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## Short Research Communications

# Larvicidal effects of crude extracts of dried ripened fruits of *Piper nigrum* against *Culex quinquefasciatus* larval instars

K. Vasudevan, R. Malarmagal, H. Charulatha, Venkata Lakshmi Saraswatula & K. Prabakaran

*Department of Zoology, Annamalai University, Annamalai Nagar, Tamil Nadu, India*

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The medical importance of mosquitoes as vectors for transmission of serious diseases that cause morbidity, mortality, economic loss, and social disruption such as malaria, lymphatic filariasis, and viral diseases is well-documented[1]. Mosquitoes not only cause nuisance by their bites but also transmit deadly disease like malaria, filariasis, yellow fever, dengue and Japanese encephalitis, contribute significantly to poverty and social debility in tropical countries[2]. The mosquito *Culex quinquefasciatus* act as a vector for *Wuchereria bancrofti* responsible for filariasis in India. However, control of such diseases are becoming increasingly difficult because the overproduction of detoxifying mechanisms of chemical insecticides has been reported for *Culex* species[3].

Humans have used plant parts, products and metabolites in pest control since early historical times. Plants are the chemical factories of nature, producing many chemicals, some of which have medicinal and pesticidal properties. By using plant parts in early historical times and plant extracts and concentrated components in more recent times, man has been able to control certain pests with these remedies quite successfully. The current use and future potential of plants for pest control on farms and homes are detailed in an FAO document[4]. Sukumar *et al*[5] published an extensive review of phytochemicals from plants with activity against mosquitoes. Members of the plant families—Asteraceae, Cladophoraceae, Labiateas, Miliaceae, Oocystaceae and Rutaceae possess various types of activity against many species of mosquitoes[5]. Wood[6] lists some important photochemical products such as pyrethrum, derris, quassia, nicotine, hellebore, anabasine, azadirachtin, d-limonene, camphor and terpenes that have been used as insecticides. These are major groups of insecticides of plant origin that were used in developed countries before the advent of synthetic organic insecticides[7]. Ethanolic extract of *Centella asiatica* leaves is promising as larvicide and adult emergence inhibitor against *Cx. quinquefasciatus* larvae[8].

More than 2000 plant species have been known to produce chemical factors and metabolites of value in pest control programmes[9]. One of the most studied botanical species with good pesticidal attributes, is the neem tree (*Azadirachta indica* A. Juss), whose extracts have shown considerable activity and multiple modes of action against agricultural pests, forestry insects, and insects of public health[10,11]. Larvicide constituents from *Lanta viburnodis* sp have reported by Innocent *et al*[12]. However, studies against the pest of public health importance are totally lacking. In the present study, the extracts of dried fruits of *Piper nigrum* have been evaluated against the larvae of important vector of filariasis and the findings are summarised here.

For evaluating larvicidal activity of *Piper nigrum*, dried ripened fruits were obtained from Tamil Nadu Agricultural Cooperative Society, a voucher specimen deposited at the Department of Botany, Annamalai University. Aqueous (PnAE) and ethanolic extracts (PnEE) of *P. nigrum* were used in the experiments. Each one kg of thoroughly cleaned air-dried in the shade, powdered material of *P. nigrum* was soaked in 3 L of double distilled water (PnAE) and 95% ethanol (PnEE) at room temperature for 48 h was suction filtered through a Buchner funnel and concentrated to dryness with rotary evaporator at 60°C, until the solvents completely evaporated. The aqueous (PnAE) and ethanolic (PnEE) extract was thus obtained, lyophilised and then refrigerated at –20°C until testing for larval instars toxicities.

The test organism, namely *Cx. quinquefasciatus* larvae, which were derived from the Medical Entomology Field Station (ICMR), Vriddhachalam and Vector Control Research Centre (ICMR), Puducherry where colonized and maintained in a laboratory free of exposure to pathogens, insecticides or repellents. The laboratory colony was maintained at 25–30°C and 80–97% relative humidity under a photoperiod of 14:10 h (light/dark) in the insectary, Faculty of Science, Annamalai University, Annamalai Nagar. Under these conditions, the full development from egg to adult lasted about three weeks. Larvae were fed on finely ground dog biscuits. The adult colony was provided with 10% sucrose and 10% multivitamin syrup and it was periodically blood-fed on restrained rabbits. Four developmental stages of larvae were available for the experiments. Under these conditions the life cycle of the mosquito from egg to adult took about 3–4 weeks. Early IV instar larvae were used in the experiments.

The larvicidal bioassay followed the WHO standard protocols[13] with slight modifications. Each pepper extract (PnAE and PnEE) was dissolved in absolute ethanol to prepare graded series of concentration. The stock solution (500 mg/ml) of aqueous (PnAE) and ethanolic extract (PnEE) of *P. nigrum* was volumetrically diluted to 250 ml filtered tap water to obtain the test solutions of 20, 40, 60, 80, 100 and 120 mg/l for PnAE; 5, 10, 20, 40, 60, 80, and 100 mg/l of PnEE in a 500 ml enamel bowl, which was shaken lightly to ensure a homogenous solution. Then 20 early IV instar larvae of the mosquito in each 25 ml of distilled water were transferred to that bowl. Each experiment was performed in six replicates for each concentration.

Symptoms of treated larvae were observed and recorded immediately and at time intervals, and no food was offered to the larvae. Mortality and survival were registered after 24 h of exposure period. The moribund and dead larvae in six replicates were combined and expressed as a percentage of larval mortality of each concentration. Dead larvae were identified when they failed to move after probing with needle in the siphon or cervical region. Moribund larvae were those incapable of riding to the surface. They might also show discolouration unnatural positions, tremors, uncoordination or rigor. The experiments were replicated for six times.

