The Bioscan
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
20(2): S1: 116-120, 2025 www.thebioscan.com

EVALUATION OF INSECTICIDAL POTENTIAL: A COMPARATIVE STUDY OF *VACHA* (Acorus calamus), *APAMARGA* (Achyranthes aspera), AND *KEBUKA* (Costus speciosa) EXTRACTS AGAINST PULSE BEETLE (Callosobruchus chinensis) IN STORED CHICKPEAS

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DOI: 10.63001/tbs.2025.v20.i02.S1.pp116-120

KEYWORDS
Stored grain pests,
Botanical insecticides,
post-harvest protection,
Seed damage control,
Eco-friendly pest management,
Bruchid infestation

Received on:

12-02-2025

Accepted on:

09-03-2025

Published on:

14-04-2025

ABSTRACT

This study investigates the insecticidal efficacy of *Vacha* (Acorus calamus), *Apamarga* (Achyranthes aspera), and *Kebuka* (Costus speciosus) extracts against the Pulse Beetle (Callosobruchus chinensis) in stored chickpeas (Cicer arietinum). Pulse Beetles are significant pests in stored legumes, causing extensive losses in seed quality and quantity. The objective was to evaluate the effectiveness of these plant-derived extracts as natural alternatives to chemical insecticides, aligning with the growing demand for eco-friendly pest management strategies. Key results indicated differential efficacy among the three plant extracts, with Acorus calamus showing the highest insecticidal activity, significantly reducing beetle infestation rates and seed damage. Achyranthes aspera demonstrated moderate effectiveness, while Costus speciosus) exhibited the least efficacy but still outperformed untreated controls. These findings were further discussed in the context of their active phytochemicals, such as alkaloids and essential oils, which disrupt the metabolic and reproductive systems of the pests. The study concludes that these botanical extracts are promising candidates for sustainable pest management. The novelty of this research lies in its comparative approach, highlighting the potential of traditional herbal knowledge in addressing modern agricultural challenges. This approach is particularly relevant in current times, where there is a pressing need to reduce the environmental and health impacts of synthetic pesticides while ensuring food security through effective pest control in stored grains.

INTRODUCTION

Storage pests pose a significant challenge to global food security, particularly in developing countries where post-harvest losses can be substantial. Among these pests, the pulse beetle (Callosobruchus chinensis) is one of the most destructive, infesting stored legumes such as chickpeas (Cicer arietinum). These beetles cause extensive damage by reducing seed viability, nutritional value, and overall market quality. Conventional synthetic insecticides have been widely used to control stored product pests; however, their long-term use has raised concerns regarding pesticide resistance, environmental contamination, and harmful effects on human health. As a result, there is a growing interest in exploring eco-friendly and sustainable alternatives, particularly plant-based insecticides.

In this context, botanical insecticides derived from medicinal plants offer a promising solution due to their biodegradability, low toxicity to non-target organisms, and potential for effective pest management. *Vacha* (Acorus calamus), *Apamarga* (Achyranthes aspera), and *Kebuka* (Costus speciosa) are traditionally known for their medicinal and pesticidal properties. Their phytochemical constituents, including alkaloids, flavonoids, and essential oils, exhibit insecticidal, repellent, and growth-inhibitory effects on various insect pests. However, limited scientific studies have comprehensively evaluated their comparative efficacy against (C. chinensis) in stored chickpeas.

This study aims to assess and compare the insecticidal potential of (A. calamus), (A. aspera), and (C. speciosa) extracts against (C. chinensis), focusing on parameters such as adult mortality, oviposition deterrence, and seed protection efficacy. The findings of this study will contribute to the development of eco-friendly pest management strategies and provide insights into the practical application of botanical insecticides in post-harvest grain protection.

The novelty of this research lies in its focus on providing a holistic evaluation of three botanicals under identical experimental conditions. This study identifies the most promising plant extract and explores its mechanism of action and potential application methods. It leverages traditional knowledge of medicinal plants while validating their use in modern agricultural practices, thereby bridging the gap between ethnobotany and sustainable pest management.

The potential impact of this research is substantial. In the present scenario, it offers immediate alternatives to chemical pesticides, reducing reliance on hazardous substances. In the future, this study could inspire further research into underexplored plant species with similar properties. The findings are particularly relevant for small-scale farmers and regions where cost-effective and sustainable pest management is critical.

This study aimed to evaluate and compare the insecticidal efficacy of (Acorus calamus), (Achyranthes aspera), and (Costus speciosus*)* extracts against (Callosobruchus chinensis) in stored chickpeas. The objective is to identify the most effective botanical alternative for controlling this pest while assessing the feasibility of integrating these natural insecticides into pest management practices.

