethane extraction was extracted with 5 liters of methanol. The resulting solution was filtered, evaporated at 60°C and lyophilized. Four fractions isolated from *K. galanga* were kept at -20°C until testing.

The test for larvicidal effect followed the WHO standard method (WHO, 1970) with some modifications. The larva groups of *Cx. quinquefasciatus* were exposed in water to which test solutions had already been added. Mortality was assessed 24 hours later. The fraction which exhibited a pronounced larvicidal activity was chosen for further laboratory test.

Adulticidal effect of the fraction of *K. galanga* was tested by using the standard WHO method (WHO, 1970) with slight modifications. The adult females of *Cx. quinquefasciatus* were exposed to the fraction on the impregnated paper for 1 hour and mortality was scored at the end of a 24 hour holding period.

In the laboratory repellent test, the fraction of *K. galanga* was evaluated against *Ae. aegypti* when applied to human skin. Observations were made on the response of the mosquitos to a graded series of dosages and the ED_{50} and ED_{95} values were computed. This test provided an estimation of the amount of repellent to be applied to the skin to produce a given level of effectiveness against a mosquito test population. The procedure for determining effective dosages of the plant fraction against laboratory-reared mosquito was a modification of the American Society for Testing and Materials Standard ED 951-83 (ASTM, 1983). Tests were based on the variable dose-fixed time, "free choice method" described by Buescher *et al* (1982) and were similar to the method described by Coleman *et al* (1993, 1994).

Four human volunteers (all males) were hired during this trial which was conducted by exposing the fraction-treated forearms to the nulliparous 5-10-day-old females. The results were subjected to a log probit regression analysis to obtain ED_{50} and ED_{95} values. Laboratory tests also were conducted to determine the duration of efficacy of the fraction. To minimize the number of bites in each volunteer, subsequent tests were terminated when a given fraction provided < 75% protection.

Under field conditions, the repellent trials were carried out at various places harboring large mosquito populations in McCormick Hospital, Chiang Mai Province. Trees, shrubs and grass were ubiquitous with human habitations dotting the landscapes in these places. Moreover, the area immediately adjacent to the sewage pond had standing water containing higher aquatic plants and animals. These habitats were capable of harboring large mosquito populations. Preliminary surveys by human-baited-trap indicated that *Armigeres subalbatus* was the predominant mosquito present at this site.

Sunset at the test site occurred at ~ 18.00 hours local time during the test period (December 10-30, 1996). Preliminary biting counts made during the period December 1-7 indicated that a 60-minute period of intense mosquito biting activity commenced 3-5 minutes after sunset. Because biting decreased markedly after 19.00 hours, the study was designed so that volunteers were exposed to natural populations of mosquitos between 18.00-19.00 hours.

Each evening, the fraction of *K. galanga* was applied on two of four volunteers (two males, two females), it was tested twice by each subject in the course of the study. Treatments were applied as 2 ml aliquots of 25% (w/v) solution of the fraction in absolute ethanol and were spread evenly from the knee to the ankle of a volunteer's leg. The other

Table 1
Various fractions obtained from extraction and fractionation of *Kaempferia galanga.*

Fraction	Yields (%)
Fr_1 : hexane fraction (nonpolar material)	3.58
Fr_2 : dichloromethane fraction (non- alkaloids)	0.63
Fr_3 : dichloromethane fraction (crude alkaloids)	0.12
Fr_4 : methanolic fraction (highly polar material)	1.62

Table 2
Larvicidal activity of various fractions of *Kaempferia galanga* against *Culex quinquefasciatus.*

Fraction	Larvicidal activity (ppm)		
	LC_{50}	LC_{95}	LC_{99}
Fr_1 : hexane fraction (nonpolar material)	42.33	62.25	77.27
Fr_2 : dichloromethane fraction (non- alkaloids)	75.91	105.48	126.84
Fr_3 : dichloromethane fraction (crude alkaloids)	141.51	238.22	318.99
Fr_4 : methanolic fraction (highly polar material)	1,052.0	1,995.70	2,857.40

Table 3
Initial repellency of hexane fraction of *Kaempferia galanga* against *Aedes aegypti.*

Repellent	ED_{50} (95% CL)[a]	ED_{95} (95% CL)[a]
Hexane fraction	30.73 (24.96-36.52)	243.53 (149.56-612.46)

[a] μg repellent / cm^2 skin
CL : confidence limits

leg acting as a control was treated with 2 ml of absolute ethanol. Other exposed untreated parts of body were protected against mosquito attack by wearing head-net, gloves and jacket.

