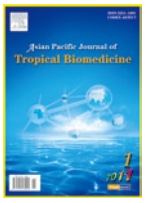
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb



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Smoke repellency and mosquito larvicidal potentiality of *Mesua ferra* L. leaf extract against filarial vector *Culex quinquefasciatus* Say

Someshwar Singha, Utpal Adhikari, Goutam Chandra*

Department of zoology, mosquito and microbiology research units, parasitology laboratory, the university of burdwan, burdwan, west bengal, India, 713104

ARTICLE INFO

Article history:
Received 2 July 2011
Received in revised form 28 July 2011
Accepted 18 August 2011
Available online 10 September 2011

Keywords:
Mesua ferra
Culex quinquefasciatus
Smoke toxicity
Larvicidal activity
Ecofriendly
Mosquito control
Filariasis

ABSTRACT

Objective: Present study was made to evaluate the smoke repellent potentiality and mosquito larvicidal activity of *Mesua ferra* (*M. ferra*) leaves against filarial vector *Culex quinquefasciatus* (*Cx. quinquefasciatus*). **Methods:** Crude, petroleum-ether, chloroform: methanol (1:1 v/v) and ethyl acetate extracts of mature plant leaves were investigated to establish its biocontrol potentiality under laboratory condition against larvae of *Cx. quinquefasciatus* at different concentrations *i.e.* 25 ppm, 50 ppm and 75 ppm. Mosquito coil prepared from *M. ferra* leaves powder were tested for smoke toxicity effect against *Cx. quinquefasciatus* adult mosquitoes. **Results:** The mortality rates of crude extract at 0.5% concentration were higher than all other concentrations tested against the first, second, third and fourth instars larvae at 24 h, 48 h and 72 h of exposure. Larval mortality rate in chloroform: methanol (1:1 v/v) extract was significantly higher ($P<0.05$) than other extracts. LC_{50} value of petroleum ether, chloroform: methanol (1:1 v/v) and ethyl acetate extracts were 195.33 ppm, 27.28 ppm and 74.19 ppm respectively, after 48 h of exposure. Smoke exposed gravid females oviposited fewer eggs when compared to non exposed female mosquitoes. **Conclusions:** In conclusion *M. ferra* crude and chloroform: methanol (1:1) extract can be used effectively against mosquito control programmes. Smoke from *M. ferra* can play an important role in the interruption of transmission of those diseases where mosquitoes act as vector at the individual level.

1. Introduction

Mosquito is one of the notorious creatures in the animal kingdom. They are vectors of several disease causing pathogens having the potentiality to kill more than a million victims annually around the world[1]. In tropical developing countries human filariasis is a socioeconomic problem and the pathogen of filariasis is transmitted by *Culex quinquefasciatus* (*Cx. quinquefasciatus*) mosquito. Lymphatic filariasis is a tropical disease infecting about 120 million people worldwide within which 44 million have chronic manifestations[2]. In Indian subcontinent the pathogen of lymphatic filariasis is *Wuchereria bancrofti* which is transmitted by female *Cx. quinquefasciatus* mosquitoes[3].

Therefore, the only efficacious approach of minimizing the incidence of these diseases is to control mosquito population by application of insecticides at larval habitats. Mosquito in the larval stage are attractive target for control operation due to their low mobility in the breeding habitats and the easiness to control in these habitats[4]. One of the methods available for the control of mosquito population is over and injudicious application of persistent synthetic insecticides, resulting undesirable effect including biomagnification through food chain, development of insecticides resistance, toxic effect in human and other non target organisms. More detailed studies on naturally occurring insecticides are needed to avoid the adverse effect of synthetic insecticides.

In recent years, the top priority in finding a new insecticide is that, they must be plant origin and does not have any ill effect on ecosystem. Research has proved the effectiveness of plant derived secondary compounds, such as saponine[5], steroids[6,7], isoflavonoids[8], essential oil[9], alkoloids and tannins[10] as mosquito larvicides. Plant compounds and their synthetic derivatives *i.e.* essential oils provide alternative source of mosquito repellents agent[11].

