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Larvicidal activity of invasive weed *Prosopis juliflora* against mosquito species *Anopheles subpictus*, *Culex quinquefasciatus* and *Aedes aegypti*

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Abstract

Mosquitoes are well known as vectors of several disease causing pathogens. The extensive use of synthetic insecticides in the mosquito control strategies resulted to the development of pesticide resistance and fostered environmental deterioration. Hence in recent years plants become alternative source of mosquito control agents. The present study assessed the larvicidal activity of *Prosopis juliflora*, a dominant invasive weed in wastelands of Madhya Pradesh region, India. Leaf extracts in methanol was used in the range of 10-200 ppm in the laboratory bioassays against third instar larvae of *Anopheles subpictus*, *Culex quinquefasciatus* and *Aedes aegypti*. The mortality data were recorded after 24 hrs and subjected to probit analysis to determine the lethal concentrations (LC₅₀ and LC₉₀). Amongst all mosquito species *An. subpictus* larvae showed highest mortality with LC₅₀ 39.19 ppm and LC₉₀ 175.24 ppm followed by *Cx. quinquefasciatus* with the LC₅₀ and LC₉₀ values 59.37 and 243.20 ppm respectively. In case of *Ae. aegypti*, less mortality was observed in larvae as compared to the other mosquitoes with LC₅₀ 126.79 and LC₉₀ 457.32 ppm. The results indicate that *P. juliflora* leaf methanol extract have the potential to be used as an ecofriendly approach for the control of *An. subpictus* and *Culex quinquefasciatus*. Further studies on the screening, isolation and purification of bioactive phytochemical constituents/compounds followed by in-depth laboratory and field bioassays are needed.

Keywords: Larvicide, Weed, *Prosopis juliflora*, Leaf extract, Vector Management.

1. Introduction

Mosquito transmits a number of diseases and remains a major source of morbidity and mortality worldwide. Malaria is an important cause of death and illness in children and adults, especially in tropical countries, transmitted from one human to another by the bite of infected *Anopheles* mosquito. According to WHO 2013 [1], there are 97 countries and territories with ongoing malaria transmission and 7 countries in the prevention of reintroduction phase, making a total of 104 countries and territories where malaria is presently considered endemic. Accordingly is estimated that 3.4 billion people are at risk of malaria globally. India contributes approximately two third of all confirmed malaria cases in the South-East Asia Region, with five states Orissa, Chhattisgarh, Madhya Pradesh, Jharkhand and West Bengal. *Anopheles subpictus* is considered as a secondary vector of malaria with wider distribution, a prolific breeder in most part of India. Sibling species A of *An. subpictus* has been incriminated and established as a primary vector of malaria in some parts of India. Japanese encephalitis virus in India has also been isolated from 16 mosquito species including *An. Subpictus* [2]. *Ae. aegypti* is vector of Dengue and Chikungunya. Approximately 3.5 billion people live in dengue endemic countries which are located in the tropical and subtropical regions of the world [3]. Lymphatic filariasis, commonly known as elephantiasis is so far a neglected tropical disease. The infection occurs when filarial parasites are transmitted to humans through *Culex quinquefasciatus*. More than 1.3 billion people in eighty-one countries worldwide are threatened by lymphatic filariasis [4]. One of the methods to manage these diseases is to control the vectors for bringing about interruption in disease transmission. The control of mosquitoes at larval stage is considered as an efficient way in the integrated vector management [5].

Therefore the ideal method for controlling mosquito infestation is the prevention of mosquito breeding by using appropriate larvicides. Owing to their quick action, synthetic insecticides are the first line of action, but their continuous use may lead to the development of resistance and permanent residual effect on the bioenvironment which can be detrimental to animals including human [6, 7]. These factors have created a need for search of easily biodegradable alternative insecticides.

Plants contain many chemicals which are important in their defense against insects. Insecticides of plant origin have been extensively used on agricultural pests and to a very limited extent against vectors of public health importance. The use of plant extracts for vector control has several appealing features as they are easily degradable, less hazardous and rich stock house of chemicals of diverse biological activity. These are considered environmentally safe and economical as well as practical in application. Therefore biologically active plant materials have attracted considerable interest in mosquito control programs in the recent time [8].

Weeds become dominant due to their wide adaptability to adverse environmental conditions, resistant to microbes (not a host to plant pathogen) and insect predators. Using weeds as botanical larvicide against mosquitoes have several advantages, as these are easily available, required little technical input and time for cultivation and procurement.

