**Estimation of level of resistance of Chemical Insecticides, Biopesticides, and Botanicals Against Rice Weevil (*Sitophilus oryzae*) Across Multiple Progenies**

**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 Prayagraj, India. Mortality rates, lethal concentrations (LC50/LC90), dose-response slopes, and relative potency were analyzed at 24, 48, and 72 hours after treatment (HAT). Synthetic insecticides (Emamectin benzoate, Deltamethrin, Spinosad) demonstrated superior efficacy with LC50 ≤2.5 ppm, while biopesticides (*Beauveria bassiana, Verticillium lecani*) and botanicals (Custard apple, 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**

**INTRODUCTION**

post-harvest losses that 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(ref???), have severe economic consequences for farmers and food supply chains. Historically, chemical insecticides such as pyrethroids (e.g., deltamethrin), organophosphates (e.g., chlorpyriphos-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, rendering many conventional insecticides ineffective and necessitating higher doses that pose environmental and health risks.

In light of these challenges, sustainable pest management strategies that integrate chemical, biological, and botanical solutions are increasingly critical. 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. Similarly, plant-derived botanicals like custard apple leaves and eucalyptus oil, which contain bioactive compounds such as alkaloids and terpenoids(reference????), have shown potential as natural insecticides. 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. 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 (LC50/LC90), 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. t

**MATERIALS AND METHODS**

**Insect Rearing and Colony Maintenance**

* **Source and Initial Colony:** Adult *S. oryzae* were collected from infested wheat stocks in Prayagraj, India, and identified morphologically using taxonomic keys(reference??). A founder colony was established in a controlled-environment chamber.
* **Rearing Conditions:** Insects were reared on whole wheat grains (12–14% moisture content) in ventilated glass jars (1 L) at 28 ± 1°C, 70 ± 5% relative humidity, and a 12:12 light-dark cycle.
* **Progeny Generation:** Four successive progeny generations (P1–P4) were produced by transferring 200 unsexed adults to fresh wheat grains every 30 days. Progenies were maintained separately to prevent cross-generational contamination.
* **Selection for Bioassays:** Adults aged 7–14 days from each progeny were used in experiments to ensure uniform physiological status.

**Treatment Preparation**

* **Synthetic Insecticides:** Commercial formulations were used: Emamectin benzoate 5% SG (Syngenta), Deltamethrin 2.8% EC (Bayer), Spinosad 12% SC (Corteva), and Chlorpyriphos-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 × 10⁸ CFU/g) and *Verticillium lecani* (strain Vl-09, 1 × 10⁸ CFU/g) were procured from the National Bureau of Agricultural 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.

**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.

**Statistical Analysis**

* **Probit Analysis:** Mortality data were analyzed using PoloPlus 2.0 (LeOra Software) to estimate LC₅₀, LC₉₀, slopes, and 95% confidence intervals. Model adequacy was validated via chi-square goodness-of-fit (χ² *p* > 0.05) and coefficient of variation (CV < 15%).
* **Relative Potency:** Calculated as the ratio of LC₅₀ of the reference treatment (Ginger rhizome) to LC₅₀ of test treatments.
* **Resistance Ratio (RR):** Determined for progeny P4 relative to P1 (RR = LC₅₀ P4 / LC₅₀ P1).
* **Data Visualization:** Dose-response curves and heatmaps for progeny trends were generated using GraphPad Prism 9.0.

**RESULTS**

**Synthetic Insecticides: Highest Efficacy**

* Emamectin benzoate showed peak potency (LC50: 0.3–1.6 ppm; Relative Potency: 99–419x botanicals).
* Deltamethrin and Spinosad achieved rapid mortality (slopes 1.72–2.93), with LC90 ≤10 ppm.
* Time Dependency: LC50 decreased by 58–63% from 24HAT to 72HAT (e.g., Spinosad: 5.8 ppm → 0.9 ppm).

**Biopesticides: Moderate Efficiency**

* Beauveria bassiana outperformed Verticillium (LC50: 5.6–35.5 ppm vs. 10–63.1 ppm).
* Steeper slopes (1.29–1.53) indicated faster action than botanicals.

**Botanicals: Limited but Notable Activity**

* Custard apple (LC50: 15.8–125.9 ppm) and Eucalyptus (31.6–199.5 ppm) were top performers.
* Flatter slopes (0.34–0.85) suggested gradual effects.

**Resistance Trends Across Progenies**

* Progeny 4 showed higher LC50 for synthetics (e.g., Emamectin: 1.2–3.2 ppm) vs. Progeny 1 (0.3–0.8 ppm).
* Botanicals required 2–3× higher doses in later progenies.

**Comparative Efficacy of Treatments Across Progenies (P1–P4)**

*(LC50 in ppm; Slopes (b); Relative Potency vs. Ginger Rhizome Powder (1.0x))*

*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** |
| **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** |

**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:

1. **Superiority of Synthetic Insecticides**
	* **Emamectin benzoate**, **Deltamethrin**, and **Spinosad** exhibited the highest potency, with LC50 values ≤2.5 ppm in early progenies (P1–P2). Their steep dose-response slopes (1.72–2.93) confirmed rapid knockdown effects.
	* However, **resistance escalation** was observed in P4 (e.g., Emamectin LC50 rose from 0.5 ppm in P1 to 2.3 ppm in P4), highlighting the need for rotational use to delay resistance.
2. **Biopesticides as Sustainable Alternatives**
	* **Beauveria bassiana** (LC50: 7.1–20.5 ppm) demonstrated efficacy comparable to some synthetics, particularly in P3, where it outperformed Verticillium (LC50: 12.8–42.7 ppm).
	* The steeper slopes of biopesticides (1.19–1.50 vs. 0.34–0.85 for botanicals) suggest faster mortality progression, making them viable for integrated pest management (IPM).
3. **Botanicals: Limited but Strategic Potential**
	* **Custard apple leaves** (LC50: 44.8–217.4 ppm) and **Eucalyptus leaves** (LC50: 63.1–269.5 ppm) were the most effective botanicals but required 10–100× higher doses than synthetics.
	* Their flatter slopes indicate slower action, but their low environmental toxicity supports use in **synergistic mixtures** or organic systems.
4. **Progeny-Specific Resistance Trends**
	* Progressive resistance was evident in P4, where LC50 values for synthetics increased **2–10×** compared to P1 (e.g., Deltamethrin: 0.8 ppm → 7.8 ppm).
	* Botanicals showed less resistance escalation, suggesting their utility in resistance management programs.
5. **Time-Dependent Efficacy**
	* Delayed mortality assessments (72HAT) revealed **40–63% reductions in LC50** for most treatments (e.g., Spinosad: 12.6 ppm → 1.3 ppm in P4), emphasizing the need for extended exposure periods in bioassays.

**CONCLUSION**

The study confirms synthetic insecticides' high efficacy (LC50 ≤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.

**SUMMARY**

The study 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 (LC₅₀: 0.3–7.8 ppm) against *Sitophilus oryzae* but faced resistance escalation in progeny 4 (e.g., Emamectin LC₅₀ rose 4.6×). Biopesticides, particularly *Beauveria bassiana* (moderate LC₅₀: 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 LC₅₀ 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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