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Research Article

BIOEFFICACY OF SELECTED FABACEAE SEED EXTRACTS AS LARVICIDES AGAINST *CULEX QUINQUEFASCIATUS*

Shri Mathi K¹, James D¹, Abinaya R¹, Niranjankumar M¹, A. Jeyasankar² and Premalatha S^{1*}

Department of Botany, Government Arts College (Autonomous),
Coimbatore, Tamil Nadu.641031

Corresponding Author: S. Premalatha

Email- premasankar2020@gmail.com



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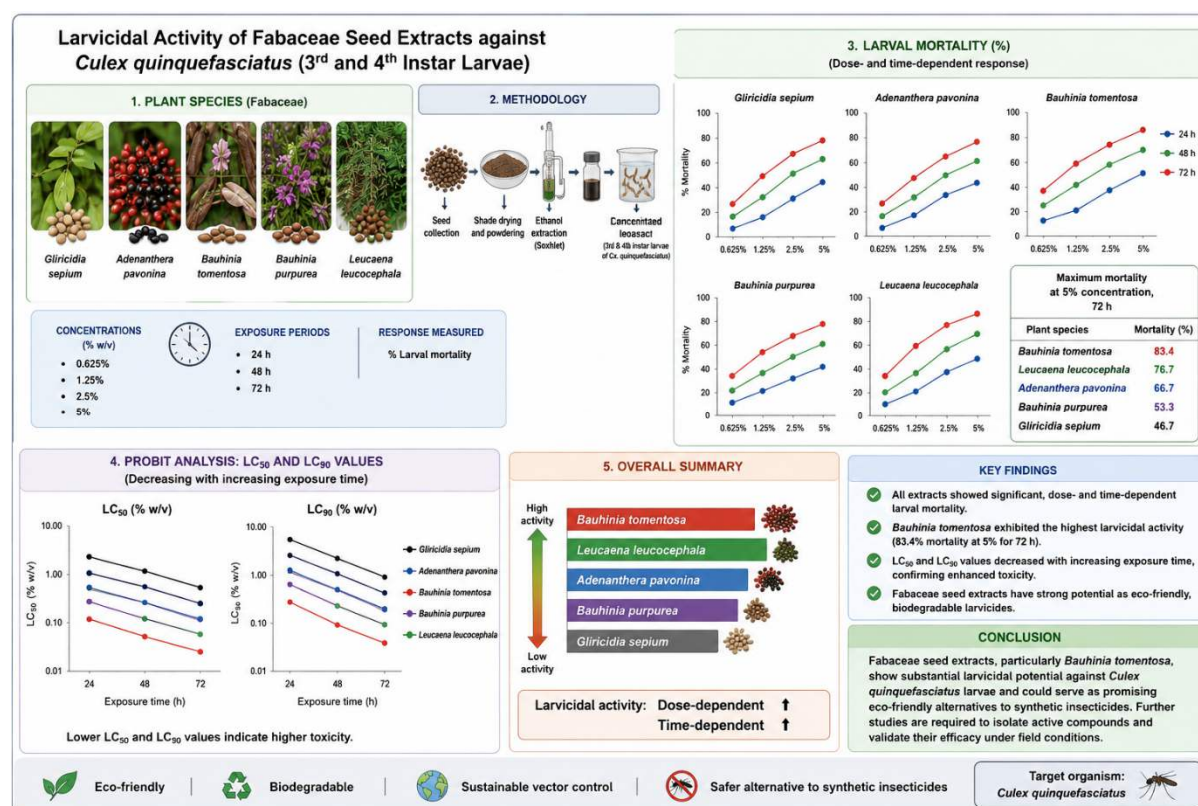
Abstract

