B. N. SEAL JOURNAL OF SCIENCE
ISSN: 0975-5624
PEER-REVIEWED JOURNAL
https://bnsealjournal.ac.in/

RESEARCH ARTICLE

Received: 17-03-2025
Accepted: 23-06-2025
Published: 30-06-2025

Citation: Dutta M, Chandra, G. Mosquito larvicidal efficacy of *Michelia champaca* seed extracts against *Culex vishnui*. B. N. Seal Journal of Science 2025, 13:14-22. https://doi.org/10.5281/zenodo.17553422

DOI: 10.5281/zenodo.17553422

***Corresponding Author:**
Email:
goutamchandra63@yahoo.co.in

Funding: None

Conflict of Interests: None

Published by:
Office of the Principal,
Acharya Brojendra Nath Seal College, Cooch Behar, West Bengal, India-736101

Mosquito Larvicidal Efficacy of *Michelia champaca* Seed Extracts Against *Culex vishnui*

Manali Dutta[1,2], Goutam Chandra[2*]

[1]P.G. Department of Zoology, Krishnagar Government College, Krishnagar, Nadia, West Bengal, India-741101
[2]Mosquito, Microbiology and Nanotechnology Research Units, Parasitology Laboratory, Department of Zoology, The University of Burdwan, Burdwan, West Bengal, India-713104

Abstract: The purpose of the study is to evaluate the larvicidal effectiveness of solvent-based seed extracts from *Michelia champaca* against the Japanese encephalitis causing rice field mosquito, *Culex vishnui*. The methanolic extract obtained from *Michelia champaca* seeds showed potent larvicidal effect. With a regression coefficient value close to 1 in each instance, the rate of mortality was positively correlated with the increasing concentrations of solvent extracts, indicating a clear positive dose-dependent mortality. For the extractive, the lowest LC50 and LC90 values was exhibited to be 63 ppm and 114 ppm, respectively against second instar larvae after 72 hours of exposure in a larvicidal bioassay. For chemical profiling of the methanolic extractive, FT-IR analysis was relied upon. Non target organisms such as *Chironomous* sp. and *Diplonychus* sp. were marked safe upon treatment with solvent extract.

Keywords: *Culex vishnui*, FT-IR analysis, Japanese encephalitis, LC50 and LC90, *Michelia champaca*

Introduction

Mosquitoes, the most notorious vector, transmit several pathogenic diseases all over the tropics and subtropics [1]. Dangerous diseases such as Filariasis, Japanese Encephalitis, Malaria, Yellow fever, Dengue and others are accounted in this list [2-6]. With increasing global temperature, their zone of occurrence is also expanding which is of grave concern to mankind. These mosquito-borne diseases cause about millions of deaths every year. They not only serve as the vector of these diseases but also cause allergic manifestations upon biting [7]. Preventive techniques including the use of larvicides, pupicides, and mosquito repellents are crucial in lowering mosquito population density because there is currently no widely used vaccination or preventative treatment for the control of these diseases. Chemically manufactured insecticides and repellents cause a variety of ecological problems, such as biomagnification and the evolution of insect strains that are resistant to them. Because of their intrinsic biodegradability, target specificity, and reduced harm to non-target organisms, natural products are usually preferred [8]. Additionally, plant items helped to manage helminths and harmful microorganisms from ancient times [9-10]. The *Culex vishnui* group, which includes *Cx. vishnui* Theobald, *Cx. pseudovishnui* Colless, and *Cx. tritaeniorhynchus* Giles, serves as a vector for the Japanese Encephalitis (JE) virus, a member of the Flaviviridae family. This virus is responsible for causing Japanese Encephalitis across various regions of India [11]. Several plant-derived compounds have demonstrated significant mosquitocidal properties. These include **nicotine** from tobacco

leaves, **rotenone** obtained from *Derris elliptica* [12] **anabasine** and **lupinine** extracted from *Anabasis aphylla* [13], **capillin** from *Artemisia nilagirica*, and **goniothalamin** derived from *Bryonopsis laciniosa* [14]. Additionally, **pyrethrums** from *Chrysanthemum cinererifolium* flowers [15] and **glucosinolate** from *Solanum nigrum* leaves [16] have also been identified as effective mosquito control agents. The tall, evergreen tree *Michelia champaca* L. is native to South and Southeast Asia and is mostly grown for its timber. During the monsoon season, it is especially well-known for its aromatic flowers. Birds find this tree's aril-coated seeds highly attractive [17]. According to traditional literature, it is used as a febrifuge and to treat leprosy, fever, labour, and postpartum protection. It is also known to have anti-inflammatory and antipyretic properties [18].

