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Sustainable mosquito control: Assessing the larvicidal activity of *Murraya koenigii* extracts against *Aedes vittatus*

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Abstract
Mosquito-borne diseases pose a significant threat to global public health and socio-economic development. Aedes species mosquitoes, including *Aedes vittatus*, act as vectors for various diseases such as dengue fever, chikungunya, zika fever, and yellow fever. In this study, we aimed to evaluate the larvicidal efficacy of *Murraya koenigii* extracts against fourth-instar larvae of *Ae. vittatus*. The use of natural products for vector control has gained attention due to the drawbacks associated with synthetic insecticides. *M. koenigii*, known as curry tree, contains bioactive compounds with larvicidal properties. Phytochemical screening of *M. koenigii* extracts revealed the presence of alkaloids, saponins, tannins, flavonoids, and glycosides. The aqueous extracts of leaves, bark, and roots were prepared and tested against *Ae. vittatus* larvae. The larvicidal bioassay followed the World Health Organization guidelines. The results showed that all three extracts exhibited larvicidal activity. Leaf extracts demonstrated the highest efficacy, with 88.33 ± 1.15% mortality at the maximum tested concentration of 400 ppm. Bark and root extracts also exhibited significant larvicidal activity starting from 200 ppm concentrations. Concentration-dependent results were observed in all bioassays. The findings suggest that *M. koenigii* extracts, particularly leaf extracts, could be effective in controlling *Ae. vittatus* larvae. These results support previous studies demonstrating the larvicidal efficacy of *M. koenigii* extracts against different mosquito species. Further research is warranted to identify and isolate the active compounds responsible for larvicidal activity and assess the potential of M. koenigii extracts as a sustainable and environmentally friendly approach for mosquito control.

Keywords: mosquito control, biological control, vector control, curry leaf extracts, vector-borne diseases

Introduction

Mosquitoes are responsible for transmitting serious human diseases, resulting in millions of deaths annually (Suganaya *et al*., 2013) [11]. *Aedes* species mosquitoes act as vectors for various diseases such as dengue fever, chikungunya, zika fever, and yellow fever. The spread of these arthropod-borne diseases is increasing due to factors like globalization and climate change (Ansari, 2000; Tawatsin *et al*., 2001) [2, 6]. These diseases not only pose a significant public health problem worldwide but also hinder socio-economic development in endemic countries (Karunamoorthi and Sabesan, 2010) [4].

Ae. vittatus, a mosquito species found in Africa, tropical Asia, and southern Europe, is known to transmit yellow fever, Zika, chikungunya, and dengue virus (Alarcón-Elbal, *et al*., 2020) [1]. Recently, *Ae. vittatus* is considered as an emerging threat to human health due to its potential to spread arboviruses like Zika virus (ZIKV), yellow fever virus (YFV), dengue virus (DENV), and chikungunya virus (CHIKV). It has also been reported to be the potential vector for Japanese encephalitis (JEV), West Nile (WNV), Chandipura (CHPV), and Chittoor (CHITV) viruses (Sudeep, *et al*. 2020; Outammassine, *et al*. 2022) [14, 15]. *Ae. vittatus* mosquitoes are susceptible to all four types of dengue viruses and were able to facilitate the growth of the DEN-2 virus (Mavale, *et al*. 1992) [16]. In addition, *Ae. vittatus* was discovered to vector *Setaria digitata* (Varma *et al*., 1971) [17].

The use of synthetic insecticides for mosquito control has led to resistance, environmental damage, and high costs. As a result, researchers have turned their attention to natural products for vector control (Suganaya *et al*., 2013) [11]. *Murraya koenigii*, which is known as curry leaves tree, is well known for its medicinal properties. Phytochemical screening of *M. koenigii* extracts revealed the presence of alkaloids, saponins, tannins, flavonoids, and glycosides in the methanol extract (Harith *et al*., 2016) [3] and several carbazole alkaloids, such as mahanimbine, girinimbine, murrayacine, murrayanine, murrayafoline-A, and 3-methylcarbazole, with larvicidal activity against *Ae. aegypti* (Sukari *et al*., 2013) [12]. The presence of bioactive compounds in *M. koenigii* supports its potential as an effective mosquito larvicide (Harith *et al*., 2016; Sukari *et al*., 2013) [3, 12].

M. koenigii extracts were found to be effective in the control of *Anopheles gambiae* (Mangera *et al*., 2021), *Aedes albopictus* (Nishan and Subramaniam 2015) [8], *Aedes aegypti* (Sukari *et al*., 2013) [12], *Culex quinquefasciatus* (Kovendan *et al*., 2012) [5]. Shivhare *et al*. (2018) [10] developed a carbopol 940-based mosquito repellent gel formulation using essential oils from various plants, including *M. koenigii*. Sai Shankar *et al*. (2013) [9] reported mosquito repellent efficacy of *M. koenigii* plant powders when they were burnt on charcoal. The present study was aimed at testing the larvicidal efficacy of *M. koenigii* aqueous extracts of leaves, bark, and roots against the fourth instar larvae of Ae. vittatus.

Materials & methods

Test Insect Culture

Early-stage larvae of *Aedes vittatus* were collected from the lake and rockpools within the Osmania University campus

in Hyderabad, Telangana State, India. The larvae were reared in glass troughs with a diet of yeast and dog biscuits in a 3:1 ratio. Fourth instar larvae were used for the larvicidal bioassay.

Extracts Preparation

High-quality leaves, bark, and roots of *Murraya koenigii* were collected from Sangareddy town, Telangana, India. They were washed with running tap water and distilled water, and shade dried for 15 days. The dried plant parts were then powdered using an electric blender. To prepare the aqueous extracts, 100 gr. of the powder was added to 500 ml of distilled water and boiled at 50^0 C for 30 minutes and subsequent filtration. Then the filtrate was poured into a plate and allowed to dry under a fan for two days. The semi-solid extracts obtained thus were stored in a plastic bottle until usage.

