Int J Pharm Bio Sci 2016 April; 7(2): (B) 162 - 167

Original Research Article **Allied Science**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

LARVICIDAL EFFICACY OF MATURE LEAF EXTRACT OF *NICOTIANA PLUMBAGINIFOLIA* VIV. (SOLANACEAE) AGAINST SOUTHERN HOUSE MOSQUITO

ANIKET SINGH[1], KUNTAL BHATTACHARYA[1,2], ANUSHREE SINGH RAY[1] AND GOUTAM CHANDRA*[1]

[1]Mosquito, Microbiology and Nanotechnology Research Units, Parasitology Laboratory, Department of Zoology, The University of Burdwan, Burdwan-713104, West Bengal, India
[2] Durgapur Government College, Durgapur, West Bengal, India, PIN- 713214

ABSTRACT

The small iniquitous fly mosquito is responsible for transmitting various deadly diseases, so it is inevitable to control mosquito vector population. Due to resistance of the synthetic insecticides, use of botanicals is a new trend to control the mosquito species. Present study was carried out to examine the efficacy of mature leaf extract of *Nicotiana plumbaginifolia* on larval forms of filarial vector *Culex quinquefasciatus*. All larval instars treated with crude and three different solvent extracts of *N. plumbaginifolia* leaves. LC_{50} and LC_{90} values were determined by log-probit analyses. Statistical justifications were carried out through regression and ANOVA analyses. Cent percent larval mortality was found against 1st instar larvae at 0.20% concentration of crude extract and 60 ppm concentration of acetone solvent extract at 72h of post exposure. Non-target organisms were non-responsive to both crude and acetone solvent extracts. Thus, *N. plumbaginifolia* may act as potential larvicidal agent against *Cx. quinquefasciatus.*

KEYWORDS: ***Nicotiana plumbaginifolia, Culex quinquefasciatus,*** **larvicide, non-target organism.**

GOUTAM CHANDRA
Mosquito, Microbiology and Nanotechnology Research Units, Parasitology Laboratory, Department of Zoology, The University of Burdwan, Burdwan-713104, West Bengal, India

***Corresponding author**

INTRODUCTION

Mosquito, the little winged devil can spread different life threatening obnoxious diseases[1] like malaria, lymphatic filariasis, Japanese encephalitis, dengue, chikungunya, yellow fever, Rift Valley fever etc. About 40 million people are affected by mosquito borne deadly diseases in India every year[2]. *Culex quinquefasciatus*, also known as southern house mosquito, belongs to the family Culicidae is the chief vector of lymphatic filariasis. Almost more than 1.2 billion people facing a threat[3] across the globe of this socioeconomic crisis transmitted by female *Cx. quinquefasciatus*. Besides, it can transmit avian malaria. Adult females prefer obtaining blood from birds after emergence, but as they age, the species are extremely anthropophilic. Peak biting time of *Cx. quinquefasciatus* is at the 3rd quadrant of night and they rest in the houses in morning and at dusk[4]. It prefers to breed in stagnant drains, septic tanks, muddy water etc. and take about eight days to complete its life cycle[5]. Filariasis, a parasitic disease caused by three nematode worms *Wuchereria bancrofti, Brugia timori and Brugia malayi.* To control these obnoxious diseases, mosquito vector control is the primary and foremost step[6]. In this regard wrigglers control is the best way to diminish mosquito population than adult mosquito control. Till now different synthetic insecticides have been used to control mosquito. The use of insecticides to control mosquito population has various drawbacks. It is non-biodegradable in nature causing biomagnifying hazards, as well as, it harms the other non-target organisms which live in the same habitat with mosquito larvae. Moreover, due to prolonged use of the chemical insecticides mosquito larvae got resistance over it. So, it is obvious to find a new trend to control mosquito larvae by botanicals. Researchers have identified phytochemicals derived from plant parts having larval control efficacy[7, 8, 9]. Plant products are easily available, cost effective, target specific, biodegradable and eco-friendly in nature [10, 11, 12, 13]. *Nicotiana plumbaginifolia* Viv. (Solanaceae), generally called as wild tobacco plant, belongs to solanaceae family. It is an annual herb, 1-3 ft tall, leaves are stalk less, and margins are wavy, pointed 15-23 cm long. It is previously reported that the plant exhibited antibacterial and mosquito larvicidal activities[14, 15]. The purpose of the present experiment was to assess the larvicidal activity of crude and solvent extracts of *N. plumbaginifolia* leaves against the *Cx. quinquefasciatus* larvae. This is the first ever report of this plant as source of target specific, eco-friendly larvicidal agent under laboratory conditions against *Cx. quinquefasciatus.*