The specific goal of the current investigation was to assess the larvicidal potential of the methanolic extract of *M. champaca* seeds against *Cx. vishnui* larvae.

Materials and Methods

Rearing of mosquitoes

The current investigation was conducted in Burdwan, West Bengal, India, between July and September of 2024 (23° 16' N, 87° 54' E). *Cx. vishnui* larvae were collected from the surrounding paddy fields, and a laboratory colony was kept at the University of Burdwan's Mosquito Research Unit, Department of Zoology. No viruses, pesticides, or repellents were allowed to come into contact with the colony. Under 13:11 light:dark cycles, the temperature kept between 25°C to 30°C and the relative humidity between 80 and 85%, the larvae were reared. They were given a 3:1 powdered combination of dried yeast powder and dog biscuits.

Solvent extract preparation

Fresh *M. champaca* fruits were picked at random from plants growing on the university premises during the experiment. For accurate identification, a voucher specimen of the plant was sent to the Department of Botany, The University of Burdwan having Specimen Id: GCMD/2018/S002. Distilled water was used to rinse the collected fruits. Seeds were obtained after slitting the fruits. About 200 g of mature seeds were gathered, washed, and allowed to dry. The extract was made by putting the dried seeds in the thimble of the Soxhlet apparatus and adding 2000 millilitres of methanol to the still pot over the course of 72 hours. The eluted extract was concentrated by evaporating it in a rotary evaporator set at 40 °C. To create a powdered form for the larvicidal bioassay, the extract was lyophilized. To get increasing concentrations in parts per million, the extract was dissolved in tween 20.

Larvicidal bioassay

With a few modifications, the larvicidal bioassay was conducted in compliance with WHO standard guidelines [19] Graded methanolic extract concentrations between 50ppm to 250 ppm were made for the experiments and put in glass beakers that had been sterilised and filled with 100 millilitres of distilled water each. Twenty-five individuals of each *Cx. vishnui* larval instar were put into separate beakers with the graded concentrations of crude extract. Larval mortality was assessed 24h, 48h, and 72h following exposure. Larvae were considered dead when they were pierced in the syphon or cervical region with a sharp needle and did not respond. Every experiment was conducted three times in the same lab environment, with a temperature of 25–30 °C and a relative humidity of 80–85%.

Phytochemical analyses

To verify the presence of tannins, terpenoids, steroids, saponins, alkaloids, flavonoids, anthraquinones, and glycosides in the methanolic extract of *M. champaca* seeds, a phytochemical screening was conducted using the methods used by Harborne [20] and Sofowara [21].

Thin layer chromatography

The bioactive components were separated using thin-layer chromatography on glass plates coated with Silica-gel "G" (Merck, India) that had a thickness of 0.5 mm. The mobile phase for this separation was a 1:1 v/v combination of methanol and chloroform. Following the full run, the plates were removed from the TLC chamber, left to air dry, and examined under a UV lamp to accurately identify the bioactive bands. The Rf value of the found spot was calculated. After that, bands with comparable Rf values were scraped for IR analysis and further studies.

FT-IR analysis

FTIR analysis is a very useful technique for figuring out the chemical makeup, chemical bonds, or functional groups of the bioactive chemicals found in plant extract. Dried powdered methanol extract from *M. champaca* seeds was used for this analysis. 100 mg of KBr pellet and 10 mg of dried material were mixed in a hydraulic press to produce tiny, discoidal tablets. A set of pure KBr discs was used as a blank reference. The analysis was conducted within the 400–4000 cm-1 scan range.

