Evaluation of Repellent and Larvicidal Activity of *Cymbopogon Citratus* (Lemon Grass) Against Filarial Vector*, Culex Quinquefasciatus*

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ABSTRACT

In search for a suitable alternative biocontrol technique to chemical insecticides, the larvicidal and repellent activity of Cymbopogon citratus (Lemon grass), against filarial mosquito vector, Culex quinquefasciatus larvae and adults was evaluated between April 2010 and March, 2011. Cymbopogon citratus (bush minth) belonging to the family, Poarceae is common and widely distributed in the South-eastern, Nigeria. The repellent and larvicidal activity of the ethanol extract of this plant against the filarial vector, Culex quinquefasciatus 3rd intar larvae and adults respectively was investigated for 48 hours. The extract was screened chemically for phytochemicals and showed presence of Alkaloids, Flavonoids, Tannins, Saponins and Steroids. The extract showed larvicidal activity with Lethal Concentration $LC_{50,}$ LC_{90} values of 91.765mg/l and 174.156mg/l respectively; and repellent activity with Effective Dose, ED_{50}, ED_{90} values of 40.541mg/l and 330.728mg/l respectively. Adult mosquito repellency and larval mosquito mortality significantly and positively correlated with the concentrations of the ethanol extract ($p<0.05$). This study revealed that an indigenous plant, Cymbopogon citratus could be considered as potential natural larvicidal and repellent agent against mosquitoes.

Key Words*:* Poarceae, Culex quinquefasciatus, Cymbopogon citratus, larvicidal activity.

INTRODUCTION

Mosquito-borne diseases such as malaria, filariasis, dengue, yellow fever and Japanese encephalitis are major public health problems in tropical and subtropical regions (Dua *et al.*, 2010). The tropical house mosquito *Culex quinquefasciatus* is the principal vector of lymphatic filariasis (Ramaiah *et al.,* 2000). The control of these diseases is largely dependent on spraying of chemical insecticides to kill mosquito adults or larvae. Larviciding is an effective method to reduce the mosquito densities before they emerge as adults and synthetic insecticides have been widely used for this purpose (TiwaryI *et al.,* 2007). The control of mosquito at the larval stage is necessary and efficient in integrated mosquito management.

Herbal products with proven potential as repellants can play an important role in the interruption of mosquito borne disease at both the individual and community level. However, the discovery, development and the use of synthetic chemicals with persistent residual action have not only overshadowed the use of herbal products against mosquito but has also become the major weapon for mosquito control. The repeated use of these synthetic insecticides produces widespread insecticide resistant mosquitoes (Hemingway *et al.*, 2000), causes undesirable effect on non-target organism, pollutes the environment and poses health risk to man (Cheng *et al.,* 2009).

This has necessitated the need for research and development of environmentally safe, biodegradable, low cost indigenous methods for vector control which can be used. Hence, there is a renewed interest in the exploration and use of plant products with insecticidal properties for mosquito control.

MATERIALS AND METHODS

Collection, Identification and Preparation of plant materials

The leaves of *Cymbopogon citratus* (lemon grass) were collected, identified by plant taxonomist, washed with distilled water and dried indoor for two weeks. The dried leaves were ground into fine powder using electric grinder and sieved. The fine powder was wrapped in extraction thimbles and put in soxhlet apparatus and extracted using absolute ethanol. The extract was concentrated in water bath at 100^0C to evaporate the ethanol which yielded dark residue that was further reduced to pastes by heating. Stock solutions were prepared according to WHO (2005) guidelines by dissolving 200mg each of the extracts in 20mls of water to make 1% stock solution from which series of concentrations were prepared. Three drops of acetone were added to dissolve the oil. The stock solution was kept in a screw cap vial, with aluminum foil over the mouth of the vial.

Rearing of mosquito larvae

Eggs of *Culex quinquefasciatus* were collected from breeding sites and identified at Arbovirus Vector Research Centre, Federal Ministry of health Enugu. After hatching larvae were reared in plastic buckets half filled with tap water and temperature maintained at 25-27^0C, 75-85% relative humidity under 12:12 (light and dark) photo period cycle. The larvae were fed with food consisting of Quaker oats and brewer's yeast in the ratio (3:1), once a day initially and twice a day during the later stages of development. Water in the rearing container was refreshed everyday by removing a little quantity of water from the rearing buckets and replacing with fresh water. This was aimed at preventing scum from forming on the water surface. Adult mosquitoes that emerged were fed with 10% sucrose solution.