MATERIALS AND METHODS

Assemblage of plant materials

Fresh, mature, green leaves of *N. plumbaginifolia* were harvested randomly during May-June 2013 from the University campus (23°16´N, 87°54´E), West Bengal, India. After proper identification a voucher specimen (Voucher No. GCZAS-04) was submitted at the Mosquito, Microbiology and Nanotechnology Research Units, Department of Zoology, The University of Burdwan.

Rearing of larvae

Larvae of *Cx. quinquefasciatus* were taken from a well maintained mosquito colony of the Mosquito, Microbiology and Nanotechnology Research Units, Department of Zoology, The University of Burdwan. The colony was kept free from all kinds of contamination of insecticides, pathogens and repellents and maintained in laboratory at 27±1^{0}C and 80±2% Relative Humidity with a photoperiod of 13:11 hour light and dark cycles respectively[17]. The larvae were reared in plastic trays and fed with a mixture of dried Brewer's yeast powder, dog biscuits and algae mixture in the ratio of 3:1:1.

Preparation of crude extracts

Fresh mature leaves of *N. plumbaginifolia* were rinsed in tap water and then washed with distilled water and soaked on a paper towel. Then the leaves were minced by electric grinder and the liquid was filtered by Whatman's no-1 filter paper. The filtrate solution was considered as the stock crude solution (100% concentration) and stored in refrigerator at 4^{o}C for future use.

Preparation of solvent extraction

Processed leaves of *N. plumbaginifolia* were dried for a few days in shed. 120 g dried leaves of *N. plumbaginifolia* were severed in small pieces and put into the 'thimble' of the Soxhlet apparatus and following the 1:10 ratio 1200 ml solvent was loaded into the 'still pot'. Three different solvents in a non-polar to polar fashion viz. petroleum ether, ethyl acetate and acetone were passed through the same column one after another. The extraction phase was set for 8 hours a day with a highest extraction period of 72 hours for each solvent. Elutes were collected from solvent chamber and concentrated through evaporation in a rotary evaporator and the residue obtained was stored at 4°C in a refrigerator.

Dose dependent larvicidal bioassay

According to the WHO standard protocol[18], larvicidal bioassay was done at the Mosquito, Microbiology and Nano Technology Research Units, Parasitology Laboratory, The University of Burdwan. Instars wise 25 larvae of *Cx. quinquefasciatus* were taken in Petri-dishes of 9 cm diameter (150 ml capacity) filled with 100 ml of tap water. For larvicidal bioassay crude extracts were applied at 0.05%, 0.10%, 0.15%, 0.20% and 0.25% concentrations and solvent extracts were applied at 20 ppm, 40 ppm, 60 ppm, 80 ppm and 100 ppm concentration in different Petri dishes against each instar. Each experiment was performed in a triplicate (n=75) with a set of control. Petri dishes were kept at room temperature (29 ± 2°C), within 88±2% relative humidity range for a total observation period of 72 hours. The larvae were assumed dead when they didn't show any movement by pricking with a sharp needle in the siphon or cervical region or they were unable to reach the water

surface[19]. Larval mortality was recorded after 24h, 48h and 72h of post exposure.

Effects on non-target populations

The little organisms sharing the same habitat with mosquitoes are considered to be the most risk groups. *Chironomus circumdatus* larvae (insect) were taken as non target organism to detect the effect of phytochemicals on it. They were exposed to crude and solvent extracts to concentration level of LC_{50} value (72 hours of post-exposure) of 3rd instars larvae to study the mortality and other irregularities such as sluggishness of swimming activity up to 72 h of exposure.