Larvicidal bioassay using bioactive compound

The chosen band scrapings were dissolved in 20 millilitres of pure alcohol (60°C –65°C) and then heated in a water bath for five minutes. The clear solution was moved to a beaker, and the precipitate containing the silica gel was disposed of. The solid lump at the bottom of the beaker was collected and weighed once the alcohol had evaporated. The acquired bioactive component was combined with distilled water to create graded concentrations of 5 ppm, 10 ppm, 15 ppm, and 20 ppm. Twenty-five *Cx. vishnui* larvae in their third instar were released to the active bio component's graded concentration for the experiment. The larval mortality rate was reported at 24, 48, and 72-hour intervals.

Effect on non-target organisms

Chironomous sp. and *Diplonychus* sp. were chosen to be the non-target organism used in this study. According to Suwannee et al. [22] protocol, twenty-five each of *Chironomous*larvae and *Diplonychus* nymphs were exposed to the solvent extract's LC50 concentration. Larval and nymphal death rates were measured 24 hours, 48 hours, and 72 hours after exposure. After the assays were performed three times, each time using a different group of controls, the average mortality was determined.

Statistical analyses

Abbott's methodology [23] was used to correct the measured percentage mortality during the bioassay. Furthermore, statistical analyses of the collected data were performed using MS Excel 2010 to ascertain the regression equations and regression coefficient value. The "Stat Plus 2009 Professional" program was used to perform the three-way ANOVA comparing different concentrations, different exposure times, and different larval instars, as well as the Probit analysis to ascertain the LC50 and LC90 values.

Results

In the 72-hour bioassay, the methanol extract from mature *M. champaca* seeds caused the highest larval mortality in all instars of *Cx. vishnui* at 250 ppm (Table 1). Mortality increased progressively with higher extract concentrations and span of exposure. Log probit analysis revealed an inverse correlation between exposure duration and LC values (95% confidence level). The lowest LC50 (63.00 ppm) and LC90 (114.50 ppm) values were observed in second-instar larvae after 72 hours. For third-instar larvae, these values were 72.20 ppm (LC50) and 120.91 ppm (LC90).

Regression analysis indicated a strong positive relationship ($R^2 \approx 1$) between extract concentration (X) and mortality rate (Y) (Table 2). A three-way ANOVA confirmed significant variations ($p < 0.05$) in mortality rates across concentrations, exposure times, and larval instars (Table 3).

Table 1. Percent mortality of all the four instars of *Culex vishnui* upon exposure to methanol extractive of *M. champaca*

Larval Instars	Concentration (ppm)	Percent Mortality (Mean±SE)		
		24h	48h	72h
First	50	55.33 ± 0.00	62.67 ± 0.57	72.33 ± 0.67
	100	65.00 ± 0.00	76.67 ± 0.57	83.00 ± 0.00
	150	81.33 ± 0.67	90.67 ± 0.33	98.33 ± 0.33
	200	92.33 ± 0.57	**100.00 ± 0.00**	**100.00 ± 0.00**
	250	**100.00 ± 0.00**	**100.00 ± 0.00**	**100.00 ± 0.00**
Second	50	53.00 ± 0.00	64.33 ± 0.33	73.33 ± 0.33
	100	65.00 ± 0.33	73.00 ± 0.33	85.33 ± 0.33
	150	77.67 ± 0.57	85.67 ± 0.00	94.67 ± 0.00
	200	88.67 ± 0.33	95.57 ± 0.57	**100.00 ± 0.00**
	250	96.00 ± 0.00	**100.00 ± 0.00**	**100.00 ± 0.00**
Third	50	50.00 ± 0.57	61.00 ± 0.00	73.00 ± 0.58
	100	59.33 ± 0.67	66.00 ± 0.58	80.33 ± 0.00
	150	68.00 ± 1.20	76.33 ± 0.33	94.67 ± 0.33
	200	77.33 ± 0.33	88.00 ± 0.00	**100.00 ± 0.00**
	250	88.67 ± 0.00	96.00 ± 0.57	**100.00 ± 0.00**
Fourth	50	46.33 ± 0.57	54.00 ± 0.58	68.67 ± 0.33
	100	57.00 ± 0.67	68.33 ± 0.33	75.67 ± 0.57
	150	66.00 ± 0.00	75.00 ± 0.00	89.00 ± 1.20
	200	74.33 ± 0.67	86.67 ± 0.57	95.00 ± 0.67
	250	85.67 ± 0.00	94.00 ± 0.67	**100.00 ± 0.00**