Mosquito-borne diseases remain a major global health concern, necessitating the development of eco-friendly and effective vector control strategies. The present study evaluated the larvicidal activity of ethanol seed extracts of selected Fabaceae plant species—*Gliricidia sepium*, *Adenanthera pavonina*, *Bauhinia tomentosa*, *Bauhinia purpurea*, and *Leucaena leucocephala*—against third and fourth instar larvae of *Culex quinquefasciatus*. Larvicidal bioassays were conducted at different concentrations (0.625%, 1.25%, 2.5%, and 5%) and exposure periods (24, 48, and 72 hours), following standard protocols. The results revealed a clear dose-dependent and time-dependent increase in larval mortality across all tested extracts. Among the plants studied, *Bauhinia tomentosa* exhibited the highest larvicidal activity, with a maximum mortality of 83.4% at 5% concentration at 72 hours, followed by *Leucaena leucocephala* (76.7%) and *Adenanthera pavonina* (66.7%). Probit analysis indicated decreasing LC₅₀ and LC₉₀ values with increasing exposure time, confirming enhanced toxicity over prolonged exposure. The

findings suggest that Fabaceae seed extracts, particularly *Bauhinia tomentosa*, possess significant larvicidal potential and could serve as eco-friendly, biodegradable alternatives to synthetic insecticides. Further studies are required to isolate active compounds and validate their efficacy under field conditions.

Keywords: Larvicidal activity, Fabaceae, seed extracts, *Culex quinquefasciatus*, bioinsecticide, probit analysis

Graphical Abstract:



Introduction

Mosquito-borne illnesses such as Dengue fever, Zika virus disease, and Chikungunya continue to pose serious threats to public health, particularly in tropical and subtropical regions. It was anticipated that close to half of the world's population would be at risk of arboviral infections by the year 2050 (Kraemer *et al.*, 2019). Despite ongoing attempts to control mosquitoes, the

spread of mosquito-borne diseases continues to endanger the health of billions of people globally and limit economic development (Sarma *et al.*, 2019).

Among various control strategies, targeting mosquito larvae is considered one of the most effective approaches, as larvae are confined to aquatic habitats and are less mobile compared to adults (Fillinger and Lindsay, 2011; Pavela, 2015). Consequently, larval control plays a crucial role in reducing mosquito populations and interrupting disease transmission (Yacoob *et al.*, 2013). The genus *Culex* is one of the most widespread mosquito groups and serves as a vector for several diseases, including Japanese encephalitis, West Nile fever, and filariasis (Soh and Aik, 2021; Madhav *et al.*, 2024). Species such as *Culex quinquefasciatus* are commonly found in urban and semi-urban environments, where favorable ecological conditions promote their proliferation (Bakran-Lebl *et al.*, 2023).

Since vaccines are unavailable for most mosquito-borne diseases, effective mosquito control measures are essential for reducing disease transmission (Valarmathi *et al.*, 2022). Chemical control management using pyrethroids and organophosphates is often challenged by high costs, limited community adoption, and slow operational implementation (Moyes *et al.*, 2017; Weetman *et al.*, 2018). Furthermore, the continued use of Chemical repellents such as N,N-diethyl-meta-toluamide, Picaridin, Ethyl butylacetylaminopropionate, and Permethrin has resulted in the biological magnification of toxic substances through the food chain due to their slow degradation, resulting in negative effects on environmental quality and non-target organisms, including humans (Liu *et al.*, 2018) such as skin irritation, neurological effects and respiratory problems. Furthermore, its extensive usage reduces biodiversity and disrupts ecosystems. However, traditional mosquito control methods are becoming less successful due to the increasing resistance of mosquito populations to chemical pesticides (Sonali *et al.*, 2023). This rising resistance highlights the urgent need for innovative and long term solutions to effectively control mosquito populations while minimizing environmental and health risks (Onen *et al.*, 2023).

Among existing options, the use plant derived pesticides is considered one of the most promising and ecologically acceptable approaches (Dubey, 2011; Subramaniyan *et al.*, 2021). Plant metabolites can influence insect behavior by disrupting feeding activity, repelling pests, interfering with physiological processes like molting, reducing fecundity, inhibiting respiration and growth and disrupting cuticle formation (Pavela, 2011; Attia *et al.*, 2013; Benelli *et al.*, 2013). Plant based insecticide are considered as environmentally friendly as they are biodegradable and pose a lower risk of harmful effects on non-target organisms and ecosystems (Pavela *et al.*, 2013; Pavela, 2014).