The objective of the present study was to observe the larvicidal activity of *Mesua ferra* (*M. ferra*) leaves crude, petroleum ether, ethyl acetate and chloroform: methanol (1:1 v/v) extracts against larvae of *Cx. quinquefasciatus* mosquitoes and repell potentiality of smoke of *M. ferra* leaf powder against adult *Cx. quinquefasciatus* mosquitoes.

*Corresponding author: Goutam Chandra, Ph.D., D.Sc., Professor, Department of Zoology, Mosquito and Microbiology Research Units, Parasitology Laboratory, the University of Burdwan, Golapbag, Burdwan 713104, West bengal, India.
Tel: +91- 9434573881
E-mail: goutamchandra63@yahoo.co.in

2. Materials and methods

2.1. Preparation of crude extract

Fresh mature and green leaves of *M. ferra* were randomly collected during March and April from plants growing on the outskirts of Burdwan and the voucher specimen is deposited in the herbarium of the department of Zoology (voucher No. 116), the university of Burdwan, West Bengal, India. Crude extract of plant leaves were prepared in an electric blender and the plant juice was filtered by passing through the Whatman No. 1 filter paper. The filtrate was used as stock solution and required concentration (0.1%, 0.2%, 0.3%, 0.4%, and 0.5%) were prepared through mixing of stock extract with variable amount of distilled water.

2.2. Preparation of different solvent extracts

The dried leaves were put in a Soxhlet apparatus and the plant extracts were prepared using three solvents namely petroleum-ether, chloroform: methanol (1:1 v/v) and ethyl acetate applying one after another on same leaves. The period of extraction for each solvent was 72-h. Each extract was concentrated by evaporation in rotary evaporator. The solid residue of each extract was used for preparation of graded concentration *i.e.* 25 ppm, 50 ppm and 75 ppm.

2.3. Mosquito culture

Raft of *Cx. quinquefasciatus* eggs were collected from cemented drains surrounding the University campus. After hatching, first instar larvae were fed with small amount of flour until reaching the third instar form. The transformed pupae were separated manually with a glass dropper into a glass beaker (500 mL) containing tap water. The beaker was introduced into cages for emergence of adult mosquitoes. A cotton ball soaked in 10% glucose solution was used for glucose meal of adult mosquitoes and was periodically blood fed on immobilized pigeon.

2.4. Larvicidal bioassay

The bioassay experiments were conducted according to standard WHO procedure (1981) with slight modifications[12]. During experiment with crude extract all instars larvae were used but only the third instar larvae of *Cx. quinquefasciatus* were used during bioassay experiment of solvent extracts. Each experiment was carried out in triplicate. The larvae were put in glass Petri-dishes (9 cm diameter/150 mL capacity) containing 100 mL of tap water. Five concentrations of aqueous extract (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) and three concentrations of solvent extract (25 ppm, 50 ppm and 75 ppm) were applied into separate Petri-dishes to investigate the rate of larval mortalities. Tap water was only used in the control treatment. Larval mortalities were recorded after 24 h, 48 h and 72-h of exposure. The data of mortality in 48 h and 72 h were expressed by the addition of the mortality at 24 h and 48 h, respectively.

2.5. Preparation of mosquito coil

Mosquito coils were prepared following the methods of Saini *et al*[13] with suitable modifications. The composition of mosquito coils were 4 grams of shade dried plant powder containing active ingredients, 2 grams of sawdust and 2 grams of charcoal powder. All the materials were thoroughly mixed with distilled water to form a semi-solid paste and the paste was used for the preparation of 0.4 cm thickness mosquito coils. The mosquito coils were dried in shade and used for further repellency experiment.

