



# Estimation of Level of Resistance of Chemical Insecticides, Biopesticides, and Botanicals against Rice Weevil (*Sitophilus oryzae*) Across Multiple Progenies

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## Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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## ABSTRACT

The rice weevil, *Sitophilus oryzae* is a major pest of stored grains, necessitating effective control strategies. This study evaluated the efficacy of chemical insecticides, biopesticides, and botanicals across four progeny generations of *S. oryzae* at laboratory of department of Entomology, Sam Higginbottom University of Agriculture Technology and Sciences, Prayagraj, India. Mortality rates, lethal concentrations (LC50/LC90), dose-response slopes, and relative potency were analyzed at

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24, 48, and 72 hours after treatment (HAT). Synthetic insecticides (Emamectin benzoate, Deltamethrin, Spinosad) demonstrated superior efficacy with LC<sub>50</sub> ≤2.5 ppm, while biopesticides (*Beauveria bassiana*, *Verticillium lecani*) and botanicals (Custard apple powder, Eucalyptus) required higher doses. Time-dependent efficacy improvements and resistance patterns were observed across progenies. Results highlight the potential of integrating synthetics with bio-botanical alternatives for sustainable pest management.

**Keywords:** Concentrations; dose response; lethal; progeny; resistance; stored grain pests; time dependent.

## 1. INTRODUCTION

The rice weevil (*Sitophilus oryzae*) is a notorious pest that inflicts severe damage on stored grain supplies, leading to substantial economic and agricultural losses. Post-harvest losses caused by this pest threaten global food security, particularly in tropical and subtropical regions like India, where warm and humid conditions favor its proliferation. These losses, estimated at 20–30% of total grain production in developing countries (FAO, 2019), have severe economic consequences for farmers and food supply chains. Historically, chemical insecticides such as pyrethroids (e.g., deltamethrin), organophosphates (e.g., chlorpyrifos-methyl), and spinosyns (e.g., spinosad) have been the primary tools for controlling *S. oryzae* due to their rapid action and high efficacy. However, the overuse of these chemicals has led to widespread resistance in *S. oryzae* populations, with some strains exhibiting resistance ratios exceeding 100-fold (Opit et al., 2012; Athanassiou et al., 2005), rendering many conventional insecticides ineffective and necessitating higher doses that pose environmental and health risks. The overuse of chemicals has led to resistance in *S. oryzae*, with resistance ratios exceeding 100-fold (Opit et al., 2012; Li et al., 2022).

In light of these challenges, sustainable pest management strategies that integrate chemical, biological, and botanical solutions are increasingly critical (Nayak et al., 2020). Biopesticides, such as the entomopathogenic fungi *Beauveria bassiana* and *Verticillium lecani*, offer an eco-friendly alternative by infecting and killing pests without harming non-target organisms (Wakil et al., 2021; Kavallieratos et al., 2017). Similarly, plant-derived botanicals like custard apple leaves and eucalyptus oil, which contain bioactive compounds such as alkaloids and terpenoids (Isman, 2020), have shown potential as natural insecticides. Botanicals like custard apple and eucalyptus contain bioactive

compounds (Kumar et al., 2023; Devi et al., 2020), but their efficacy requires optimization (Keswani et al., 2021; Rajendran, 2005).

However, their efficacy is often inconsistent, requiring further research to optimize their use. Despite the availability of these alternatives, there remains a lack of comprehensive studies comparing their effectiveness across multiple progeny generations of *S. oryzae*, particularly in terms of resistance development, time-dependent mortality, and relative potency (Phillips & Throne, 2010).

Sustainable strategies integrating chemical, biological, and botanical solutions are critical (Fields & White, 2002; Nayak et al., 2020; Guedes et al., 2016).

This study aims to bridge these gaps by evaluating the efficacy of 12 treatments—four synthetic insecticides, two biopesticides, and six botanicals—against four successive progeny generations (P1–P4) of *S. oryzae*. Key objectives include analyzing dose-response relationships (LC<sub>50</sub>/LC<sub>90</sub>), assessing time-mortality trends over 24–72 hours, and identifying resistance patterns across generations. The findings will provide valuable insights for developing integrated pest management (IPM) strategies that balance effectiveness, sustainability, and resistance mitigation.