Statistical analyses

The percentage mortalities (%M) were précised by Abbott's formula[20] during the observation of the larvicidal potentiality of the plant extracts. The LC_{50} and LC_{90} values of crude and solvent extracts, regression analyses and ANOVA analyses were done by using "MS Excel 2007" and Stat plus 2009 professional.

RESULTS

N. plumbaginifolia was found to be effective as mosquito larvicidal agent against *Cx. quinquefasciatus* larvae. The larval mortality rate of all instars of at 0.25% concentration was significantly higher ($p< 0.05$) than the mortality rates at 0.05%, 0.10%, 0.15% and 0.20% concentrations of crude plant extract at 24, 48 and 72 hours of post exposure (Table 1). 100% mortality was found against 1st instars larvae at 0.20% concentration of crude extract after 72h of post exposure. Among three different solvent extracts acetone extract exhibited highest mosquito larvicidal potentiality against *Cx. quinquefasciatus.* 100% mortality was found in 1st instar larvae at 60 ppm concentration of acetone extract at 72h of post exposure (Table 2). The mortality gradually increased with an increase in time of exposure in each larval instar with both crude and acetone extracts. The results of log probit analyses (95% confidence level) revealed that LC_{50} and LC_{90} values gradually decreased with the increase in time of exposure having the lowest value at 72 h of exposure in each larval instar. Lowest LC_{50} and LC_{90} values (5.75 and 19.10 respectively) were found against 1st instars larvae at 72h of exposure followed by 2nd, 3rd and 4th instars larvae. The results of regression analyses revealed that the mortality rate (Y) was positively correlated with the concentration of exposure (X) having a regression coefficient (R^2) close to 1 in each case (Table 3). The result of the three-way factorial ANOVA (Table 4) of acetone extract of leaves of *N. plumbaginifolia* carried out with different concentrations, different time interval and different instars revealed significant difference in larval mortality ($p<0.05$) with respect to these three parameters. No mortality or abnormality related to sluggishness or swimming activity was observed in non-target organism after 72h of exposure.

DISCUSSION

Several plant products are now employed as suitable alternative to synthetic insecticides to control mosquito vector population. To protect human population from mosquito borne deadly diseases mosquito vector control is foremost and crucial step. Use of phytochemicals to control mosquito vector population is an innovative trend in research world. Plant derived phytochemicals are so exceptional due to their easy availability, cost effectiveness, target specificity, biodegradability and ecofriendly nature. Amongst all the life stages (egg, larva, pupa and adult) of mosquito, larvae are most vulnerable due to their confinement to water bodies and extremely stumpy rate of scatterings. So, wrigglers control is the best way to manage vector population. Many researchers have reported a large no of plants that has mosquito larvicidal,[19, 20, 21, 22, 23] pupicidal, adulticidal, phagodeterrence, oviposition deterrence, repellent and smoke toxicity properties[24, 25, 26]. Singha Ray *et al.* (2015)[27] stated that ethyl acetate extract of *Capparis zeylanica* leaves exhibit larvicidal efficiency against *Cx. quinquefasciatus.* LC_{50} and LC_{90} values were 12.44 and 33.88 respectively against 1st instar larvae after 72h of post exposure. Bhattacharya *et al.* (2014)[28] reported that chloroform: methanol (1:1 v/v) extract of *Ravenala madagascariensis* leaves showed 100% mortality against 1st instars larvae of *Cx. quinquefasciatus* after 72 h of exposure at 150 ppm concentration with LC_{50} and LC_{90} value 25.41 ppm and 90.98 ppm respectively. In present experiment, acetone extract was found effective and showed 100% mortality against 1st instars larvae of *Cx. quinquefasciatus* at very low concentration (only 60 ppm) at 72h of post exposure with very less LC_{50} and LC_{90} values 5.75 and 19.10 respectively. In another work, Rawani *et al.* (2013)[29] showed 80% larval mortality against 3rd instar larvae of *Cx. quinquefasciatus* with 120 mg/ml concentration of chloroform: methanol (1:1 v/v) extract of *Solanum nigrum* berry with LC_{50} and LC_{90} values were 23.54 ppm and 515.45 ppm respectively. Kundu *et al.* (2013)[30] showed that 1% concentration of crude extract was able to kill all the larval instars and 480 ppm concentration of ethyl acetate solvent extract was responsible for 100% larval death of 1st instar larvae of *Cx. quinquefasciatus.* It also previously reported that *N. plumbaginifolia* have effective larvicidal property against *Anopheles stephensi*, cent percent mortality of 1st instars larvae was found at 40 ppm concentration of ethyl acetate extract at 72h of exposure with lowest LC_{50} and LC_{90} values 8.43 and 19.42 respectively. Rahuman *et al.* (2009)[31] reported that methanol extract of *Cedrus deodara* stem bark was effective against *Cx. quinquefasciatus* with the LC_{50} value of 95.19 ppm. Singha et al. (2012) also showed the larvicidal effect of acetone extracts of *Holoptelea integrifolia* against *Cx. vishnui* group where cent percent mortality was found at 400 ppm concentrations at 72h of exposure in case of 3rd instar larvae. The non-target organism was completely safe after exposure.

