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Larvicidal activity of *Lawsonia inermis* and *Murraya exotica* leaves extract on filarial vector, *Culex quinquefasciatus*

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ABSTRACT
Extensive use of synthetic and chemical insecticides to control mosquitoes result in environment hazards and development of resistance in vector species. This research work is about an alternative mosquito control method that is considered as safe to environment and non-target species and also bio-degradable. Hence an attempt was made to study the larvicidal effect of the extract of *Lawsonia inermis* and *Murraya exotica* leaves on III and IV instar larva and pupa of *Culex quinquefasciatus*. The LC_{50} value of *Murraya exotica* for III and IV instar larvae and pupae is 135.539 ppm, 154.361 ppm and 178.571 ppm respectively. Likewise for *Lawsonia inermis* it is 139.057 for III instar, 163.630 for IV instar and 188.151 for the pupa. Of these, two plants *Murraya exotica* plant extract is more effective than the *Lawsonia inermis.*

Keywords: Larvicides, *Culex quinquefasciatus, Lawsonia inermis*, *Murraya exotica,* Plant extract.

1. Introduction
Mosquitoes are major public health pests throughout the world. Of the 3000 species of mosquitoes recorded worldwide more than hundred species are capable of transmitting various diseases to human [1]. Mosquitoes transmit many medically important pathogens and parasites such as viruses, bacteria, protozoan and nematodes [2]. Mosquito borne diseases are one of the major public health problems today [3]. These diseases are responsible for morbidity, mortality, economic loss and social disruption [4].
Various measures have been taken to control mosquito menace and one such approach is by killing the mosquitoes at it is larval stages. This is mortality on the basis of using of synthetic insecticides. Even though these insecticides are effective in controlling mosquitoes they created many environment problems like insecticide resistance [5, 6]. Insecticides residual problem together with the insect resistance pose us in the environment to seek attention towards alternative methods [7]. This has necessitated the need of research and development on environmental safe, bio-degradable and indigenous method for vector control. Many herbal products have been evaluated and used as natural insecticides early before the use of synthetic insecticides [3]. The co-evolution of plants with insect has equipped them with plethora of chemical defense which can be used against insect [8]. A considerable work has been done and the use of botanical derivatives against mosquitoes and have been reviewed [9].
About 80% of the world's population use plant as their primary source of medicine. Natural products are generally preferred to control insect since they are harmless, have no effect on non-target organisms and their bio-degradability [10, 12].

2. Materials and methods
2.1 Preparation of plant extract
The healthy leaves of *Lawsonia inermis* (Lythraceae), *Murraya exotica* (Rutaceae) were collected from Karambayam, Thanjavur district, Tamilnadu, India and the leaves were washed with running tap water and dried in a shady place for 7-14 days at an ambient environment temperature (27-37 °C).The dried leaves were powdered mechanically using commercial electrical stainless steel blender. The plant powder extract was derived with the help of a Soxhlet apparatus using methanol as solvent (Boiling temperature (45-50 °C) for 8 hours [13]. The extracts were filtered through a Buchner funnel with Whatman number 1 filter paper and concentrated; the residue obtained was stored at 4 °C for further use [14, 15].

2.2 Larvicidal bioassay

Mosquito larvicidal bio-assay were carried out according to WHO [16] standard procedures with slight modification. The 200 ml of tap water was taken in a series of 250 ml beakers. The residue was redissolved in a known volume of acetone and the test concentration ranging between 50 ppm-300 ppm of the methanol extract of *Lawsonia inermis* and *Murraya exotica* were made with 200 ml tap water. A control was also maintained separately by adding 2 ml of acetone to 200 ml of water. 10 larvae per concentration were used for all the experiments. The number of dead larvae at the end of 24 hr was recorded. Dead larvae were removed as soon as possible in order to prevent decomposition. This experiment was repeated 3 times. Finally, mortality percentage value was calculated by Abbott's formula [17].

2.3 Statistical analysis

The larval mortality data were subjected to Probit analysis [18] for calculating LC_{50} and LC_{90} and Chi-square value were calculated by using SPSS 21 version for windows. The significant level was set at 0.05 level.

3. Results and Discussion

Susceptibility of filarial vector *Culex quinquefasciatus* against the methanolic extract of the leaves of *Lawsonia inermis* and *Murraya exotica* were studied. The LC_{50} and LC_{90} value obtained in the study are presented in Table 1.

Table 1: The LC_{50} and LC_{90} values of the methanolic extract of *Lawsonia inermis* and *Murraya exotica* leaf against the III and IV instar larva and pupa of *Culex quinquefasciatus* under 24 hrs exposure.

Plant species	Larval stage	LC $_{50}$(ppm) (UCL-LCL)	LC $_{90}$(ppm) (UCL-LCL)	χ^2	Regression equation
Lawsonia inermis	III instar	139.057 (141.2 – 136.914)	241.518 (243.901 – 239.135)	1.565	Y= -1.289 + 0.0403X
	IV instar	163.63 (165.844 – 161.416)	270.65 (273.082 – 268.218)	.427	Y= -2.265 + 0.0414X
	Pupa	188.151 (190.426 – 185.876)	339.356 (290.902 – 286.124)	.231	Y= -2.114 + 0.0361X
Murraya exotica	III instar	135.539 (137.692 – 133.380)	219.259 (221.6 – 216.918)	.321	Y= -1.33 + 0.419X
	IV instar	154.361 (156.55 – 152.172)	270.448 (272.88 – 268.016)	.604	Y= -1.67 + 0.0396X
	Pupa	178.57 (180.823 – 176.319)	288.513 (290.902 – 286.124)	2.057	Y= -3.266 + 0.0434X

- Significant at 0.05 level.