## 2. MATERIALS AND METHODS

### 2.1 Insect Rearing and Colony Maintenance

Adult *Sitophilus oryzae* were collected from infested wheat stocks obtained from local vendors in Prayagraj, India, and identified morphologically using taxonomic keys (Haines, 1991). A founder colony was subsequently established in a controlled-environment chamber. Insects were reared on whole wheat grains (12–14% moisture content) in ventilated glass jars (1

L) under standardized conditions of  $28 \pm 1^\circ\text{C}$ ,  $70 \pm 5\%$  relative humidity, and a 12:12 light-dark cycle (Nayak et al., 2020). Four successive progeny generations (P1–P4) were produced by transferring 200 unsexed adults to fresh wheat grains every 30 days, with each progeny maintained separately to prevent cross-generational contamination. For bioassays, adults aged 7–14 days from each progeny generation were selected to ensure uniform physiological status (Opit et al., 2012).

## 2.2 Treatment Preparation

- **Synthetic Insecticides:** Commercial formulations were used: Emamectin benzoate 5% SG (Syngenta), Deltamethrin 2.8% EC (Bayer), Spinosad 12% SC (Corteva), and Chlorpyrifos-methyl 50% WP (Dow AgroSciences). Stock solutions were prepared in acetone and serially diluted to 1–1,000 ppm.
- **Biopesticides:** *Beauveria bassiana* (strain Bb-12,  $1 \times 10^8$  CFU/g) and *Verticillium lecani* (strain VI-09,  $1 \times 10^8$  CFU/g) were procured from the National Bureau of Agriculture Insect Resources Important Insects (NBAIR). Spore suspensions were prepared in 0.05% Tween-80 and adjusted to 1–1,000 ppm using a hemocytometer.
- **Botanicals:** Fresh plant materials (Custard apple leaves, Eucalyptus leaves, Ginger rhizome, etc.) were shade-dried, powdered (60-mesh sieve), and extracted via Soxhlet apparatus using ethanol (95%). Crude extracts were concentrated under vacuum, and working solutions (1–1,000 ppm) were prepared in distilled water with 0.1% Triton X-100 as an emulsifier.

## 2.3 Bioassay Design

- **Experimental Setup:** Contact toxicity bioassays were conducted using a no-choice test. Wheat grains (50 g) were treated with 10 mL of each concentration, air-dried, and placed in Petri dishes (9 cm diameter). Control grains received solvent-only (acetone or 0.1% Triton X-100).
- **Insect Exposure:** Twenty adults per replicate (4 replicates per treatment) were

introduced into dishes. Mortality was assessed at 24, 48, and 72 hours post-exposure. Insects were considered dead if unresponsive to prodding.

- **Environmental Conditions:** Bioassays were conducted under the same temperature and humidity as rearing to minimize confounding effects.

## 2.4 Statistical Analysis

- **Probit Analysis:** Mortality data were analyzed using PoloPlus 2.0 (LeOra Software) to estimate  $LC_{50}$ ,  $LC_{90}$ , slopes, and 95% confidence intervals. Model adequacy was validated via chi-square goodness-of-fit ( $\chi^2 p > 0.05$ ) and coefficient of variation ( $CV < 15\%$ ).
- **Relative Potency:** Calculated as the ratio of  $LC_{50}$  of the reference treatment (Ginger rhizome) to  $LC_{50}$  of test treatments.
- **Resistance Ratio (RR):** Determined for progeny P4 relative to P1 ( $RR = LC_{50} P4 / LC_{50} P1$ ).
- **Data Visualization:** Dose-response curves and heatmaps for progeny trends were generated using GraphPad Prism 9.0.
- **Statistical Analysis:** Mortality data were analyzed using PoloPlus 2.0, with Abbott's formula for corrected mortality (Abbott, 1925) and Finney's probit analysis (Finney, 1971).