Each subject sat at least 10 m apart and exposed both legs for 60 minutes beginning at 18.00 hours. Each 60-minute period was divided into twelve 5-minute segments. The number of mosquitos coming to bite on each leg were recorded and collected using aspirators, and placed into separate cups for subsequent identification. After each 5-minute segment, the volunteers then moved to a new site at least 20 m from the last station. All the mosquitos collected were identified using the standard key of Tanaka *et al* (1979). This procedure permitted us to determine species biting, total numbers of bites during the exposure period, biting rate and percent protection provided by the fraction as compared with control [(bites on control - bites on treated leg) / bites on control x 100].

RESULTS

The extraction and fractionation of *Kaempferia galanga* provided 4 fractions (Fr_1-Fr_4) with various yields as shown in Table 1. Fr_1, hexane fraction, was nonpolar material obtained in a yield of 3.58 % w/w. Fr_2 and Fr_3, dichloromethane fractions, were non-alkaloids (0.63% w/w) and crude alkaloids (0.12% w/w), respectively. Fr_4, methanolic fraction, with the yield of 1.62% w/w was highly polar material. These fractions of *K. galanga* were tested and compared with its ethanolic extract for larvicidal effect.

Four fractions of *K. galanga* exhibited different levels of larvicidal activity as shown in Table 2. Quite marked larvicidal activity was seen in Fr_1 and Fr_2. The most active fraction, Fr_1 having LC_{50} and LC_{99} values of 42.33 and 77.27 ppm, respectively, was therefore selected for further adulticidal and repellent tests.

The hexane fraction of *K. galanga* showed no adulticidal activity when tested on *Culex quinquefasciatus*. No mortality was observed following exposure the adult mosquitos to the highest concentration (400 g%) of hexane fraction in absolute ethanol. However, the fraction was found to cause a "knockdown" effect (the rapidly and normally reversible paralysis). After exposure for about 30 minutes all mosquitos showed signs of paralysis, *ie*, unable to walk and lay at the bottom of the exposure tube. Nevertheless, when transferred from the exposure tube to the holding tube, at the end of the exposure period, the mosquitos rapidly recovered within 1 hour. Subsequent record at the end of a 24 hour holding period revealed no mortality.

Hexane fraction of *K. galanga* possessed marked repellent activity. It provided biting protection against *Aedes aegypti* with ED_{50} and ED_{95} values of 30.73 and 243.53 μg/cm^2, respectively (Table 3). However, it exerted an effective (>90%) biting protection against *Ae. aegypti* with a duration of only 3 hours (Fig 1).

Species and number of mosquitos collected from 4 human volunteers during field study are demonstrated in Table 4. A total of 852 adult biting females comprising 10 species in 6 genera were collected, and *Armigeres subalbatus* was the most predominant species (699, 82.04%). This finding confirms the results of the preliminary human-baited-trap surveys (December 1-7, 1996). *Mansonia uniformis* was the second common biting species (7.28%). Other species collected included *Culex quinquefasciatus* (3.29%), *Cx. tritaeniorhynchus* (3.29%), *Cx. gelidus* (2.70%), *Aedes aegypti* (0.35%), *Ae. lineatopennis* (0.35%), *Anopheles barbirostris* (0.35%), *An. aconitus* (0.12%) and *Coquilletidia orchracea* (0.23%). The mosquito species that were collected from the treated legs were identified, and all of them were *Ar. subalbatus* which constituted 2.04% of those collected from the controls.

Table 4
Mosquito collections on control legs of four human volunteers during field study (December 10-30, 1996).