Test Solutions Preparation

1 gr. of dried extracts were added to 1000 ml of distilled water separately, to prepare a 1000 ppm stock solution. By dilution method, five different concentrations (25, 50, 100, 200, and 400 ppm) of test solutions were prepared

Larvicidal Bioassay

The larvicidal bioassay followed the guidelines provided by the World Health Organization (WHO, 2005) [7]. Larvae were placed in three batches of 20 individuals each in 100 mL of the prepared test solutions, separately in 250 mL test cups. The number of dead larvae was recorded after 6, 12, and 24 hours of exposure, and the percentage mortality was calculated based on the average of three replicates using the formula:

Mortality % = (Number of dead larvae / Total larvae population) × 100.

Results & discussion

The results of the present study are given in Table 1 and Figure 1. All three tested extracts were proved to be efficient against the 4th instar larvae of *Ae. vittatus*. However, Leaf extracts showed better larvicidal efficacy than the remaining two extracts. In all three bioassays, concentration dependant results were observed. At the maximum tested concentration of 400 ppm, Leaf extracts yielded 88.33 ± 1.15 % mortality, whereas 76.67 ± 0.58 % and 66.67 ± 0.58 % mortalities were observed with Bark and Root extracts, respectively. At the 50 ppm concentration, Leaf extracts showed more than 50% mortality. However, more than 50% mortality was achieved with 200 ppm concentration for bark and Root extracts.

The results of the study clearly showed that the leaf extracts of *M. koenigii* are effective against the 4th instar larvae of *Ae. vittatus*. Bark and Root extracts exhibited significant mortality starting from 200 ppm concentrations.

The present study results are in line with the previous studies. Nishan and Subramaniam (2015) [8] investigated the larvicidal efficacy of methanolic extracts of Azadirachta indica and M. koenigii individually and in combination. The results demonstrated high larvicidal activity for A. indica, with a mortality rate of 96.30% at 3.75 mg/ml, while M. koenigii exhibited a mortality rate of 83.70% at the same concentration. The combination of the two extracts showed an additive effect. Sukari *et al*. (2013) [12] reported *M. koenigii* leaf and bark extracts showed more larvicidal efficacy than root extracts against the larvae of *Ae. aegyptii*.

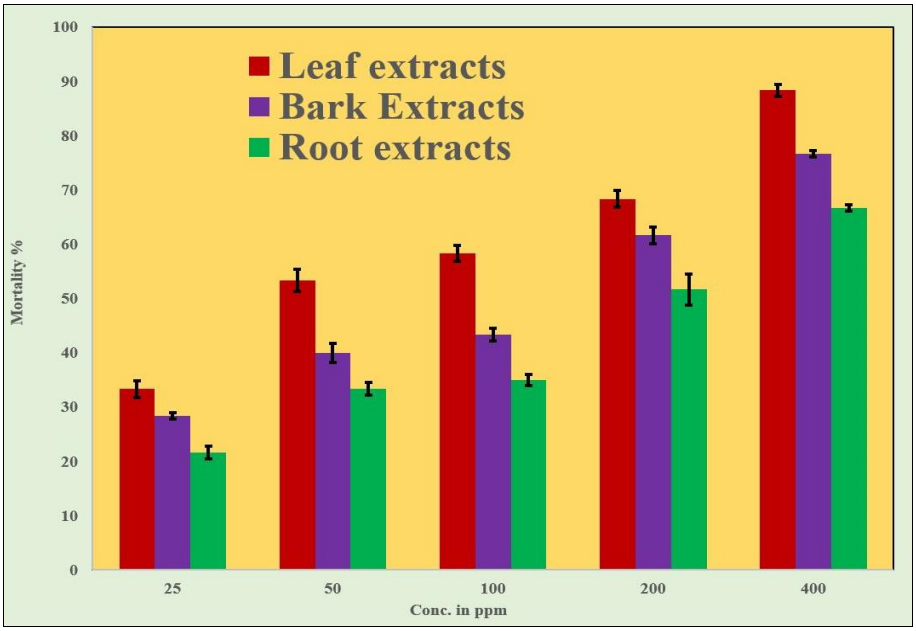

Fig 1: *M. koenigii* extracts larvicidal efficacy against the 4th instar larvae of *Ae. vittatus*

Table 1: Mean mortality rates ± SD of *M. koenigii* extracts against the 4th instar larvae of *Ae. vittatus*.

Conc. In ppm	Leaf extract	Bark extract	Root extract
0	0	0	0
25	33.33 ± 1.53	28.33 ± 0.58	21.67 ± 1.15
50	53.33 ± 2.08	40.00 ± 1.73	33.33 ± 1.15
100	58.33 ± 1.53	43.33 ± 1.15	35.00 ± 1.00
200	68.33 ± 1.53	61.67 ± 1.53	51.67 ± 2.89
400	88.33 ± 1.15	76.67 ± 0.58	66.67 ± 0.58

Conclusion

In the present study, Leaf extracts of *M. koenigii* showed efficient larvicidal properties against the 4th instar larvae of *Ae. vittatus.* The remaining two extracts showed significant results starting from 200 ppm concentration. The leaf extracts of *M. koenigii* have the potential to be used as promising larvicides against the larvae of *Ae. vittatus.* The earlier studies confirmed the presence of various secondary metabolites in *M. koenigii* extracts. These secondary metabolites are responsible for the larvicidal efficacy of *M. koenigii* against *Ae. vittatus*. To know which phytochemicals are responsible for this larvicidal efficacy, further studies are required.

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