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Shiva Ohri
 Department of Zoology, School
 of Bioengineering and
 Biosciences, Lovely Professional
 University, Punjab, India

Gautampreet Singh
 Department of Zoology, School
 of Bioengineering and
 Biosciences, Lovely Professional
 University, Punjab, India

Dr. Amaninder Kaur
 Assistant Professor, Department
 of Zoology, School of Bio
 Engineering and Bio Sciences
 Lovely Professional University,
 Punjab, India

Corresponding Author:
Dr. Amaninder Kaur
 Assistant Professor, Department
 of Zoology, School of Bio
 Engineering and Bio Sciences
 Lovely Professional University,
 Punjab, India

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Larvicidal activity of *Murraya koenigii* against *Aedes* Mosquito

Shiva Ohri, Gautampreet Singh and Dr. Amaninder Kaur

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Abstract

The control of mosquito species is a challenging task which solely depends upon chemical insecticides as there is no effective alternative approach has filled this gap. There are various plant based chemicals known as phytochemicals having compounds which can control the mosquito's population efficiently as a cost effective method. *Murraya koenigii* has been found to have insecticidal properties and was found to be effective in order to control the pest populations. We have tested the leaves and stems of this plant against the 3rd and 4th larval instars of *Aedes* mosquito which is a major vector of dengue disease. We have tested the shade dried and sun dried extracts of leaves and stems prepared by boiling method of *Murraya koenigii* at various concentrations at different time intervals. Good mortality rates were observed in shade dried leaves extract as compared to the sun dried extract of leaves and stem as well as shade dried extract of stem. Highest mortality i.e. 76.67% was shown by shade dry leaves at 200 ppm concentration after 72 hours and lowest mortality i.e. 60% was shown by sundry stem extract at 200 ppm after 72 hours.

Keywords: *Aedes aegypti*, mosquito control, larvicidal activity, phytochemicals

1. Introduction

Mosquitoes belong to family Culicidae. They are midge like-flies and are threat to human life. Species belonging to these genera are *Aedes*, *Anopheles* and *Culex*. Only the female mosquito transmits the disease. *Aedes* is considered most dangerous among all different species of mosquitoes. WHO has declared Mosquitoes as "Public enemy number one". The genera *Aedes* is primary vector for spreading dengue virus. It is an anthropophilic mosquito; it evolved a relationship with humans. There are some distinctive features which help in identification of *Aedes* like black and white markings on their body and legs. Only during day time, they are active. Their biting periods are in evening and in early morning. Different species are there of this genera like *A. aegypti*, *A. albopictus*, *A. australis*, *A. polynesiensis*, *A. rusticus*, *A. vexans*. Among all these species of mosquitoes, *A. aegypti* is responsible for spreading of Dengue. Only female mosquito transmits the disease. Except Antarctica it is found in all other continents and tropical and subtropical regions. Species like *A. albopictus* is a most invasive disease, German entomologist Johann Wilhelm Meigen in 1818, first described it and named it. Serious diseases are transmitted by some species of these genera like chikngunya, yellow fever, dengue fever and Zika virus. Human lymphatic filariasis is transmitted by *A. Polynesiensis*.

They cause local and systematic skin reactions such as angioedema, urticaria and also causing allergic reactions^[1]. In Malaysia, *A. aegypti* and *A. albopictus* are considered two main vectors of Dengue fever^[2, 3]. The presence of certain bioactive chemicals in plants may help in control of pests. Many plants are there which help in mosquito control. *Murraya koenigii* is one of the best plants which help in mosquito control. *M. koenigii* or *Berbera koengii* also called as Meethi neem belongs to Rutaceae family. Also known as Curry tree is a sub-tropical and tropical tree native to Sri Lanka and India. The leaves of this tree are used by India and many neighbouring countries in many dishes. In India, it can be found in different places like Assam, Bengal, Sikkim, and Garhwal. The tree is having different names in area of its distribution. Curry tree is cultivated for its aromatic leaves.