Species	No. of mosquitos collected
Armigeres subalbatus	699 (82.04%)
Mansonia uniformis	62 (7.28%)
Culex quinquefasciatus	28 (3.29%)
Cx. tritaeniorhynchus	28 (3.29%)
Cx. gelidus	23 (2.70%)
Aedes aegypti	3 (0.35%)
Ae. lineatopennis	3 (0.35%)
Anopheles barbirostris	3 (0.35%)
An. aconitus	1 (0.12%)
Coquilletidia orchracea	2 (0.23%)
Total	852 (100%)

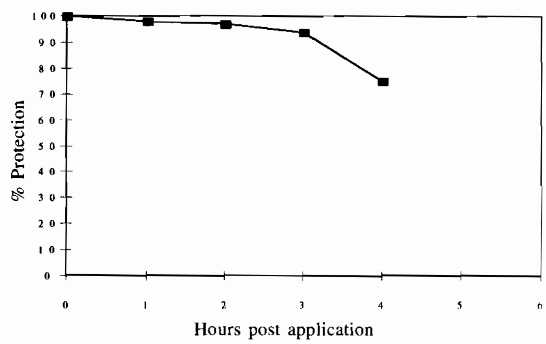

Fig 1–Duration of protection of *Kaempferia galanga* extract (hexane fraction) against bites of *Aedes aegypti*.

Table 5
Efficacy of hexane fraction of *Kaempferia galanga* as a repellent against field populations of mosquitos, mostly *Armigeres subalbatus*, tested in McCormick Hospital, Chiang Mai Province.

Treatment	Mosquito biting rate per man-5 minute	Mosquito biting rate per man-hour	% Protection (Based on biting counts during tests)[a]
Control (ethanol)	8.73 ± 9.16	104.75 ± 48.10	0
Kaempferia (25 g%)	0.22 ± 0.63[b]	1.75 ± 2.49[b]	97.96

[a] Only bites from *Armigeres subalbatus* were used in the calculations.
[b] significantly different from control ($p < 0.05$)

Table 5 summarizes the results of the field testing of *K. galanga*. There was a highly significant difference between the mean bites on the treated and control legs. The bites in the treated legs were only 0.22 ± 0.63 bites / man-5 minute and 1.75 ± 2.49 bites / man-hour whereas in the controls were 8.73 ± 9.16 bites / man-5 minute and 104.75 ± 48.10 bites / man-hour. The hexane fraction was effective in reducing bites, with 97.96 % protection. However, *Ar. subalbatus* was the only one of the biting population to bite treated legs.

DISCUSSION

Among the four fractions isolated from *Kaempferia galanga*, hexane fraction, the maximum yield (3.58%), was found to be the most potent larvicide (LD_{50}= 42.33 ppm), while the least effective one was the methanolic fraction (LD_{50}= 1,052.0 ppm). The highest larvicidal activity of the hexane fraction suggests that the active constituents are extracted in greater measures with hexane, and therefore can be classified as nonpolar compounds.

K. galanga has traditionally been used as a stomachic, carminative, stimulant and an incense. The rhizome of *K. galanga* has been reported to contain an essential oil comprising borneol, camphor, cineol and ethyl alcohol (Keys, 1976). It is also possible that chemicals other than essential oils are present in the hexane fraction of *K. galanga.* Thus it could not state which type(s) of compounds are responsible for larval mortality. However, subsequent studies by Insun *et al* (1999) have indicated that the possible site(s) of action of *K. galanga* on killing *Cx. quinquefasciatus* larva seemed to be on an anal gill, by destruction of the irregular ridge-like reticulum on the surface of gill which function as an ionic regular.

Eventhough the hexane fraction of *K. galanga* is considered as a potential larvicide (ED_{50} and ED_{95} values of 42.33 and 62.25 ppm, respectively), its potency is still much lower than currently used chemicals such as DDT, malathion, fenthion, fenitrothion, carbosulfan (OMS 3022) and OMS-33 (Velayudhan, *et al,* 1990; WHO, 1970, 1992). These chemicals exhibit the larvicidal activity with the ED_{50} and ED_{95} level of 0.00054-0.64 ppm. However, their adverse environmental effects and vector resistance have been increasingly reported (WHO, 1970, 1992).

Hexane fraction of *K. galanga* exhibiting the most potent larvicidal effect was the candidate for subsequent laboratory tests including adulticidal activity and repellent study (laboratory and also investigations of applicability at field level).

