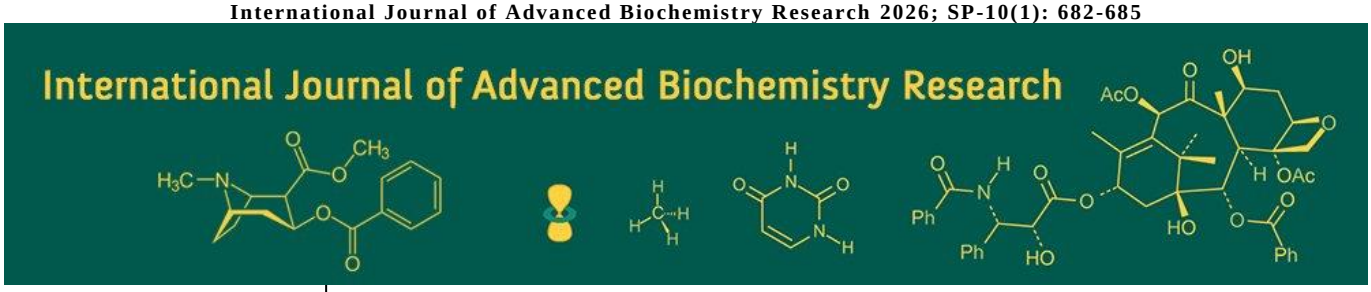


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# Custar apple seed powder as an effective alternatove for chemical pesticide

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**Abstract**
Botanical pesticides are gaining importance as alternatives to synthetic insecticides in crop protection. Custard apple seed extract (CASE) contains bioactive compounds that demonstrate insecticidal activity. This study evaluated the efficacy of CASE against *Myzus persicae* (green peach aphid) and *Plutella xylostella* (diamondback moth) under laboratory conditions. CASE was tested at four concentrations (3%, 5%, 10%, and 100%), and mortality was assessed at 24 and 72 hours after treatment. Results showed a clear dose-dependent mortality response, with complete mortality of *M. persicae* at 100% concentration and *P. xylostella* at 10% within 72 hours. These findings highlight the potential of CASE as a promising botanical insecticide for incorporation into integrated pest management (IPM) programs.

**Keywords:** *Annona squamosa*, biopesticides, *Myzus persicae*, *Plutella xylostella*, integrated pest management

## Introduction
Insect pests such as *Myzus persicae* and *Plutella xylostella* cause significant yield losses in vegetables and field crops. Conventional reliance on synthetic insecticides has resulted in multiple issues including resistance development, non-target toxicity, and environmental contamination. Botanical insecticides are considered sustainable alternatives due to their biodegradability and diverse modes of action. Among these, custard apple (*Annona squamosa* L.) seeds have been reported to possess strong insecticidal properties, attributable to bioactive compounds like acetogenins and alkaloids. Previous studies (Rajalakshmi & Mohan, 2014; Gajalakshmi *et al.*, 2017) [6, 3] have reported efficacy against sucking and chewing pests. This study aimed to evaluate the efficacy of custard apple seed extract against *M. persicae* and *P. xylostella* under laboratory conditions (Pavela, R. (2015) [5].

## Materials and Methods
Custard apple (*Annona squamosa*) seeds were collected, dried, and ground into fine powder. The seed extract was prepared using standard solvent extraction methods and diluted to obtain different concentrations (3%, 5%, 10%, and 100%). Laboratory bioassays were conducted against *Myzus persicae* and *Plutella xylostella* following leaf-dip and topical application methods. Mortality was recorded at 24-and 72-hours post-treatment. Each treatment was replicated five times, and control groups were maintained without treatment. Abbott's formula (Abbott, 1925) [1] was applied to correct mortality values.