Table 1

Dose response larvicidal bioassay using crude extract of mature leaves of Nicotiana plumbaginifolia against Culex quinquefasciatus larvae

Larval Instars	Concentration (%)	Percent Mortality(Mean ± SE)		
		24h	48h	72h
First	0.05	62.67 ± 0.33	72.00 ± 0.00	84.00 ± 0.33
	0.10	69.33 ± 0.33	78.67 ± 0.54	92.00 ± 0.00
	0.15	76.00 ± 0.00	85.33 ± 0.67	97.33 ± 1.20
	0.20	81.33 ± 0.54	93.33 ± 0.88	100.00 ± 0.00
	0.25	90.67 ± 0.54	100.00 ± 0.00	100.00 ± 0.00
Second	0.05	58.67 ± 0.33	64.00 ± 0.33	73.33 ± 0.54
	0.10	65.33 ± 0.54	72.33 ± 0.88	80.00 ± 0.00
	0.15	73.33 ± 0.33	77.33 ± 0.33	86.67 ± 0.88
	0.20	78.67 ± 0.54	84.00 ± 0.00	92.00 ± 0.67
	0.25	84.00 ± 0.00	93.33 ± 1.20	100.00 ± 0.00
Third	0.05	40.00 ± 0.00	49.33 ± 0.88	60.00 ± 0.00
	0.10	48.00 ± 0.00	54.67 ± 1.20	72.00 ± 0.00
	0.15	53.33 ± 0.33	64.00 ± 0.33	73.33 ± 0.54
	0.20	62.67 ± 0.33	70.67 ± 0.54	78.67 ± 0.33
	0.25	69.33 ± 0.88	80.00 ± 0.00	82.67 ± 0.33
Fourth	0.05	18.67 ± 0.67	25.33 ± 0.33	26.67 ± 0.33
	0.10	21.33 ± 0.33	28.00 ± 0.00	32.00 ± 0.00
	0.15	28.00 ± 0.00	33.33 ± 0.54	34.67 ± 0.33
	0.20	33.33 ± 0.33	40.00 ± 0.33	44.00 ± 0.00
	0.25	41.33 ± 0.54	46.67 ± 1.20	53.33 ± 0.67

Table 2

Dose response larvicidal bioassays using acetone extract of Nicotiana plumbaginifolia leaves against Culex quinquefasciatus larvae

