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Mosquito larvicidal activity of some common spices and vegetable waste on *Culex quinquefasciatus* and *Anopheles stephensi*

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ABSTRACT

Objective: To investigate the larvicidal activities of crude and chloroform: methanol (1:1 v/v) extracts of some common spices (*Cuminum cyminum*, *Allium sativum*, *Zingiber offinale*, *Curcuma longa*) and vegetable waste (*Solanum tuberosum* germinated tuber) against *Anopheles stephensi* and *Culex quinquefasciatus* mosquito larvae. **Methods:** Larval mortality of above mosquito species were observed after 24, 48 and 72 h of exposure to five concentrations of aqueous extract (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) and four concentrations (25, 50, 75 ppm) of chloroform: methanol (1:1 v/v) extract. The lethal concentration of individual spices or vegetable waste was determined by log-probit analysis (at 95% confidence level) and effect of crude and chloroform: methanol (1:1 v/v) extracts were recorded on non target organisms. **Results:** Relative mortality rate of both larval mosquito species were recorded in the following sequences: *Cuminum cyminum*> *Allium sativum*>*Zingiber offinale*, *Curcuma longa* > *Solanum tuberosum* germinated tuber for crude extract, and efficacy of chloroform: methanol (1:1 v/v) extract were as follows: *Curcuma longa* > *Zingiber offinale* > *Solanum tuberosum* germinated tuber > *Cuminum cyminum*> *Allium sativum*. **Conclusions:** Crude and chloroform: methanol (1:1 v/v) extract of *Cuminum cyminum*, *Allium sativum*, *Zingiber offinale*, *Curcuma longa* and *Solanum tuberosum* germinated tuber can be recommended effectively in mosquito control programmes at very low concentrations. No mortality and other abnormalities were noticed on non target organisms and further studies are needed to investigate the chemical structure of active principal which are responsible for larvicidal activity.

1. Introduction

In the past the best efficacious approach of minimizing the incidence of mosquito borne disease was to eradicate and control mosquito vector mainly by application of synthetic organophosphate and organochlorines insecticides at larval habitat[1]. Synthetic insecticides used for vector control procedure, are non-biodegradable, non-selective and cause harmful effect on beneficial organisms. Control of mosquitoes at their larval stage is effective due to their low mobility, especially where the principal breeding habitats are man made and can easily be identified[2]. Now the top priority in finding a new insecticide is to observe whether it is biodegradable and does not have any ill effect on non-target organisms[3]. The plant derived natural products have the advantage of being harmless to non-target organisms and have been used by human communities as mosquito larvicides, insect growth regulators, repellents and ovipositional attractant.

Mosquitoes are important vectors of various disease including malaria, filariasis, Japanese encephalitis, dengue, yellow fever *etc*. In tropical and subtropical countries *Anopheles stephensi (An. stephensi)* and *Culex quinquefasciatus (Cx. quinquefasciatus)* are important vectors of malaria and filariasis, respectively. Anti-mosquito measures are effective solution to the problem of mosquito borne diseases.

Phytoextracts have been successfully tested for various biocontrol programs[4-7]. Essential oil of different plants are potential mosquito repellent agents[8] .Effectiveness of plant derived secondary compounds, such as saponine[9, 10],

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steroids[11,12], isoflavonoids[13] and tannin compounds[14] were well documented for mosquito larvicidal activity.

The aims of the present study was to observe the potential larvicidal activity of some common spices (*Cuminum cyminum, Allium sativum, Zingiber offinale, Curcuma longa*) and vegetable waste (*Solanum tuberosum* germinated tuber) against *An. stephensi* and *Cx. quinquefasciatus* mosquito larvae.

2. Material and method

2.1. Test mosquitoes

The present study was conducted at Mosquito and Microbiological Research Unit, Department of Zoology, The University of Burdwan, Burdwan (23°16′N, 87°54′E) West Bengal, India. *Cx quinquefasciatus* larvae were collected from drains surrounding the university campus. *An. stephensi* larvae were collected from kolkata and the larval colony were maintained under the laboratory condition for further bioassay experiments. Larvae of both mosquito species were kept separately in different plastic trays and fed with artificial food *i.e.* mixture of dog biscuits and dried yeast powder at the ratio of 3:1. Colonies were kept free from exposure to pathogen, insecticides or repellents.