Table 2. Regression and log-probit analyses using the methanol extractive of *Michelia champaca* seeds against *Culex vishnui*

Larval Instars	Period of	LC 50	LC 90	Regression	R^2- value
First	24	83.38	172.52	y = 0.2333x + 43.797	0.99
	48	74.35	130.57	y = 0.196x + 56.605	0.92
	72	**68.35**	**115.72**	**y = 0.1447x + 68.63**	**0.87**
Second	24	78.59	201.19	y = 0.2193x + 43.167	0.99
	48	74.16	151.21	y = 0.1878x + 55.541	0.98
	72	**63.00**	**114.50**	**y = 0.136x + 70.263**	**0.89**
Third	24	84.98	312.89	y = 0.1907x + 40.064	0.99
	48	77.44	207.91	y = 0.184x + 49.866	0.98
	72	**72.20**	**120.91**	**y = 0.1473x + 67.499**	**0.89**
Fourth	24	87.64	364.41	y = 0.192x + 37.063	0.99
	48	70.41	225.61	y = 0.1967x + 46.098	0.98
	72	**67.10**	**146.21**	**y = 0.164x + 61.071**	**0.97**

Table 3. Three-way ANOVA analysis of larval mortality of *Culex vishnui* against different concentrations of methanolic extract of *Michelia champaca* seeds

Source of	Sum of squares	Degree of	Mean of	F value	p-level
Instars (I)	178.3343	3	59.4448	186.9761	0
Hours (H)	515.9796	2	257.9898	811.4746	0
Conc. (C)	1,879.6462	4	469.9116	1,478.0478	0
I × H	38.0172	6	6.3362	19.9297	0
I × C	1.7677	12	0.1473	0.4633	0.93
H × C	77.5908	8	9.6989	30.5065	0
I × H × C	19.0096	24	0.7921	2.4913	0
Within groups	37.8333	119	0.3179	0	0
Total	2,748.1788	178	15.4392	0	0

On conduction of chemical analysis of the methanol extract from *M. champaca* seeds following Harbone and Sofowara's methods, the presence of tannins, terpenoids, flavonoids, alkaloids, and glycosides were identified. (Table 4).

Table 4. Results of Phytochemical analyses of methanol extract of *Michelia Champaca* seeds

Phytochemicals	Methanol extract
Tannin	+
Terpenoids	+
Sterols	–
Saponin	–
Flavonoids	+
Alkaloids	+
Coumarins	–
Glycosides	+
Cardiac glycosides	–

Table 5 had displayed the percent mortality of third-instar mosquito larvae following treatment with different concentrations of the TLC fraction (Rf=0.67). Complete (100%) mortality had been achieved at 20 ppm after 72 hours of exposure.

Table 5. Percent mortality of third instar larvae of *Culex vishnui* treated with graded concentrations of bioactive ingredients isolated from seeds of *M. champaca*

Concentration (ppm)	Percent Mortality (Mean±SE)		
	24h	48h	72h
05	46.37 ± 0.33	55.91 ± 0.33	67.00 ± 0.57
10	59.57 ± 0.33	69.24 ± 0.33	77.00 ± 0.57
15	68.57 ± 0.33	75.24 ± 0.33	90.91 ± 0.33
20	85.00 ± 0.57	92.00 ± 0.00	100.00 ± 0.00

Several functional groups, including azides (2311.27 cm^{-1}), amine (2337.3 $cm^{-1}$and 2360.44 cm ^{1}), aromatics (1621.84 cm^{-1}), carboxylic acid (1218.79 cm^{-1}), and ether (1028.84 cm^{-1}) were detected by Infrared Spectroscopic analysis of the TLC fraction obtained from the methanol extract of *M. champaca* seeds (Figure 1)