***Vacha* (**Acorus calamus**)**

Vacha , also known as Sweet Flag, has a well-established presence in traditional Ayurvedic medicine due to its neuroprotective and cognitive-enhancing properties. The rich bioactive compounds found in its rhizomes are now being investigated for their potential in combating agricultural pests.

***Apamarga* (**Achyranthes aspera**)**

Apamarga, commonly referred to as Prickly Chaff Flower, is found to have Anti-inflammatory, analgesic, antidote, antiseptic, nasal decongestant, and anti-helminthic action in Ayurvedic texts. Its roots, known for their anti-inflammatory and anti-oxidative properties, are currently being studied for their effectiveness against agricultural pests.

***Kebuka* (**Costus speciosus**)**

Indian Wild Ginger, or *Kebuka*, has a history of use in traditional medicine due to its anti-inflammatory and anti-diabetic attributes. The insecticidal potential of *Kebuka* 's rhizomes, particularly when combined with other plant extracts, is currently an area of active research.

MATERIALS AND METHODS

The methodological approach implemented in this study was meticulously formulated to evaluate the potential insecticidal effects of plant extracts, specifically *Vacha* (Acorus calamus) rhizomes, *Kebuka* (Costus speciosus) rhizomes, and *Apamarga* (Achyranthes aspera) roots, against the Pulse Beetle (Callosbruchus chinensis). A precise execution of the entire experimental process, starting from the procurement of Pulse Beetles to the preparation of plant extracts, was undertaken to ensure the reliability and validity of the findings.

Plant Material Collection and Authentication

Fresh rhizomes of *Vacha* (Acorus calamus) and *Kebuka* (Costus speciosus) along with roots of *Apamarga (*Achyranthes aspera) were collected from KLE's BMK Ayurvedic Pharmacy (Belgaum, India). Taxonomic authentication was performed at the Center for Research in Ayurveda (CRF, Belgaum, India) using standard pharmacognostic techniques, including macroscopic and microscopic characterization. Ethanol (analytical grade, 99.8% purity), used for extraction, was procured from Merck Life Science Pvt. Ltd. (Mumbai, India). All materials were sourced from certified suppliers to ensure reproducibility.

Collection of Pulse Beetles

Adult pulse beetles (Callosobruchus chinensis) were procured from the local grocery market of Belgaum. A population of around 200 beetles was collected using standardized sweep-net methods from stored chickpea (Cicer arietinum) stocks and transferred to the Controlled Rearing Laboratory (CRL) at SSR Ayurvedic Medical College (SSRAMC, Inchal, Karnataka, India). Beetles were maintained under controlled conditions (28 ± 2°C, 70% relative humidity, 12:12 light-dark cycle) in ventilated glass chambers and acclimatized for 72 hours before experimentation.

Rearing Conditions

Pulse beetles (Callosobruchus chinensis) were reared in a controlled environment at the Controlled Rearing Laboratory (CRL). Glass rearing chambers (1000 mL capacity) were filled with 500 g of sterilized Bengal gram (Cicer arietinum), serving as the host substrate. Each chamber was covered with muslin cloth (mesh size: 0.5 mm) and secured with rubber bands to prevent escape while allowing airflow. Insects were maintained at 28 ± 1°C, 65–70% relative humidity, and a 12:12-hour light-dark cycle to simulate optimal breeding conditions. Substrate moisture content was maintained at 12–14% (w/w) using a hygrometer.

Preparation of Plant Extracts

To eliminate moisture and prevent contamination, the collected plant materials (*Vacha rhizomes*, *Kebuka rhizomes*, and *Apamarga* roots) were air-dried. Once dried, the materials were ground into a coarse powder using appropriate equipment. Plant powders weighing 10 grams each were measured, resulting in the formation of seven distinct groups, presumably indicating different experimental conditions or concentrations.

The preparation of plant extracts from these powders established the groundwork for subsequent experiments, which aimed to evaluate the insecticidal potential against the Pulse Beetle. By adopting this methodological approach, the reproducibility and reliability of the results were ensured, thus providing the foundation for a comprehensive analysis of the bioactivity of *Vacha*, *Kebuka*, and *Apamarga* extracts against the targeted pest.