Prosopis juliflora is a drought resistant, wide spread exotic weed in semi arid areas and of M.P. region (India), which has been documented for their medicinal and other pharmacological properties. This deciduous thorny shrub belongs to Fabaceae family commonly known as 'Vilayati Babul' in Hindi. Leaves of this plant have strong antifungal properties [9]. Insecticidal property of this plant has been reported [10]. Hence, the present study was aimed to determine the larvicidal effect of methanolic extracts of *P. juliflora* leaves against third instar larvae of malaria vector, *Anopheles subpictus*; Japanese encephalitis vector, *Culex quinquefasciatus* and Dengue vector *Aedes aegypti*.

2. Materials and Methods

Plant material

P. juliflora plant leaves were collected from wasteland areas of Gwalior District, Madhya Pradesh. Leaves were air dried in a shady place for 10 days to retain their active ingredients intact. Dried materials were powdered by using an electric blender. Powdered plant material (100 g) was soaked in methanol (500 ml) in airtight wide mouth bottle and kept for 7 days with periodic shaking. After that, the cold extracts from the bottle along with methanol were filtered and kept in Petri dishes for drying at room temperature [11]. Dried extracts were used for larvicidal bioassay.

Mosquito

Larvae of *Anopheles subpictus* were collected from the field location of Gwalior District and were reared in the laboratory. After 10 generations, this strain was considered a laboratory strain. *Culex quinquefasciatus* and *Aedes aegypti* were maintained separately in our laboratory at 27±2 °C room temperature and 70±10 % relative humidity. Larval stages were kept in bowls (2.5L), and yeast powder was provided to them as larval food. Adult mosquitoes were reared in wooden cages (30x30x30 inches) and were provided cotton soaked with 10% sugar solution. *An.*

subpictus and *Ae. aegypti* females were offered rabbit blood, whereas *Cx. quinquefasciatus* females offered fowl blood once in a week.

Bioassay

Test solutions were prepared with methanolic extracts at different concentrations ranging from 10, 20, 50, 100 and 200 ppm by diluting the stock solution in distilled water. Third instar larvae of all the three mosquito species were used for larvicidal bioassay with four replication of each concentration [12]. The evaluation of mortality rate was performed 24 hours after the beginning of the experiment, verifying the number of dead larvae. The environmental temperature and humidity were observed during the experiment. The control mortality was corrected by Abbott's formula (1925) [13]. LC₅₀ and LC₉₀ values at 95% confidence limit were calculated by using probit analysis [14].

3. Results

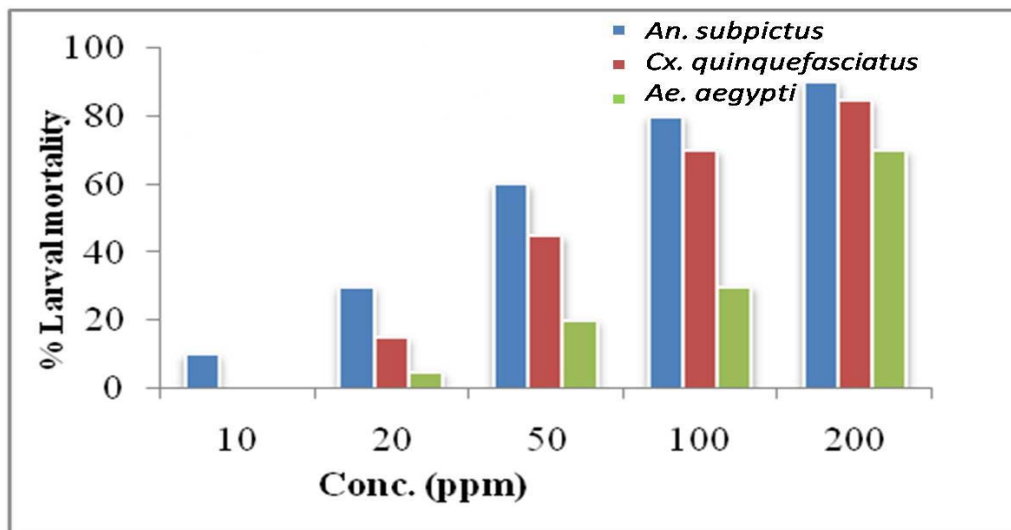
The results of the larval susceptibility of the three mosquito species using methanolic leaf extracts of *P. juliflora* are presented in Table 1. *Anopheles* larvae showed higher susceptibility with the LC₅₀ value 39.19 and LC₉₀ 175.24 ppm, followed by *Cx. quinquefasciatus* larvae which exhibited LC₅₀ as 59.37 and LC₉₀ 243.20 ppm. *Ae. aegypti* larvae reported moderate larval mortality with LC₅₀ as 126.79 and LC₉₀ 457.32 ppm. The comparative study of larval mortality in all three mosquito species *An. subpictus*, *Cx. quinquefasciatus* and *Ae. aegypti* in all the concentrations of methanolic leaf extract, represented in Fig 2. At the lowest concentration 10 ppm, only *An. subpictus* showed larval mortality. In all the other concentrations 20, 50, 100 and 200 ppm *An. subpictus* exhibited maximum mortality trend as compared to *Cx. quinquefasciatus* and *Ae. aegypti* larvae. *Ae. aegypti* reported lower larval mortality in all the concentrations.