Adulticidal activity testing revealed that, although hexane fraction was found to be very toxic to larvae of *Cx. quinquefasciatus*, adults were relatively unaffected. It only caused knockdown, a nonlethal paralysis (Sawicki, 1962), but did not show promising adulticidal effect. However, this is interesting for further laboratory repellent study, since this knockdown effect may be due to greatly irritant stimuli of insect's olfactory sensilla. The mode of action of repellent involves effects on the insect's olfactory and taste perception, *ie*, a stimulation of receptor sites and/or modification of sensory perception (Maibach *et al,* 1974).

In an effort to find new, more effective insect repellents, no compound has yet been identified that possesses all of the advantages of chemical repellents without any of the drawbacks. In the present study, hexane fraction of *K. galanga* was considered as a potential repellent. It possessed the repellency with the ED_{50} and ED_{95} values of 30.73 and 243.53 $\mu g/cm^2$, respectively, and provided biting protection for 3 hours. However, its repellency is still lower than currently used chemicals such as Deet, AI3-37220, AI3-35765 and CIC-4 (Schreck and McGovern 1985; Coleman *et al,* 1993; 1994) in both efficacy and duration. These chemical compounds provide better and longer protection against many biting insects (ED_{50} and ED_{95} level = 0.37-25.37 $\mu g/cm^2$, 3-8 hours). Nevertheless, the possible health risks associated with the use of these chemicals should be taken into consideration.

Selection of repellent for further development, however, cannot be based on the results of any one test against a single insect. Tests should be against as many different biting arthropods as possible under both laboratory and field conditions. Moreover, several methods which enhance the effectiveness of repellent such as purification of the active fraction, reduction of skin absorption and increase in the persistence and the duration of repellency need to be studied.

In field study, the hexane fraction of *K. galanga* provided personal protection against certain mosquitos. The sensitivity of mosquitos to repellents varies among species. Many mosquito species including *Armigeres subalbatus*, *Mansonia uniformis*, *Anopheles barbirostris*, *An. aconitus*, *Aedes aegypti*, *Ae. lineatopennis*, *Culex gelidus*, *Cx. quinquefasciatus*, *Cx. tritaeniorhynchus* and

Coquilletidia orhracea were collected from the controls. The only species that came to bite or landed on the treated legs was *Ar. subalbatus*, indicating that this species is slightly tolerated to hexane fraction. However, a number of *Ar. subalbatus* collected from the treated legs (14 mosquitos) were markedly lower than those from the controls (699 mosquitos). With these promising results, it indicates that hexane fraction of *K. galanga* is a potential repellent which may be attributed to an essential oil component.

The percent protection against *Ar. subalbatus* based on numbers of bites received on the controls compared to those on treatments was 97.96%. The results indicate that some bites occurred during the exposure period and this may not be considered adequate protection. However, the effectiveness in reducing bites certainly was not negligible. Moreover, the possible protection against the other species is considered satisfactory. Although *Ar. subalbatus*, the most predominant species biting, has not been implicated in disease transmission, complaints of annoyance as vicious biters should not be disregarded. Moreover, the other species which were repelled by hexane fraction are known as the vectors of many tropical diseases, malaria - *An. barbirostris, An. aconitus*- (Gould *et al*, 1967; Reid, 1968; Scanlon *et al*, 1968; Harrison and Scanlon, 1975); filariasis -*Ma. uniformis, Cx. quinquefasciatus*- (Guptavanij *et al*, 1971; Sasa, 1976) ; dengue hemorrhagic fever and yellow fever - *Ae. aegypti* and Japanese B encephalitis - *Cx. gelidus* and *Cx. tritaeniorhynchus*- (Bram, 1967; Tanaka *et al*, 1979). These mosquito-borne diseases are still major public health problems for people living in the tropics (WHO, 1992).

At present, there is no effective and practical control for these mosquito vectors. In this study, however, the effectiveness of hexane fraction against certain mosquito vectors is considered satisfactory and might be an alternative approach for personal protection. In addition, all volunteers did not complain of the odor or stickness or uncomfortable feeling with the hexane fraction.

ACKNOWLEDGEMENTS

We thank Associate Professor Dr Piya Netrawichien, Dean of Faculty of Medicine, for his interest in this research, to the Chulabhorn Research Institute for financial support, and to the Faculty of Medicine Endowment Fund for Research Publication for financial support to publish this paper.