Different uses are there of curry leaves and of oil extracted from leaves used for dysentery, blood purifier, tonic and also used as flavouring agent in curries and chutneys. Oil is used in perfume and soap industries. *Murraya koenigii* show different activities like antifungal activity, anti-inflammatory activity, antioxidant activity, antibacterial effects [4], cytotoxic activity [5] and anti diarrhoeal activity.

It is small deciduous shrub, height up to 6 meters; stem is having dots on it and colour is dark green to yellowish; Leaves are bipinnately compound, exstipulate, is about 30 cm long bearing around 24 leaflets. Flowers are white, funnel shaped, bisexual, sweetly scented, complete, stalked, regular, actinomorphic, pentamerous, and hypogynous. Flowering and fruiting season is December to July. Fruits are 1-1.2 cm in diameter and 1.4-1.6 cm long and are fully ripe, black in color with shining surface.

Alkaloids are isolated from leaves which are phyto constituents include koenine, mahanine, girinimbiol, girinimibine 5, koenimbine, O-methylmahanine, isomahanine, bismahanine, bispyrayafoline6, O-methyl murrayamine A. The oil extracted from leaves contain α -phellandrene, dipentene, D- α -terpinol and caryophyllene, D- α -pinene, D-sabinene. Main constituent which show inhibitory action against *A. aegypti* are terpinolene and carene.

Antibacterial effects against many species like *B. subtilis*, *P. vulgaris* and many other is showed by essential oil which is obtained from *Murraya koenigii* leaves. The oil obtained from *M. koenigii* leaves shows anti-fungal activity against many species like *A. niger*, *C. albicans* and *C. tropicalis*. But the ethanolic extracts of roots don't show anti-fungal activity against *Microsporum canis* and *Cryptococcus neoformans*. Repellent activity of *Murraya koenigii* is also observed. The petroleum ether and acetone extracts of *Murraya koenigii* leaves has showed the larvicidal activity against mosquito species. *Murraya koenigii* provide 6 hours protection against mosquitoes. The derivatives of *M. koenigii* plants have good results when used for vector control operation as well as it can also be used for other purposes.

2. Materials and Methods



Fig 1: *Murraya koenigii*

2.1 Collection of Plant Material: Curry plant material was collected from herbal garden of Lovely Professional University (Figure 1).

2.2 Preparation of Leaf extract: Leaves of curry plant were collected and washed with water. Leaves were kept for shade and sun dry and were crushed in Mortar pestle for powder form. Boiling method was used for preparation of extract.

In Boiling method: 10 gm of leaf powder was taken and added in 100 ml of distilled water. It was kept for boiling in heating mantle for about half an hour at 80 °C. After boiling it was allowed to cool down at room temperature and was filtered with whatman filter paper and kept in refrigerator.

2.3 Preparation of Stem extract: Both shade and sundry stem were used for preparation of extract. Boiling method was used for preparation.

In Boiling method: 10 gm of stem powder was taken in 100 ml of distilled water and kept for boiling about 30 minutes at 80 °C in heating mantle. After boiling it was cooled down and filtered with whatman filter paper and kept in refrigerator.

2.4 Collection of Larvae: Larvae of *Aedes aegypti* were collected from water sources like water cooler, pot etc. with the help of dipper in plastic bottles and kept in laboratory.

2.5 Bioassay: 3rd and 4th instar larvae of *Aedes aegypti* were treated in triplicates with different concentration of leaf and stem extracts of curry plant i.e., 50, 100, 150 and 200 ppm. 15 larvae were taken in each beaker having 250 ml of de-chlorinated water and extracts of leaves and stem were tested. In one beaker only 15 larvae were taken without adding any extract which was kept as control and examined. Mortality rate was checked after 24, 48 and 72 hours (Fig 2).



Fig 2: Treatment to *Aedes aegypti* larvae with the various concentrations of *Murraya koenigii*

3. Results

The two variable methods were used for the preparation of extracts: sun-dried and shade dried plant parts were used to check whether the sun has any deleterious effect on the phytochemicals. The mortality rates were recorded after different time intervals.