Repellency Test

The plant leaves were kept inside the room with unscreened windows and doors from 18.30 - 6.30 hours during the experimental night, and the period of protection from mosquito bites was recorded. Outside the room, plant leaves were bruised with the hand to enhance the release of repellent volatiles. The bruised leaves were rubbed on one of the legs of the volunteers, while the unrubbed leg of the volunteers served as control. All other parts of the body (except the legs) were covered. Repellency percentage was calculated as recommended by WHO, (2009), by subtracting the number of mosquitoes landing on the rubbed leg from the number of mosquitoes landing on the unrubbed leg and dividing it by the number of mosquitoes landing on unrubbed leg and multiplying by 100. The period of active repellency (protection time) was recorded. The plant extract was tested for repellency in the Laboratory. The hands of two volunteers were exposed to adult *Culex quinquefasciatus* mosquitoes in a cages (30cm X 30cm X 30cm) for one hour. Before the application of the plant extracts, the hands were washed and cleaned thoroughly with 70% ethanol. Aliquot of 0.3ml of the test solution was smeared on the dorsal side of one hand (wrist to finger tips) of the volunteer. The repellent chamber contained between 100 and 150 3-day old and 4-hour starved mosquitoes. After 30 minutes of application, the hand was placed inside the repellent chamber for 10 minutes through a hole up to the wrist and plugged with cotton wool to prevent escape of mosquitoes and to encourage the female mosquitoes to bite on. The test was repeated at 30 minutes' interval. The interval between the application of plant extract and the first two consecutive bites occurring within 30 minutes was considered as protection time against the mosquito bites (Das *et al*., 2003; WHO, 2009). The test was repeated five times for each of the concentrations. Control readings were obtained by placing untreated hand in repellant chamber. All tests were conducted at room temperature of 27 ± 2^0C and relative humidity of 75 - 80%. The dose of plant extracts providing at least 6-8hours of protection in mosquito cage was considered to be an ideal compound for use as repellents as recommended by WHO (2009). Repellency percentage was calculated using the formula (WHO, 2009) given below:

$$\frac{No.\ Landing\ on\ Negative\ control - No.\ landing\ on\ treated\ with\ repellent}{No.\ landing\ on\ negative\ control} \times \frac{100}{1}$$

Bioassay

Standard methods and guidelines for laboratory and field testing of mosquito larvicides as stipulated by WHO, (2005) were adopted. The bioassay were performed at room temperature of 25-27^{0}C and Relative humidity of between 70 and 85%, photoperiod of 12:12 (light: dark) and pH 7.0 of distilled water. The larvae were exposed to test concentrations of 25, 50,100,200,300 mg of extracts in 1000ml of water. 100mls of tap water was taken in a series of 500ml glass beaker. The measured amount of extract was dissolved in 1ml of the solvent (ethanol) and added into water in the beakers. A control was also maintained by adding 1ml of solvent ethanol to 100 ml of tap water. The total of 25, 3rd or 4th instar larvae were selected by means of strainer and rubber pipette and introduced into the 500ml beakers containing different concentrations of the plant extracts. Thus each concentration contains 25 larvae. The treatments were replicated four (4) times and each replicate set contained one control. The larvae in all the beakers were fed with equal amount of Quaker oat and yeast powder in the ratio, 3:1 every 24 hours and this was spread evenly across the water surface. Larval mortality was recorded at intervals of 24 and 48 hours exposure. The moribund and dead larvae in the four (4) replicates were combined and expressed as percentage mortality for each concentration. Larvae were declared dead when they failed to move after probing with a needle. Moribund larvae were those unable to rise to the surface within a reasonable period of time.

Phytochemical analysis

Glycosides

The presence of glycosides was determined using the method described by Evans (2002). Two drops of the plant extracts were put in small beaker. 15ml of distilled water and 3mls of 10% sulphuric acid were added and mixture boiled for 15minutes.The boiled mixture was then made alkaline by adding 10ml solution of 5% potassium hydroxide. 10% of Fehling's solution was added and the beaker boiled for three (3) minutes. The occurrence of a brick precipitate indicated the presence of glycosides.

