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Larvicidal activity of aqueous leaf extract of *Caladium bicolor* against *Anopheles* sp.

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ABSTRACT

As mosquitoes develop increasing levels of resistance to synthetic larvicides, there is a strong need to explore alternative solutions that are more environmentally friendly. In this study, we investigated the larvicidal activity of an aqueous leaf extract of *Caladium bicolor* against the malaria mosquito, *Anopheles*, which is considered the primary malaria vector. The leaf extract was prepared using Soxhlet extraction and was tested at doses of 125-500 mg/10ml following the recommended WHO (World Health Organization) protocols. The extract mixture showed substantial larvicidal activity, with an LC50 of 400 mg/10ml after 24 hours. The extract suggests that *Caladium bicolor* may be viable candidates for environmentally-friendly mosquito control. *Anopheles* larvae exposed to aqueous extract of *Caladium bicolor* gave behavioural responses to various toxic reactions such as erratic movements; air gulping with prolonged exposure resulted in dullness, inability to swim. This study concluded potential bioactive agents in *Caladium bicolor* leaf extract might have toxic and/or metabolic characteristics that are harmful to larvae. The widespread availability of *Caladium bicolor* as a plant enhances its feasibility as a sustainable larvicide. This study pioneers the use of its leaves, suggesting a novel resource for integrated vector management. Further research into active compounds and field applications is recommended to validate scalability and environmental safety.

Keywords: Larvicidal activity, *Caladium bicolor, Anopheles,* larvicidal mortality.

INTRODUCTION

Anopheles species, a major vector of malaria, is responsible for many cases each year in India and other tropical regions (WHO, 2023). Synthetic larvicides such as temephos, while effective, can be subject to resistance and have environmental toxicity (Inaba et al., 2022). Plant-based larvicides high in phytochemicals like phenolic acids and

flavonoids are made from recyclable plant materials that are inexpensive (Kamaraj et al, 2011). Aqueous extracts of plants are environmentally friendly for larvicides and are a straightforward means of extraction, but may not have the same efficacy as extracts isolated with solvents (Dua et al., 2009).

Caladium bicolor is a popular ornamental plant cultivated throughout India. It has many flavonoids and phenolic compounds and could be used as an insect repellent (Mulla & Su, 1999). There are no studies evaluating larvicidal activity of *Caladium* species against *Anopheles* species. This project evaluates the larvicidal activity of aqueous *Caladium bicolor* leaf extract against *Anopheles* sp. larvae. Soxhlet extraction was performed to promote an environmentally-safe extract containing no organic solvents.

MATERIAL AND METHODS

Plant Material Collection:

Caladium bicolor leaves were collected from various houses in Amravati region. Plants leaves were separated with scissors, washed with distilled water to remove soil, and authenticated by a botanist. Leaves were sun and air-dried at 30°C for 72 hours and stored in airtight containers.

Fig.1: *Caladium bicolor*

Scientific classification:

Kingdom: Plantae
Division: Tracheophyta
Class: Liliopsida (Monocotyledons)
Order: Alismatales (Arales)
Family: Araceae
Genus: *Caladium*
Species: *bicolor*

Extract Preparation:

The leaves separated from stem of *Caladium bicolor* were dried at environmental temperature (25-37 ^{0}C). Dried leaves were powdered separately by pestle and mortar. A *Caladium bicolor* leaf powder (50 g total) was used for extraction. The mixture was placed in a Soxhlet apparatus with 300 mL of distilled water and extracted for 12 hours at 100°C. The extract was filtered through filter paper, concentrated using a rotary evaporator at 60°C, and lyophilized to obtain a dry powder. Then distilled water was added to the powder in the proportion of approximately 125 mg, 250 mg, 375 mg and 500 mg per 10 ml of water to from solution of 300 ml each. For preparation of solution, considered amount of powder keep packed in muslin cloth pouch and dip into required warm water for 24 hours. Then pouch removed and formed solution used for experiment.

Mosquito Rearing*:* Anopheles species larvae were collected from nearby ponds, lakes and stagnant water from Amravati region and stored in beakers. These larvae were maintained at 25-30 ^{0}C, with relative humidity 60-70% under a photoperiod of (12h: 12h) following standard operating procedures for mosquito maintenance (WHO, 1975). The larvae were free of exposure to pathogens, insecticides or repellents and were maintained in beaker by providing dog biscuit or yeast powder.