2.6. Smoke toxicity test

The smoke toxicity experiment was conducted in a glass chamber measuring 140 cm×120 cm×60 cm with a door at the front of the chamber. Hundred blood fed adult mosquitoes were released into the chamber and the mosquitoes were exposed to the smoke of burning coils for 40 minutes and the data of mortality were recorded after every 10 minutes. The data of adult mosquito mortality at 20, 30 and 40 min were expressed by the addition of the mortality at 10 min, 20 min and 30 min, respectively. Survived bloods fed mosquitoes were reared in a mosquito cages measuring 45 cm×30 cm ×30 cm. Glass bowl containing water was kept inside the cage for oviposition of blood fed adult mosquitoes. The eggs from the cage were collected daily until all the mosquitoes died to compare those which were not exposed to smoke.

2.7. Effect on non target organisms

Non target organisms were those animals that share the common habitats of target mosquito larvae and some of them were natural predators of mosquito larvae. The effect of the crude, petroleum-ether, chloroform: methanol (1:1 v/v) and ethyl acetate extracts of *M. ferra* leaves were tested against non-target organisms like *Toxorhynchites* larvae (mosquito predator), *Gambusia affinis*, *Poecilia reticulata* (predatory fishes), *Diplonychus annulatum* (predatory water-bug) and *Chironomus circumdatus* larvae (insect). The predators were exposed to appropriate lethal concentration of crude and solvent extracts at 24 h to observe the mortality and other abnormalities such as sluggishness and reduced swimming activity up to 72 h of exposure.

2.8. Phytochemical analysis of the plant extract

Phytochemical analysis of the plant extract was carried out according to the methodology of Harbone (1984) [14] and Stahl (1989) [15]. One or more phytochemical usually play an active role in killing mosquito larvae. Phytochemicals includes under study were saponins, terpenoids, alkaloids, steroids, tannin, flavonoids, cardiac glycosides and free glycoside bound anthroquinones

3. Results

The results of the present study indicate that the mortality rate of all larval instars of *Cx. quinquefasciatus* at 0.5% concentration was significantly higher ($P< 0.05$) than the mortality rates at 0.1%, 0.2%, 0.3% and 0.4% concentrations of crude plant extract at 24 h, 48 h and 72 h of exposure (Table 1). Higher mortality rate was also recorded in 72 h bioassay than those in 24 and 48 h. The result of the three-way

factorial ANOVA (Table 2) of crude extract of leaves of *M. ferra* carried out at different concentrations, different time interval and different instars revealed significant difference in larval mortality ($P<0.05$). The results of regression analysis revealed that the mortality rate (Y) was positively correlated with the period of exposure (X) having a regression coefficient close to one in each case (Table 3). The results of log probit analysis (95% confidence level) revealed that LC_{50} values gradually decreased with the exposure period (Table3).

Table 1
Efficacy of *M. ferra* leaf crude extract at different concentrations on different larval instars of *Cx. quinquefasciatus* (Mean ± standard errors).

Larval instars	Concentrations (%)	Mortality rate (%)		
		24 h	48 h	72 h
First	0.1	49.67±1.45	56.33±0.89	66.67±1.85
	0.2	55.67±0.67	61.67±0.89	74.67±1.85
	0.3	69.00±1.15	70.67±0.33	80.67±0.89
	0.4	76.67±0.89	81.00±0.58	87.33±1.20
	0.5	86.33±1.20	90.33±0.33	94.33±0.67
Second	0.1	49.33±1.20	60.67±1.20	80.33±1.20
	0.2	59.33±0.89	68.33±0.89	81.33±0.67
	0.3	67.33±1.20	73.00±1.15	84.33±0.67
	0.4	78.33±0.67	81.33±0.67	91.33±0.33
	0.5	84.67±0.33	89.67±0.33	95.67±1.33
Third	0.1	52.67±0.33	61.67±0.89	67.00±0.58
	0.2	61.67±1.20	68.33±0.33	75.67±0.33
	0.3	69.33±0.33	77.67±0.89	87.00±1.15
	0.4	82.67±0.67	86.00±0.58	95.33±0.33
	0.5	90.00±0.58	95.67±0.67	100.00±0.00
Fourth	0.1	46.00±0.58	53.33±1.33	65.33±1.45
	0.2	54.67±0.33	64.33±0.67	71.67±1.20
	0.3	65.00±1.15	71.33±0.68	77.33±0.33
	0.4	75.00±0.58	80.00±0.58	88.33±0.33
	0.5	78.67±0.89	90.00±0.58	96.67±0.89

Table 2
Completely randomized three ways fractional ANOVA using different concentrations, different instars and hour as variables.