Tannins

Method described by Evans (2002) was adopted. The filtrate obtained from boiling 2g of the samples with 20ml of 45% ethanol for 5 minutes was used for these tests:

Ferric chloride test: 1ml of filtrate was diluted with 2ml of distilled water and 2 drops of ferric chloride solution added and observed for transient greenish to black colour.

Lead acetate test: To 1ml of the filtrate, 3 drops of 5% lead acetate solution was added and observed for gelatinous precipitate.

Alkaloids

Method described by Evans (2002) was also adopted.

Dragendorff's Test: Two drops of the extract were dissolved in 1% dilute sulphuric acid and boiled. The mixture was filtered hot and a drop of freshly prepared Dragendroff's reagent was added. Formation of pink or red precipitate was taken as a positive test.

Mayer's Test: Two (2) drops of the extract were dissolved in 10% dilute H_2SO_4 and boiled. The mixture was filtered hot and 1-3drops of Mayer's reagent were added. A white or yellow precipitate was taken as a positive test.

Saponin

The frothing test was used. 0.1g of the powdered sample was boiled with some distilled water for 5 minutes and decanted while hot. 1ml of the filtrate was diluted with 4ml of distilled water and the mixture shaken vigorously and observed for stable frothing on standing (Evans, 2002).

Flavonoids

The Shinoda test was used. 0.5g of the powdered sample was extracted in ethanol by boiling in a water bath for 5 minutes; this was filtered and cooled. To the filtrate was added 4 pieces of magnesium fillings followed by 1-3 drops of concentrated hydrochloric acid. A pink or red colour indicated the presence of flavonoids (Harborne, 1984).

Steroids and Terpenes
5 grams of the powdered sample was extracted by maceration with 50ml of ethyl alcohol (95%), filtered and the filtrate evaporated to dryness and used for the Liberman acid test. A portion of the organic extract was treated with drops of acetic and hydrate, and then concentrated H_2SO_4 acid was carefully added by the side of the test tube. The presence of a brown colour at the boundary of the mixture was taken as positive result (Evans, 2002).

Ethical Consideration
All the volunteers used were properly educated on the nature of the work to be done. All health matters of the volunteers were taken care of properly during the study period.

Statistical analysis
Data from all the replicates were pooled for analysis. Percentage mortality was calculated and corrections of mortality if needed were done by using the formula:

$$\text{Mortality (\%)} = \left[\frac{X-Y}{X}\right] 100$$

Where X = % survival in the untreated control; Y= % survival in the treated sample. Analysis of data was done using Probit analysis (Finney, 1971). Lethal Concentrations, LC_{50}, LC_{90} and Effective Doses, ED_{50} and ED_{90} values were calculated from log dosage-probit mortality regression line using computer soft ware SSPS. Standard deviation or confidence intervals of the means of $LC_{50,}$ $LC_{90,}$ ED_{50} and ED_{90} values were calculated. Student's t-test was used to compare the toxicity effects of the extract on larvae.

Results
The highest concentrations of ethanol extract of *Cymbopogon citratus,* 300mg/l, caused 100% mortality (Table 1.). Mortalities increased with increasing concentrations of the plant extract. There was no mortality in the controls and when the larvae were treated with 25mg/l of *Cymbopogon ciratus*. The mortalities significantly and positively correlated with the concentrations of *Cymbopogen citratus* extracts ($P<0.05$). When treated against the 3^{rd}-4^{th}instar larvae of *Culex quinquefasciatus,* the LC_{50} and $LC_{90,}$ were 91.756mg/l and 174.156 mg/l respectively.

Table 1: Percent mortality of 3^{rd}-4^{th} instar larvae of *Culex quinquesfasciatus* treated with different concentrations of ethanol extracts of *Cymbopogon citratus* (Lemon grass) for 48 hours

Plant	Concentration (mg/l)	Mortality percentage											
		3hrs	%	6hrs	%	12hrs	%	24hrs	%	48hrs	%	Total	%
Cymbopogonci trates	25	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
	50	0	0.00	0	0.00	0	0.00	0	0.00	2	8.00	2	8.00
	100	3	12.00	2	8.00	4	16.00	5	20.00	3	12.00	17	68.00
	200	3	12.00	4	16.00	5	20.00	5	20.00	5	20.00	22	88.0
	300	4	16.00	6	24.00	7	28.00	5	20.00	3	12.00	25	100
	Control	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00