RESULTS

Caladium bicolor leaf extract was tested for its larvicidal effect for *Anopheles* larvae at 125, 250, 375, and 500 mg/10 ml of the extract and at 6, 12, 18, and 24 hours of exposure time. The behavioural responses for both mortality and behaviours were observed and recorded as indicators of activity of the extract.

Responses of *Anopheles* larvae observed that the larvae were exhibiting behaviours indicative of stress. When exposed to the leaf extract, erratic movements were observed across concentrations and times, with greater behaviour generally observed with higher concentrations, and specifically, at 500 mg/10 ml, the larvae exhibited high levels of erratic behaviour. Air gulping, which is indicative of physiological stress, was clearly observed from 1 to 6 hours in *Anopheles* larvae exposed to (before the larval mortalities), in the 250, 375 and 500 mg/10 ml treatments, and only decreased as exposure to the treatment lengthened, which suggests that the larvae were becoming weaker.

Table 1: Mortality of *Anopheles* larvae exposed to extract of *Caladium bicolor* leaves for different interval of time

Concentration Mg/10ml	Total Respondent	Mortality			
		6 Hours	12 Hours	18 Hours	24 Hours
000	30	00	00	00	00
125	30	00	00	00	00
250	30	00	01	03	08
375	30	00	06	10	12
500	30	00	17	25	30

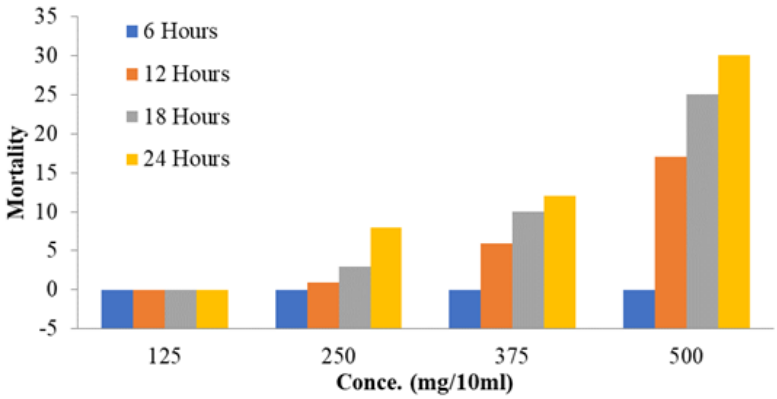

Figure 2: Mortality of *Anopheles* larvae exposed to aqueous extract of *Caladium bicolor* leaves for different interval of time

Table 2: Behavioural responses by *Anopheles* larvae exposed to *Caladium bicolor* leaves extract

Behaviors	Exposure Time																			
	1 hr					6 hrs				12 hrs				18 hrs				24 hrs			
	Concentrations																			
	0	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Erratic	+	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	+	-	-	-	-
Air Gulping	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Dullness	-	-	-	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+
Loss of reflex	-	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+
Discoloration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+