Fabaceae (Leguminosae) is one of the largest and most diverse groups, known for its rich phytochemical profile, including flavonoids, alkaloids, tannins, saponins, and fatty acids. Particularly, the seeds of Fabaceae plants have been reported to possess significant bioactive compounds. Several studies have demonstrated that seed extracts of plants like *Millettia pinnata*, *Cassia tora*, and *Delonix elata* show effective larval mortality, highlighting their potential as natural insecticides. Therefore, exploring Fabaceae seed extracts for mosquito larvicidal activity offers a promising approach toward developing cost-effective, biodegradable, and environmentally safe vector control strategies (Murugan *et al.*, 2015). Hence this current study aimed to explore the mosquito larvicidal activity of ethanolic seed extract of *Gliricidia sepium*, *Adenanthera pavonina*, *Bauhinia tomentosa*, *Bauhinia purrpurea* and *Leucaena leucocephala*.

Materials and methods

Collection and Identification of Plant Material

Fresh and mature seeds of selected Fabaceae plant species *Gliricidia sepium*, *Adenanthera pavonina*, *Bauhinia tomentosa*, *Bauhinia purrpurea* and *Leucaena leucocephala* were collected during January 2026 from different locations in Coimbatore district, Tamil Nadu, India. The collected seeds were taxonomically identified and brought to the laboratory for further processing. The seed materials were thoroughly washed under running tap water followed by distilled water to remove dust, debris, and surface contaminants. The cleaned seeds were shade-dried at room temperature for several days until complete drying was achieved. After drying, the seeds were mechanically ground into a fine powder using a laboratory grinder. The powdered seed samples were stored in airtight containers at room temperature and used for subsequent extraction and larvicidal studies.

Extraction of seed

Fifty grams of powdered seed material from selected Fabaceae plant species were used for extraction. The powdered samples were subjected to extraction using 250 mL Ethanol employing the maceration method. The mixtures were kept at room temperature for 72 hours with intermittent shaking to ensure proper extraction of bioactive compounds. After the extraction period, the samples were filtered using Whatman No. 1 filter paper to remove solid residues. The filtrates were then concentrated under reduced pressure using a rotary vacuum evaporator to obtain crude extracts. The concentrated extracts were further air-dried until a thick, viscous paste or dry extract was obtained. The dried crude extracts were collected and used to prepare a 1% stock solution

using the respective solvents. From the stock solution, different concentrations such as 0.625%, 1.25%, 2.5%, and 5% were prepared by diluting with distilled water for larvicidal bioassay.

Collection of Mosquito Larvae

Larvae of *Culex quinquefasciatus* were collected from stagnant water sources such as drains, sewage water, and temporary water collections in Coimbatore district, Tamil Nadu, India, during the study period. Larvae were collected using a standard dipper and transferred into clean plastic containers containing the same habitat water to avoid stress during transportation. The collected larvae were brought to the laboratory and maintained temporarily under room temperature conditions. They were used for larvicidal bioassays within 24 hours of collection.

Larvicidal activity:

The larvicidal activity of crude seed extracts of selected Fabaceae plants was evaluated following the standard protocol recommended by World Health Organization 2005.

Larvae of *Culex quinquefasciatus* were collected from stagnant water sources and used for the bioassay. Only healthy third and fourth instar larvae were selected for the experiment. Different concentrations of the plant extracts (0.625%, 1.25%, 2.5%, and 5%) were prepared from the stock solution. For each concentration, ten larvae were introduced into 100 mL plastic cups containing 99mL of water, and 1 mL of the respective extract was added to obtain the desired concentration. A control was maintained simultaneously with 99mL of water and 1 mL of solvent (without plant extract). Each experiment was conducted under laboratory conditions at room temperature. Larval mortality was recorded after 24, 48, and 72 hours of exposure. Larvae were considered dead if they showed no movement when probed gently.

The percentage mortality was calculated using the formula:

$$\text{Mortality \%} = \text{Number of dead larvae} \div \text{Number of larvae introduced} \times 100$$

Statistical analysis:

The obtained data were subjected to log–probit regression analysis to determine LC₅₀ and LC₉₀ values along with 95% confidence limits. Statistical analysis was carried out using IBM SPSS Statistics (Version 23). Larval mortality data were expressed as mean ± standard deviation (SD) of three independent experiments (n = 3). Lethal concentration (LC₅₀) represents the concentration of the test substance required to cause 50% mortality of the test organisms within a specified exposure period, while LC₉₀ represents the concentration required to cause 90% mortality. These

values were calculated using probit analysis. The goodness of fit of the probit model was assessed using the chi-square (χ^2) test, and p-values greater than 0.05 were considered to indicate an acceptable fit of the model.