NB: Values are means of four replicates

The highest mean protection time and repellency percentages was recorded against the highest concentration, 300mg/l of the plants extract (Table 2). This includes 372 minutes and 97.33% for *Cymbopogon citratus* respectively. Protection time and repellency percentage increased with concentration. There was no significant difference in the mean protection time and repellency percentage ($P>0.05$). There was very strong and significant positive correlation between the concentrations of the plant extract and mean protection time and repellency ($P<0.05$). The Effective Dose, ED_{50} and ED_{90} for *Cymbopogon citratus* were 40.541mg/l and 330.728mg/l respectively.

Table 2: Relative repellency and protection time of the three (3) herbs against *Culex quinquefascintus* in the laboratory

Herb	Concentration in mg/L	Mean of the protection time (in minutes)	Means of the number of bites	Repellency percentage
Cymbopogon citratus	25	16.2	24.2	46.22
	50	33	22.8	49.33
	100	66	15.8	64.89
	200	123.8	9.8	78.22
	300	372	1.2	97.33
	Control	---	45	-----

The indoor and outdoor repellent activities of the herb against the mosquitoes are shown in Table 3. Long protection time of 42 minutes was recorded for *Cymbopogon citratus* outdoors. This was more than time of 34.8 minutes recorded indoors; but the percent repellency was greater outdoors (69.01%) than indoors (20.86%). There was no significant difference in the mean protection time of the herb against indoor and outdoor man-biting mosquitoes (P>0.05).

Table 3: Repellent activities of *Cymbopogon citratus* against Indoor and Outdoor Man-biting mosquitoes

Herbs	Indoor Biting Mosquitoes				Outdoor Biting Mosquitoes			
	Means no. of mosquito bite Test	Control	Mean protection time in hours	Repellency percentage	Means no. of mosquito bite Test	Control	Mean protection time in hours	Repellency percentage
Cymbopogon citrates	36.80	46.50	0.58	20.86	4.40	14.20	0.75	69.01

The phytochemcial compound, glycosides, was not present whereas alkaloids flavionoids, tannins, saponins and steroids were present in *Cymbopogon citratus*, (Table 4).

Table 4: Qualitative analysis of phytochemicals of ethanol extracts *Cymbopogon citratus.*

Phytochemcials compounds	*C. citratus*
Alkaloids	+
Flavonoids	+
Glycosides	-
Tannins	+
Saponin	+
Steroids	+

KEY: +: Indicate Present - : Indicate Absent

DISCUSSION

The repellency of plant materials had been exploited for hundreds of years by man in houses simply by hanging bruised plants in houses; a practice that is still in wide use throughout developing countries (Moore *et al*., 2006). Plant materials are still extensively used in the tropics because they are the only means of protection from mosquito bites that are available for many of the poorest communities (Moore *et al.,* 2007). Many plant volatile components released by these plants are deterrent or repellents because they have high vapour toxicity to insects (Gershen and Dudareva, 2007); and also have shown strong responses to mosquito odour receptors (Carey *et al*., 2010). Data obtained in this study showed *Cymbopogon cittratus* had larvicidal and repellent activity against the larvae of *Culex quinquefasciatus* The larvicidal and repellent activities varied with the concentrations of the plant extract.

This study confirms larvicidal and repellent activities of natural products of plants origin, with insecticidal properties against mosquitoes as reported by different researchers. Equally proven is the fact that the the herb studied have insecticidal/mosquitocidal properties and can be used as useful, cost effective, inexpensive, safe and environmentally friendly control measure, an alternative to chemical insecticides. This study has revealed presence of some phytochemical substances in the herb. These phytochemicals may be associated with the larvicidal and repellent activities recorded in this herb.

Results of this study indicated that as the concentration of extract increased, mortality of *Culex quinquefasicatus* larvae also increased. The highest mortality of 100% was recorded in the highest concentration of 300mg/l, of the plant extract. Mortality with the various concentrations of plant extracts was significantly higher than that in the control ($P<0.05$). These could have been bioactive compounds responsible for larval mortality.