2.2. Preparation of crude extract

Fresh mature *Cuminum cyminum, Allium sativum, Zingiber offinale* , *Curcuma longa* and *Solanum tuberosum* germinated tuber were collected from Debipur, Burdwan, West Bengal, India. All the spices and vegetable waste were initially rinsed with tap water and then distilled water, dried by paper towel. Finally the spices and vegetable waste were chopped into small pieces of approximately 1 cm size by sharp razor and crushed with a mixer-grinder machine and each juice was filtered by Whatman No.1 filter paper. The filtrate of each sample was used as stock solution (100% concentration) for further bioassay experiments and required concentrations *i.e.* 0.1%,0.2%,0.3%, 0.4% and 0.5% were prepared through mixing of stock solution with variable amount of distilled water.

2.3. Chloroform: methanol (1:1) extract

Semidried chopped small pieces of plant material were individually put in Sox- let apparatus and the extract was prepared using chloroform: methanol (1:1 v/v) as solvent. The extraction period of each material was 72 h and the temperature was maintained at nearly 40 ℃. After extraction procedure of each material, the Sox let apparatus was washed with distilled water. The eluent of each extract was concentrated by evaporation in rotary evaporator. Graded concentrations (25, 50, 75 ppm) were prepared from solid residue of each extract for further bioassay experiment.

2.4. Larvicidal bioassay

The larvicidal bioassay followed the World Health Organization (WHO)[15] standard protocol with slight modifications. Aqueous extract of each sample at five concentrations (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) and solvent extract at four concentrations (25, 50, 75 ppm) were applied for bioassay experiment. Earlier prepared concentrations of each sample was transferred into the sterile glass Petri dishes (9 cm diameter/150 mL capacity) containing 100 mL of tap water. Twenty third instar larvae of *Cx. quinquefasciatus* and *An. stephensi* were separately introduced into different Petri dishes containing appropriate concentration. All the experiments were conducted in triplicate and control were performed at parallel condition in each series of experiment. Tap water was used in the control experiment. No food was provided for the larvae. Larvae were considered dead if they were unrousable within a period of time, even when gently prodded. Larval mortality was recorded at 24, 48, and 72 h of exposure. The data of mortality at 48 and 72 h were expressed by the addition of mortality at 24 and 48 h, respectively.

2.5. Effect on non target organisms

Non targets were animals who share the common habitats of target mosquito larvae and some of them were natural predators of mosquito larvae. The effect of the crude and chloroform: methanol(1:1 v/v) extract of *Cuminum cyminum, Allium sativum, Zingiber offinale, Curcuma longa* and *Solanum tuberosum* germinated tuber were tested against non-target organisms, *Toxorhynchites splendens* (mosquito predator), *Gambusia affinis, Poecilia reticulate* (predatory fishes), *Diplonychus indicus, Diplonychus annulatum* (predatory water-bug), *Anisops bouvieri* (*Notonecta* sp.) and *Chironomus circumdatus* (fourth instars larval form). They were collected from field and maintained for a few weeks in cemented tanks at Mosquito and Microbiology Laboratory, the University of Burdwan. The predators were exposed to appropriate lethal concentration at 24 h (both crude and solvent extract) of each material to observed the mortality and other abnormalities such as sluggishness and reduced swimming activity up to 72 h exposure. Five replicates were performed for each test concentration along with two replicates of untreated control.

2.6. Statistical analysis

Statistical analysis of the experimental data was performed using the computer soft wares Statplus 2007, MS Excel 2003 and SPSS to find out the LC_{50}, LC_{90} lethal dose, regression equations, co-efficient values, mean larval mortality, standard error *etc*.

Table 1

Efficacy of crude extracts of *Allium sativum*, *Cuminum cyminum*, *Zingiber offinale*, *Curcuma longa*, *Solanum tuberosum* germinated tuber on instar larvae.