Results

The larvicidal activity of ethanol extracts of selected fabaceae seeds was evaluated against *Culex quinquefasciatus* at different concentrations and exposure time. The bioassay results of *Gliricidia sepium*, *Adenanthera pavonina*, *Bauhinia tomentosa*, *Bauhinia purrpurea* and *Leucaena leucocephala* demonstrated a clear dose-dependent and time dependent increase in mortality across all treatments. Mortality percentages increased progressively with increasing concentration (0.625, 1.25, 2.5 and 5) and exposure time (24, 48 and 72 hours).

At 0.625% and 1.25% lower concentrations, mortality percent was minimal at 24 hours but increased moderately at 48 and 72 hours. Conversely, higher concentrations (2.5% and 5%) showed significant mortality even at 24 hours, which further increased with time. The positive control showed 100% mortality across all time intervals, confirming the validity of experimental conditions. In NC (Negative Control) 0% mortality was recorded confirming that the observed larvicidal activity was due to the plant extract (**Table 1**).

At 5% highest concentration more over all plant shows maximum larvicidal activity. Among the tested plants, *Bauhinia tomentosa* exhibited the highest larvicidal activity, showing the maximum mortality of 83.4% at 5% concentration at 72 hours exposure, followed by *Leucaena leucocephala* 76.7% and *Adenanthera pavonina* 66.7%. Moderate activity was recorded in *Bauhinia purrpurea* 56.7% and *Gliricidia sepium* 36.7% at the same concentration and exposure time.

At lowest concentration 0.625%, some plant extract such as *Bauhinia tomentosa* and *Leucaena leucocephala* showed noticeable larval mortality even at 24 hours, indicating their effectiveness at low doses. This effect further increased with exposure time at 72 hours the mortality recorded was around 26.7% in *Bauhinia tomentosa* at same low concentration. Whereas other plants showed little or no effect at this stage at 24 hours and effectiveness observed over prolonged exposure.

Table 1: Percentage mortality of *Culex quinquefasciatus* larvae exposed to Ethanol extracts of selected Fabaceae seeds at different concentration and exposure periods

Plant species	Concentration (%)	24 hours (%)	48 hours (%)	72 hours (%)
<i>Gliricidia sepium</i>	0.625	0±0	0±0	3.4±0.5
	1.25	0±0	0±0	6.7±1.1
	2.5	6.7±0.5	16.7±1.5	23.4±1.1
	5	13.3±1.5	26.7±0.5	36.7±0.5
<i>Adenanthera pavonina</i>	0.625	0±0	3.3±0.5	20±1
	1.25	3.4±0.5	13.4±1.1	30±1
	2.5	10±0	26.7±0.5	43.4±0.5
	5	23.4±0.5	60±1	66.7±0.5
<i>Bauhinia tomentosa</i>	0.625	10±1	20±1	26.7±0.5
	1.25	10±1	33.4±1.5	46.7±0.5
	2.5	40±1	50±1	70±1
	5	56.7±0.5	73.4±0.5	83.4±0.5
<i>Bauhinia purrpurea</i>	0.625	0±0	6.7±1.1	13.4±0.5
	1.25	0±0	13.4±0.5	23.4±1.1
	2.5	6.7±0.5	30±1	36.7±1.1
	5	16.7±0.5	43.4±0.5	56.7±0.5
<i>Leucaena leucocephala</i>	0.625	3.4±0.5	6.7±1.1	16.7±0.5
	1.25	6.7±0.5	26.7±0.5	40±1
	2.5	33.4±2	46.7±2	63.4±1.1
	5	43.4±1.1	56.7±0.5	76.7±1.1
PC	5	100±0	100±0	100±0
NC	5	0±0	0±0	0±0

Values are expressed as Mean±Standard deviation (SD) of three independent experiments (n=3)

PC- Positive control; NC- Negative Control

At 1.25% and 2.5% concentration a clear increase in larval mortality was observed in all exposure periods. Mortality was relatively low at 24 hours but increased at 48 and 72 hours. This indicates that the larvicidal compounds present in the extracts require sufficient exposure time to exert their full toxic effect.