: + Present/ - Absent

*: 0 Control; A 125 mg/10ml; B 250 mg/10ml; C 375 mg/10ml; D 500 mg/10ml

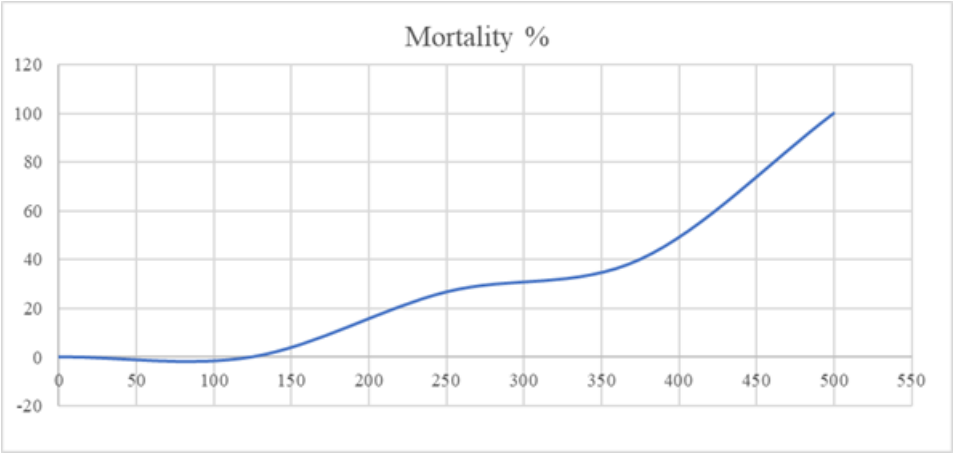

Figure 2: Mortality Analysis for LC_{50} for 24 hours

Lethargy as a behavioural indicator was absent in the control and lower dose treatments, but was first observed after 6 hours in the 375 and 500 mg/10 ml concentrations. Loss of reflex activity, which is indicative of high toxicity, was first recorded at 12 hours in the 375 and 500 mg/10 ml concentrations. Discoloration as an indication of cellular or internal damage was not observed at the earliest times of exposure or at lower doses, but had occurred at 18 hours in the 500 mg/10 ml treatment, with the severity worsening by the 24 hours collection.

There were patterns in mortality that clearly exhibited a dose- and time dependent effect. No mortality was recorded in the control group or at 125 mg/10 ml at any time point. At 250 mg/10 ml, there was some mortality, one larva dead at 12 hours and increasing to 8 dead at 24 hours. Mortality was more pronounced at 375 mg/10 ml, where there were 6 dead at 12 hours and on the increase with 10 dead at 18 hours and 12 dead at 24 hours. The highest concentration at 500 mg/10 ml exhibited a steep change, where there were 17 dead at 12 hours, 25 dead at 18 hours, and all mortality (30 larvae) at 24 hours. These patterns can be summarized in a bar graph, which provides visual representation to confirm that mortality was increasing in a time-related and dose-related manner, and it also highlights the active removal potential of the extract at that behaved most effectively as a larvicidal agent at 375 and 500 mg/10 ml dose.

The LC50 value of the leaf extract at 24 hours was about 400 mg/10 ml, therefore this is the minimum concentration of leaf extract used to achieve 50% mortality of larvae within the 24 hours observation time.

The leaf extract of *Caladium bicolor* have remarkable larvicidal activity on *Anopheles* larvae, confirming their potential role as sustainable larvicides. Behavioural disturbances for the larvae included erratic movements, gulping of air, lethargy, the loss of reflexes, loss of pigmentation, which closely matched mortality, indicating both physiological and biochemical disruptions. The activity is likely due to chlorogenic acid and apigenin, which disrupt larval physiology by inhibiting detoxifying enzymes like glutathione S-transferase (Inaba et al., 2022). The greater effectiveness of the leaves compared to the leaves indicates distinct phytochemical content, potentially influenced by metabolites from the soil.

The distinct advantage of *Caladium bicolor* is that this is the plant which many people have access to. Thus, it offers a resource that would biodegrade, resulting in fewer adverse environmental side effects compared to a synthetic larvicide. If the mechanism of action has a rapid effect, it might be useful to look at field settings, such as urban drain systems that support *Anopheles* populations to facilitate rapid application.

In contrast to commercial legacies, *Caladium bicolor* leaf extract may provide a source at higher concentrations compared to other sources, but its labour-intensive and time-intensive process of collection from leaves pose some limitations to credibility and scalability. Future investigations must also isolate the active components of *Caladium bicolor* and its capability of synergistic effects with other botanicals such as *Piper nigrum*. Non-target toxicity may also be assessed on aquatic species (Ghosh et al., 2012). Ultimately, field assessments in situ are important to assess practical impact.

The leaf extract of *Caladium bicolor* possess potent larvicidal effects against *Anopheles* larvae with behavioural effects and larvicidal mortality at 375 and 500 mg/10 ml in the limited testing conducted in this investigation. Aside from the ecological benefits of being used as a larvicide to control filariasis vectors, this offers an economic aspect in proportional and scalable resource in regards to the cost-free potential of a locally obtainable natural resource that can increases community initiatives to reduce dependence on chemical insecticides.

CONCLUSIONS

The aqueous leaf extract combination of Caladium bicolor provides potential larvicidal activity against Anopheles sp. in vitro, utilizing chlorogenic acid and apigenin as bioactive compounds. The study demonstrates the potential for using plant extract combinations to produce an eco-friendly, more sustainable mosquito control strategy using a Soxhlet extraction method. More work on extraction optimization, field validation, and environmental safety is needed.

Conflict of Interest: The authors declare no conflict of interest in relation to this research.

Data Availability Statement: Not applicable.
Correspondence and requests for materials should be addressed to **Vaibhao Thakare**

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