Source	Sum of suares	df	Mean square	*F* value	*P* value
Instar	1058.772	3	352.924	147.393	0.001
Hours	7224.411	2	3612.206	1508.578	0.001
Conc	23652.644	4	5913.161	2469.534	0.003
Instar * Hours	193.411	6	32.235	13.462	0.002
Instar * Conc	284.644	12	23.720	9.906	0.001
Hours * Conc	368.756	8	46.094	19.251	0.002
Instar * Hours * Conc	339.422	24	14.143	5.906	0.003
Residual	287.333	120	2.394	–	–
Total	33409.394	179	–	–	–

Table 3
Log probit analysis and regression analysis of larvicidal activity of *M. ferra* leaf crude extract against different larval instars of *Cx. quinquefasciatus*.

Larval instars	Period of bioassay (h)	LC_{50} (% extract)	LC_{90} (% extract)	Regression equations	*R* value
First	24	0.1223	0.9194	Y= 94.333X + 39.167	0.9766
	48	0.0956	0.7654	Y= 87.333X + 45.8	0.9837
	72	0.0561	0.4788	Y=68X + 60.333	0.9587
Second	24	0.1176	0.9493	Y=89.667X + 40.9	0.9843
	48	0.0703	0.7987	Y=71X + 53.3	0.9759
	72	0.0177	0.3635	Y=40.667X + 79.4	0.8930
Third	24	0.1077	0.7077	Y=95.667X + 42.567	0.9866
	48	0.0789	0.5029	Y=85.667X + 52.167	0.9901
	72	0.0732	0.2893	Y=85.667X + 59.3	0.9776
Fourth	24	0.1362	1.3177	Y=85.667X + 38.167	0.9734
	48	0.1003	0.7371	Y=89X + 45.1	0.9875
	72	0.0682	0.4625	Y=79.333X + 56.067	0.9714

Table 4
Result of larval mortality by three solvent extract of *M. ferra* leaf at different concentrations on third instar larvae of *Cx. quinquefasciatus*.

Solvent extract	Concentrations (ppm)	Mean mortality rate (%)		
		24 h	48 h	72 h
Petroleum ether	25	0.00±0.00	0.00±0.00	3.67±0.67
	50	3.67±0.33	5.00±0.00	6.67±0.33
	75	5.33±0.33	9.67±0.33	13.67±0.67
Ethyl acetate	25	13.67±0.33	24.33±0.33	30.67±0.67
	50	28.33±0.33	36.33±0.67	42.00±0.58
	75	42.33±0.67	52.33±0.67	58.67±0.33
Chloroform: methanol	25	36.33±0.67	45.67±0.67	54.33±0.67
	50	64.33±0.67	78.33±1.67	81.67±1.67
	75	87.67±0.67	91.67±0.67	100.00±0.00

Table 5
Smoke toxicity effect of *M. ferra* leaf powder, commercial mosquito coils and mosquito coil without any plant materials on *Cx. quinquefasciatus* adult mosquitoes.

Time of observation after burning of mosquito coil	*M. ferra* leaf mosquito coil		No. of mosquito died by commercial mosquito coil	No. of mosquito died by control mosquito coil*
	No. of dropped down mosquito	No. of death		
After 10 minutes	32.670±1.450	17.67±1.45	51.33±0.33	1.67±0.33
After 20 minutes	67.000±2.050	31.33±1.45	100.00±0.00	3.00±0.58
After 30 minutes	77.670±1.850	62.33±1.77	100.00±0.00	4.67±0.33
After 40 minutes	94.000±2.309	91.67±0.89	100.00±0.00	6.33±0.33

The result of third-instar larval mortality with petroleum ether, chloroform: methanol (1:1 v/v) and ethyl acetate solvent extracts was presented in Table 4. Higher mortality was observed in chloroform: methanol (1:1 v/v) extract at 75 ppm at 24 h, and it is statistically different ($P<0.05$) from mortalities at 25 ppm, and 50 ppm concentrations. LC_{50} value of petroleum ether, chloroform: methanol (1:1 v/v), and ethyl acetate were 195.33 ppm, 27.28 ppm and 74.19 ppm respectively, after 48 h of exposure.