Table 2: Probit analysis showing LC₅₀ and LC₉₀ values, confidence limits, Slope, Chi-square and p-value of ethanol extracts of selected Fabaceae seeds against *Culex quinquefasciatus* larvae at different exposure periods

Plant Species	Exposure Time (hours)	LC 50 (%) (LCL-UCL)	LC 90(%) (LCL-UCL)	Slope±SE	X ²	p- value
<i>Gliricidia sepium</i>	24	8.178 (5.95-26.65)	12.1 (8.23-46.43)	0.326±0.13	1.804	0.406
	48	6.366 (5.08-10.22)	9.84 (7.45-17.87)	0.368±0.104	5.033	0.081
	72	5.83 (4.56-9.46)	10.06 (7.46-18.52)	0.303±0.083	1.905	0.386
<i>Adenanthera pavonina</i>	24	7.069 (5.41-13.49)	11.01 (7.95-23.92)	0.325±0.104	1.077	0.584
	48	4.263 (3.55-5.47)	7.39 (6.01-10.37)	0.410±0.081	0.972	0.615
	72	3.323 (2.47-4.82)	7.94 (5.94-13.81)	0.277±0.072	0.261	0.878
<i>Bauhinia tomentosa</i>	24	4.18 (3.38-5.69)	7.935 (6.22-12.12)	0.342±0.076	3.08	0.214
	48	2.831 (2.07-3.85)	6.885 (5.33-10.88)	0.316±0.07	0.66	0.719
	72	1.737 (0.84-2.44)	5.411 (4.24-8.22)	0.349±0.08	2.58	0.274
<i>Bauhinia purrpurea</i>	24	7.471 (5.71-16.75)	10.965 (7.86-28.71)	0.367±0.13	1.537	0.464
	48	5.297 (4.08-8.79)	10.02 (7.31-19.26)	0.271±0.07	1.561	0.458
	72	4.179 (3.21-6.43)	8.94 (6.6-16.32)	0.269±0.07	0.618	0.734
<i>Leucaena leucocephala</i>	24	5.067 (4.07-7.33)	8.931 (6.8-14.56)	0.332±0.08	4.96	0.081
	48	3.888 (3.02-5.6)	8.268 (6.26-13.95)	0.293±0.07	5.18	0.075
	72	2.374 (1.63-3.16)	6.080 (4.79-9.11)	0.346±0.07	4.19	0.123

LC₅₀ and LC₉₀- lethal concentration; LCL-Lower Confidence limit; UCL-Upper Confidence limit; X² – Chi-square; p>0.05 indicates good model fit.

Probit analysis (Table 2) further supported these findings, as the LC₅₀ and LC₉₀ values decreased with increasing exposure time for all plant extracts, indicating enhanced toxicity over

time. The plant extract with the lowest LC_{50} and LC_{90} value exhibited the highest larvicidal activity. Among the tested plants, *Bauhinia tomentosa* showed the lowest LC_{50} value (1.737, 2.831 and 4.180) at all exposure time (24, 48 and 72 hours), suggesting it is the most potent larvicidal agent. The chi-square values were low and the p-values were greater than 0.05, indicating a good fit of the probit model and reliability of the results (Rui et al., 2024).

Discussion

The use of biologically active plant-based products and phytochemicals with insecticidal properties has attracted considerable interest worldwide due to their biodegradable nature and relative safety to humans and non-target organisms. Extensive studies have been carried out to identify plant extracts effective against mosquito vectors, especially due to restrictions on synthetic chemical pesticides. Similar findings have been reported by various authors (Pavela, 2015; Benelli et al., 2016).

The regular use of chemical insecticides in the control of *Ae. aegypti* can result in insecticide resistance, environmental pollution, and hazards to human and other organisms (AlShebly et al., 2017). On the other hand, the high cost, increasing multidrug resistance, and side effects of chemotherapeutic agents limit the use of these in the cancer treatment (Gautam et al., 2014). In this way, these problems direct the search for alternative larvicidal. As it demonstrated that, plant essential oils are potential sources of larvicidal compounds, including those from *Bauhinia* species (Gois et al., 2011; de Sousa et al., 2016).

The current study demonstrated that the ethanol extracts of selected Fabaceae seeds possess substantial larvicidal activity against larvae *Culex quinquefasciatus*. The results clearly revealed that larval mortality increased with increasing concentration and exposure time, indicating a dose-dependent and time-dependent impact. This pattern is consistent with earlier reports on plant-based larvicidal activity, where higher concentrations and longer exposure periods resulted in increased mortality of mosquito larvae (Pavela, 2015).