### Preparation of Custard Apple Seed Extract (CASE)
Mature and healthy custard apple (*Annona squamosa*) fruits were collected from local sources. Seeds were separated manually, thoroughly washed, and shade-dried for 7-10 days. The dried seeds were pulverized using a mechanical grinder to obtain a fine powder. For extraction, 100 g of seed powder was soaked in 500 mL of ethanol (95%) for 48 hours at room temperature with intermittent shaking. The mixture was filtered through muslin cloth followed by Whatman No.1 filter paper. The filtrate was concentrated using a rotary evaporator at 40 °C under reduced pressure to yield a semi-solid crude extract. This crude extract was further diluted using distilled water to prepare different concentrations of the test solution: 3%, 5%, 10%, and 100% (undiluted crude extract). A distilled water treatment served as the negative control (Srinivasan, G., & Regupathy, A. (2017) [9]

**Test Insect Culture**

The diamondback moth (*Plutella xylostella*), a major cruciferous pest, was used for the bioassay. The colony was maintained under laboratory conditions (25±2 °C, 65±5% RH, and a photoperiod of 12:12 h L:D) on pesticide-free cabbage (*Brassica oleracea*) leaves. Second to third instar larvae were selected for the experiment, as they represent actively feeding stages crucial for bioefficacy testing.

**Leaf-Dip Bioassay Method**

The bioassay was conducted using the leaf-dip method. Fresh cabbage leaves were cut into uniform discs (approximately 5 cm in diameter) and dipped individually into the respective CASE treatments for 10 seconds. After treatment, the leaf discs were air-dried at room temperature to remove excess moisture and placed on moist filter paper inside sterile Petri plates (9 cm diameter). Ten larvae of second to third instar stage were introduced into each Petri plate.

Each treatment, including the control, was replicated five times (n = 5), with ten larvae per replicate, totaling 50 larvae per treatment. The experimental setup was maintained under controlled laboratory conditions as described earlier (Abbott, W. S. (1925) [1]

Custard apple seeds were shade-dried, ground, and extracted with ethanol. The crude extract was concentrated and diluted to 3%, 5%, 10%, and 100% concentrations. Laboratory bioassays were conducted against M. persicae and P. xylostella using leaf-dip and feeding assays. Mortality was assessed at 24 and 72 hours. Abbott's formula (Abbott, 1925) [1] was applied for corrected mortality. Data were analyzed using ANOVA, and treatment means were compared using Tukey's HSD test at $p<0.05$.

**Mortality Assessment**

Larval mortality was assessed 72 hours after treatment. Larvae were considered dead if they did not respond to gentle probing with a fine brush. The percentage of larval mortality was calculated using the following formula (Sahayaraj, K., & Kombiah, P. (2010) [8].

$$\text{Mortality (\%)} = \frac{\text{Number of dead larvae}}{\text{Total number of larvae}} \times 100$$

If mortality in the control treatment exceeded 5%, the observed mortality was corrected using Abbott's formula (Abbott, 1925) [1]:

$$\text{Corrected Mortality (\%)} = \frac{\text{T-C}}{\text{100-C}} \times 100$$

where:

- T = observed mortality in the treatment (%)
- C = observed mortality in the control (%)

**Data Analysis**

Mortality data were subjected to arcsine square root transformation to normalize variance before statistical analysis. One-way analysis of variance (ANOVA) was performed to determine significant differences among treatments, followed by Tukey's Honestly Significant Difference (HSD) test at a 5% significance level using statistical software (e.g., SPSS, R, or SAS).

**Results and Discussion**

The efficacy of CACE against *Myzus persicae* and *Plutella xylostella* is presented in Table 1. Mortality increased with higher concentrations and longer exposure times. At 3% concentration, mortality was moderate (45.5%), while 5% and 10% concentrations caused over 87% and 90% mortality, respectively. Complete mortality (100%) was achieved at 100% concentration. Against *P. xylostella*, CASE at 10% caused 100% mortality within 72 hours, comparable to the results of Gajalakshmi *et al.* (2017) [3]. These findings confirm earlier reports that *Annona squamosa* seed extracts are effective against both sucking and lepidopteran pests.