3.1 Leaf extracts

The leaves of *M. koenigii* were used to prepare the extracts to record its effective against *Aedes* larvae. After the treatment of different concentrations of *M. koenigii* extracts i.e 50, 100, 150 and 200 ppm. The results depicted the increase in mortality rate as concentration of sundry leaf extract and time increases. At 72 hours, 200 ppm concentration shows highest mortality rate i.e. 73.34%±1.41 and lowest mortality was 53.34%±0.57 at 50 ppm concentration after 24 hours. The bars depict Standard error (Fig. 3).

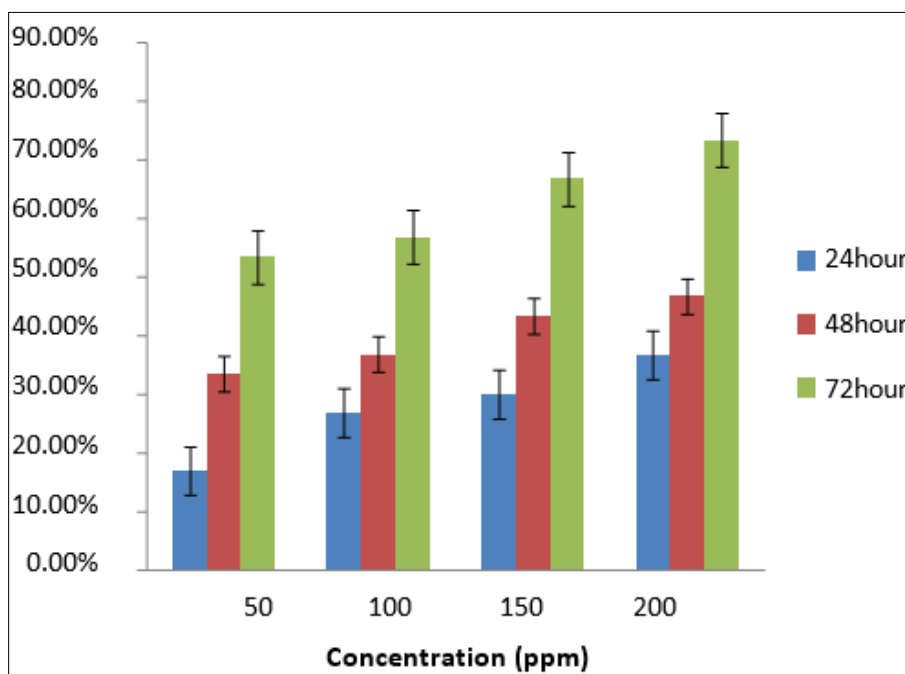


Fig 3: Mortality rate of *Aedes aegypti* larvae at different time interval of sundry boiling leaf extract

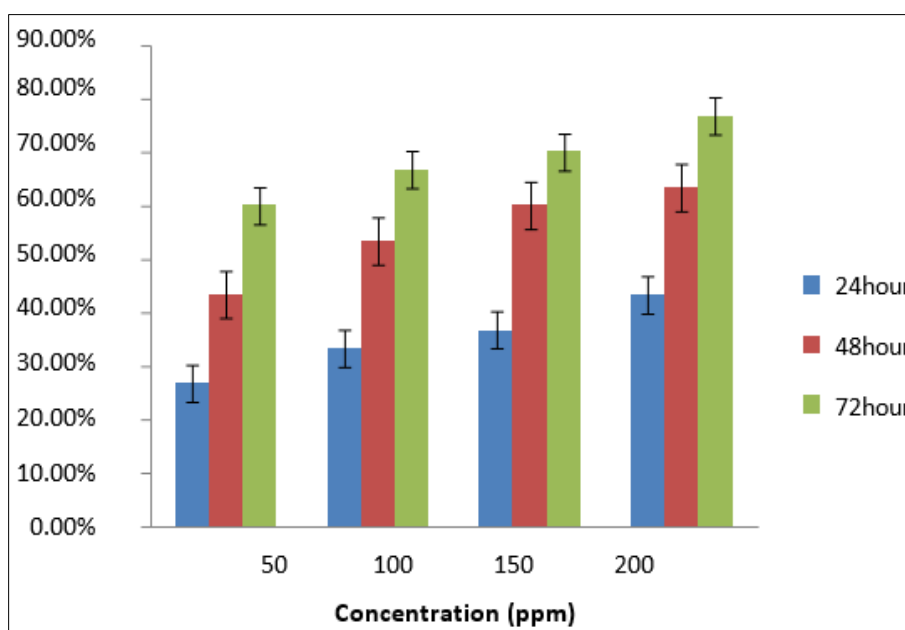


Fig 4: Mortality rate of *Aedes aegypti* larvae at different time interval of shade dry boiling leaf extract