Larvae	Samples	Concentration (%)	Mortality(%)		
			24 h	48 h	72 h
An. stephensi	*Allium sativum*	0.1	5.67±0.89	12.00±1.00	14.33±0.33
		0.2	8.00±0.58	13.67±0.33	16.67±0.33
		0.3	11.67±1.33	17.67±0.67	11.67±0.33
		0.4	13.67±0.67	19.67±0.33	20.00±0.00
		0.5	14.67±0.33	20.00±0.00	20.00±0.00
	Cuminum cyminum	0.1	9.00±0.58	12.67±0.33	17.67±0.67
		0.2	9.67±0.33	14.00±1.00	18.67±0.67
		0.3	13.67±0.89	19.67±0.33	20.00±0.00
		0.4	14.00±0.58	20.00±0.00	20.00±0.00
		0.5	16.33±0.33	20.00±0.00	20.00±0.00
	Zingiber offinale	0.1	3.67±0.89	6.67±0.33	10.00±0.58
		0.2	4.67±0.33	9.33±1.33	12.33±1.20
		0.3	8.00±0.58	11.00±0.58	14.00±0.00
		0.4	7.67±0.89	12.33±1.20	16.67±0.33
		0.5	9.67±0.33	15.00±1.00	17.00±0.00
	Curcuma longa	0.1	1.33±0.67	4.33±0.33	6.33±0.33
		0.2	4.67±0.67	6.67±0.89	10.00±0.58
		0.3	6.33±0.33	10.00±1.00	11.00±0.58
		0.4	7.33±0.33	12.33±0.33	14.00±1.00
		0.5	9.33±0.33	12.33±1.20	14.33±0.33
	Solanum tuberosum germinated tuber	0.1	0.00±0.00	0.00±0.00	1.00±0.00
		0.2	0.33±0.33	0.67±0.33	1.67±0.33
		0.3	0.33±0.33	1.33±.33	2.33±0.33
		0.4	1.33±0.33	1.67±0.67	4.67±0.33
		0.5	2.67±0.33	6.33±0.67	8.00±0.58
Cx. quinquefasciatus	*Allium sativum*	0.1	4.00±0.58	10.33±0.33	12.00±0.58
		0.2	4.67±0.33	13.67±0.67	16.33±0.33
		0.3	9.67±0.33	16.33±0.67	19.67±0.33
		0.4	10.67±0.33	19.67±0.33	20.00±0.00
		0.5	11.67±0.33	20.00±0.00	20.00±0.00
	Cuminum cyminum	0.1	7.67±0.89	11.00±1.00	15.33±0.89
		0.2	8.33±0.33	13.33±0.33	16.33±0.33
		0.3	10.33±0.33	17.00±0.58	20.00±0.00
		0.4	14.00±0.58	19.33±0.33	20.00±0.00
		0.5	15.00±0.00	20.00±0.00	20.00±0.00
	Zingiber offinale	0.1	3.33±0.33	4.33±0.67	10.33±1.20
		0.2	4.67±0.67	6.33±0.33	12.00±0.58
		0.3	5.33±0.33	7.67±0.33	12.00±1.00
		0.4	7.00±0.58	10.00±1.15	12.67±0.33
		0.5	8.33±0.33	9.67±0.89	15.00±0.58
	Curcuma longa	0.1	1.33±0.33	3.67±0.33	7.67±0.67
		0.2	3.67±0.33	7.00±0.00	11.00±1.52
		0.3	5.00±0.58	9.67±1.20	11.33±0.67
		0.4	7.00±0.00	11.00±0.58	12.33±0.67
		0.5	9.00±0.58	12.33±0.33	12.67±0.33
	Solanum tuberosum germinated tuber	0.1	0.00±0.00	0.33±0.33	0.67±0.33
		0.2	0.33±0.33	0.67±0.33	2.00±0.00
		0.3	0.67±0.33	1.33±0.33	2.33±0.33
		0.4	2.00±0.58	4.33±0.33	6.33±0.89
		0.5	2.33±0.33	7.67±0.33	9.00±0.58

Table 2
Log probit analysis and regression analysis of larvicidal activity of *Allium sativum*, *Cuminum cyminum*, *Zingiber offinale*, *Curcuma longa*, *Solanum tuberosum* germinated tuber on third instar larvae.