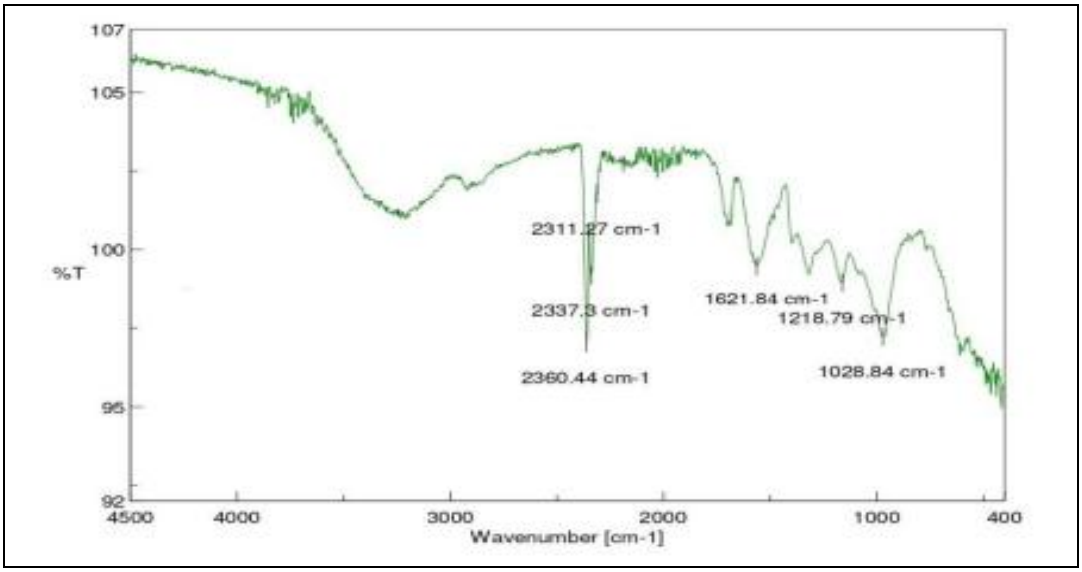

Figure 1. FT-IR plot of the TLC scrapings obtained from methanolic extract of *M. champaca* seeds

No change in the survival rate or swimming behaviour of nontarget organisms, including Chironomid larvae and *Diplonychus* sp. nymphs, was observed after 72 hours of exposure to the methanolic extract of *M. champaca* seeds at the LC50 concentration (for the third instar mosquito larvae at 72 hours) (Table 6).

Table 6. Effect of methanol extract of *Michelia Champaca* seeds on non-target organisms at laboratory conditions

Non-Target Organism (Nymphs)	Percent Mortality (Mean±SE)		
	24h	48h	72h
***Diplonychus* sp.**	0.00 ± 0.00	1.00 ± 0.00	1.33 ± 0.00
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
***Chironomus* sp.**	0.00 ± 0.00	0.33 ± 0.00	1.00 ± 0.00
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Discussion

Natural pesticides derived from plants have gained attention recently as an alternate approach to managing arthropods that are essential to public health because they are biodegradable and environmentally benign. Plant-based remedies have long been used to control pests. Natural and environmentally friendly insecticidal agents are continuously being sought after and studied. The plant products can be extracted from the entire plant or a particular area using a variety of solvents, including water, methanol, chloroform, and hexane, depending on the polarity of the phytochemicals. Therefore, a botanical larvicide should be harmless for people and other non-target animals. Using it instead of synthetic ones would be more cost-effective and have less of an adverse environmental effect [18].

In the present investigation, when first instar larvae were subjected to 200 ppm of methanolic extract of *M. champaca* seeds for 72 hours of exposure resulted in 100% death. With a regression coefficient near 1, methanol extract regression investigations showed a positive relationship between mortality and extract content.

Log-probit analyses [24] show that the LC50 and LC90 values progressively decreased as the concentration and exposure duration increased. Following 72 hours of exposure, LC50 and LC90 values against first-instar larvae were 68ppm and 115ppm respectively. Using the same extract and exposure period, the lowest LC50 and LC90 values were found to be 63 ppm and 114 ppm, respectively for second instar.

