IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 9, Issue 1 Ver. I (Jan. 2016), PP 36-39
www.iosrjournals.org

Studies on the control of filarial vector, *Culex quinquefasciatus* by using locally available plant extracts

Subarna Ghosh, Sabir Hossain Molla, Mandira Saha, Sourav Ghosh and Probir K.Bandyopadhyay

Parasitology Laboratory, Department of Zoology, University of Kalyani, Kalyani-741235

Abstract: *The effect of whole plant extracts of Lantana camara, Ocimum sanctum and Adhatoda vasica plants in acetone against the larvae of Culex quinquefasciatus mosquito was studied. 3rd - 4thinstar larvae of Culex quinquefasciatus mosquitoes were exposed to 50, 75, 100, 125, 150, 175 and 200mg/l acetone extract of whole plant for a period of 24 hours. However, among the three plants Adhatoda vasica showed the highest larvicidal activity. Based on Probit analysis, the LC50 values of acetone extract of Lantana camara, Ocimum sanctum and Adhatoda vasica for the 3rd - 4thlarvae was found to be 131.27 ppm, 153.81 ppm and 61.74 ppm respectively . The results suggest that the whole plant extracts of Lantana camara, Ocimum sanctum and Adhatoda vasica could be used as an effective herbal larvicidal agent against Culex quinquefasciatus without causing damage to the environment.*

Keywords: *Culex quinquefasciatus, Lantana camara, Ocimum sanctum, Adhatoda vasica, LC50, larvae*

I. Introduction

Mosquitoes can transmit more diseases than any other group of arthropods and affect millions of people throughout the world. Mosquito borne diseases are prevalent in more than 100 countries across the world, acting as vector for most of the life threatening diseases like malaria, yellow fever, dengue fever, chikungunya fever, filariasis, encephalitis, in almost all tropical and subtropical countries and many other parts of the world. The major tool in mosquito control operation was the application of synthetic insecticides such as organochlorine and organophosphate compounds. But this has not been very successful because it has harmful effect on human health and other non-target populations. Biological control using natural plant products is a simple and sustainable method of mosquito control [1]. Plant derived insecticides act on both behavioral and physiological processes, thus there is very little chance of developing resistance to such substances. *Culex quinquefasciatus* is a cosmopolitan mosquito species found in tropical, subtropical, and warm temperate regions. *Cx. quinquefasciatus* acts as a vector of filarial worms, protozoan parasites and various arboviruses. In India, *Cx.quinquefasciatus* is the principal vector of bancroftial filariasis [2]. Recently, Japanese encephalitis virus has also been isolated from *Culex quinquefasciatus* from West Bengal [3]. The vector is highly resistant to DDT, malathion and other insecticides [4]. Ghosh et al. [5] has showed the efficacy of three plant extract against *Cx vishnui* the potent vector of JE. The plant extracts may act as alternative sources of mosquito control agents, since they constitute a rich source of bioactive compounds which are biodegradable into nontoxic products and potentially suitable for use in controlling mosquitoes. Plant extracts in general have been recognized as an important natural source of insecticides [6-8]. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities. Toxicity of phytochemicals in mosquitoes was first reported by Campbell et al. (1933) [9]. In a review paper, Ghosh et al. (2012) summarized a list of several plant species of different families that possessed either larvicidal or pupicidal and/or adulticidal activity against mosquitoes based on different extraction solvents [10]. Therefore, crude extracts of *Lantana camara, Ocimum sanctum* and *Adhatoda vasica* leaves extracts were tested against the late third and fourth instar larvae of *Culex quinquefasciatus* in laboratory and under natural condition. The plants were selected on the basis of their potentiality in controlling the vector. *Osimum sanctum* is an aromatic plant under the family Lamiaceae which is native to the Indian subcontinent. The dried leaves are mixed with stored grains to repel insects. This plant has been used as a mosquito repellent in many countries. Some of the main chemical constituents of tulsi are: Ursolic acid, oleanolic acid, rosmarinic acid, eugenol, carvacrol, linalool etc. [11].Along with this plant *Lantana camara* belonging to the family Verbenaceae and *Adhatoda vasica* of Acanthaceae family has been selected. *Lantana camara* is a notorious weed found throughout the world. *Lantana* leaves can display antimicrobial, fungicidal and insecticidal properties [12]. *L.* camara has also been used in traditional herbal medicines for treating variety of ailments. *Adhatoda vasica* has also been used which is plant native to Asia, widely used in Ayurvedic and Unani systems of medicine. The leaves of *Adhatoda vasica* contain phytochemicals such as alkaloids, tannins, saponins, phenolics and flavonoids [13]. The most important is quinazoline, vasicine, and alkaloid.

