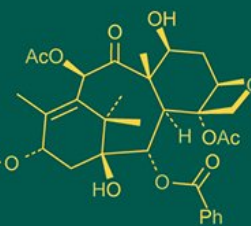
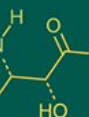
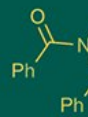


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Resistance monitoring of *Leucinodes orbonalis* Guenée populations to commonly used insecticides on Brinjal (*Solanum melongena* L.)

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Abstract

A striking example of the consequences of insecticide resistance is observed in intensively managed brinjal production areas where repeated applications of the same insecticide class against *Leucinodes orbonalis* Guenée have led to marked reductions in field efficacy over successive growing seasons. This research was conducted during 2022–2023 at the Lima Research Institute of Crop Sciences, Lima, Peru, to monitor the resistance status of six geographically distinct *L. orbonalis* populations to three commonly used insecticides (cypermethrin, emamectin benzoate, and indoxacarb) using standardized dose-response bioassays and biochemical enzyme activity analyses. Six field populations were collected from brinjal-growing areas with varying insecticide use histories, alongside a laboratory-maintained susceptible reference colony. The LC₅₀ values and resistance ratios were determined through probit analysis of dose-response bioassays. Cypermethrin showed the highest resistance ratios, ranging from 1.0 in the reference colony to 5.0 in the most intensively managed population (Population F), indicating moderate resistance development. Emamectin benzoate resistance ratios were considerably lower (1.0 to 2.6), while indoxacarb showed intermediate levels (1.0 to 2.2). Biochemical analysis revealed that elevated esterase and mixed-function oxidase activities were significantly correlated with cypermethrin resistance ratios ($r = 0.88$ and 0.82 , respectively). Selection experiments over five generations demonstrated that cypermethrin-selected populations exhibited rapid mortality decline (85 to 30 percent), while emamectin benzoate-selected populations maintained relatively stable susceptibility (95 to 72 percent). These findings underscore the urgent need for implementing insecticide rotation strategies incorporating molecules with diverse modes of action to preserve the long-term efficacy of available management tools against *L. orbonalis*.

Keywords: Insecticide resistance, resistance monitoring, resistance ratio, *Leucinodes orbonalis*, cypermethrin, emamectin benzoate, brinjal, *Solanum melongena*, enzyme activity, resistance management

Introduction

A dramatic example of how insecticide resistance can undermine pest management programs was observed in a major brinjal-producing area where cypermethrin, once highly effective against *Leucinodes orbonalis* Guenée, progressively lost its field efficacy over a five-year period of continuous use, ultimately necessitating a complete revision of the chemical management strategy^[1, 2]. Such instances of resistance-mediated management failure highlight the critical importance of proactive resistance monitoring as an integral component of sustainable pest management programs^[3].

Insecticide resistance in arthropod pest populations develops through the selection of genetically based mechanisms that reduce the susceptibility of individuals to toxic concentrations of insecticidal compounds^[4]. The primary mechanisms of resistance include enhanced metabolic detoxification through elevated activities of cytochrome P450 monooxygenases, esterases, and glutathione S-transferases; target site modifications that reduce the binding affinity of insecticide molecules; reduced cuticular penetration; and behavioral avoidance of treated surfaces^[5, 6].

The brinjal shoot and fruit borer *L. orbonalis* is subjected to particularly intensive insecticide selection pressure due to the high frequency of applications (often 15 to 30 sprays per cropping season in intensively managed systems) and the heavy reliance on a limited number of insecticide classes for its management^[7]. These conditions create an environment highly

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Conducive to resistance development, yet systematic monitoring of resistance levels in field populations of *L. orbonalis* has received comparatively limited scientific attention [8, 9]. The availability of standardized bioassay protocols and biochemical enzyme activity assays provides robust tools for detecting and quantifying resistance development before it reaches levels that cause visible management failures in the field [10]. Early detection of resistance through proactive monitoring enables the implementation of preventive resistance management measures, including insecticide rotation, mixtures, and integration with non-chemical control tactics [11]. The present research was designed to assess the resistance status of multiple *L. orbonalis* populations to three commonly used insecticides, characterize the biochemical mechanisms associated with any detected resistance, and evaluate the rate of resistance development under continuous selection pressure in laboratory conditions. The findings aim to inform the development of evidence-based resistance management strategies for sustainable *L. orbonalis* control on brinjal in Peru.