Larvicidal activity of PnAE and PnEE against early IV instars of the mosquito were registered. In cases control mortality, observed mortality was corrected by using Abbott's formula[14]. $LC_{50}$ and $LC_{90}$ confidence intervals were analysed by means of computerized probit analysis (SPSS 12.0), yielding a level of effectiveness at 50 and 90% mortality, and 95% confidence interval were used to measure differences between test samples.

Aqueous (PnAE) and ethanolic (PnEE) extracts of *P. nigrum* with a yield of 2.18% (w/w) and 3.24% (w/w), were semisolid, light yellow brownish and strong aromatic. Larvicidal activity of PnAE against early IV instar larvae of *Cx. quinquefasciatus* is shown in Table 1.

In this study, early IV instar larvae of *Cx. quinquefasciatus*, under laboratory conditions, were subjected to a dose-dependent efficacy of *P. nigrum* **(**PnAE and PnEE) extracts.

**Table 1. Larvicidal activities of extracts of *P. nigrum* against early IV larval instars of *Cx. quinquefasciatus***

| Type of extract | $LC_{50}$ mg/l | 95% confidence limit (mg/l) | $LC_{90}$ mg/l (in water) | 95% confidence limit (mg/l) | Regression equation | $\chi^2$ |
|---|---|---|---|---|---|---|
| Aqueous | 63.82 | 59.81–67.90 | 108.90 | 102.47–116.78 | Y = 47.057+0.823 x | 10.426* |
| Ethanolic | 29.11 | 22.25–36.50 | 62.37 | 52.21–79.16 | Y = 19.213+0.978 x | 22.143† |

*$p < 0.05$; †$p < 0.001$; Each value (mean ± S.D.) represents mean of six values.

Aqueous extracts of *P. nigrum* (PnAE) caused mortality against early IV larval instars of *Cx. quinquefasciatus*. However, the sensitivity of larval instars was positively correlated dose-dependent level with PnAE (Table 1). Following the treatment of increasing dosages of PnAE from 20–120 mg/l. The 24 h $LC_{50}$ and $LC_{90}$ against early IV larval instars were 63.82 and 108.90 mg/l, respectively. The results showed that this extract PnAE can be used to control larval instars of the mosquitoes. Ethanolic extracts of *P. nigrum* (PnEE), observations on the results of the larval susceptibility were given in Table 1. With the IV instars of the mosquito species, mortality was dose-dependent—mortality increased with increase in concentration of PnEE. $LC_{50}$ and $LC_{90}$ values along with their fiducial limits, regression equation and chi-square were calculated. $LC_{50}$ and $LC_{90}$ values as observed for early IV larval instars of *Cx. quinquefasciatus* were 29.11 and 62.37 mg/l, respectively (Table 1). Results of the experiments envisaged larvicidal property in both PnAE and PnEE extracts of *P. nigrum.* As the dried fruits of *P. nigrum* are available most of the time throughout the country, the larvicidal properties of the plant species can be well-utilized, while planning alternate vector control programmes.

Today, the environmental safety is considered to be of paramount importance. An insecticide does not have to cause high mortality on target organisms in order to be acceptable[14]. Phytochemicals may serve as they are relatively safe inexpensive, and are readily available in many parts of the world. According to Bowers *et al*[15], the screening of locally available medicinal plants for mosquito control would generate local employment, reduce dependence on expensive and imported products, and to stimulate local efforts to enhance public health system.

The crude extracts of dried fruits of *P. nigrum* both aqueous (PnAE) and ethanolic (PnEE) have been found to possess larvicidal effect against the IV larval instars of the mosquito *Cx. quinquefasciatus.* The biological activity of the PnAE and PnEE might be due to the various compounds including potentially insecticidal compounds[16,17]. Some of the piper species, *P. longum, P. guanacastensis* and their bioactive constituents are reported to have remarkable larvicidal activity against various mosquito species such as *Cx. pipiens pallens*, *Ae. aegypti*, *Ae. togoi* and *Ae. atropalpus*[18,19]. In this study, early IV instar larvae of *Cx. quinquefasciatus* under laboratory conditions, were subjected to a dose-dependent efficacy of the two piper extracts (Table 1). Following the treatment of increasing the dosages of PnAE and PnEE of *P. nigrum*, the larval mortality increased significantly as complete mortality occurred. In the case of the control or untreated group mortality observed within 24 h was within admissible range and the IV larvae development into pupae and then to adults with 48–72 h.

Among the extracts, PnEE showed more remarkable larvicidal potency than PnAE. Variety of types and levels of active constituents (pipernoneline and piperidine alkaloid)[18], (methly 4-hydroxyl-3-(3’-methyl 2’ butenyl) benzoate)[19] in the piper species may be responsible for the variability in their potential against *Cx. quinquefasciatus* larvae.

Although the larvicidal potential of crude aqueous extracts derived from *P. nigrum* dried fruits observed in the study was significantly lower than that of ethanolic extracts (PnEE) further studies for the isolation and identification of bioactive compounds would be useful in developing new types of mosquito larvicides. However, this study with PnAE and PnEE demonstrated the potential of *P. nigrum* extracts against IV instar larvae of *Cx. quinquefasciatus* and its benefits to developing cost-effective and, environment friendly new type of larvicide for mosquito control needs to be studied.

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*Corresponding author:* Dr K. Vasudevan, Reader in Zoology, Department of Zoology (DDE), Annamalai University, Annamalai Nagar–608 002, India.
E-mail: drkvasudevan@yahoo.com

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