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Larvicidal Activity of *Crotalaria biflora* Methanolic Extract Against *Culex Pipiens*: An Implication for Vector Control

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ABSTRACT

Vector-borne diseases like malaria, dengue, filariasis, and Japanese encephalitis continue to pose significant public health challenges globally. The use of synthetic larvicides raises concerns regarding environmental hazards and the development of insecticide resistance, highlighting the necessity for environmentally friendly alternatives. This study investigated the larvicidal properties of the methanolic extract of Crotalaria biflora against fourth-instar larvae of Culex pipiens. The entire fresh plants were shade-dried, ground into a powder, and then extracted using methanol through maceration over a period of seven days. Solutions of concentrations 2, 4, 6, and 8 mg/mL were prepared and introduced to the mosquito larvae for intervals of 24, 48, and 72 hours. Mortality rates were recorded, and LC_{50} values were estimated using an interpolation method. The extract exhibited larvicidal activity that depended on both concentration and exposure time. The highest mortality rate of 90% was recorded at a concentration of 8 mg/mL after 72 hours, while LC_{50} values were.

KEYWORDS

Crotalaria biflora, *Culex pipiens*, Larvicidal activity, Methanolic extract, Botanical larvicide, LC_{50}, Vector control.

I. INTRODUCTION

Mosquitoes are important vectors of many human diseases and remain a major public health concern worldwide. Female mosquitoes require blood meals for egg production, while males feed mainly on plant nectar. Different mosquito genera are responsible for transmitting specific diseases: *Anopheles* spreads malaria, *Aedes* transmits dengue, chikungunya, and Zika virus, and *Culex* carries diseases such as West Nile fever and Japanese encephalitis. These mosquito-borne diseases contribute significantly to illness, mortality, and economic burden, especially in tropical countries like India.

In India, diseases such as malaria, dengue, chikungunya, and Japanese encephalitis continue to affect large populations, particularly during and after monsoon seasons. Control measures are coordinated through the National Vector Borne Disease Control Programme (NVBDCP). Although synthetic insecticides are widely used to manage mosquito populations, their repeated use has led to environmental pollution, harmful effects on human health, and the development of insecticide resistance among mosquitoes.

Because of these limitations, attention has shifted toward plant-based alternatives for mosquito control. Natural plant extracts are considered eco-friendly, biodegradable, and less harmful to non-target organisms. Many medicinal plants also possess antimicrobial and insecticidal properties that may help reduce mosquito populations effectively.

Based on this approach, the present study investigates the larvicidal activity of methanolic extracts of *Crotalaria biflora* against *Culex pipiens* larvae. Different concentrations of the extract (2, 4, 6, and 8 mg/mL in 50 mL water) were tested over 24, 48, and 72 hours to evaluate larval mortality, behavioural changes, and developmental effects using standard experimental methods. [1,2,3]

PLANT PROFILE

Several species belonging to the genus *Crotalaria* have demonstrated promising potential in mosquito vector management. Certain species, such as *Crotalaria spectabilis*, are known to contribute to

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mosquito control by supporting the activity of natural mosquito predators. However, there is a lack of documented scientific evidence regarding the larvicidal efficacy of *Crotalaria biflora* L. against mosquito larvae, particularly under Indian conditions. This existing research gap emphasizes the significance of the present investigation, which aims to evaluate the larvicidal potential of *C. biflora* extracts as an environmentally sustainable alternative for mosquito vector control.

Crotalaria biflora is a hardy and drought-tolerant species widely distributed in tropical and subtropical regions. The plant commonly occurs in grasslands, roadsides, wastelands, and dry agricultural lands, reflecting its adaptability to diverse environmental conditions. It grows optimally in well-drained sandy to loamy soils, although its robust taproot system also enables survival in moderately clayey soils. As a member of the Fabaceae family, the plant possesses nitrogen-fixing root nodules that improve soil fertility and support growth in nutrient-deficient environments without requiring extensive fertilization. Propagation primarily occurs through seeds, which exhibit rapid germination under warm climatic conditions. In addition, the species requires minimal irrigation, thrives under full sunlight, and exhibits considerable resistance to pests and abiotic stress. These characteristics make *C. biflora* a suitable candidate for sustainable cultivation and further pharmacological and phytochemical investigations. [4,5,6,]

II. MATERIAL AND METHODS

Methodology: Larvicidal Bioassay

1. Plant Material Collection and Preparation Fresh Crotalaria biflora plants were collected from local habitats and authenticated. The whole plant was washed thoroughly, shade-dried for several days, and powdered using a mechanical grinder. The powdered material was stored in airtight containers until extraction.