Normally, low LC_{50} and LC_{90} values indicate higher toxicity. *Cymbopogon citratus* was toxic to *Culex quinquefaciatus* with LC_{50} of 91.756mg/l. Data obtained were in consonance with the findings of several researchers who worked on the effect of same plants, and other plants against *Culex quinquefaciatus*. Pushpanathan *et al*., (2006) recorded that *Cymbopogon citratus'* essential oil exhibited toxicity to *Culex quinquefasciatus* larvae, with LC_{50} values of 165.70±1.2 and 184.18±0.8ppm for 3rd and 4thinstar respectively. They recorded 100% larvicidal activity at the highest concentration of 300ppm. Among 100 Indian coastal plants screened for larvicidal activity against *Culex quinquefasciatus* larvae, *Cymbopogon citratus* emerged the most toxic with LC_{50} value of the 24±0.06 and was recommended for massive mosquito control programme (Nazar *et al*., 2009).

Kalu *et al*., (2010) reported that ethanol extract from garlic bulb exhibited effective larvicidal properties, with LC_{50} of 184.18±0.8ppm, against *Culex quinquefasciatus* 4th Instar larvae. Aidaross *et al*., (2005) recorded 62% mortality at 10,000ppm aqueous extracts after 24 hours post exposure to *Cymbopogon citratus* against *Culex quinquefasciatus*. The essential oil obtained from lemon grass, has lemon flavor and contains citral as the major constituent. Bipul *et al*., (2012) reported that the essential oil hydrolates of *C. citratus* had the least larvicidal activity among the four plants tested, having LC_{50} and LC_{90} of 33.7%v/v and 49.2%v/v againt *Aedes albopictus* respectively and 38.8%v/v and 61.3%v/v against *Culex quinquefaciatus* respectively. Mgbemena (2010) also recorded *Cyumbopogen citratus* as having the least larvicidal activity against *Aedes aegypti* among three plants, *Azadirichta indica, Ocimium gratissimium* and *Cymbopogon citratus* studied with LC_{50} of 8.32 mg/ml, 19.50 mg/ml and 34.67mg/ml respectively. S Larval mortalities increased with concentration and confirms the report of Shadia *et al*., (2007), that there is a positive correlation between concentration and the percentage of larval mortality.

The toxicity of the plant extract could be due to the presence of to a wide range of chemicals including alkaloids, glycosides, tannins, quinines, terpenoids, non-metabolized amino acids which are part of the chemical resistance mechanisms of various plant groups against degradation of herbivorous insects (Hedin, 1983). Presence of the phytochemicals recorded in this study confirms the reports by Edeoga *et al*., (2006); Mgbemena (2010) and Gopieshkhanna and Kannabiran (2007) who recorded presence of Alkaloids, Tannins, Phenols, Saponins, flavonoids, Steroids, carbohydrates, phytosterols in various plant species including *Hyptis suaveolen, Cumbopogon citratus, Allium sativum, ocimum gratissimum* and *Azadrichta indica. Cymbopogan citratus* showed significant repellent activity against *Culex quinquefasciatus,* with ED_{50} and ED_{90} of 34.658mg/l and 337.571mg/l. The repellency and protection time increased with increasing concentrations, due to increased phytochemical contents. Fradin and Day (2002) also reported that effects of a solution containing 8% of the oil persisted and repelled up to 97.56% of mosquitoes by 5 hours post application.

Pushpanathan *et al*., (2006) reported that *Cymbopogon citratus* had repellency activity against adult mosquito *Culex quinquefaciatus* and offered a mean protection of 5hours and 100% repellency obtained at 5.0mg/cm^2. Data obtained in this study agrees with Aidaross *et al*., (2005) who recorded 100% repellent activity by *C. citratus* i.e zero landed mosquito for all exposed volunteers' arms. *C. citratus* oil has also been reported to have good repellent protection against mosquito bite (Oyedele *et al*., (2002). The repellent activities of the herbs studied is attributed to their phytochemical content. The mode of action of these photochemicals cannot be unconnected with the suggestions by Ansari and Razdam (1995). They are of the opinion that the active ingredients (alkaloids, flavonoids, saponins, phenolics, and tannins) present in phytochemical extracts from the three herbs might have exerted some inhibitory effects on lactic acid receptor cells by masking or changing the lactic acid that normally

attracts mosquitoes thereby confusing or distracting the mostquitoes. Thus, the blood-feeding contact or response is prevented. Consequently, with the application of the phytochemicals extracts on the skins, the mosquitoes could not bite because the active ingredients did not let them smell the attractant (Lactic acid) and could not therefore identify the human as their source of meal. This implies that the active ingredients confused the olfactory receptors and the mosquito simply could not smell the host.