Numerous researches claim that phytochemicals have been effectively employed as larvicidal treatments to lower mosquito populations [25-26]. Numerous research has already examined the effect of phytosteroid on various mosquito larvae. *Aedes aegypti* and *Cx. quinquefasciatus* were significantly killed by the *Ocimum sanctum* leaf extract in the past, with LC50 values of 425.94 and 592.60 ppm, respectively [27]. With LC50 values of 167.00 and 99.03 ppm, *Aegle marmelos* (L) leaves shown strong larvicidal activity against *Anopheles subpictus* and *Culex tritaeniorhynchus,* as per Elango et al. [28]. Banerjee et al. examined the efficacy of chloroform:methanol (1:1 v/v) extracts of mature *Limonia acidissima* leaves against the larval form of *Cx. quinquefasciatus*. The bioactive compounds in the mature plant's leaves had LC50 values of 1.73, 5.01, 17.37, and 29.19 ppm for *Cx. quinquefasciatus* larvae in their first, second, third, and fourth in stars, respectively, following 72 hours of exposure [29].

However, the current work concentrated on the selective mortality of *Cx. vishnui* larval instars following treatment of *M. champaca* seeds. For, second instar larvae LC50 and LC90 values after 72 hours of exposure are found to be noticeably lower than those previously published. The bioactive compounds found in the analysis showed 100% larval mortality at a concentration of 20 ppm after 72 hours of exposure. Furthermore, the experiment demonstrated the extract's target specificity by revealing the safety of non-target organisms.

Conclusion

In conclusion, it can be said that *M. champaca* seeds extracts are environmentally beneficial because they have a strong larvicidal ability against *Cx. vishnui* and do not harm other living things. In accordance with the previously reported findings, this phyto-extract can thus function as potent mosquito larvicidal agent.

Acknowledgements

The authors are indebted to Prof. Dr. A. Mukhopadhyay, Department of Botany, the University of Burdwan, for his kind assistance in plant identification.

References

1. Udonsi JK. The status of Human filariasis in relation to clinical signs in endemic areas of the Niger delta. Annals of