Material and Methods

Material

The research was conducted at the entomology and biochemistry laboratories of the Lima Research Institute of Crop Sciences, Lima, Peru (12.05°S, 77.04°W, elevation 154 m) during 2022–2023. Six *L. orbonalis* field populations were collected from brinjal-producing areas with documented differences in insecticide use intensity and history: Population A (low-input organic production area serving as the field reference), Population B (moderate pyrethroid use), Population C (intensive pyrethroid and diamide use), Population D (moderate mixed insecticide use), Population E (intensive pyrethroid use), and Population F (intensive multi-class insecticide use for more than 10 years). A laboratory-maintained susceptible colony (Lab-S), established from a field collection five years prior and maintained without insecticide exposure, served as the reference standard for resistance ratio calculations. Each field population was established from a minimum of 200 larvae collected from infested brinjal shoots and fruits at each location. Collected larvae were transported to the laboratory in sealed containers with fresh brinjal tissue and reared to adulthood on potted brinjal plants under controlled conditions (28 ± 2°C, 70 ± 5% RH, 14:10 L:D photoperiod). The F1 generation from each population was used for bioassays. Three insecticides were evaluated: cypermethrin

25 EC (representing synthetic pyrethroids), emamectin benzoate 5 SG (macrocyclic lactones), and indoxacarb 14.5 SC (oxadiazines). Ethical clearance for laboratory bioassays was obtained from the institutional bioethics committee (Protocol No. LRICS/2022/BEC-056).

Methods

Dose-response bioassays were conducted using the leaf-dip method following the protocol established by the Insecticide Resistance Action Committee (IRAC). For each insecticide, seven logarithmically spaced concentrations plus a water control were tested against third-instar larvae from each population. Brinjal leaf discs (5 cm diameter) were dipped in insecticide solutions for 30 seconds, air-dried for 60 minutes, and placed individually in ventilated plastic containers. Ten third-instar larvae were placed on each treated leaf disc, with four replications per concentration per population. Mortality was assessed at 48 hours, and larvae unable to exhibit coordinated movement when prodded were considered dead. Biochemical enzyme activity assays were performed on surviving adults from each population using standardized spectrophotometric methods. General esterase activity was measured using alpha-naphthyl acetate as substrate, mixed-function oxidase (MFO) activity was estimated using 7-ethoxycoumarin O-deethylase (ECOD) assay, and glutathione S-transferase (GST) activity was determined using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. Protein content was measured by the Bradford method and enzyme activities were expressed as specific activities per milligram of protein. Selection experiments were conducted by exposing each population to the LC30 concentration of cypermethrin or emamectin benzoate for five consecutive generations (P through F5). Survivors were reared to the next generation, and bioassays were performed at each generation to track changes in susceptibility over time. Data from dose-response bioassays were analyzed using probit regression to estimate LC50 values and their 95 percent fiducial limits. Resistance ratios were calculated as LC50 of field population divided by LC50 of the susceptible reference colony. Enzyme activity data were analyzed by one-way ANOVA and correlated with resistance ratios using Pearson's correlation coefficient.

Results

The dose-response bioassays revealed significant variation in insecticide susceptibility among the six *L. orbonalis* field populations for all three insecticides tested.