Till date, no information is available on the larvicidal activities of the experimental plant species against *C. quinquefasciatus.* Therefore, the study focuses on control the larval stages of *C. quinquefasciatus* using acetone as a medium for extraction.

II. Materials and methods

Collection of plants

All the plant samples namely *Ocimum sanctum, Lantana camara,* and *Adhatoda vasica* were collected from the locality of University of Kalyani campus. The samples were authenticated by the taxonomist of the Department of Botany, University of Kalyani, Kalyani. The leaves of the plants were then dried for about 15-20 days, and powdered mechanically by using a commercial stainless steel blender. Powders of dried plant were then sieved using a fine sieved cloth and prepared for extraction through Soxhlet apparatus.

Preparation of plant extract

For each of the whole plant extract 30 gm of powder was used in 1:10 ratio with acetone as extracting solvent in the Soxhlet apparatus. After a run of 6 hours at 40-50°C the extract was filtered using Buchner funnel with Whatman no.1 filter paper. The filtrate was then processed further to concentrate the extract using Rota evaporatorat 50-55°C, which was followed by drying in air or incubator. Finally, 1% stock solution was prepared by dissolving 1 gm of crude extract in 100ml of acetone and preserved in 4° C for further usein bioassay.

Rearing and maintenance of life cycle

The present study was conducted at the Parasitology Laboratory, University of Kalyani, West Bengal, India during November 2014-April 2015. The culture of mosquito was done by following the method of Spitzen and Takken(2005) [14] with some modifications. Larvae of *Culex quinquefasciatus* were collected from open sewage dumping ponds from several regions of Kalyani sub-division, West Bengal. The larvae were maintained at different relative humidity, 27±20^{o}C temperature and a photoperiod of 14:10 h (light/dark) in the insectaries. Larval cultures were prepared by placing hundred larvae in each bowl containing distilled water and small 'pinch' of finely ground larval food, (consisting of equal parts, finely ground biscuit, yeast extract, in the ratio of 3:1) until the completion of pupation. The larval stages completed in 9-35 days (mean twenty one days) at a water temperature of 23^{0}C. Pupa stage lasts for 1-2 days at a water temperature of 23^{0}C. The adults of *Culex quinquefasciatus* were reared in the net cages of 30 x 30 x 30 cm dimension and adult colony was provided with 10 per cent sucrose solution with periodically blood-fed on restrained rats. After three days, the eggs were collected and transferred to the trays. Two developmental stages, larvae and pupae were collected and used for determining the toxicity of the plant extract on them.

Bioassay

A preliminary screening of different doses was performed on 3rd-4th instars larvae and pupae to find the range of doses. 100 ml of tap water was taken in a series of 250 ml beakers and then 50, 75, 100, 125, 150, 175 and 200mg/l acetone extract of plant leaves was used against 3rd - 4th instar larvae. A control was also maintained separately by adding 1 ml of acetone to 100 ml of water. 10 larvae per concentration were used for all the experiments. The number of dead larvae at the end of 24 hours was recorded. Dead larvae were removed as soon as possible in order to prevent decomposition. This experiment was repeated 3 times for each dose. No food was provided during the treatment. Finally, corrected mortality percentage was calculated by Schneider-Orelli's formula. Based on the percent mortality of *Cx. Quinquefasciatus,* LC50 values of plant extracts were obtained by calculating the regression line employing Probit analysis.

III. Results and discussion

Result of Plant Bio-assay Experiment

Plants assayed in this study are commonly used as medicinal plants in different areas of India and other parts of the world. The mortality rate of 3rd-4th instar larvae of *Culex quinquefasciatus* have been observed for 24 hours upon application of acetone extracts of plants namely *Ocimum sanctum, Lantana camara,* and *Adhatoda vasica*. The LC50 values of *Ocimum sanctum, Lantana camara, and Adhatoda vasica* against those larvae of *Cx. quinquefasciatus* is 153.81, 131.27 and 61.74 ppm, respectively (Table 1).