2. Preparation of Plant Extract The whole plant of Crotalaria biflora was shade-dried, coarsely powdered, and subjected to maceration using methanol as the solvent. The powdered material was soaked in methanol for 7 days with occasional stirring. After maceration, the extract was filtered and the filtrate was concentrated under reduced pressure to obtain the methanolic extract, which was stored in an airtight container until use.

3. Preparation of Test Solutions A stock solution was prepared by dissolving 400 mg of the methanolic extract in distilled water to obtain the required test concentrations. From this stock, different concentrations were prepared by dilution with distilled water to a final volume of 20 mL as follows: 2 mg/mL, 4 mg/mL, 6 mg/mL, 8 mg/mL, Distilled water without extract served as the negative control. Copper sulphate is taken as positive control.

4. Collection and Maintenance of Larvae Healthy fourth-instar mosquito larvae were collected and maintained under laboratory conditions at room temperature. The larvae were acclimatized for 24 hours prior to experimentation and were fed a standard larval diet. Only active and healthy larvae were used for the bioassay.

5. Larvicidal Bioassay Procedure The larvicidal activity was evaluated following a standard larval immersion method. For each concentration, 10 fourth-instar larvae were introduced into beakers containing 20 mL of the respective test solution. Each concentration was tested separately, and the control group contained larvae in distilled water only. Larvae were exposed to the test solutions for a total duration of 72 hours, and mortality was recorded at 24, 48, and 72 hours of exposure.

6. Observation of Mortality Larval mortality was assessed by observing the larvae for movement. Larvae were considered dead if they showed no movement even after gentle probing with a glass rod,for concentrations of 2, 4, 6, and 8 mg/mL, respectively.

7. Data Analysis The percentage mortality was calculated for each concentration and time interval using the formula: Percentage mortality (Total number of larvae/Number of dead larvae) ×100 The results were used to assess the dose- and time-dependent larvicidal activity of the methanolic extract of Crotalaria biflora. Mortality in the control group was monitored to ensure exp. [7,8,9,10)]

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III. RESULT AND DISCUSSION

Percentage mortality

Concentration (mg/mL)	24 h (%)	48 h (%)	72 h (%)
2	10	30	50
4	40	60	70
6	60	70	80
8	70	80	90
Control+	80	90	90

Table 1: percentage mortality

Exposure Time	LC_{50} (mg/mL)
24 hours	5.0
48 hours	3.33
72 hours	2.0

Table 2: Lc 50

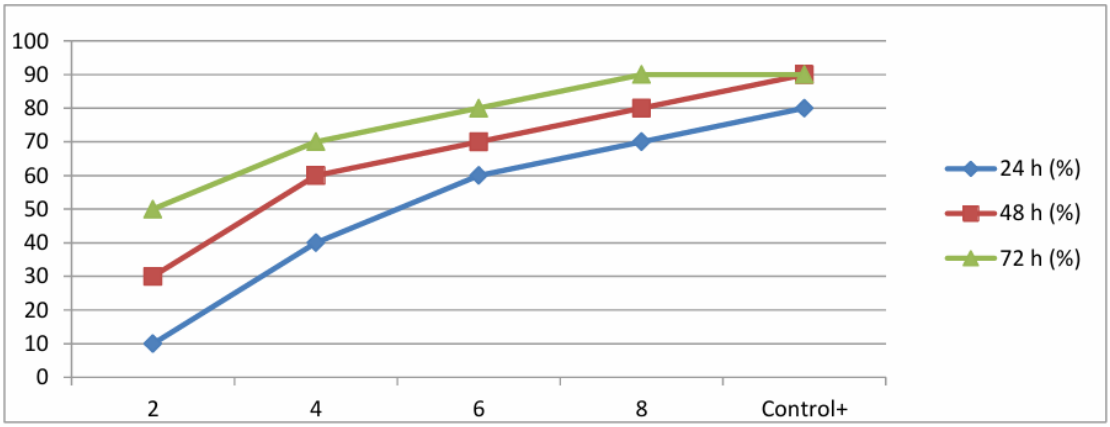

Figure 1: different concentrations with different times.