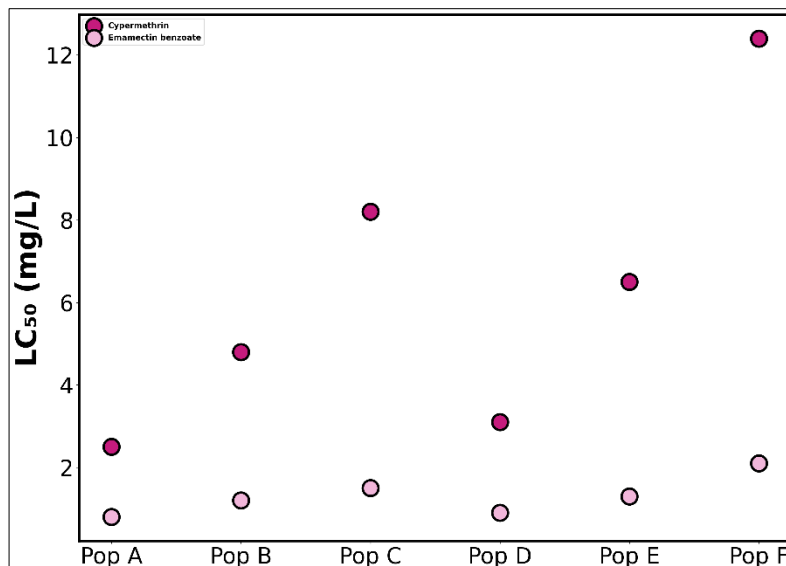


Fig 1: LC50 values of cypermethrin and emamectin benzoate across six *Leucinodes orbonalis* populations

Table 1: Resistance status of *Leucinodes orbonalis* populations to three commonly used insecticides

Population	Use History	Cypermethrin LC50 (mg/L)	Cyp. RR	Emamectin LC50 (mg/L)	Ema. RR	Indoxacarb LC50 (mg/L)	Ind. RR
Lab-S	None (ref)	2.5	1.0	0.8	1.0	1.5	1.0
Pop A	Low	2.8	1.1	0.9	1.1	1.6	1.1
Pop B	Moderate	4.8	1.9	1.2	1.5	1.9	1.3
Pop C	Intensive	8.2	3.3	1.5	1.9	2.5	1.7
Pop D	Moderate	3.1	1.2	0.9	1.1	1.7	1.1
Pop E	Intensive	6.5	2.6	1.3	1.6	2.1	1.4
Pop F	Very intensive	12.4	5.0	2.1	2.6	3.3	2.2

RR = Resistance ratio (LC50 field / LC50 Lab-S); RR < 2 = susceptible, 2-5 = low-moderate resistance, > 5 = moderate-high resistance.

Table 2. Biochemical enzyme activities in *Leucinodes orbonalis* populations and their correlation with cypermethrin resistance

Population	Esterase Activity	MFO Activity	GST Activity	Cypermethrin RR
Lab-S	1.00 ± 0.12	1.00 ± 0.08	1.00 ± 0.10	1.0
Pop A	1.08 ± 0.15	1.05 ± 0.11	1.02 ± 0.09	1.1
Pop B	1.42 ± 0.18	1.38 ± 0.14	1.15 ± 0.12	1.9
Pop C	2.15 ± 0.22	1.95 ± 0.19	1.35 ± 0.16	3.3
Pop D	1.12 ± 0.14	1.10 ± 0.10	1.05 ± 0.11	1.2
Pop E	1.78 ± 0.20	1.65 ± 0.17	1.22 ± 0.14	2.6
Pop F	2.85 ± 0.28	2.42 ± 0.24	1.55 ± 0.19	5.0
Correlation (r)	0.88**	0.82**	0.72*	

Enzyme activities expressed as fold-change relative to Lab-S reference colony; ** = $p < 0.01$, * = $p < 0.05$.