	3^{rd} – 4^{th} Instar Larvae				
Plant extract	Conc. (mg/L)	% mortality	% corrected mortality	LC50 (mg/L)	Regression equation (y = a+bx)
Ocimum sanctum	Control	4.44			
	100	6.66	2.32		
	125	15	11.05		
	150	36.67	33.73	153.81	a = -14.19
	175	83.33	82.56		b = 1.07
	200	100	100		
Lantana camara	Control	4.44			
	50	10	5.82		
	75	16.67	12.80		
	100	30	26.75		a = -29.81
	125	46.67	44.19	131.27	b = 0.61
	150	56.67	54.66		
	175	90	89.54		
	200	96.67	96.52		
	225	100	100		
Adhatoda vasica	Control	4.44			
	50	46.66	44.19		
	75	53.33	51.16	61.74	a = 12.1
	100	80	79.07		b = 0.61
	125	93.33	93.02		
	150	100	100		

Table 1: **Mortality of *Cx. quinquefasciatus* 3^{rd} – 4^{th} instars Larvae at 24 hours of exposure**

These results are quite impressive in terms of mortality rate, rendering higher susceptibility to plant extracts to the larvae (Figure 1).The results emanated from the present study are comparable with few earlier reports. Jayapriya and Shoba [15] observed the effect of *Adhatoda vasica* leaf extract in petroleum ether, chloroform, ethyl acetate and methanol, showed LC50 values 151.05, 156.34, 156.04 and 146.54 ppm, respectively. Whereas, the study on whole plant extract of *A. vasica* in acetone showing LC50 at 61.74 ppm. The acetone, chloroform, ethylacetate, hexane, and methanol leaf and flower extracts of *Ocimum sanctum* were studied by Anees (2008) [16] against larvae of *Cx. quinquefasciatus*. The LC50 values of *O. sanctum* against the larvae of *Cx. quinquefasciatus* were 592.60, 93.92, 212.36, 76.61,and 82.12 ppm, respectively. The whole plant acetone extract of *L. camara* have shownLC50 at 153.81 ppm. Senthilnathan [17] studied the methanol and ethanol flower extractof *Lantana camara* which shows high larvicidal activity against *Cx. quinquefasciatus*, whereas, the study depicts LC50 at 131.27 ppm. This study clearly depicts that the acetone extract of *A. vasica* is the most toxic to *Cx. quinquefasciatus* larvae.

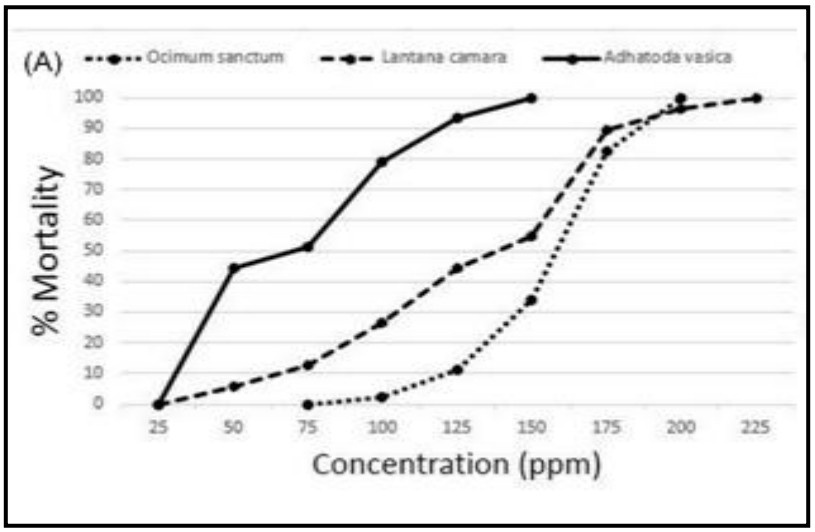

Figure 1:
Graphical representation of mortality in 3^{rd} – 4^{th} instars larvae (A) of *Cx. quinquefasciatus* in response to different concentration of doses at 24 hours of exposure

However, from the present study, the detailed structure of the active principle or the combinations of active principles responsible for larval mortality cannot be ascertained, and further study is necessary in this respect.