CONCLUSION

This study revealed larvicidal and repellent activity of *Cymbopogon cittratus* against *Culex quinquefasciatus* larvae which increased with the concentrations of the plant extract and may be associated with the phytochemical substances present in the herb. This study confirms larvicidal and repellent activities of natural products of plants origin, with insecticidal properties against mosquitoes as reported by different researchers. Therefore, with this insecticidal/mosquitocidal properties Cymbopogon *cittratus* can be used as useful, cost effective, inexpensive, safe and environmentally friendly control measure, an alternative to chemical insecticides.

REFERENCES

Abagali, A.Z. & Alavo, T.B.C. (2011). Essential oil from Bush mint, *Hyptis suavelens*, is as effective as DEET for personal protection against mosquito bites. *The Open Entomology Journal, 5:* 45-48.

Aidarosis, M., Kokob, W. & Galalb, M. (2005). Evaluation of repellent and larvicidal activity of *Ocimum basilicum* L and *Cymbopogon citratus* against *Culex quinquefasciatus. Intl, chern. Pharm. Medical Journal, 2 (2):* 243-246.

Akram, W., Khan, H.A.A., Hafeez, F., Bilal, H., Kim, Y.K. & Lee, J. (2010). Potential of citrus seed extracts against dangue fever mosquito, *Aedes albopictus* (skuse) (Culicidae: Deptera). *Pakistan Journal of Botany, 42(4):* 3343–3348.

Amusan, A.A.S., Idowu, A.B. & Arowolo, F.S (2005). Comparative toxicity effect of bush tea leaves (*Hyptis suaveolens*) and orange peels (*Citrus sinesis)* oil extracts on larvae of the yellow fever mosquito, *Aedes aegypti. Tanzania Health Research, Bulletin, 7:* 174-178.

Ansari, M.A. & Razdam, R.K. (1995). Relative efficacy of various oils in repelling mosquito. *Indian Journal of Malariaology, 32:*104.

Aranda C, Eritja R. & Roiz D, (2006). First record and establishment of the mosquito *Aedes albopictus* in Spain. *Medical and Veterinary Entomology, 20:*150-152.

Bipul, R., Reji, G., India, B. & Singh, L. (2012). Larvicidal activity of some essential oil hydrolates against dengue and filariasis vectors. *E3 Journal of Medical Research, 1:*14–16.

Carey, A.F., Wang, G., Su, C.Y., Zwiebel, L.J. & Carison, J.R (2010). Odorant reception in the malaria Mosquito-*Anopheles gambiae*. *Nature, 464:* 66-71.

Cheng, S.S., Huang, C.G., Chen, Y.Y.J., Yu, J.J., Chen, W.J. & Chang, S.T. (2009). Chemical compositions and larvicidal activities of leaf essential oils from two eucalyptus species. *Bioresource Technol.*,100: 452–456.

Das, N.G., Goswami, D. & Rabha, B. (2007). Preliminary evaluation of mosquito larvicidal efficacy of plants extracts. *Journal of Vector Borne Diseases, 44:* 145-148.

Dua, V.K, Pandey, A.C. & Dash, A.P.P. (2010). Adulticidal activity of essential oil of *Lantana camara* leaves against mosquitoes. *Indian Journal of Medical Research.,131:*434-439.

Edeoga, H.O., Omosun, G. & Uche, L.C. (2006). Chemical composition of *Hyptis suaveolens* and *Ocimum gratissimum* hybrids from Nigeria. *African Journal of Biotechnology, 5 (10):* 892-895.

Evans, W.C. (2002). *Trease and Evans pharmagnosy* (15th Edition). W.B Saunders company Ltd. P.135-150.

Finney, D.J. (1971). *Probit analysis* (third edition). Cambridge university press. UK. 38p.

Gershen, J. & Dudareva, N. (2007). The function of terpene natural products in the natural world. *Nature Chemical Biology, 3:*408-414.