REFERENCES

American Society for Testing and Materials (ASTM). Standard test methods for laboratory testing of non-commercial mosquito repellent formulations of the skin. Standard E951-83, Annual Book of ASTM Standards. Philadelphia, PA: American Society for Testing and Materials, 1983.

Arnason JT, Philogene BJR, Morand P. Insecticides of Plant Orgin. USA: American Chemical Society, 1989.

Bram RA. The Genus *Culex* in Thailand (Diptera: Culicidae). *Contrib Am Entomol Inst* 1967; 12: 272pp.

Buescher MD, Rutledge LC, Wirtz RA, Glackin KB, Moussa MA. Laboratory tests of repellents against *Lutzomyia longipalpis* (Diptera: Psychodidae). *J Med Entomol* 1982; 19: 176-80.

Coleman RE, Robert LL, Roberts LW. Laboratory evaluation of repellents against four anopheline mosquitos (Diptera: Culicidae) and two phebotomine sand flies (Diptera: Psychodidae). *J Med Entomol* 1993; 3: 499-502.

Coleman RE, Richards AL, Magaon GJ. Laboratory and field trials of four repellents with *Culex pipiens* (Diptera: Culicidae) *J Med Entomol* 1994; 31: 17-22.

Gould DJ, Esah S, Pranith U. Relation of *Anopheles aconitus* to malaria transmission in the central plain of Thailand. *Trans R Soc Trop Med Hyg* 1967; 60: 441-2.

Guptavanij P, Harinasuta C, Vutikes S, Deesin T. The vectors of periodic and subperiodic *Brugia malayi* in Thailand. *Southeast Asian J Trop Med Public Health* 1971; 2: 589-90.

Harrison BA, Scanlon JE. Medical entomology studies II. The subgenus *Anopheles* in Thailand (Diptera: Culicidae). *Contrib Am Entomol Inst* 1975; 12: 307 pp.

Insun D, Choochote W, Jitpakdi A. Possible site of action of *Kaempferia galanga* on killing *Culex quinquefasciatus* larva. *Southeast Asian J Trop Med Public Health* 1999; 30: 195-9.

Keys JD. *Chinese herbs (their botany, chemistry* and *pharmacodynamics).* Tokyo: Charles E Tuttle, 1976.

Maibach HI, Khan AA, Akers CW. Use of insect repellents for maximum efficacy. *Arch Dermatol* 1974; 109: 32-5.

Pitasawat B, Choochote W, Kanjanapothi D. Screening for larvicidal activity of ten carminative plants. *Southeast Asian J Trop Med Public Health* 1998; 29: 660-2.

Reid JA. Anopheline mosquitos of Malaya and Borneo. *Stud Inst Med Res Malaya* 1968; 31: 520 pp.

Sasa M. Human filariasis: A global survey of epidemiology and control. University Tokyo Press 1976; 819 pp.

Sawicki RM. Insecticidal activity of pyrethrum extract and its four insecticidal constitutents against house flies. III. Knockdown and recovery of flies treated with pyrethrum extract with and without piperonyl butoxide. *J Sci Food Agric* 1962; 13: 283-92.

Scanlon JE, Peyton EL, Gould DJ. A annotated checklist of *Anopheles* of Thailand. *Thai Natt Sci Pap Fauna Ser* 1968; 2: 1-35.

Schreck CE, McGovern TP. Repellent test in the field and laboratory against wild populations of *Mansonia titillans* (Diptera: Culicidae) *J Med Entomol* 1985; 22: 658-62.

Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: a review. *J Am Mosq Control Assoc* 1991; 7: 210-36.

Tanaka K, Mizusawa K, Saugstad E. Mosquitos of Japan and Korea. *Contrib Am Entomol Inst* 1979; 16: 987 pp.

Velayudhan R, Amalraj D, Arunachalam N, Das PK. Insecticidal activity of carbosulfan (OMS 3022) and pyraclofos (OMS 3040) against mosquitos. *J Commun Dis* 1990; 22: 140-7.

World Health Organization. Insecticide resistance and vector control. 17th Report WHO Expert Committee on Insecticides. *WHO Tech Rep Ser* 1970; 443: 47-71.

World Health Organization. Vectror resistance for pesticides. *WHO Tech Rep Ser* 1992; 818.