Larvae	Samples	Time	Regression equations	r^2 value	LC_{50}	LC_{90}
An. stephensi	*Allium sativum*	24 h	Y=23.667X+3.6333	0.86	0.23	1.19
		48 h	Y=22X+10	0.88	0.10	0.29
		72 h	Y=14.667X+13.733	0.81	0.07	0.19
	Cuminum cyminum	24 h	Y=19X+6.8333	0.86	0.15	1.21
		48 h	Y=20.667X+11.067	0.78	0.09	0.23
		72 h	Y=6X+17.467	0.57	0.04	0.12
	Zingiber offinale	24 h	Y=15X+2.2333	0.78	0.59	6.26
		48 h	Y=19.667X+4.9667	0.80	0.22	1.69
		72 h	Y=18.333X+8.5	0.87	0.11	0.78
	Curcuma longa	24 h	Y=18.667X+0.2	0.90	0.56	2.70
		48 h	Y=21.667X+2.6333	0.82	0.31	1.75
		72 h	Y=20X+5.1333	0.86	0.21	1.44
	Solanum tuberosum germinated tuber	24 h	Y=6.333X−0.9667	0.71	1.18	3.02
		48 h	Y=13.667X−2.1	0.68	0.75	1.68
		72 h	Y=17X−1.5667	0.85	0.81	3.26
Cx. quinquefasciatus	*Allium sativum*	24 h	Y=21.333X+1.733	0.88	0.37	2.14
		48 h	Y=25.333X+8.4	0.93	0.11	0.32
		72 h	Y=19.667X+11.7	0.78	0.09	0.21
	Cuminum cyminum	24 h	Y=20.333X+4.9667	0.90	0.21	1.65
		48 h	Y=24X+8.9333	0.91	0.11	0.32
		72 h	Y=13X+14.433	0.73	0.06	0.18
	Zingiber offinale	24 h	Y=12.333X+2.0333	0.86	0.92	14.87
		48 h	Y=14.333X+3.3	0.74	0.49	6.35
		72 h	Y=10X+9.4	0.56	0.10	5.59
	Curcuma longa	24 h	Y=18.667X−0.4	0.95	0.62	2.87
		48 h	Y=21.333X+2.3333	0.88	0.33	1.85
		72 h	Y=11.333X+7.6	0.55	0.19	5.18
	Solanum tuberosum germinated tuber	24 h	Y=6.3333X−0.8333	0.71	1.30	3.87
		48 h	Y=6.3333X−0.8333	0.71	0.68	1.65
		72 h	Y=21X−2.2333	0.87	0.62	1.90

3. Results

The results of the present study indicated that the mortality rate of *An. stephensi* and *Cx. quinquefasciatus* third-instar larvae treated with 0.5% crude extracts of spices and vegetable wastes was significantly higher than the mortality rates at 0.1%, 0.2%, 0.3%, and 0.4% concentrations of each extract at 24, 48 and 72 h of exposure (Table 1). The results of regression analysis revealed that the mortality rate (Y) was positively co-related with the period of exposure(X) with regression co-efficient close to 1. The result of log-probit analysis (at 95% confidence level) revealed that LC_{50} values gradually decreased with the exposure period with the lowest value at 72 h of exposure (Table 2). Relative mosquito larvicidal efficacy of the spices and vegetable waste crude extract was as follows: *Cuminum cyminum*> *Allium sativum* >*Zingiber offinale* > *Curcuma longa* > *Solanum tuberosum* germinated tuber(Table 1), while chloroform: methanol (1:1 v/v) extract showed different mortality sequence, as follows: *Curcuma longa* > *Zingiber offinale* > *Solanum tuberosum* germinated tuber > *Cuminum cyminum*> *Allium sativum* (Table 3). No mortality or other abnormality was recorded on the non-target organisms.