M. ferra leaf powder demonstrates smoke toxicity effect on adult *Cx. quinquefasciatus* mosquitoes. Mortality rates of different smoke exposed adult mosquitoes were recorded in the following sequences: commercial mosquito coil> mosquito coil containing powder *M. ferra* leaf > mosquito coil without any plant materials (Table 5).

After treatment with *M. ferra* leaf smoke on 100 fed mosquitoes only 8 fed mosquitoes were survived, ovipositing a total of 782 eggs, of which only 337 eggs hatched. In the negative control, 8 mosquitoes laid 871 eggs and only 589 eggs hatched. Smoke exposed adults mosquitoes oviposited fewer eggs in compareision with negative control group because the vaporized form of plant active compounds affected the fecundity of female mosquitoes. Phytochemical analysis of *M. ferra* leaves revealed the presence of many bioactive principles including tannin, steroid, flavonoid, and free glycoside-bound anthraquinones, which might be responsible for mosquito larvicidal potentiality. No change in the swimming behaviors and survivality were observed when crude and solvent extracts were studied on non-target organisms at appropriate lethal concentration of 24 h and the observation were continued up to 72 h.

4. Discussion

From ecological point of view, insecticides of plant origin are efficient, biodegradable as well as suitable alternative for mosquito control[4]. The life cycle of the mosquito has to be understood before any control method is applied. The control methods should aim at the weakest link of the life cycle of the mosquito, which is the larva stage. Mosquito control at the larval stage can be effective procedure due to the low mobility of larvae in their breeding habitats in respect to time[5]. The pest control methods were directed to the use of insecticides of plant origin. Plants are rich source of bioactive compound and offer an advantage over synthetic insecticides as are less prone to development of resistance, easily biodegradable and less toxic to natural environment. Plant derived natural products have the advantage of being harmless to beneficial non-target organisms. Shaalan *et al*[16] reviewed on different plants species having growth retrading, reproduction inhibiting, ovicides, additive, and antagonistic action of botanical mixture. In fact many researches have reported that essential oils of different plant extract haveing good larvicidal potentiality[17-20]. The hexane extract of *Spilanthes acmilla*, *Spilanthes calva*, *Spilanthes paniculata*[21], the petroleum ether extract of *Abutilon indicum*[22], leaves of *Artemisia annua* and *Azadirachta indica*[23], acetone extract of *Nerium indicum* and *Thuja orientalis*[24] and *Ferula asafaetida*, *Trigonella foenum graecum*[25] have been used against *Cx. quinquefasciatus* and *Aedes aegypti* (*A. aegypti*) larvae.

However, control of adult mosquitoes has to be considered too, either by adulticiding or by preventing methods such as repellency or mosquito coil burning. There are four major type of insecticidal products used by general people in their residences like, aerosols, mosquito coils, liquid vaporizers and vaporizing mats, out of which mosquito coils are preferred as anti-mosquito product in low income communities of India. The common active ingredients in coils are various pyrethroids, different toxic chemicals and frequently contain octa-chlorodipropyl ether, bis-(chloromethyl) ether (BCME), which are harmful to exposed person. Volatile constituents of octa-chlorodipropyl ether

include undefined genotoxic agents[26]. Plant derived smoke contains an array of chemicals which has been used since early time to deter mosquitoes and it is cheap, target specific, self sustained and highly toxic to adult mosquito at low doses. Murugan *et al*[27] reported that smoke from *Albizza amara* was more toxic and effective repellent agent against *Ae. aegypti* than *Ocimum bascilicum*. Smoke produced from powder of *Azadirachta indica*, *Ocimum sanctum* and *Adhatoda rasica* leaves were used against *Cx. quinquefasciatus* and *Armigeres subalatus* biting activity for 6-8h.