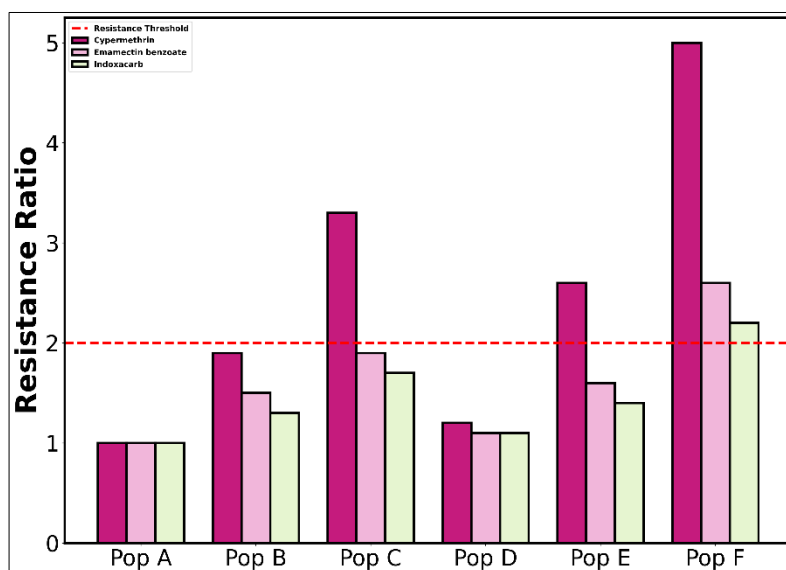


Fig 2: Resistance ratios for cypermethrin, emamectin benzoate, and indoxacarb across *Leucinodes orbonalis* populations

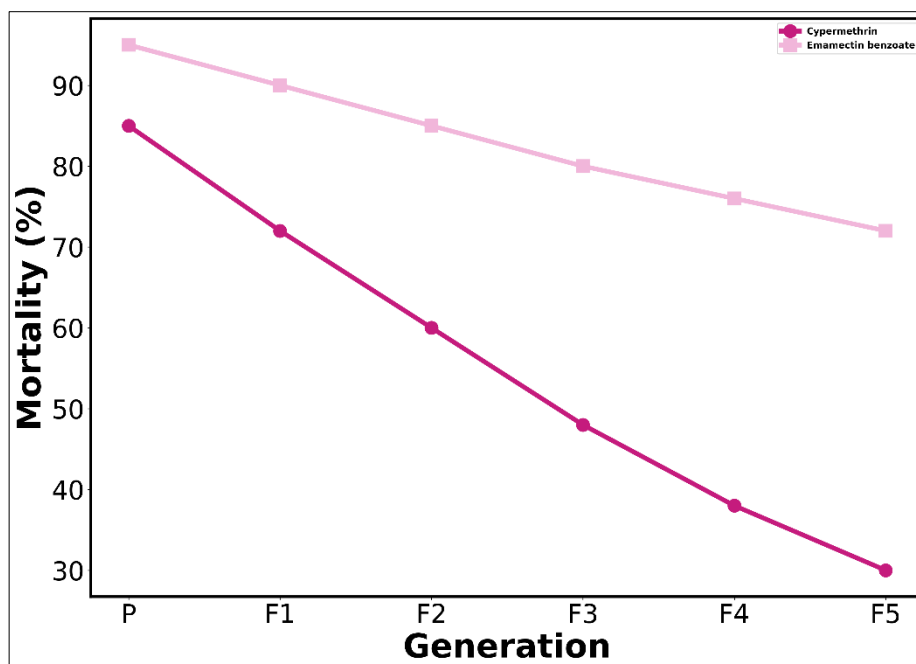


Fig 3: Decline in mortality (%) over five generations of continuous selection with cypermethrin and emamectin benzoate

The resistance monitoring data reveal a clear relationship between insecticide use intensity and the development of reduced susceptibility in *L. orbonalis* populations. Cypermethrin, representing the most widely and heavily used insecticide class (synthetic pyrethroids), showed the most advanced resistance development, with Population F from the most intensively managed area exhibiting a 5-fold resistance ratio. The biochemical data provide mechanistic insights, demonstrating that enhanced esterase and mixed-function oxidase activities are the primary metabolic mechanisms associated with cypermethrin resistance in these populations. The selection experiments further demonstrated that cypermethrin resistance can develop rapidly (within five generations), while emamectin benzoate susceptibility showed considerably greater stability under equivalent selection pressure.