4. Discussion

The results of the study revealed that the methanolic leaf extract of plant material was effective against larvae of mosquito. In earlier studies Baskararan and Narayanasamy (1995) [15] have been reported the insecticidal activity of *P. juliflora* against some agricultural pests. In the present study Malaria vector *An. subpictus* larvae showed higher susceptibility as compared to *Cx. quinquefasciatus* and *Ae. aegypti*. Furthermore, the effect of larval mortality was depended on the concentration of leaf extract. Sakthivadivel and Daniel (2008) [16] have been reported that petroleum ether extracts of flowers of *P. juliflora* were also quite effective against *Cx. quinquefasciatus* followed by *An. stephensi* and *Ae. aegypti*. Senthil kumar *et al.* (2009) [17] also evaluated the larvicidal efficacy of 10% leaf extracts of *P. juliflora* and showed that these were effective (LC₅₀ 9.3 mg/lit) against larvae of *An. stephensi*. It is notable that the larvicidal activity of *P. juliflora* extract is varying in different studies. Reason of this variation might be due to plant part used, geographical location of the plant, photosensitivity of some of the compounds in the plant extract, the solvent of extraction and the process of extraction and finally the species responses to the specified extracts (Sukumar *et al.*, 1991) [18]. The susceptibility of mosquito strain and the larval stage taken may also affect the study.

Table 1: LC₅₀, LC₉₀, and X² analysis of methanol leaf extract of *P. juliflora* against third instar larvae of *Anopheles subpictus*, *Culex quinquefasciatus* and *Aedes aegypti*.

Mosquito Species	LC ₅₀ (ppm) (95% confidential limit)	LC ₉₀ (ppm) (95% confidential limit)	X ²
<i>Anopheles subpictus</i>	39.19(33.61- 45.48)	175.24(138.95-236.375)	1.233
<i>Culex quinquefasciatus</i>	59.37(52.05-50.68)	243.20(189.24 -344.92)	0.497
<i>Aedes aegypti</i>	126.79(90.61- 183.27)	457.32(285.52- 1153.3)	6.906

**Fig 2:** Graph showing the comparative larvicidal activity of *P. juliflora* leaf extracts against the third instar larvae of *Anopheles subpictus*, *Culex quinquefasciatus* and *Aedes aegypti*

Plant extract might have some complex mixture of biocidal active compounds, including phenolics, terpenoids, flavonoids and alkaloids which may jointly or independently contribute to produce mortality and delayed growth of larvae. This may be the cause of larvicidal activity in the present study. A study reported that acetone extracts phenols and flavonols, while methanol extracts flavones, terpenoids, tannins and polyphenols [19], which contains larvicidal activity. The mode of action and site of effect for larvicidal phytochemicals has received little attention. Ray *et al.* (1999) [20] and David *et al.* (2000) [21] found that botanical derivatives primarily affect the midgut epithelium and secondarily affect the gastric caeca and the malpighian tubules in mosquito larvae.

Our results revealed significant larvicidal property of methanol leaf extract of *P. juliflora* against *An. subpictus* and *Cx. quinquefasciatus*. This study may also contribute to assess the possibility of using large biomass of weeds available in the wastelands of northern India as potential insecticides in spite of using other medicinal or cultivable plants which are facing extinction or severe genetic loss. For improving the potency and stability of the products, in depth investigation on the active compound of this plant is needed to be further elucidated.

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