In conclusion *M. ferra* crude and chloroform: methanol (1:1) extract can be used effectively against mosquito control programmes. The plant extracts were safe to those non-target organisms that share the same habitat of *Cx. quinquefasciatus* mosquito larvae. Further study is needed to know the chemical structure of the active principal involve in larvicidal activity. Smoke from *M. ferra* can play an important role in the interruption of transmission of those diseases where mosquitoes act as vector at the individual level.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Vatandoost H, Vaziri M. Larvicidal activity of neem extract (*Azadirachta indica*) against mosquito larvae in Iran. *Pestol* 2001; **25**: 69-72.

[2] Bernhard L, Bernhard P, Magnussen P. Management of patient with lymphoedema caused by filariasis in north- eastern Tanzania: alternative approaches. *Physiother* 2003; **89**: 743-749.

[3] 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.

[4] 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.

[5] Wiseman 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.

[6] 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 .

[7] 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.

[8] 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.

[9] Cavalcanti ESB, Morais SM, Lima MAA, Santana EWP. Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti* L. *Mem Inst Oswaldo Cruz* 2004; **99**: 541-544.

[10] 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.

[11] Yang YC, Le EH, Lee HS, Lee DK, Ahn YJ. Repellency of aromatic medicinal plant extracts to *Aedes aegypti*. *J Am Mosq Control Assoc* 2004; **20**(2): 146-149.

[12] World Health Organization. Instruction for determining the susceptibility or resistance of mosquito larvae to insecticides. Geneva: WHO; 1981.

[13] Saini HK, Sharma RM, Bami HL, Sidhu KS. Preliminary study on constituents of mosquito coil smoke. *Pesticides* 1986; **20**: 15-18.

[14] Harborne JB. phytochemical methods. *A guide to modern technique of plant analysis*. London: Chapman and Hall; 1984, pp. 49-188.

[15] Stahl E . *Thin layer chromatography a laboratory handbook*. 2nd edn. Berlin: Springer; 1989.

[16] 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.

[17] Sharma P, Mohan L, Srivastava CN. Phytoextract-induced developmental deformities in malaria vector. *Bioresour Technol* 2006; **97**(14): 1599-1604.

[18] 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.

[19] 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* 2004; **52**(11): 1349-1352.

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

[21] 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.

[22] Rahuman AA, Gopalakrishnan G, Venkatesan P, Geetha K. Isolation and identification of mosquito larvicidal compound from *Abutilon indicum* (Linn.) Sweet. *Parasitol Res* 2008; **102**(5): 981-988.

[23] Tonk S, Bartarya R, Maharaj Kumari K, Bhatnagar VP, Srivastava SS. Effective method for extraction of larvicidal component from leaves of *Azadirachta indica* and *Artemisia annua* Linn. *J Environ Biol* 2006; **27**(1): 103-105.

[24] Sharma P, Mohan L, Srivastava CN. Larvicidal potential of *Nerium indicum* and *Thuja oriertelis* extracts against malaria and Japanese encephalitis vector. *J Environ Biol* 2005; **26**(4) :657-660.

[25] Harve G, Kamath V. Larvicidal activity of plant extracts used alone and in combination with known synthetic larvicidal agents against *Aedes aegypti*. *Indian J Exp Biol* 2004; **42**(12): 1216-1219.

[26] Pauluhn J, Mohr U. Inhalation studies in laboratory ani- mals- current concepts and alternatives. *Toxicol Pathol* 2000; **28**: 734-753.

[27] Murugan K, Murugan P, Noortheen A. Larvicidal and repellent potential of *Albizzia amara* Boivin and *Ocimum basilicum* Linn against dengue vector, *Aedes aegypti* (Insecta: Diptera: Culicidae). *Bioresour Technol* 2007; **98**(1): 198-201.