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Larvicidal Activity of Anopheles Subpictus and Culex Quinquefasiatus Mosquitoes from Crude Plant Extract of Phyllanthus Niruri

Sandhya .C, Dr. Mary Josephine Rani.A

Deptof Zoology, Auxilium college, Vellore, Tamil Nadu, India.

Mob no: 9442260137

Email: sandhyaack@gmail.com,

Postal add: Auxiliumcollege, Gandhi nagar, Vellore, TamilNadu, India.

ABSTRACT

The Chloroform , Ethyl alcohol , Petroleum ether and leaf extract of Phyllanthus niruri were tested against fourth instar larvae. It is reared in the laboratory and used for larvicidal bioassay against malarial vector, Anopheles subpictus (LC_{50}=153.88) (LC_{90}=649.74) and filarial vector , Culex quinquefasiatus (LC_{50}=182.04) (LC_{90}=739.09).And higher mortality of larvae is measured at 1000 ppm concentration., the larval mortality were observed after 24 hr of exposure. These result suggested that higest mortality of Anopheles subpictus and culex quinquefasiatus was found in Phyllanthus ethyl alcohol extract. This study investigates the larvicidal potential of indigenous plant extracts from commonly used medicinal plants as an environmentally safe measure to control mosquito larvae.

Keywords: Larvicidal, *Phyllanthus niruri, Anopheles subpictus and Culex quinquefasiatus.*

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INTRODUCTION

The world's prime choice to control mosquitoes or their transmission of parasitic or arboviral disease continues to be the selective application of residual synthetic insecticides. The public health benefit delivered by these, both in tropical resource-poor settings, as well as in temperate zones, cannot be over-emphasized – they save thousands of lives each year. (Hemingway and Ranson 2000; Brooke *et al.* 2002; Chandre *et al.* 1999).

Mosquitoes are arthropods (in the PhylumArthropoda). The virus diseases they can transmit are often referred to as arboviruses (arthropod-borne viruses). Mosquitoes belong to the Class Insecta, theOrder Diptera (flies) and, within the order, all mosquitoes belong to the Family group called Culicidae.Within the family there are Sub families and then Genera. Mosquito genera important to human disease transmission include *Aedes, Culex and Anopheles*.Water is an essential

requirement for the larval stages. (Lounibos *et al*. 1985).

Anopheles subpictus is known to transmit malaria (Hoedojo *et al.* 1980); Amerasinghe 1999). *Anopheles subpictus* breeds profusely in rainwater accumulations and fallow rice fields, (Dhanda and Kaul 1980), waste water disposal systems, and irrigated sites (Mukhtar *et al*. 2003), and is also associated with floating and submerged aquatic vegetation in the vicinity of rice plants (Kant *et al*. 1996). (Tyagi and Yadav 2001). Malaria is a highly prevalent mortality causing tropical disease which affects over 103 endemic countries with a combined population of over 2.5 billion people and causes one to three million deaths per year (Jain *et al*. 2000).

Malaria afflicts 36% of the world population that is 2020 million in 107 countries and territories

situated in the tropical and subtropical regions. In the South East Asian Region of WHO, out of about 1.4 billion people living in 11 countries, 1.2 billion (85.7%) are exposed to the risk of malaria and most of whom live in India . Of the 2.5 million reported cases in the South East Asia, India alone contributes about 70% of the total cases. Currently, 80.5% of the 109 billion population of India lives in malaria risk areas. The Global Malaria Eradication Programme of WHO launched in the 1950s was a huge success in India (Sharma *et al.* 1996). One of the ways of controlling malaria in the tropics is by attacking the vector of the disease; mosquito. (Jbilou *et al.* 2006).