Gopieshkhanna, V. & Kannabiran, K. (2007). Larvicidal effect of *Hemidesmus indicus, Gymnema sylvestre and Edipta prostrate* against *Culex quinqurefasciatus* mosquito larva. *African journal of Biotechnology, 6(3):* 307-311.

Harborne, J.B. (1984). *Phytochemical methods.* Chapman and Hall, London. p166-226.

Hedin, P.A. (1983). *Plant resistance to insecticides.* American chemistry society, washinton. p370.

Hemingway, J, Ranson H, (2000). Insecticide Resistance in Insect Vectors of Human Disease. *Annual Review of Entomology*, 45: 371-391.

Kalu, I.G., Ofoegbu, U., Eroegbusi, J., Nwachukwu, C.U. & Ibeh, B. (2010). Larvicidal activities of ethanol extract of *Allium sativum* (garilic bulb) against the filarial vector, *Culex quinquefasciatus*. *Journal of Medicinal Plants Research, 4(6):* 496–498.

Kumar, M.S & Maneemegalai, S. (2008). Evaluation of larvicidal effects of *Lantana camara Linn* against mosquito species *Aedes aegypti and Culex quinquefasciatus. Advances in Biological Research, 2(3-4):* 39-43.

Logan, J.G., Stanczyk, N.M., Hassanali, A., Kemei, J., Santa, A.E.G., Ribeiro, K.A.L., Pickett, J.A.& Mordane, L.A. (2010). Arm-in-cage testing of natural human-derived mosquito repellents. *Malaria journal, 9:* 239.

Mgbemena, I.C. (2010). Comparative evaluation of larvicidal potentials of three plants extracts on *Aedes aegypti. Journal of American Science, 6(10):* 435-440.

Moore, S.J., Lenglet, A. & Hill, N. (2006). In: *Insect repellents: principles, methods and use*. Debboun, M., Francis, S.P. and Sickman, D. edition. Boca Raton Floid: CRC press; Plant-based insect repellents: Herodotus: the histories. Penguin. P 91.

Nazar, S., Ravikumar, S., Prakash, G.W., Syed, A. M. & Sauganthi, P. (2009). Screening of Indian coastal plant extracts for larvicidal activity of *Culex quinquefasciatus. Indian Journal of Science and Technology, **2** (3):* 24-27.

Oyedele, A., Gbolade, A., Sosan, M., Adewoyin, F., Soyelu, O. & Orafidiya, O. (2002). Formation of an effective mosquito repellent topical product from lemon grass. *Phytomedicine, 9:* 359-362.

Pushpanathan, T., Jebanesan, A. & Govindarajan, M. (2006). Larvicidal, ovicidal and repellent activities of *Cymbopogon citratus* (Graminae) essential oil against the filarial mosquito, *Culex quinquefasciatus* (say) (Diptera: Culicidae). *Tropical Biomedicine, **23**(2):* 208–212.

Ramaiah KD Das PK, Michael E. & Guyatt H (2006). The Economic Burden of *Lymphatic filariasis* in India. *Parasitol Today*, 16 (6): 251-253
Ranaweera, S. (1996). Mosquito larvicidal activity of some Sri Lankan plants. *Journal of National Science Council of Sri Lanka, 24(2):* 63-70.
Shadia, E.A., Elsayed, A.O. & Aly, S.S (2007). Chemical composition of *Ocimum americanum* essential oils and biological effect against *Agrotis ipsilon* (Lepidoptera: noctuidea). *Research Journal of Agriculture and Biological Science, **3** (6):* 740-747.
Tiwary M, Naik SN, Tewary DK, Mittal P.K. & Yadav S (2007). Chemical composition and larvicidal activities of the essential oil of *Zanthoxylum armatum* DC (Rutaceae) against three mosquito vectors. *Journal of Vector-Borne Diseases, 44:* 198–204.
WHO (2005). *Guidelines for laboratory and field testing of mosquito larvicides*. WHO pesticide evaluation scheme. WHO/CDS/WHOPES/GCD.
WHO Geneva. Pp 40.
WHO (2009). *Guideline for efficacy testing of mosquito repellents for human skin*. World Health Organization Pesticide Evaluation Scheme (WHOPES). WHO/HTM/NTD/WHOPES Pp. 1–30