## 3. RESULTS

### 3.1 Efficacy of Treatments Across Progenies

Synthetic insecticides demonstrated the highest efficacy, with emamectin benzoate exhibiting peak potency ( $LC_{50}$ : 0.3–1.6 ppm; relative potency 99–419× botanicals), consistent with its broad-spectrum neurotoxic action (Agrafioti et al., 2019). Deltamethrin and spinosad achieved rapid mortality (slopes 1.72–2.93), with  $LC_{90}$  values  $\leq 10$  ppm, though their effectiveness diminished over time as  $LC_{50}$  decreased by 58–63% from 24 to 72 hours post-treatment (Haddi et al., 2017). Biopesticides showed moderate efficiency: *Beauveria bassiana* outperformed *Verticillium lecani* ( $LC_{50}$ : 5.6–35.5 ppm vs. 10–63.1 ppm), with steeper slopes (1.29–1.53)

**Table 1. Comparative efficacy of treatments across progenies**

| Treatment            | Type         | Progeny | 24HAT (LC50) | 48HAT (LC50) | 72HAT (LC50) | Avg. LC50 | Slope (b) | Avg. LC90 | Relative Potency |
|----------------------|--------------|---------|--------------|--------------|--------------|-----------|-----------|-----------|------------------|
| Emamectin benzoate   | Synthetic    | P1      | 0.8          | 0.5          | 0.3          | 0.5       | 2.13      | 2.0       | 198–290x         |
|                      |              | P2      | 1.6          | 0.6          | 0.6          | 0.9       | 1.87      | 3.2       | 249–419x         |
|                      |              | P3      | 1.8          | 1.1          | 0.6          | 1.2       | 1.23      | 2.5       | 132–280x         |
|                      |              | P4      | 3.2          | 2.5          | 1.2          | 2.3       | 1.19      | 6.3       | 72–99x           |
| Deltamethrin         | Synthetic    | P1      | 1.3          | 0.8          | 0.3          | 0.8       | 2.51      | 3.2       | 122–290x         |
|                      |              | P2      | 2.0          | 1.0          | 0.4          | 1.1       | 2.15      | 4.0       | 199–264x         |
|                      |              | P3      | 3.2          | 2.1          | 1.2          | 2.2       | 0.97      | 19.9      | 35–198x          |
|                      |              | P4      | 12.6         | 7.9          | 2.8          | 7.8       | 0.78      | 50.1      | 10–35x           |
| Spinosad             | Synthetic    | P1      | 3.2          | 1.6          | 0.6          | 1.8       | 1.97      | 10.0      | 50–145x          |
|                      |              | P2      | 5.0          | 2.5          | 0.8          | 2.8       | 2.64      | 19.9      | 79–99x           |
|                      |              | P3      | 5.8          | 2.7          | 0.9          | 3.1       | 1.72      | 19.9      | 49–96x           |
|                      |              | P4      | 12.6         | 5.0          | 1.3          | 6.3       | 1.74      | 50.1      | 20–74x           |
| Beauveria bassiana   | Biopesticide | P1      | 15.8         | 10.0         | 5.0          | 10.3      | 1.38      | 50.1      | 8–17x            |
|                      |              | P2      | 31.6         | 19.9         | 10.0         | 20.5      | 1.35      | 63.1      | 7–14x            |
|                      |              | P3      | 12.6         | 6.3          | 2.4          | 7.1       | 1.50      | 25.1      | 12–75x           |
|                      |              | P4      | 35.5         | 20.0         | 5.6          | 20.4      | 1.41      | 141.3     | 6–12x            |
| Verticillium lecani  | Biopesticide | P1      | 31.6         | 19.9         | 10.0         | 20.5      | 1.05      | 125.9     | 5–8x             |
|                      |              | P2      | 63.1         | 39.8         | 25.1         | 42.7      | 1.03      | 251.2     | 3–7x             |
|                      |              | P3      | 23.4         | 9.8          | 5.1          | 12.8      | 1.19      | 100.0     | 8–20x            |
|                      |              | P4      | 57.5         | 39.8         | 10.0         | 35.8      | 1.12      | 275.4     | 3–6x             |
| Custard apple leaves | Botanical    | P1      | 125.9        | 50.1         | 25.1         | 67.0      | 0.51      | 794.3     | 1.3–1.8x         |
|                      |              | P2      | 251.2        | 125.9        | 63.1         | 146.7     | 0.53      | 1,000.0   | 1.0–2.0x         |
|                      |              | P3      | 75.9         | 42.7         | 15.8         | 44.8      | 0.76      | 199.5     | 1.8–2.8x         |
|                      |              | P4      | 501.2        | 125.9        | 25.1         | 217.4     | 0.73      | 1,584.9   | 0.6–2.8x         |
| Eucalyptus leaves    | Botanical    | P1      | 79.4         | 63.1         | 46.8         | 63.1      | 0.54      | 501.2     | 1.4–2.0x         |
|                      |              | P2      | 199.5        | 141.3        | 31.6         | 124.1     | 0.59      | 794.3     | 1.0–1.9x         |
|                      |              | P3      | 100.0        | 72.4         | 31.6         | 68.0      | 0.73      | 316.2     | 1.3–1.6x         |
|                      |              | P4      | 562.3        | 199.5        | 46.8         | 269.5     | 0.69      | 3,162.3   | 0.3–1.8x         |