4. Discussion

Mosquito control at the larval stage is effective procedure because they are localized in space and time[2] resulting in less-dangerous out comes to non-target organisms, while the fight against adult is temporary and unsatisfactory. During the last three decades, mosquito controls were directed to the use of insecticide of plant origin. Environmental safety of insecticides is of first and foremost criterion for mosquito control programmes[16]. Long term extensive application of synthetic insecticides results to the accumulated side effects of toxicant and environmental contamination. Plant extracts have promising larvicidal efficacies because they are rich in bioactive organic chemicals, more beneficial over synthetic

Table 3

Efficacy of *Allium sativum*, *Cuminum cyminum*, *Zingiber offinale*, *Curcuma longa* and *Solanum tuberosum* germinated tuber chloroform: methanol (1:1 v/v) extract on instar larvae .

Larvae	Chloroform: methanol (1:1 v/v) extract	Concentrations (ppm)	Mean mortality rate (%)		
			24 h	48 h	72 h
An. stephensi	*Curcuma longa*	25	6.67±0.33	11.00±0.58	13.67±0.67
		50	9.33±0.33	16.67±0.33	17.33±0.33
		75	17.67±0.33	19.33±0.33	20.00±0.00
	Zingiber offinale	25	3.67±0.33	6.67±0.33	7.67±0.33
		50	6.67±0.33	8.67±0.67	11.67±0.33
		75	9.00±.0.58	11.00±0.58	14.33±0.33
	Solanum tuberosum germinated tuber	25	2.67±0.33	5.00±0.58	6.33±0.33
		50	5.33±0.33	6.33±0.33	7.33±0.33
		75	6.33±0.33	7.67±0.33	8.67±0.33
	Cuminum cyminum	25	0.00±0.00	0.67±0.33	1.33±0.33
		50	1.33±0.33	1.67±0.33	3.67±0.89
		75	2.33±0.33	3.67±0.33	5.00±0.58
	Allium sativum	25	0.00±0.00	0.00±0.00	0.33±0.33
		50	0.33±0.33	0.67±0.33	2.33±0.33
		75	1.67±0.33	2.33±0.33	3.33±0.33
Cx. quinquefasciatus	*Curcuma longa*	25	5.67±0.33	8.67±0.33	12.67±0.33
		50	7.67±0.33	14.33±0.67	15.67±0.33
		75	15.33±0.33	17.67±0.67	18.67±0.33
	Zingiber offinale	25	2.33±0.33	5.67±0.67	7.33±0.67
		50	6.33±0.33	8.33±0.67	11.33±0.67
		75	8.33±0.33	9.67±0.33	12.67±0.33
	Solanum tuberosum germinated tuber	25	1.67±0.33	3.67±0.33	5.33±0.67
		50	4.33±0.33	5.33±0.33	6.67±0.33
		75	4.67±0.33	5.00±0.00	8.00±0.58
	Cuminum cyminum	25	0.00±0.00	0.33±0.33	0.67±0.33
		50	1.00±0.00	2.00±0.58	3.00±0.58
		75	1.67±0.33	3.33±0.67	4.67±0.33
	Allium sativum	25	0.00±0.00	0.00±0.00	0.33±0.33
		50	0.33±0.33	0.33±0.33	1.67±0.33
		75	2.00±0.00	2.67±0.33	3.67±0.33

insecticides as easily bio-degradable and less toxic to environment. Shaalan *et al*[17] reviewed different plants species, which can retard growth, inhibit reproduction, and have function as ovicides, additive, and antagonistic action of botanical mixture. In fact, many researches have reported that essential oils of different plant extract with potential larvicidal activity[18-21].The hexane extract of *Spilanthes acmilla*, *Spilanthes calva*, *Spilanthes paniculata*[22-26], the petroleum ether extract of *Abutilon indicum*[27] leaves of *Artemisia annua* and *Azadirachta*. Ethanol extract of *Allium sativum* (garlic bulb) acts as good control agent against the filarial vector, *Cx. quinquefasciatus*[28]. *Curcuma longa* L. (Zingiberaceae), *Allium sativum L. Alliaceae*, *Zingiber montanum* (Koen) aqueous extracts were reported to be good larvicidal agents against *Cx. quinquefasciatus* and *Ae aegypti* mosquito larvae[29]. But here the effect of *Allium sativum*, *Curcuma longa*, *Zingiber montanum* crude and chloroform: methanol (1:1 v/v) extracts were studied on *Cx. quinquefasciatus* and *An. stephensi* mosquito larvae.