Larval Instars	Concentration (ppm)	Percent Mortality (Mean ± SE)		
		24h	48h	72h
1st	20	62.67±0.33	82.67±0.33	94.67±0.33
	40	72.00±0.00	88.00±0.00	98.67±0.54
	60	78.67±0.58	94.67±0.33	100.00±0.00
	80	86.67±0.33	100.00±0.00	100.00±0.00
	100	94.67±0.33	100.00±0.00	100.00±0.00
2nd	20	58.67±0.58	73.33±1.20	85.33±0.33
	40	66.67±0.67	80.00±0.00	93.33±0.67
	60	73.33±0.33	85.33±0.33	100.00±0.00
	80	81.33±0.33	93.33±0.54	100.00±0.00
	100	89.33±0.33	100.00±0.00	100.00±0.00
3rd	20	61.33±0.33	66.67±0.58	76.00±0.00
	40	68.00±0.00	74.67±0.33	82.67±0.33
	60	74.67±0.67	80.00±0.00	92.00±0.00
	80	81.33±0.54	86.67±0.33	100.00±0.00
	100	88.00±0.33	100.00±0.00	100.00±0.00
4th	20	28.00±0.54	32.00±0.00	36.00±0.00
	40	33.33±0.33	37.33±0.33	40.00±0.00
	60	38.67±0.33	44.00±0.00	49.33±0.67
	80	45.33±0.33	50.67±0.67	57.33±0.33
	100	52.00±0.00	58.67±0.33	64.00±0.00

Table 3

Assessment of LC_{50} and LC_{90} values through log-probit and regression analyses using acetone extract of Nicotiana plumbaginifolia leaves

Larval Instars	Period of Exposure	LC_{50}	LC_{90}	Regression	R^2- value
1st	24	14.56	119.98	0.098x + 13.83	0.99
	48	8.95	36.76	0.058x + 19.77	0.94
	72	5.75	19.10	0.014x + 23.77	0.67
2nd	24	17.92	167.12	0.094x + 12.77	0.99
	48	10.55	62.24	0.083x + 16.59	0.99
	72	9.58	27.16	0.045x + 21.22	0.77
3rd	24	14.62	184.09	0.083x + 13.66	0.99
	48	13.90	87.31	0.098x + 14.50	0.97
	72	11.68	43.46	0.081x + 17.63	0.94
4th	24	116.28	3500.35	0.075x + 5.366	0.99
	48	83.48	2598.75	0.083x + 6.13	0.99
	72	56.06	1066.06	0.091x + 6.33	0.98

Table 4
Completely randomized three way ANOVA analyses of the larvicidal activity using concentration (C), hour (H) and instars (I) as three independent parameters

Source of variation	Sum of squares (SS)	Degree of freedom (df)	Mean of squares (MS)	F value	p-level
Instars (I)	3,733.36	3	1,244.45	1,647.07	0.00
Hours (H)	525.28	2	262.64	347.61	0.00
Conc. (C)	829.20	4	207.30	274.37	0.00
I × H	50.14	6	8.35	11.06	0.00
I × C	24.53	12	2.04	2.70	0.0029
H × C	28.83	8	3.60	4.77	0.0000
I × H × C	51.30	24	2.14	2.82	0.0001
Within groups	90.67	120	0.76	---	---
Total	5,333.31	179	29.80	----	---

CONCLUSION

This study reveals that leaves of the plant *N. plumbaginifolia* has good larvicidal properties against the vector of lymphatic filariasis *Cx. quinquefasciatus* larvae. So, this bioactive portion can be used as an effective larvicidal agent in near future. Further detail experiments are compulsory to determine the proper bioactive fractions responsible for larval death.

CONFLICT OF INTEREST

The authors have no conflict of interest.

ACKNOWLEDGEMENT

Authors are greatly indebted to Professor A. Mukhopadhyay, Botany Department, The University of Burdwan, for his kind assistance in identification of plant species. We are indebted to UGC for providing Rajiv Gandhi Fellowship (RGNF-2013-14-ST-WES-52307) and UGC-DRS for providing financial support.