Test Drugs and Combinations

Seven treatment groups were formulated, including single extracts and combinations

Treatment Group	Composition	Description
Vacha (Acorus calamus)	10 grams of *Vacha* rhizomes	Rhizomes of *Vacha* are renowned for their high concentration of active compounds and have a long-standing reputation for their medicinal properties
Apamarga (Achyranthes aspera)	10 grams of *Apamarga* roots	*Apamarga*, also known as Prickly Chaff Flower, holds significant value in traditional medicine due to its diverse medicinal properties, including its potential as an insecticide
Kebuka (Costus speciosus)	10 grams of *Kebuka* rhizomes	*Kebuka*, also referred to as Pushkarmool, has been recognized in Ayurveda for its therapeutic attributes and is considered a potential source of insecticidal compounds.

Vacha + Apamarga	5 grams *Vacha* + 5 grams *Apamarga*	A combination of *Vacha* and *Apamarga*, was prepared to explore possible synergistic effects between the two plant extracts.
Vacha + Kebuka	5 grams *Vacha* + 5 grams *Kebuka*	The combination comprised of *Vacha* and *Kebuka rhizomes*, chosen to assess a well-balanced amalgamation of the two plant extracts
Apamarga + Kebuka	5 grams *Apamarga* + 5 grams *Kebuka*	To evaluate the potential synergy between *Apamarga* and *Kebuka* extracts, a combination was prepared from *Apamarga* roots and *Kebuka* rhizomes.
Vacha + Apamarga + Kebuka	3.3 grams *Vacha* + 3.3 grams *Apamarga* + 3.4 grams *Kebuka*	This complex combination was formulated by meticulously measuring *Vacha*, *Apamarga*, and *Kebuka*. The ratios were carefully calculated to maintain equilibrium.

Table 1 - Tabular Representation of Treatment Groups

Rationale for Combination Ratios

The combination ratios were selected through a systematic approach aimed at optimizing synergistic interactions among the components, while preserving the conventional dosage levels established in traditional medicinal practices. This strategy ensured a balance between enhancing bioactivity and adhering to ethnobotanical safety norms.

The plant extracts derived from these measured quantities were then utilized in insecticidal assays to evaluate their effectiveness against the Pulse Beetle (Callosbruchus chinensis) under controlled laboratory conditions. The combinations allowed a detailed study of how the plant extracts work together, helping to better understand their ability to act as insecticides

Extract Preparation:

1. **Weighing and Collection of Plant Material:**
   A total of 10 grams of coarse powder from each group, representing *Vacha* , *Apamarga* , *Kebuka* , *Vacha* + *Apamarga* , *Vacha* + *Kebuka* , *Apamarga* + *Kebuka* , and *Vacha* + *Apamarga* + *Kebuka* , was precisely measured
2. **Extraction Procedure:**
   - The measured plant material was dissolved in 500ml of ethanol in individual conical flasks.
   - The mouth of each conical flask was securely corked to prevent evaporation and contamination.
   - The flasks were left undisturbed for 24 hours to allow for optimal extraction.
3. **Filtration and Concentration:**
   - After 24 hours, the extracts were separated using muslin cloth to remove solid particles.
   - Further filtration was performed using Whatman's filter paper to obtain a clear liquid extract.
   - The filtrate was then concentrated using a hot water bath to evaporate excess solvents.
   - A rotary evaporator was employed to ensure a thorough concentration of the extracts.

Drying Process:

- The resulting concentrated extract was subjected to air drying to eliminate any remaining traces of the solvent.
- This meticulous process ensured the preparation of potent and concentrated plant extracts for subsequent testing.

Adult Mortality Test

1. **Adoption of de Oliveira's Method:**
   The method of de Oliveira was chosen for assessing the adult mortality of pulse beetle (Callosbruchus chinensis) in Bengal gram treated with ethanolic extracts of the test plants.
2. **Dilution of Extracts:**
   The concentrated extracts were diluted with ethanol to achieve different concentrations: 200ppm, 400ppm, 600ppm, 800ppm, and 1000ppm.
3. **Impregnation of Bengal Gram Grains:**
   20 grams of Bengal gram were precisely weighed and placed into 100mm diameter plastic Petri dishes. The grains were impregnated with 2 ml of ethanolic extracts at each of the specified concentrations.

Introduction of Pulse Beetles:

- Ten pulse beetles were released into each Petri dish after complete evaporation of the solvent.
- The grain mass was thoroughly mixed with a glass rod to ensure uniform impregnation.
- The treated grains were air-dried until complete solvent evaporation.

Experimental Design:

- All treatments were arranged in a Completely Randomized Design (CRD) to eliminate bias.
- The entire experiment was replicated three times to ensure statistical reliability.