In conclusion, crude and chloroform: methanol (1:1 v/v) extracts of some common spices (*Cuminum cyminum*, *Allium sativum*, *Zingiber offinale*, *Curcuma longa*) and vegetable waste (*Solanum tuberosum* germinated tuber) can be recommended effectively in mosquito control programmes. Crude plant extracts were more cost effective and may be employed in localized situation. Chloroform: methanol (1:1 v/v) extract of these materials were very effective as mosquito larvicide at very low concentration. From ecological view point the extract of studied materials were safe for field application because no mortality and other abnormality were noticed on non target organisms. However, further studies are needed to investigate the chemical structure and action mechanism of the active principle , which have mosquito larvicidal activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Yang YC, Lee SG, Lee HK, Kim MK, Lee SH, Lee HS. A piperidine amide extracted from *Piper longum* L. fruit shows activity against *Aedes aegypti* mosquito larvae. *J Agric Food Chem* 2002; **50**: 3765-3767.

[2] Howard AFB, Zhou G, Omlin FX. Malaria mosquito control using edible fish in western Kenya: preliminary findings of a controlled study. *BMC Public Health* 2007; **7**: 199-204.

[3] Redwane A, Lazrek HB, Bouallam S, Markouk M, Amarouch H, Jana M. Larvicidal activity of extract from *Querus lusitania* var infectoria galls (oliv). *J Ethnopharmacol* 2002; **79**: 261-226.

[4] Jeyabalan D, Arul N, Thangamathi P. Studies on effect of *Pelagonium citrosa* leaf extracts on malarial vector, *Anopheles stephensi* Liston. *Bioresources Technol* 2003; **89**: 185-189.

[5] Sharma P, Mohan L, Srivastava CN. Larval susceptibility of *Ajuga remota* against anopheline and culicine mosquitoes. *Southeast Asian J Trop Med & Public Health* 2004; **35**: 608-610.

[6] Choochote N, Tuetun B, Kanjanapothi D. Potential of crude seed extract of celey, *Apium graveolens* L, against the mosquito *Aedes aegypti* (L) (Diptera: Culicidal). *J Vector Ecol* 2004; **29**: 340-346.

[7] Sharma P, Mohan L, Srivastava CN. Phytoextract-induce developmental deformities in malarial vector. *Bioresource Technol* 2006; **97**: 1599-1604.

[8] Yang YC, LeEH, Lee HS,Lee DK, Ahn YJ. Repellency of aromatic medicinal plant extracts to *Aedes aegypti*. *J Am Mosquito Control Ass* 2004; **20**(2): 146-149.

[9] Pelah D, Abramovich Z, Markus A, Wiesman Z. The use of commercial saponin from *Quillaja saponaria* bark as a natural larvicidal agent against *Aedes aegypti* and *Culex pipiens*. *J Ethnopharmacol* 2002; **81**(2): 407-409.

[10]Wiesman Z, Chapagain BP. Larvicidal effects of aqueous extracts of *Balanites aegyptiaca* (desert date) against the larvae of *Culex pipiens* mosquitoes. *Afr J Biotechnol* 2005; **4**(11): 1351-1354.

[11]Chowdhury N, Ghosh A, Chandra G. Mosquito larvicidal activities of *Solanum villosum* berry extract against the dengue vector *Stegomyia aegypti*. *BMC Complement Altern Med* 2008; **8**: 10.

[12]Ghosh A, Chowdhury N, Chandra G. Laboratory evaluation of a phytosteroid compound of mature leaves of day jasmine (Solanaceae: Solanales) against larvae of *Culex quinquefasciatus* (Diptera: Culicidae) and nontarget organisms. *Parasitol Res* 2008; **103**: 221-277.

[13]Joseph CC, Ndoile MM, Malima RC, Nkunya MH. Larvicidal and mosquitocidal extracts, a coumarin, isoflavonoids and pterocarpans from *Neorautanenia mitis*. *Trans R Soc Trop Med Hyg* 2004; **98**(8): 451-455.

[14]Khanna VG, Kannabiran K. Larvicidal effect of *Hemidesmus indicus, Gymnema sylvestre*, and *Eclipta prostrata* against *Culex qinquifaciatus* mosquito larvae. *Afr J Biotechnol* 2007; **3**: 307-311.