Culex quinquefasciatus, a domestic mosquito mainly found in urban areas is a vector of human filariasis in India.*Culex quinquefasciatus* acts as a vector of *Wucherreria bancrafti, Brugia malayi* and *Brugia timori,* which are responsible for lymphatic filariasis, a prevalent disease in India. Filariasis is an endemic disease in many parts of India especially in Kerala, Mysore, Tamil Nadu, Andhra Pradesh and Maharashtra states. Lymphatic filariasis infects 80 million people annually, of which 30 million cases exist in chronic infection. In India, 45 million cases of lymphatic filariasis have been recorded (Bowers *et al*. 1995).The infected people carry Overall, it is estimated that US $842 million are lost to patient and households every year in India from treatment costs and reduced working time (Ramaiah *et al.* 2000). Control of the mosquito larvae is frequently dependent on continued

applications of organophosphates and insect growth regulators (Yang *et al.* 2002). An obvious method for the control of mosquito-borne diseases is the use of insecticides, and many synthetic agents have been developed and employed in the field with considerable success. It has also provoked undesirable effects, including toxicity to non-target organisms, and fostered environmental and human health concerns (Lee *et al*. 2001).

These problems have highlighted the need for the development of new strategies for selective mosquito larval control. Extracts or essential oils from plants may be alternative sources of mosquito larval control agents, since they constitute a rich source of bioactive compounds that are biodegradable into nontoxic products and potentially suitable for use in control of mosquito larvae. In fact, many researchers have reported on the effectiveness of plant extracts or essential oils against mosquito larvae (Rasheed *et al*. 2005; Amer and Mehlhorn 2006a, b; Rahuman *et al.* 2008a, b, c, d). The man has dependent on nature, particularly on the plants for its substances and survival since his existence on earth. In ancient times, he knew how to relieve his sufferings by using the plants growing around him. The civilizations records show that a number of drugs used today were already in use during ancient times (Brahman and Saxena, 1989).

Wild edible plants play an important role in the food and nutritional security of large section of Indian population living in remote area. These plants are a good source of minerals and vitamins essential to take the edge off malnutrition of the tribal people living in harsh environments. The Himalayan Region of India is well known for biodiversity of wild edible plants especially consumed by local people in the form of food, medicine, fuel, fodder, timber, fiber and for other purposes (Samant *et al.* 1998a); (Samant and Pant, 2006).

Phyllanthus niruri (Euphorbiaceae) is a small herb distributed throughout the tropical and subtropical regions of both hemispheres. This plant is popular in folk medicine, whole plant, fresh leaves and fruits are used in the treatment of various diseases, particularly hepatitis and other viral infection (Chopra *et al.* 1986); (Wang 2000).Hepatitis B is one of the major diseases inflicting human population. Conventional treatment with interferon – alpha is very expensive and has many serious side effects. Alternative herbal medicine using extracts of *Phyllanthus*

niruri have been reported to be effective against Hepatitis B and other viral infections (Meixa *et al.* 1995).

The plant is of medicinal importance for numerous ailments like dysentery, influenza, vaginitis, tumors, diabetes, diuretics, jaundice, kidney stone, dyspepsia, antihepatotoxic, antihepatitis-B, antihyperglycemic and also as antiviral and antibacterial (Chopra *et al.* 1986). *Phyllanthus niruri* extract has been shown to inhibit DNA polymerase of hepatitis B virus and related hepatitis viruses (Blumberg *et al.* 1990) and the extract of this plant contains several bioactive molecules such as lignans, phyllanthin, hypophyllanthin, flavonoids, glycosides and tannins (Rajeshkumar *et al.* 2002). *Phyllanthus niruri* was used for treating liver ailment (Kapur *et al*.1994) and possess hypolipidemic (Khanna *et al.* 2002), antiviral (Jayaram *et al.* 1997), anticarcinogenic (Rajeshkumar *et a*l.2002), antioxidant (Harish and Shivanandappa, 2006), antinociceptive and antispasmodic activities as well as its role in the inhibition of calcium oxalate formation in kidney (Santos *et al*.1995, Qian-Cutrone *et al*. 1996, Freitas *et al*. 2002).

This study of medicinal plants is used for the effectiveness to control mosquito larva.

MATERIALS AND METHODS

Collection of plant material:

The Leaves of *phyllanthus niruri linn.* (Euphorbiaceae) were collected from Jawadhu Hills, Tiruvannamalai region (altitude 705 m), Tamil Nadu, South India. In the present study, the experimental plants were selected based on the ethnobotanical information collected through different literature sources. The taxonomic identification of plants was made by Dr.C.Hema, Department of Botany, Arignar Anna Government Arts College for Women , Walajapet, India. The voucher specimen was numbered and kept in our research laboratory for further reference.