REFERENCES

1. Tolle MA. Mosquito-borne diseases. Current Problems in Pediatric and Adolescent Health Care. 2009; 39: 97-140.
2. Ghosh A, Chowdhury N, Chandra G. Plant extracts as potential mosquito larvicide. Indian J Med Res. 2012; 135: 581-598.
3. Bockarie JM, Erling MP, Graham BW, Michael E. Role of Vector Control in the Global Program to Eliminate Lymphatic Filariasis. Annu Rev Entomol. 2009; 54: 469-487.
4. Chandra G. Peak period of filarial transmission. American J Trop Med Hyg. 1995; 53: 378-379.
5. Hill S, Roxanne C. Features Creatures: Southern house mosquito. University of Florida. 2009; Retrieved 19 March 2014.
6. Aktar W, Sengupta D, Chowdhury A. Impact of pesticides use in agriculture: their benefits and hazards. Interdisciplinary Toxicol. 2009; 2: 1-12.
7. Bhattacharya K, Chandra I, Kundu P, Ray S, Halder D, Chandra G. Larval control of *Culex vishnui* group through bio-active fraction of traveller's tree, *Ravenala madagascariensis Sonn*. (Strelitziaceae). J Mosq Res. 2014; 4(15): 1-6.
8. Chowdhury N, Chatterjee SK, Laskar S, Chandra G. Larvicidal activity of *Solanum villosum* Mill (Solanaceae: Solanales) leaves to *Anopheles subpictus* Grassi (Diptera: Culicidae) with effect on non-target *Chironomus circumdatus* Kieffer (Diptera: Chironomidae). J Pest Sci. 2009; 82: 13-18.
9. Adhikari A, Singha S, Chandra G. In vitro repellent and larvicidal efficacy of *Swietenia mahagoni* against the larval forms of Culex quinquefasciatus Say. Asian Pac J Trop Biomed. 2013; 2(1): s260-s264.
10. Singha S, Adhikari U, Ghosh A, Chandra G. Mosquito Larvicidal Potentiality of *Holoptelea integrifolia* Leaf Extract against Japanese Encephalitis Vector*, Culex vishuni* Group. J Mosq Res. 2012; 2(4): 25-31.
11. Bhattacharya K, Chandra G. Bioactivity of *Acyranthes aspera* (Amaranthaceae) Foliage against the Japanese Encephalitis Vector *Culex vishnui* Group. J Mosq Res. 2013; 3(13): 89-96.
12. Chakraborty S, Singha S, Bhattacharya K, Chandra G. Control of human filarial vector, *Culex quinquefasciatus* Say 1823 (Diptera:Culicidae) through bioactive fraction of *Cayratia trifolia* leaf. Asian Pac J Trop Biomed. 2013; 3(12): 980-84.
13. Singha Ray A, Bhattacharya K, Singh A, Chandra G. Larvicidal Activity of *Nelumbo nucifera* Gaertn. (Nymphaeaceae) against *Anopheles stephensi* (Liston 1901) and its Effect on Non-target Organisms. J Mosq Res. 2014; 4(10): 1-7.
14. Hossain E, Rawani A, Chandra G, Mandal SC, Gupta JK. Larvicidal activity of *Dregea volubilis* and *Bombax malabaricum* leaf extracts against the filarial vector *Culex quinquefasciatus.* Asian Pac J Trop Med. 2011; 4(6): 436-441.
15. Singh KP, Daboriya V, Kumar S, Singh S. Antibacterial activity and phytochemical Investigations on *Nicotiana plumbaginifolia* viv. (Wild tobacco). Rom J Biol-plant biol. 2010; 55(2): 135-142.