[15]World Health Organization. *Instruction for determining the susceptibility or resistance of mosquito larvae to insecticides .WHO\VBC\81.807 WHO*. Geneva: WHO; 1981.

[16]Rajkumar S, Jebanesan A. Larvicidal and adult emergence inhibition effect of *Centella asiatica* Brahmi (Umbelliferae) against mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae). *Afr J Biomed Res* 2005; **8**: 31-33.

[17]Shaalan EHS,Canyonb D, Younese MWF, Abdel-wahaba H, Mansoura AH. A review of botanical phytochemicals with mosquitocidal potential. *Environ Int* 2005; **31**: 1149-1166.

[18]Sharma P, Mohan L, Srivastava CN. Phytoextract-induce developmental deformities in malarial vector. *Bioresource Technol* 2006; **97**: 1599-1604.

[19]Rasheed M, Afshan F, Tariq RM, Siddiqui BS, Gulzar T, Mahmood A, et al. Phytochemical studies on the seed extract of *Piper nigrum* Linn. *Nat Prod Res* 2005; **19**(7): 703-712.

[20]Siddiqui BS, Gulzar T, Mahmood A, Begum S, Khan B, Afshan F. New insecticidal amides from petroleum ether extract of dried *Piper nigrum* L. whole fruits. *Chem Pharm Bull* (Tokyo) 2004; **52**(11): 1349-1352.

[21]Amer A, Mehlhorn H. Larvicidal effects of various essential oils against *Aedes, Anopheles*, and *Culex larvae* (Diptera, Culicidae). *Parasitol Res* 2006a; **99**: 466-472.

[22]Pandey V, Agrawal V, Raghavendra K, Dash AP. Strong larvicidal activity of three species of *Spilanthes* (Akarkara) against malaria (*Anopheles stephensi* Liston, *Anopheles culicifacies*, species C) and filaria vector (*Culex quinquefasciatus* Say). *Parasitol Res* 2007; **102**(1): 171-174.

[23]Govindarajan M, Mathivanan T, Elumalai K, Krishnappa K, Anandan A. Ovicidal and repellent activities of botanical extracts against *Culex quinquefasciatus, Aedes aegypti* and *Anopheles stephensi* (Diptera: Culicidae). *Asian Pac J Trop Biomed* 2011; **1**(1): 43-48.

[24]Kitvatanachai S, Apiwathnasorn C, Leemingsawat S, Wongwit W, Overgaard HJ. Lead levels of *Culex mosquito* larvae inhabiting lead utilizing factory. *Asian Pac J Trop Biomed* 2011; **1**(1): 64-68.

[25]Kumar S, Wahab N, Warikoo R. Bioefficacy of *Mentha piperita* essential oil against dengue fever mosquito *Aedes aegypti* L. *Asian Pac J Trop Biomed* 2011; **1**(2): 85-88.

[26]Prabhu K, Murugan K, Nareshkumar A, Ramasubramanian N, Bragadeeswaran S. Larvicidal and repellent potential of *Moringa oleifera* against malarial vector, *Anopheles stephensi* Liston (Insecta: Diptera: Culicidae). *Asian Pac J Trop Biomed* 2011; **1**(2): 124-129.

[27]Rahuman AA, Gopalakrishnan G, Venkatesan P, Geetha K. Isolation and identification of mosquito larvicidal compound from *Abutilon indicum* (Linn.) Sweet. *Parasitol Res* 2008b. DOI 10.1007/s00436-007-0864-5.

[28]Kalu IG, Ofoegbu U, Eroegbusi J, Nwachukwu C U and Ibeh B: Larvicidal activities of ethanol extract of *Allium sativum* (garlic bulb) against the filarial vector, *Culex quinquefasciatus*. *J Med Plants Res* 2010; **4**(6): 496-498.

[29]Chansang U, Zahiri NS, Bansiddhi J, Boonruad T, Thongsrirak P, Mingmuang J, et al. Mosquito larvicidal activity of aqueous extracts of long pepper (*Piper retrofractum* Vahl) from Thailand. *J Vector Ecol* 2005; **30**(2): 195.