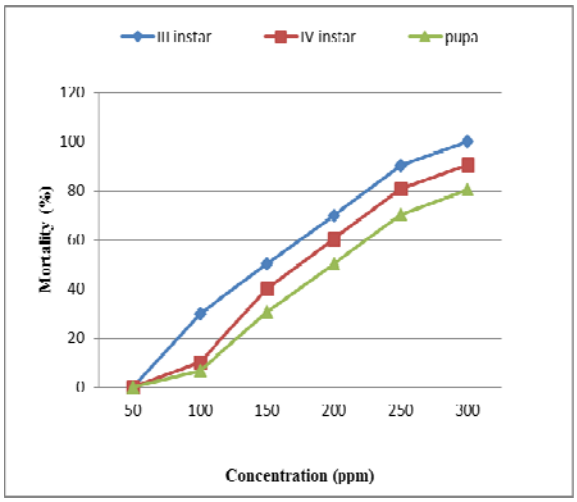

Fig 1: Mortality rate of III instar, IV instar and pupa of *Culex quinquefasciatus* against *Lawsonia inermis* plant extract.

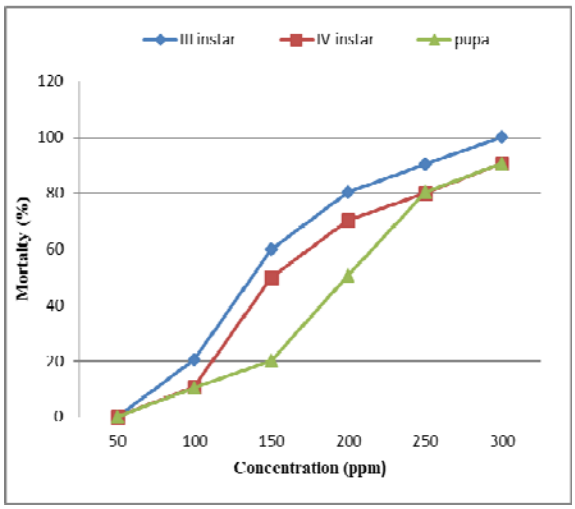

Fig 2: Mortality rate in III instar, IV instar and pupa of *Culex quinquefasciatus* against *Murraya exotica* plant extract.

The mortality rate observed for 24 hrs *Culex quinquefasciatus* III instar, IV instar and pupa against *Lawsonia inermis* and *Murraya exotica* extract used (Figure 1, 2). The 24 hrs LC_{50} value for III, IV instars and pupa for *Lawsonia inermis* is 139.057 ppm, 163.630 ppm and 188.151 ppm respectively; for *Murraya exotica* The LC_{50} value is 135.539 ppm, 154.361 ppm and 178.57 ppm for III, IV and pupa respectively. From this study it is known that an III instar larva is more susceptible than IV instar and pupa. Mosquitoeborne diseases are one of the most public health issues in the developing countries. Controlling the mosquitoes in larval stage is important and effective. To control larvae of mosquito various plant extracts are studied extensively and these studies shows the activity of such plant extracts based on the various significantly plant part, age of plant part, solvent used extraction and mosquito species [9]. Studies on focus on herbs and other medicinal plants due to the historical experimental knowledge and some scientific studies have shown them to be particularly active

against certain organisms [19]. Senthilnathan [20] studied the methanol and ethanol flower extract of *Lantana camara* which shows high larvicidal activity against *Ae. aegypti* and *Culex quinquefasciatus*. The different solvent extracts of *Strychnos nuxvomia* L. (Loganiaceae) leaf extracts control the filarial vector *Culex quinquefasciatus* [21] and extracts of *M. citrifolia* leaf extract against to *Culex quinquefasciatus* and *An. Stephensi***.** These studies show that methanol extract is highly toxic to *Culex quinquefasciatus* when compare with others [22]. Likewise *Spillanthus acmella* flower extract and *Andrographis paniculata* leaf extract *Spillanthus acmella* flower extract actively control the *Ae. aegypti* mosquito larvae [23]. In the present study, two plant extracts were used against *Culex quinquefasciatus*; *Murraya exotica* extract is effective than *Lawsonia inermis* extract used.

4. Conclusion

Larvicidal activity of *Lawsonia inermis, Murraya exotica* leaves were studied against III instar, IV instar and pupa of *Culex quinquefasciatus*. It is observed that *Murraya exotica* is more toxic against *Culex quinquefasciatus* as a potentential larvicide and isolation of active principle from these plants will help to control mosquitoe population.

Appendix

Ppm: Parts per million
hrs: Hours
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
LCL: Lower Confidence Limit
^{0}C: Degree Celsius
χ^2 : Chi-square

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