Preparation of plant extracts:

The leaves were dried for 7-10 days in the shade at the environmental temperatures (27-37^{o} C day time). The leaves (500 g) were powdered mechanically using commercial electrical stainless steel blender and extracted with chloroform (1,000 ml, Fine Chemicals, Qualigens), ethyl acetate (1,500 ml, Qualigens, Fine Chemicals, Mumbai, India), and petroleum ether (1,800 ml, Qualigens), in a soxhlet apparatus (boiling

point range 60–80°C) for 6hr. The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper. The extract was concentrated under reduced pressure 22 - 26 mm Hg at 45^{o}C and the residue obtained was stored at 4^{o}C.The residues were then made in to a 1 per cent stock solution with acetone (stock solution). From the stock solution, 1000-31ppm, dilutions were prepared with dechlorinated tap water. Polysorbate 80 (Qualigens) was used as an emulsifier at the concentration of 0.05per cent in the final test solution(Mehra and Hiradhar 2000).

Mosquito culture:

Anopheles subpictus and Culex quinquefasciatus were collected from rice field and stagnant water areas of Melvisharam and identified in Zonal Entomological Research Centre, Vellore, and TamilNadu. To start the colony and larvae were kept in plastic and enamel trays containing tap water. They were maintained and all the experiments were carried out at27 ± 2°C and 75–85 percent relative humidity under14:10 h light and dark cycles. Larvae were fed a diet of Brewer's yeast, dog biscuits and algae collected from ponds in a ratio of 3:1:1, respectively as per the method of (Kamaraj *et al*. 2009).

Larvicidal bioassay:

Early fourth-instar larvae were used for bioassay test. A total of 100 larvae were exposed in five replicates of 20 larvae each. Experiments were conducted for 24 h at room temperature (28±2°C). The control was setup with solvent and polysorbate 80. The experimental media, in which 100% mortality of larvae occurs alone, were selected. The different fractions isolated were tested against the early fourth-instar larvae of mosquitoes by the procedure of WHO (1996) with some modification and as per the method of (Rahuman *et al.* 2000). For Bioassay test, larvae were taken in five batches of 20 in 249 ml of water and 1.0 ml of plant extract concentration. The numbers of dead larvae were counted after 24 hr of exposure, and the percentage mortality was reported from the average of five replicates.

RESULTS AND DISCUSSION

The activity of crude plant extracts is often attributed to the complex mixture of active compounds .Hence larvicidal activity of different solvent that is chloroform, ethyl alcohol and petroleum ether extracts of *phyllanthus niruri* were

tested against *Anopheles subpictus* and *culex quinquefasciatus*.

Larvicidal activity of different concentrations leaves ethyl alcohol extracts against

Anopheles subpictus* and *culex quinquefasciatus.

The preliminary screening is a better mean of larvicidal activity of different solvents crude leaf extracts of *phllanthus niruri* are noted and presented(Table 1).Among the crude extracts tested larvicidal activity showed that the leaf *phllanthus niruri* with ethyl alcohol extract shows 100% mortality (Table 1) against *Anopheles subpictus*(Table 2) Lc_{50}=153.88 and Lc_{90} =649.74 and *culex quinquefasciatus*(Table 2) Lc_{50}=182.04 and LD_{90} =739.09 at 1000ppm (Highest concentration) .31.25(lowest concentration) *phyllanthus niruri* extract against *Anopheles subpictus* (Table 1) 11%Mortality and *culex quinquefasciatus* 8% Mortality.