As the concentration and time interval increases mortality rate of larvae also increases. After 72 hours mortality rate was $60\% \pm 0.57$ at 50 ppm concentration but as concentration increased mortality rate also increased i.e. after 72 hours mortality rate was $76.67\% \pm 1.73$ at 200 ppm concentration. These bars depict Standard error (Fig. 4).

3.2 Stem extracts

The stem part were used to check the efficacy against

mosquito larvae. The sun dried and shade dried both methods were used to prepare the extracts. The graph describes about relationship between mortality rate and concentration of sundry stem extract (Fig. 5). After 72 hours $46.67\% \pm 1.00$ mortality was observed at 50 ppm concentration but as concentration increased mortality also increased i.e. at 200 ppm $60\% \pm 0.57$ of mortality was observed. The bars depict Standard error.

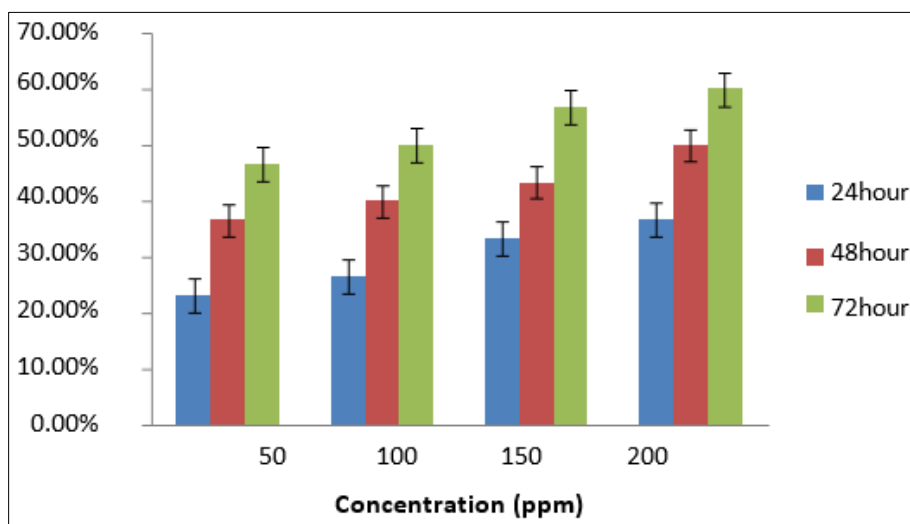


Fig 5: Mortality rate of *Aedes aegypti* larvae at different time interval of sundry boiling stem extract