| <b>Treatment</b> | <b>Type</b> | <b>Progeny</b> | <b>24HAT (LC50)</b> | <b>48HAT (LC50)</b> | <b>72HAT (LC50)</b> | <b>Avg. LC50</b> | <b>Slope (b)</b> | <b>Avg. LC90</b> | <b>Relative Potency</b> |
|------------------|-------------|----------------|---------------------|---------------------|---------------------|------------------|------------------|------------------|-------------------------|
| Ginger rhizome   | Botanical   | P1             | 158.5               | 89.1                | 74.1                | 107.2            | 0.47             | 1,000.0          | 1.0x (Baseline)         |
|                  |             | P2             | 398.1               | 251.2               | 79.4                | 242.9            | 0.45             | 1,584.9          | 0.8–1.0x                |
|                  |             | P3             | 428.6               | 148.2               | 32.5                | 203.1            | 0.62             | 1,258.9          | 0.6–1.0x                |
|                  |             | P4             | 1,258.9             | 316.2               | 74.1                | 549.7            | 0.43             | 6,309.6          | 0.2–1.0x                |

indicating faster fungal pathogenesis compared to botanicals (Wakil et al., 2021).

Synthetic insecticides like spinosad showed resistance escalation, consistent with findings in *Helicoverpa armigera* (Wang et al., 2020).

Among botanicals, custard apple (LC50: 15.8–125.9 ppm) and eucalyptus oil (31.6–199.5 ppm) were the most effective, though their flatter slopes (0.34–0.85) highlighted gradual bioactivity, likely due to volatile compound degradation (Isman, 2020). Botanicals such as custard apple exhibited gradual bioactivity, aligning with phytochemical profiles (Kumar et al., 2023). Resistance escalated significantly across progenies: by P4, synthetic insecticides required 1.2–3.2 ppm (emamectin) versus 0.3–0.8 ppm in P1, while botanicals necessitated 2–3× higher doses, underscoring adaptive resistance mechanisms (Nayak et al., 2020). Comparatively, synthetic treatments maintained superior relative potency (up to 419×) against ginger rhizome powder (1.0× baseline), though biopesticides and botanicals offered sustainable alternatives with lower cross-resistance risks (Table 1).

#### 4. DISCUSSION

The study systematically evaluated the efficacy of chemical insecticides, biopesticides, and botanicals against *S. oryzae* across four progeny generations (P1–P4). Key findings include: Synthetic insecticides, including emamectin benzoate, deltamethrin, and spinosad, demonstrated superior potency in early progeny generations (P1–P2), with LC50 values  $\leq 2.5$  ppm and steep dose-response slopes (1.72–2.93) indicative of rapid knockdown effects (Agrafioti et al., 2019). However, resistance escalated markedly by P4, as seen in emamectin benzoate (LC50: 0.5 ppm in P1 vs. 2.3 ppm in P4), underscoring the need for rotational strategies to mitigate resistance development (Opit et al., 2012). Resistance in P4 progenies mirrors trends observed in Indian granaries (Chaudhary & Tripathi, 2022) and phosphine-resistant populations (Sousa et al., 2020). Biopesticides emerged as sustainable alternatives: *Beauveria bassiana* (LC50: 7.1–20.5 ppm) exhibited efficacy comparable to synthetic insecticides in P3, outperforming *Verticillium lecani* (LC50: 12.8–42.7 ppm), with steeper slopes (1.19–1.50) suggesting faster mortality progression suitable for integrated pest management (IPM) frameworks (Wakil et al., 2021). *Beauveria*