Table1: Larvicidal activity of different concentrations leaves ethyl alcohol extracts against *Anopheles subpictus and Culex quinquefasciatus.*

Plant name	Concentrations(ppm)	Percent mortality ±SD*	Percent mortality ±SD*
Phyllanthus niruri Linn. (Euphorbiaceae)	1000	100±0.00	100±0.00
	500	82±1.42	78±1.41
	250	63±1.82	56±1.82
	125	40±1.90	35±1.92
	62.5	21±1.00	17±1.01
	31.25	11±1.00	08±1.00

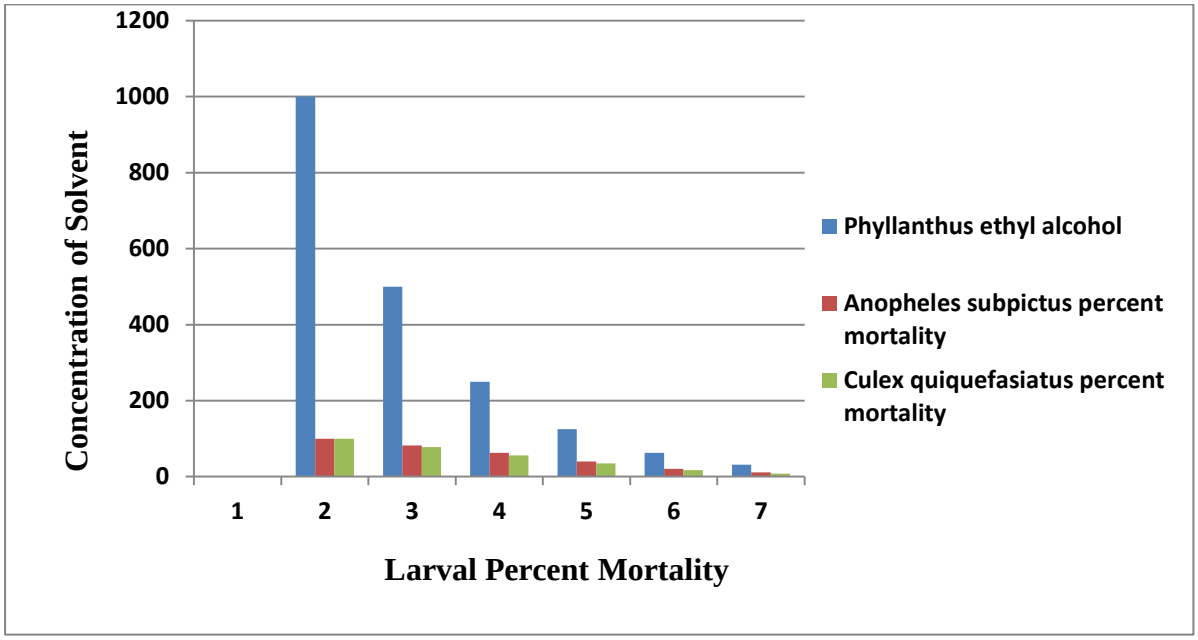
Control—nil mortality.* Mean value of five replicates ± SD standard deviation.

Table 2:LC_{50}, LC_{90}, and other statistical analysis of different test samples larvicidal activity of different concentrations leaves of chloroform, ethyl alcohol and petroleum ether extracts against *Anopheles subpictus and Culex quinquefasciatus* .

S.No.	Plant name	Extract	Parasite	Lc_{50}± SE	LC_{90}±SE	χ2 (df =4)
1.	*phyllanthus niruri*	Ethyl alcohol	*Culex quinquefasciatus*	182.04±12.23	739.09±85.30	9.50
2.	*phyllanthus niruri*	Ethyl alcohol	*Anopheles subpictus*	153.88±10.53	649.74±74.69	7.65

LC_{50}- Lethal concentration that kills 50% of the exposed larvae, LC_{90} - Lethal concentration that kills 90% of the exposed larvae, UCL= Upper confidence Limit, LC = Lower confidence Limit, χ2 -Chi-square, df-Degree of freedom, Significant at P< 0.05 level.

Fig1 : Larvicidal Activity of *Anopheles subpictus* and *Culex quinquefasciatus* in *Phyllanthus* Ethyl alcohol extract. (1000 ppm – 100% mortality).



CONCLUSION

The present experimental result showed that the fractionated Ethyl alcohol extract possess effective larvicidal properties against *Culex* larvae and *Anopheles* larvae. Whereas Chloroform and petroleum ether is non-effective against Culex species of mosquito larvae and *Anopheles* species of mosquito larvae. Hence *Phyllanthus niruri* can be used as a botanical insecticide for treating the mosquito larvae as it is a

commonly available plant and easily affordable.

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