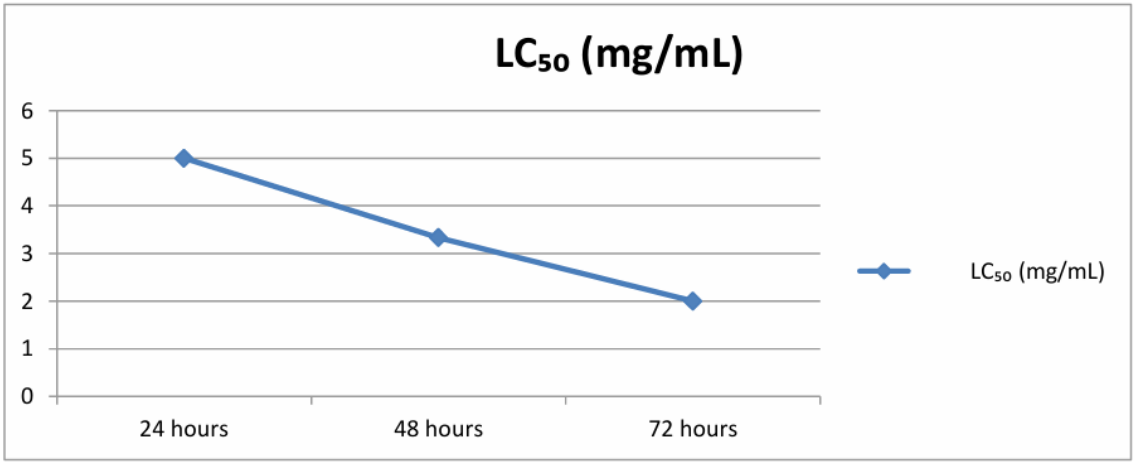

Figure 2:Lc 50 decreases as time increases

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Mosquito-borne infections remain a serious public health problem, especially in tropical and subtropical regions. The extensive use of synthetic larvicides has resulted in environmental contamination and the emergence of insecticide-resistant mosquito populations, creating the need for safer and more sustainable alternatives. Botanical larvicides are increasingly considered effective substitutes because they are biodegradable, environmentally friendly, and less likely to induce resistance. The present study investigated the larvicidal effectiveness of the methanolic extract of *Crotalaria biflora* against fourth-instar larvae of *Culex pipiens*.

The results revealed that the extract produced noticeable larval mortality, which increased with both concentration and exposure time. The LC_{50} values declined progressively over 24, 48, and 72 hours, indicating greater toxicity with prolonged exposure. These findings demonstrate that the plant extract possesses strong larvicidal potential and can effectively interfere with larval survival and development.

The observed activity may be associated with the presence of biologically active phytochemicals such as alkaloids, flavonoids, tannins, saponins, phenolic compounds, and terpenoids. These compounds may affect multiple physiological systems in mosquito larvae, including nervous, respiratory, and digestive functions, ultimately leading to larval death. The use of multiple mechanisms of action may also reduce the possibility of resistance development.

Since *Culex pipiens* is an important disease-transmitting mosquito and often shows resistance to conventional insecticides, the effectiveness of *Crotalaria biflora* against this species suggests its potential usefulness in mosquito control programs. Overall, the study supports the possibility of using *Crotalaria biflora* as a natural, eco-friendly larvicide. Further investigations are necessary to isolate the active compounds, evaluate environmental safety, and confirm its effectiveness under field conditions.

IV. CONCLUSION

The current research aimed to assess the larvicidal properties of the methanolic extract derived from Crotalaria biflora against fourth-instar larvae of Culex pipiens. Diseases transmitted by mosquitoes represent a significant public health issue, and the drawbacks of synthetic larvicides have heightened the demand for safer, plant-based alternatives. The methanolic extract of Crotalaria biflora demonstrated considerable larvicidal effects in a manner that was both concentration- and time-dependent. The mortality rate of larvae increased with both higher concentrations of the extract and longer periods of exposure. The LC_{50} values were determined to be 5.0 mg/mL at the 24-hour mark, 3.33 mg/mL at 48 hours, and 2.0 mg/mL at 72 hours, showing that larvicidal potency improves with extended exposure. The larvicidal activity observed may be linked to the existence of bioactive phytochemicals, including alkaloids, flavonoids, tannins, phenolic compounds, and saponins.

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