**Table 1:** Efficacy of Custard Apple Seed Extract (CASE) Against *Myzus persicae* and *Plutella xylostella*

| Sl. No | Treatment | Mortality (%)- *M. persicae* (24h) | Mortality (%)- *M. persicae* (72h | Mortality (%)- *P. xylostella* (72h) |
|---|---|---|---|---|
| 1 | $T_1$: CECAS @ 3% | 45.4±0.67 | 77.2±0.49 | 77±0.57 |
| 2 | $T_2$: CECAS @ 5% | 87.2±0.97 | 94.4±0.74 | 91.25±4.11 |
| 3 | $T_3$: CECAS @ 10% | 90.8±0.58 | 99.6±0.4 | 97±1.73 |
| 4 | $T_4$: CECAS @ 100% | 99.6±0.4 | 0±0 | 100±0 |
| 5 | T5: Control | 0±0 | 0±0 | 100±0 |
| | C.D | 1.614 | 1.379 | 6.096 |
| | SE(m) | 0.534 | 0.456 | 1.957 |
| | SE (d) | 0.755 | 0.645 | 2.767 |
| | C.V | 1.848 | 1.88 | 4.206 |

**Note:** Dash (-) indicates no data recorded.

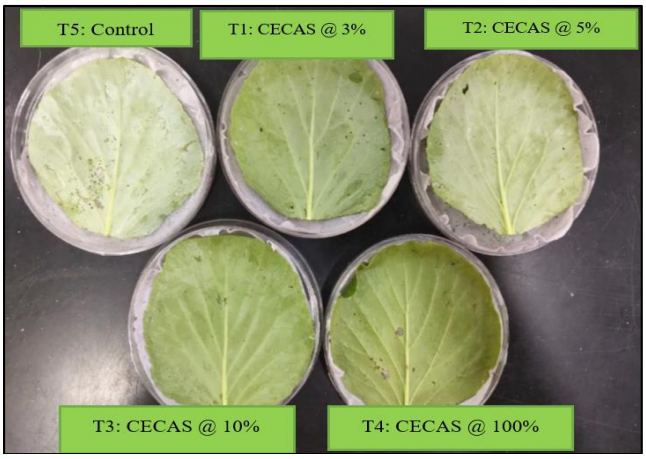


**Fig 1:** Efficacy of CECAS against *M. persicae* and *P. xylostella*

The efficacy of CASE against *M. persicae* and *P. xylostella* is presented in Table 1. Mortality of *M. persicae* was 45.5% at 3% concentration after 24 h, increasing to 77.2% after 72 h. Higher concentrations (5% and 10%) resulted in over 87% mortality, while 100% extract achieved complete mortality.

The insecticidal activity of CASE against *P. xylostella* larvae at 72 hours post-treatment is also shown in Table 1. A similar dose-dependent pattern was observed. The 3% concentration resulted in 50% larval mortality, while 5% and 10% caused 75% and 87.50% mortality, respectively. The highest concentration (100%) again achieved 100% mortality, indicating potent larvicidal activity. No mortality occurred in the untreated control.