Tropical Medicine & Parasitology 1986, 8(4):423–425

2. Vatandoost H, Vaziri M. Larvicidal activity of neem extract (*Azadirachta indica*) against mosquito larvae in Iran, 2000. Pestology 2001, 25(1):69–72
3. Youdeowei A, Service MW. Pest and vector management in the tropics: With particular reference to insects, ticks, mites, and snails. The University of Wisconsin, Madison, 1983, pp. 399
4. Chatterjee SN, Chandra G. Role of *Anopheles subpictus* as a primary vector of malaria in an area in India. Japanese Journal of Tropical Medicine and Hygiene, 2000, 28:177-181 http://dx.doi.org/10.2149/tmh1973.28.177
5. De SK, Chandra G. Studies on the filariasis vector-*Culex quinquefasciatus* at Kanchrapara, West Bengal, India. Indian. Journal of Medical Research 1994, 99:255-258 PMid:8088885
6. Collins FH, Paskewitz SM. 1995. Malaria: current and future prospects for control, Annual Review of Entomology 1995, 40: 195-219 http://dx.doi.org/10.1146/annurev.en.40.010195.001211 PMid:7810986
7. Peng Z, Yang J, Wang H, Simons FER. 1999. Production and characterization of monoclonal antibodies to two new mosquito *Aedes aegypti* salivary proteins. Insect Biochemistry and Molecular Biology 1999, 29: 909-914 http://dx.doi.org/10.1016/S0965-1748(99)00066-1
8. Prabakar K, Jebanesan A. Larvicidal efficacy of some Cucurbitaceous plant leaf extracts against *Culex quinquefasciatus* (Say). Bioresource Technology 2004, 95:113-114 http://dx.doi.org/10.1016/j.biortech.2003.05.001 PMid:15207304
9. Burman S, Bhattacharya K, Mukherjee D, Chandra G. 2018. Antibacterial efficacy of leaf extracts of *Combretum album* Pers. against some pathogenic bacteria. BMC Complementary Medicine and Therapies 2018, 18:1-18. Doi: 10.1186/s12906-018-2271-0
10. Hossain E, Chandra G, Nandy AP, Mondal SC, Gupta JK. Antihelminthic effect of a methanol extract of leaves of *Bombax malabaricum* on *Paramphistomum explanatum*. Parasitology Research 2012, 110(3): 1097-1102. doi: 10.1007/s00436-011-2594-y
11. Hati AK. Vector of Japanese Encephalitis in India and their bionomics and control. Bulletin of the Calcutta School of Tropical Medicine 1981, 29:87–88.
12. Zubairi SI, Sarmidi MR, Aziz RA. A study into the effect of concentration process on the yield of rotenone from the extract of local plant species. In: Proceedings of Seminar Sebatian Smulajadi Malaysia Ke-20, Unimas, Sarawak 2004.
13. Campbell FL, Sullivan WW, Smith LN. The relative toxicity of nicotine, anabasine, methyl anabasine and lupine for Culicine mosquito larvae. Journal of Economic Entomology 1993, 26:500. https://doi.org/10.1093/jee/26.2.500
14. Shaalan EAS, Canyon D, Younes MWF, Abdel-Wahab H, Mansour AH. A review of botanical phytochemicals with mosquitocidal potential. Environment International 2005, 31:1149–1166. doi:10.1016/j.envint.2005.03.003
15. Hartzell A, Wilcoxon F. A survey of plant products for insecticidal properties. Contributions from the Boyce Thompson Institute 1941, 12:127–141.
16. Rawani A, Ghosh A, Laskar S, Chandra G. Glucosinolate from leaf of *Solanum nigrum* L. (Solanaceae) as a new mosquito larvicide. Parasitology Research 2014, 113:4423–4430. DOI:10.1007/s00436-014-4120-5
17. Frisch JD, Frisch CD. Aves Brasileiras e Plantas que as Atraem. 3rd ed. 2005, p. 374.
18. Vimala R, Nagarajan S, Alam M, Susan T, Joy S. Anti-inflammatory and antipyretic activity of *Michelia champaca* Linn. (white variety), *Ixora brachiata* Roxb. and *Rhynchosia cana* (Willd.) D.C. flower extract. Indian Journal of Experimental Biology 1997, 35:1310–1314.
19. World Health Organization. Vector resistance to pesticides. Fifteenth Report of the WHO Expert Committee on Vector Biology and Control. WHO Technical Report Series 1992, 818:1–62.
20. Harborne JB. Phytochemical Methods. 2nd ed. Chapman & Hall: London 1984, p. 85–196.
21. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Ltd., Ibadan 1993, p. 150–153.
22. Suwannee P, Amara N, Maleeya K, Usavadee T. Evaluations of larvicidal activity of medicinal plant extracts to *Aedes aegypti* (Diptera: Culicidae) and other effects on a non-target fish. Insect Science 2006, 13:179–188.
23. Abbott WS. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 1925, 18:265–266.
24. Finney DJ. Probit Analysis. Cambridge University Press: Cambridge 1971, p. 333.
25. Chowdhury N, Bhattacharjee I, Laskar S, Chandra G. Efficacy of *Solanum villosum* Mill. (Solanaceae: Solanales) as a biocontrol agent against fourth instar larvae of *Culex quinquefasciatus* Say. Turkish Journal of Zoology 2007, 31(4):365–370.
26. Singha S, Adhikari U, Chandra G. Smoke repellency and mosquito larvicidal potentiality of *Mesua ferrea* L. leaf extract against filarial vector *Culex quinquefasciatus* Say. Asian Pacific Journal of Tropical Biomedicine 2011, 1(Suppl 1):119–123.

27. Anees AM. Larvicidal activity of *Ocimum sanctum* Linn. (Labiatae) against *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say). Parasitology Research 2008, 103(6):1451–1453.
28. Elango G, Rahuman AA, Bagavan A, Kamaraj C, Zahir AA, Venkatesan C. Laboratory study on larvicidal activity of indigenous plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchus*. Parasitology Research 2009, 104(6):1381–1388.
29. Banerjee S, Singha S, Laskar S, Chandra G. Efficacy of *Limonia acidissima* L. (Rutaceae) leaf extract on larval immatures of *Culex quinquefasciatus* Say 1823. Journal of Tropical Medicine 2011, 4:711–716.