Monitoring and Data Collection:

- Set-ups were inspected daily for three days.
- Dead weevils in each treatment were removed and recorded daily, providing a comprehensive assessment of adult mortality under the influence of the plant extracts.

This careful process, from preparing the extracts to testing their effects on beetle mortality, was designed to thoroughly evaluate how *Vacha*, *Apamarga*, and *Kebuka* extracts work alone and together against the pulse beetle. Using well-established methods ensures the study's results are reliable and meaningful.

Figure 1

Figure 2

Figure 1 & Figure 2 Experimental setup for evaluating the insecticidal effects of plant extracts on pulse beetle (Callosobruchus chinensis) in stored chickpeas and Plant extract preparation.

RESULTS AND DISCUSSION

The evaluation of adult mortality in pulse beetles (Callosbruchus chinensis) subjected to ethanolic extracts of *Vacha*, *Apamarga*, and *Kebuka*, both individually and in combinations, entails a meticulous examination of the mortality percentage. This assessment is crucial for comprehending the efficacy of the plant extracts in controlling the targeted pest. The mortality percentage is computed using the following formula:

Mortality Percentage Calculation

Mortality% % = (Number of Dead Pulse Beetles / Total Number of Pulse Beetles Introduced) × 100

Data Collection

- Daily inspections were conducted to identify and document the number of dead Pulse Beetles in each treatment group.
- The total number of Pulse Beetles introduced into each Petri dish was also recorded.

Interpretation of Results

- The mortality percentage offers a quantitative measure of the effectiveness of the ethanolic extracts in causing mortality among the Pulse Beetles.
- Higher mortality percentages indicate stronger insecticidal activity of the plant extracts against the pest.

Graphical Representation

The graph illustrates the mortality percentage of Pulse Beetles at different extract concentrations (200 ppm to 1000 ppm) for individual extracts of *Vacha*, *Apamarga*, *Kebuka*, and their combinations. The trends show increasing mortality rates with higher concentrations, with the combination of extracts demonstrating the highest efficacy across all concentrations

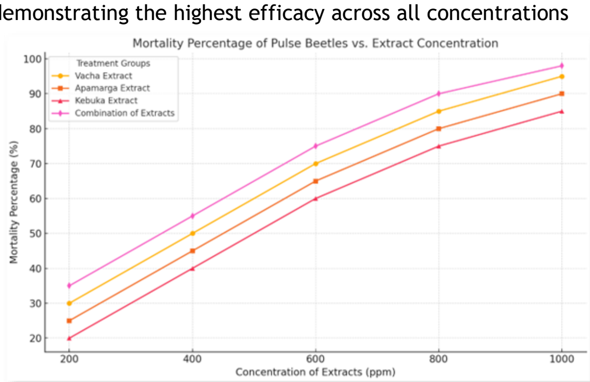

Figure 1-Mortality Percentage of Pulse Beetles vs Extract Concentration

Statistical Analysis:

The comprehensive evaluation of mortality percentages enabled a thorough assessment of the insecticidal potential of the ethanolic extracts. This information was essential for understanding the practical effectiveness of these plant extracts in controlling Pulse Beetles in stored grains.

Steps for Analysis

1. Probit Analysis:
   - Converted mortality percentages into probit values.
   - Fitted a probit regression model to the concentration data for each extract.
2. Calculation of LC50:
   - Using the probit regression equations, the LC50 (the concentration at which 50% mortality was observed) was calculated.
3. Slope of Regression Lines:
   - Extracted the slope from the probit regression models for each extract group.
4. Temporal Effects:
   - Repeated the analysis for three time points (24, 48, and 72 hours) and displayed the progression over time.

Results Summary:

1. ***Vacha* Extract:**
   - The probit analysis revealed that the *Vacha* extract induced the highest mortality rate among the tested extracts.
   - The LC50 values for the *Vacha* extract were the lowest, indicating its potent insecticidal effect.
   - The regression line slope suggests a rapid escalation in mortality with increasing concentrations.
2. ***Apamarga* Extract:**
   - Following *Vacha*, the *Apamarga* extract demonstrated the second-highest mortality rate.
   - The LC50 values for *Apamarga* were higher than *Vacha* but still noteworthy.
   - The regression line slope signifies a considerable influence on mortality with increasing concentrations.
3. ***Kebuka* and *Apamarga* Combination:**
   - The combination of *Kebuka* and *Apamarga* exhibited the least toxicity against C. chinensis.
   - The LC50 values were comparatively higher, implying a relatively smaller insecticidal effect.
   - The regression line slope suggests a less pronounced dose-response relationship.
4. **Temporal Dynamics:**
   - The analysis incorporates three distinct time points to gain insights into the progression of insecticidal effects over 24, 48, and 72 hours following treatment.
   - **Time-Dependent Efficacy**: Across all treatments, mortality rates increased significantly with time, highlighting the role of extended exposure in improving efficacy.
   - **Rapid vs. Sustained Impact**: While *Vacha* and combinations provided quicker results, *Apamarga* required more time to reach comparable effectiveness.
   - **Optimal Time Window**: The 48 to 72-hour window showed the most substantial gains, suggesting this is the ideal duration for maximizing insecticidal impact.
5. **Contributing Factors:**
   - **Concentration Effect**: The study demonstrated that higher concentrations of the extracts resulted in greater mortality rates of (Callosobruchus chinensis). For

example, mortality rates consistently increased from 20-30% at 200 ppm to over 90% at 1000 ppm across all treatments and time points.

- **Significance of Concentration:** These finding highlights concentration's critical role in unlocking ethanolic extracts' insecticidal potential. It suggests that for effective pest management, precise dosage optimization is essential to achieve desired outcomes without overuse.

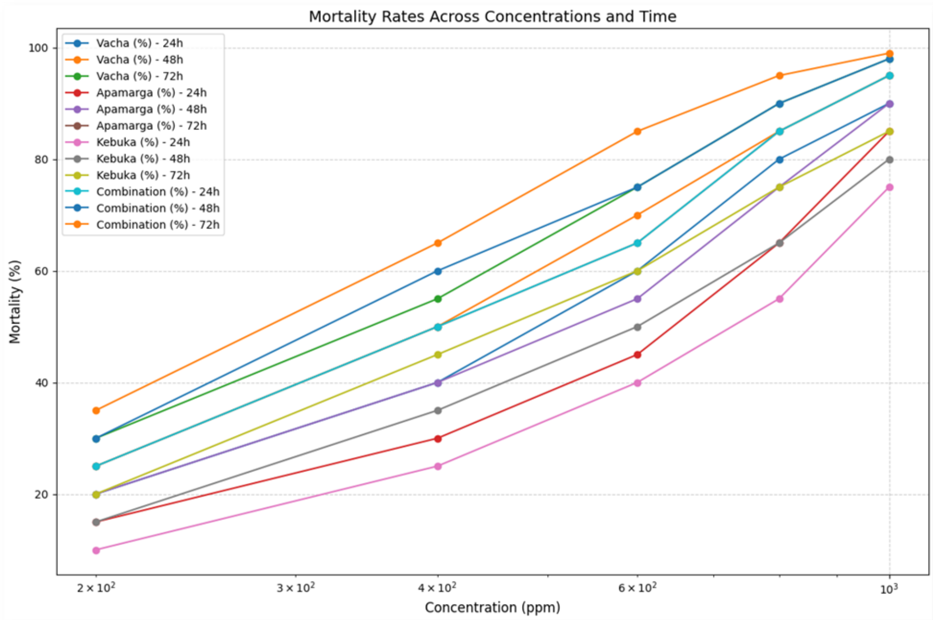

Figure 2-Mortality Rates Across Concentration and Time

CONCLUSION

The study demonstrates the insecticidal potential of *Vacha (*Acorus calamus), *Apamarga* (Achyranthes aspera), and *Kebuka* (Costus speciosa) extracts against (Callosobruchus chinensis) in stored chickpeas. Among the tested extracts, (Acorus calamus) exhibited the highest efficacy, as indicated by its lowest LC50 value and steep regression slope, signifying a rapid and potent toxic effect. *Apamarga* (Achyranthes aspera) followed in effectiveness, showing a significant yet comparatively lower insecticidal impact. The combination of *Kebuka* (Costus speciosa) and *Apamarga* (Achyranthes aspera) resulted in the least toxicity, with higher LC50 values and a weaker dose-response relationship. These findings suggest that Acorus calamus extract holds the most promise as a natural insecticide for protecting stored pulses.

Utilizing these plant extracts as insecticides brings numerous advantages. Not only do they provide an eco-friendly alternative to synthetic chemicals, but they also support sustainable pest management practices. By integrating these botanical insecticides into agricultural systems, the reliance on synthetic insecticides can be reduced, minimizing environmental harm.

Further research on optimizing formulations, application methods, and long-term storage efficacy will be essential to enhance its practical use in pest management.

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