*bassiana* efficacy aligns with nano-encapsulation studies (Ahmad et al., 2023) and global IPM perspectives (Ngegba et al., 2023). Botanicals, though less potent, showed strategic potential; custard apple leaves (LC50: 44.8–217.4 ppm) and eucalyptus leaves (LC50: 63.1–269.5 ppm) required 10–100× higher doses than synthetics but exhibited minimal environmental toxicity, supporting their use in synergistic mixtures or organic systems (Isman, 2020). Synergistic effects of botanicals and diatomaceous earth (El-Sayed & Mahmoud, 2023) support their integration into IPM. Resistance trends varied across progenies: synthetic insecticides faced 2–10× higher LC50 values in P4 (e.g., deltamethrin: 0.8 ppm → 7.8 ppm), while botanicals showed less resistance escalation, highlighting their role in resistance management programs (Nayak et al., 2020). Time-dependent efficacy assessments revealed significant LC50 reductions (40–63%) at 72 hours post-treatment (e.g., spinosad: 12.6 ppm → 1.3 ppm in P4), emphasizing the importance of extended exposure periods in bioassay design (Haddi et al., 2017). Challenges in formulating botanicals (Keswani et al., 2021) and their field validation in regions like Nepal (Khanal et al., 2021) underscore the need for optimization. Molecular insights into detoxification mechanisms (Li et al., 2022) and stored-product entomology evolution (Hagstrum & Phillips, 2017) highlight future research directions (Kavallieratos et al., 2017).

#### 5. CONCLUSION

The study confirms synthetic insecticides' high efficacy (LC50  $\leq 2.5$  ppm) against *Sitophilus oryzae*, but escalating resistance in later progenies demands strategic rotation with alternatives. Biopesticides like *Beauveria bassiana* (LC50 7–20 ppm) offer sustainable, rapid-action solutions, while botanicals (e.g., custard apple) mitigate resistance despite requiring higher doses. Progeny-specific resistance trends highlight the need for annual monitoring and molecular insights into detoxification mechanisms. Extended exposure (72HAT) enhances efficacy, emphasizing revised bioassay protocols and farmer training. A holistic IPM approach—integrating synthetics for acute control, biopesticides for suppression, and botanicals for organic/resistance management—is critical. Future efforts should optimize synergies (e.g., biopesticide-botanical blends) and validate field scalability to balance efficacy, sustainability, and ecological safety.

The study advocates for IPM strategies incorporating botanicals (Prakash & Rao, 2019) and microbial biopesticides (Ngegba et al., 2023), validated through resistance monitoring and CRISPR-Cas9 insights (Li et al., 2022).

The study also provides a comprehensive evaluation of chemical insecticides, biopesticides, and botanicals in managing *Sitophilus oryzae* across four progeny generations (P1–P4) in Prayagraj, India. Synthetic insecticides (Emamectin benzoate, Deltamethrin, Spinosad) showed superior efficacy (LC<sub>50</sub>: 0.3–7.8 ppm) against *Sitophilus oryzae* but faced resistance escalation in progeny 4 (e.g., Emamectin LC<sub>50</sub> rose 4.6×). Biopesticides, particularly *Beauveria bassiana* (moderate LC<sub>50</sub>: 7.1–20.5 ppm), offered sustainable alternatives with faster action than botanicals. Botanicals like custard apple and eucalyptus leaves required 10–100× higher doses but showed minimal resistance, ideal for preventive or organic use. Resistance trends highlighted the need for annual progeny monitoring, while extended exposure (72HAT) significantly enhanced efficacy (e.g., Spinosad LC<sub>50</sub> dropped 90% in P4). An integrated IPM strategy should combine synthetics (acute outbreaks), biopesticides (sustainable control), and botanicals (resistance mitigation), supported by rotation and resistance tracking for balanced pest management.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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