16. Singh A, Bhattacharya K, Chandra G. Efficacy of *Nicotiana plumbaginifolia* (solanaceae) leaf extracts as larvicide against malarial vector *Anopheles stephensi* Liston 1901. Int J Pharma Biosci. 2015; 6(1): (B) 860- 868.
17. Sharma M, Saxena RC. Phytotoxicologial evaluation of *Tegetes erectes* on aquatic stages of *Anopheles stephensi*. Indian J Malariol. 1994; 31: 21-26.
18. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. 2005; WHO, Geneva WHO/CDS/WHOPES/GCDPP/ 13 pp.
19. Macedo M, Consoli RAGB, Grandi TSM, Des AAMG, De OAB, Mendes MM, Queiroz RO, Zani CL. Screening of Asteraceae (Compositae) plant extract for larvicidal activity against *Aedes fluviatilis* (Diptera: Culicidae). Mem Inst Oswaldo Cruz. 1997; 92: 565-570.
20. Abott WS. A method of computing the effectiveness of an insecticide. J Econ Enotomol. 1925; 18(2): 265-267.
21. Mallick S, Bhattacharya K, Chandra G. Mosquito larvicidal potentiality of wild turmeric, *Curcuma aromatica* rhizome, extracts against Japanese Encephalitis vector *Culex vishnui* group. J Mosq Res. 2014; 4 (19): 1-6.
22. Kamaraj C, Rahuman AA, Bagavan A, Zahir AA, Elango G, Kandan P, Rajakumar G, Marimuthu S, Santhoshkumar T. Larvicidal efficacy of medicinal plant extracts against *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera: Culicidae). Trop Biomed. 2010; 27(2): 211–219.
23. Singha S, Chandra G. Mosquito larvicidal activity of some common spices and vegetable waste on *Culex quinquefasciatus* and *Anopheles stephensi*. Asian Pac J Trop Med. 2011; 4(4): 288-293.
24. Haldar KM, Ghosh P, Chandra G. Evaluation of target specific larvicidal activity of the leaf extract of *Typhonium trilobatum* against *Culex quinquefasciatus* Say. Asian Pac J Trop Biomed. 2011; 1(2): S199-203.
25. Bhattacharya K, Chandra G. Biocontrol efficacy of *Operculina turpethum* (L.) (Convolvulaceae) leaf extractives against larval form of malarial mosquito *Anopheles stephensi* (Liston 1901). Int J Pharm Bio Sci. 2015; 6(3): (B) 460-468.
26. Bhattacharya K, Chandra G. Phagodeterrence, larvicidal and oviposition deterrence activity of *Tragia involucrata L*. (Euphorbiaceae) root extractives against vector of lymphatic filariasis *Culex quinquefasciatus (*Diptera: Culicidae). Asian Pac J of Trop Dis. 2014; 4 (Suppl 1): S226-S232.
27. Singha S, Adhikari U, Chandra G. Smoke repellency and mosquito larvicidal potentiality of *Mesua ferra* L. leaf extract against filarial vector *Culex quinquefasciatus* Say. Asian Pac J Trop Biomed. 2011; 1(1): S119-S123.
28. Rawani A, Ghosh A, Laskar S, Chandra G. Aliphatic Amide from Seeds of *Carica papaya* as Mosquito Larvicide, Pupicide, Adulticide, Repellent and Smoke Toxicant. J Mosq Res. 2012; 2(2): 8-18.
29. Singha Ray A, Bhattacharya K, Chandra G. Target specific larvicidal effect of *Capparis zeylanica* L. (capparaceae) foliages against lymphatic filarial vector *Culex quinquefasciatus* say (1823). Int J Pharma Biosci. 2015; 6(3): (B) 139-148.
30. Bhattacharya K, Burman S, Nandi S, Roy P, Chatterjee D, Chandra G. Phytochemical extractions from the leaves of *Ravenala madagascariensis* from Sundarban area and its effect on southern house mosquito *Culex quinquefasciatus* Say (1823) larvae. J Mosq Res. 2014; 4(12): 1-6.
31. Rawani A, Chowdhury N, Ghosh A, Laskar S, Chandra G. Mosquito larvicidal activity of *Solanum nigrum* berry extracts. Indian J Med Res. 2013; 137: 972-976.
32. Kundu M, Rawani A, Chandra G. Evaluation of Mosquito Larvicidal Activities of Seed Coat Extract of *Cassia sophera* L. J Mosq Res. 2013; 3(11): 76-81.
33. Rahuman A, Bagavan A, Kamaraj C, Vadivelu M, Zahir A, Elango G, Pandiyan G. Evaluation of indigenous plant extracts against larvae of *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol Res. 2009; 104: 637-643.