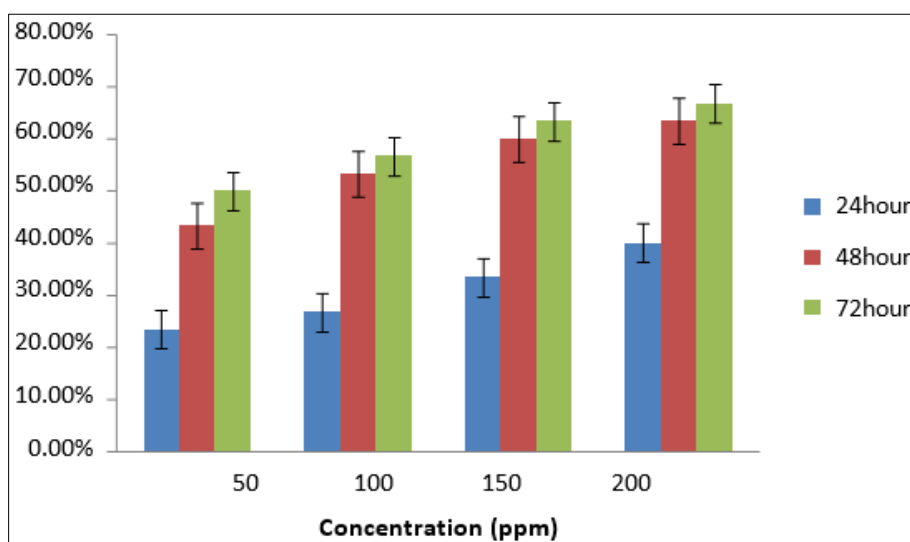


Fig 6: Mortality rate of *Aedes aegypti* larvae at different time interval of shade dry boiling stem extract

The graph depicts about increase in mortality rate as concentration of shade dry stem increases (Fig. 6). After 24 hours lowest mortality rate was observed i.e. $40\% \pm 0.57$ at 200 ppm concentration but as time increased mortality rate also increased i.e. $66.67\% \pm 1.73$ mortality rate was shown by *Aedes* larvae at 200 ppm concentration after 72 hours.

4. Discussion

This study is about larvicidal activity of *Murraya koenigii* plant against *Aedes aegypti* larvae. The parts used were leaves and stem. Shade and sundry leaves and stem extracts were prepared by boiling method. After preparation their effect was checked against *Aedes aegypti* larvae.

The toxicity of shade, sundry leaves and stem was checked on 3rd and 4th instar larvae of *Aedes aegypti* at different time intervals. Various concentrations were used of both the extracts. Good mortality rate was observed in leaves then stem. But as comparison to shade and sundry leaves; good result was in shade dry leaves same results were observed in case of stem; shade dry stem showed better results than sundry stem. Highest mortality i.e. 76.67% was shown by shade dry leaves at 200 ppm concentration after 72 hours and lowest mortality i.e. 60% was shown by sundry stem extract at 200 ppm after 72 hours.

The boiling extract of shade and sundry leaves after 72 hours at highest concentration i.e. 200 ppm showed 76.67% and 73.34% mortality rate respectively. Another study was conducted by Noel *et al.*, (2016) they tested the larvicidal activity of ethanolic and aqueous extracts of *Murraya koenigii* plant against *Aedes* [6]. D.K. Kocher and A.K. Riat (2017) checked larvicidal potential of *Eucalyptus globulus* against *Aedes* and *Culex*. 100% mortality rate was observed on *Aedes* larvae at 100 ppm concentration after 3hrs whereas in *Culex*, 100% mortality was shown after 12 hours at 100 ppm concentration [7].

Petroleum ether and acetone extracts of *Murraya koenigii* leaves has been used against *Aedes aegypti* larva to determine the larvicidal activity with a concentration range of 250 ppm – 900 ppm [8]. The plant extracts has been used of different plants like *Murraya koenigii*, *Coriander sativam*, *Ferula asafoetida* and *Trigonella foenum graceum* against larvae of two genera of mosquito i.e. *Aedes* and *Culex*. The effective results were found [9]. The plant extract of *Murraya koenigii* has been used, was prepared using the solvent chloroform against 1st, 2nd, 3rd, and 4th instar larvae of *Aedes aegypti*. The LC₅₀ values were 1.263%, 1.871%, 2.446% and 3.168% respectively. This study showed the presence of alkaloids, saponins, steroids and flavanoids in chloroform extract of

leaves of *M. koenigii* ^[10].

5. Conclusion

The present study suggests the bio-pesticides should be used rather than using chemical insecticides which are harmful to crops and to non-targeted insects. Plant based chemicals are safer to environment and to other organisms. Pests are more prone to develop resistance against synthetic insecticides. So, it is good approach to use bio-insecticides. Many phytochemicals are present inside the plant having a great potential to kill mosquito larvae. These results correlate with that of the present report conduct to check the efficacy of *Murraya koenigii* against *Aedes aegypti*.

6. Declaration of Interest

The authors declare that they have no conflicts of interest.

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