IV. Conclusion

All the tested plants possessed different range of larvicidal property which may be used as a traditional mosquito control agent. On the basis of the present investigation we can conclude that the acetone extract of *A. vasica* contains the most potent larvicidal bioactive principles.

References

[1]. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control a review; J Am Mosq Control Assoc 1991; 7:210-237.

[2]. Das M.Vectors of filariasis with special reference to India; The Journal of Communicable Diseases, 1976,8:101–109,.

[3]. Thenmozhi V., Mariappan T., Krishnamoorthy R., Baskarn G., Krishnamoorthi R., Balaji T., Tyagi BK.A first note on Japanese encephalitis virus isolation from *Culex quinquefasciatus* Say in Northern West Bengal; IJMR 2014; 1 (1): 1-4

[4]. Kumar K., Sharma A.K., Kumar S., Patel S., Sarkar M., Chauhan LS. Multiple insecticide resistance/susceptibility status of *Culex quinquefasciatus*, principal vector of bancroftian filariasis from filaria endemic areas of northern India; Asian Pacific Journal of Tropical Medicine (2011); 426-429.

[5]. Ghosh S. , Bandopadhyay PK. Identification of Culex vishnui (Diptheria: Culicidae) the Potent Vector of Japanese Encephalitis by SEM with Special Reference to its Gene Control; Modern Biotechniques and Biotechnology, 2014, 71-80.

[6]. Bagavan A., Kamraj C., Rahuman A.A, Elango G., Zahir A.A and Pandiyan G. Evaluation of Larvicidal and Nymphicidal Potential of Plant Extracts against *Anopheles subpictus* Grassi, *Culex tritaeniorhynchus* Giles and *Aphis gossypii* Glover. *Parasitol Res*. 2009, 104(5): 1109-1117.

[7]. Rahuman AA, Gopalakrishnan G., Venkatesan P, Geetha K. Larvicidal Activity of some Euphorbiaceae Plant Extracts against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera:Culicidae). *Parasitol Res*. 2008a, 1025: 867-873.

[8]. Kamraj C., Bagavan A, Rahuman AA, Zahir AA, Elango G and Pandiyan G. Larvicidal Potential of Medicinal Plant Extracts against *Anopheles subpictus* Grassi and *Culex tritaeniorhynchus* Giles (Diptera: Culicidae). *Parasitol Res*. 2009, 1045: 1163-1171.

[9]. Campbell FL, Sullivan WW, Smith LN. The relative toxicity of nicotine, anabasine, methyl anabasine and lupinine for Culicinae mosquitoes; *J Econ Entomol*. 1933 26: 500– 509.

[10]. Ghosh A., Chowdhury N., Chandra G. Plant extracts as potential mosquito larvicides; Indian *J Med Res* 2012; 35:581-598.

[11]. Padalia, Rajendra C.; Verma, Ram S. Comparative volatile oil composition of four *Ocimum* species from northern India; *Natural Product Research*, 2011, 25 (6): 569–575.

[12]. Chavan SR, Nikam ST. Investigation of Lantana camara Linn (Verbenaceae) leaves for larvicidal, activity; Bull Haffkin Inst. 1982, 10: 21-22.

[13]. Singh D. The *Culex pipiens fatigans* problem in South-East Asia with special reference to urbanization; Bulletin of the World Health Organization, 1967, 37: (2) 239–243.

[14]. Spitzen J, Takken W. Malaria mosquito rearing–Maintaining quality and quantity of laboratory-reared insects. *Proc. Neth. Entomol. Soc. Meet*. 2005; 16:96–100.

[15]. Jayapriya G. and Shoba G. Larvicidal, ovicidal, adulticidal and repellent activity of *Justicia Adhatoda* Linn (Acanthaceae) against Aedes *aegypti* Linn and *Culex quinquefasciatus* say; JEZS 2015; 3 (1): 154-159.

[16]. Anees AM. Larvicidal activity of *Ocimum sanctum* Linn. (Labiatae) against *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say). Parasitol Res. 2008 Nov; 103(6):1451-3. Doi: 10.1007/s00436-008-0991-7. Epub 2008 Aug 14.

[17]. Senthilnathan S. The use of *Eucalyptus tereticornis* Sm. (Myrtaceae) oil (Leaf extract) as a natural larvicidal agent against the malaria vector *Anopheles stephensi Liston* (Diptera Culicidae); Biosource Tech 2007; 98:1856-1860.