At lower concentrations (0.625%), certain plant extracts such as *Bauhinia tomentosa* and *Leucaena leucocephala* exhibited noticeable larvicidal activity even at 24 hours ($LC_{50} \leq 5\text{ppm}$),

and the mortality increased further at 72 hours, reaching up to approximately 26.7% in *Bauhinia tomentosa* ($LC_{50} \leq 1\text{ppm}$). This suggests that even at low concentrations, prolonged exposure enhances the toxic effect of the plant extracts. Similar findings have been reported in previous studies, where plant extracts showed gradual larvicidal effects over time due to cumulative toxicity (Noorazlan *et al.*, 2024).

Among the tested plants, *Bauhinia tomentosa* exhibited the highest larvicidal activity, as indicated by its highest percentage mortality ($83.4\% \pm 0.5$) and lowest LC_{50} (LCL-UCL) value 1.737 (0.84-2.44), followed by *Leucaena leucocephala* and *Adenanthera pavonina*. The higher efficacy of *Bauhinia tomentosa* may be attributed to the presence of potent bioactive compounds. Members of the genus *Bauhinia* have been previously reported to possess significant larvicidal properties against *Aedes aegypti* mosquito larvae (Silva *et al.*, 2020). In contrast, *Gliricidia sepium* showed comparatively lower activity, which may be due to differences in phytochemical composition.

The larvicidal activity observed in the present study may be attributed to the presence of secondary metabolites such as alkaloids, flavonoids, tannins, and saponins, which are known to exhibit insecticidal activities. These phyto-compounds may interfere with the physiological and metabolic processes of mosquito larvae, leading to their mortality. Similar mechanisms have been reported in earlier studies on plant-derived larvicides (Baz *et al.*, 2024).

Probit analysis confirming the findings, as the LC_{50} and LC_{90} values decreased with increasing exposure time, indicating increased toxicity over time. The plant extract with the lowest LC_{50} value showed the highest larvicidal activity, confirming its effectiveness against larvae. The chi-square values were low and the p-values were greater than 0.05, indicating a good fit of the probit model and reliability of the experimental data (Ruiu *et al.*, 2024).

The positive control showed 100% mortality, while no mortality was observed in the negative control, confirming that the observed larvicidal activity was due to the plant extracts and not external factors. Despite numerous studies on plant-based larvicides, there are limited reports on the larvicidal activity of seeds of selected Fabaceae species. Therefore, the present study provides new insights into their potential as eco-friendly mosquito control agents. Overall, the

findings of this study are in agreement with previous reports and suggest that Fabaceae seed extracts *Gliricidia sepium*, *Adenanthera pavonina*, *Bauhinia tomentosa*, *Bauhinia purpurea* and *Leucaena leucocephala*, particularly *Bauhinia tomentosa*, have strong potential as eco-friendly and effective alternatives for mosquito larval control.

Conclusion

The present study confirms that ethanol extracts of selected Fabaceae seeds—*Gliricidia sepium*, *Adenanthera pavonina*, *Bauhinia tomentosa*, *Bauhinia purpurea*, and *Leucaena leucocephala* exhibit significant larvicidal activity against *Culex quinquefasciatus*. Larval mortality increased with increasing concentration and exposure time, indicating a clear dose- and time-dependent response. Among the tested species, *Bauhinia tomentosa* showed the highest larvicidal efficacy, followed by *Leucaena leucocephala* and *Adenanthera pavonina*, while *Bauhinia purpurea* and *Gliricidia sepium* exhibited comparatively moderate activity. The progressive decrease in LC_{50} and LC_{90} values with increasing exposure time further confirms the enhanced effectiveness of the seed extracts. Notably, certain extracts demonstrated measurable larvicidal activity even at lower concentrations upon prolonged exposure, suggesting cumulative toxic effects. As studies focusing specifically on the larvicidal potential of Fabaceae seed extracts are limited, the present investigation provides valuable baseline data. Overall, these findings indicate that the tested plant extracts, particularly *Bauhinia tomentosa*, have strong potential as environmentally friendly, biodegradable, and cost-effective alternatives to synthetic insecticides. However, further studies are required to isolate active compounds, elucidate their mechanisms of action, and evaluate their efficacy under field conditions.

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