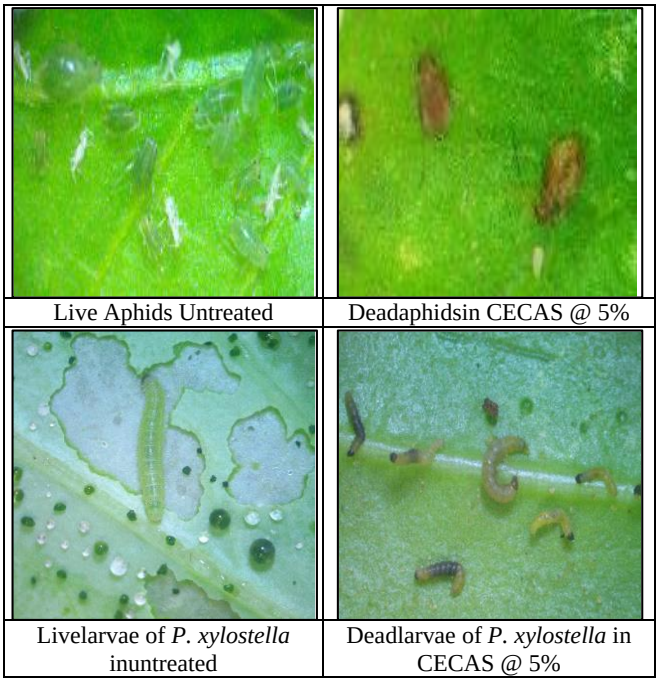


**Fig 2:** Microscopic vied of pest before and after treatment.

## Discussion

The present study confirmed that CASE exhibits strong insecticidal potential against both *M. persicae* and *P. xylostella*. Mortality was dose-dependent and rapid, consistent with previous findings (Rajalakshmi & Mohan, 2014; Gajalakshmi *et al.*, 2017) [6, 3]. The bioactive compounds in *A. squamosa*, such as annonaceous acetogenins, are believed to disrupt mitochondrial electron transport, causing energy depletion and insect mortality. The complete mortality observed at relatively low concentrations against *P. xylostella* demonstrates the extract's effectiveness against lepidopteran larvae. These results support the inclusion of CASE in integrated pest management programs as a sustainable alternative to chemical insecticides. However, field validation, toxicity studies on beneficial insects, and formulation standardization are needed for wider application (Ribeiro, L. P. *et al.* 2014) [7]

The mortality data of *Myzus persicae* at 24-and 72-hours post-treatment are presented in Table 1. The results revealed a dose-dependent response to CASE treatments. At 24 hours, the lowest concentration (3%) caused 45.50% mortality, which increased significantly to 87.50% and 90.90% at 5% and 10% concentrations, respectively. The highest mortality (100%) was observed at 100% crude extract (Amoabeng, B. W *et al.*, 2014) [2]

At 72 hours, mortality further increased across all concentrations. The 3% treatment caused 77.27% mortality, and 5% reached 94.20%. Complete mortality (100%) was recorded at 10% and 100% concentrations. No mortality was observed in the control treatment.

These findings indicate that CASE exhibits strong insecticidal activity against aphids, particularly at higher concentrations. The rapid increase in mortality over time suggests both acute and residual toxicity effects of the extract. The presence of bioactive compounds such as acetogenins, alkaloids, and fatty acids in custard apple seeds may contribute to its antifeedant and toxic effects, consistent with earlier reports (Rajalakshmi & Mohan, 2014) [6].

### Efficacy of CASE against *Plutella xylostella*

The data clearly demonstrate that CECAS is effective against DBM larvae, with significant mortality at concentrations ≥5%. These results corroborate the findings of prior studies that reported botanical insecticides, particularly those containing annonaceous acetogenins, possess strong ovicidal and larvicidal properties against lepidopteran pests (Gajalakshmi *et al.*, 2017) [3].

The mode of action may include disruption of mitochondrial function, inhibition of feeding, or interference with nervous system activity, as suggested by the rapid onset of mortality. Moreover, the use of seed extracts offers a sustainable and eco-friendly alternative to synthetic pesticides, potentially reducing the risk of pesticide resistance in DBM, a species well known for developing resistance to conventional insecticides (Isman, M. B. (2006) [4]

## Conclusion

The present study demonstrates the high efficacy of custard apple seed extract (CECAS/CASE) against two economically important pests—*Myzus persicae* and *Plutella xylostella*. Mortality was clearly dose-dependent, increasing with both concentration and exposure time, with the 10% and 100% treatments providing near or complete control within 72 hours. These findings highlight the strong insecticidal potential of custard apple seed extract as a botanical pesticide, offering a safer, ecofriendly, and environmentally sound alternative to chemical pesticides for use in integrated pest management programs.

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