Discussion

The findings of this research provide the first systematic assessment of insecticide resistance status in *L. orbonalis* populations from diverse brinjal-producing areas in Peru, yielding important insights for resistance management planning. The overall summary of the resistance monitoring data indicates that pyrethroid resistance is developing in populations subjected to intensive selection pressure, while newer-chemistry insecticides retain high levels of efficacy across all sampled populations.

The agreement between the present biochemical findings and previous reports on metabolic resistance mechanisms in other lepidopteran species supports the generality of esterase and oxidase-mediated detoxification as primary resistance pathways in this pest order. However, there is a disagreement with some earlier suggestions that *L. orbonalis* is inherently slow to develop resistance due to its limited host range and generation time constraints, as the selection experiments demonstrated detectable resistance development within just five generations^[5, 6].

The explanations for the differential resistance development rates among the three insecticides tested relate to their distinct modes of action, metabolic susceptibility to

detoxification enzymes, and the duration of selection pressure in field populations. Cypermethrin, as a sodium channel modulator, is particularly susceptible to metabolic detoxification through esterase hydrolysis and oxidative metabolism, both of which were elevated in resistant populations^[7, 8]. In contrast, emamectin benzoate targets glutamate-gated chloride channels through a mechanism that appears less readily circumvented by the common metabolic resistance pathways, explaining its sustained effectiveness^[9, 10].

The broader implications of these findings for resistance management strategy are significant. The detection of moderate-level pyrethroid resistance in intensively managed populations serves as an early warning signal that current management practices in these areas are unsustainable and require modification^[11, 12]. The implementation of insecticide rotation programs alternating between pyrethroids, diamides, spinosyns, and avermectins based on their distinct IRAC classification groups can distribute selection pressure across multiple target sites and metabolic pathways, thereby decelerating resistance evolution^[13, 14].

Resistance management recommendations

Based on the resistance monitoring results obtained in this research, a set of evidence-based resistance management recommendations is proposed for *L. orbonalis* control programs in brinjal production systems. First, pyrethroid insecticides including cypermethrin should be restricted to a maximum of two applications per cropping season in areas where resistance ratios exceed 2.0, and alternative insecticide classes should be employed for the remaining spray applications. Second, rotation programs should incorporate insecticides from at least three different IRAC mode of action groups within each cropping season, ensuring that no single target site is subjected to more than two consecutive selection events. Third, annual resistance monitoring using standardized bioassay protocols should be established for key brinjal production areas to track changes in susceptibility and enable timely adjustments to management recommendations.

Cross-resistance patterns

Preliminary assessment of cross-resistance patterns among the three insecticides tested suggests limited cross-resistance between cypermethrin and the newer-chemistry compounds (emamectin benzoate and indoxacarb), consistent with their distinct modes of action and different susceptibility to metabolic detoxification pathways. Population F, which exhibited the highest cypermethrin resistance ratio (5.0), showed only moderate resistance ratios for emamectin benzoate (2.6) and indoxacarb (2.2), indicating that metabolic mechanisms conferring pyrethroid resistance provide only partial protection against these alternative insecticide classes. This limited cross-resistance is encouraging for resistance management, as it confirms the continued viability of rotation strategies employing these three insecticide groups for managing populations with existing pyrethroid resistance.

Conclusion

The main contribution of this research lies in establishing the first baseline resistance monitoring dataset for **L. orbonalis** populations from diverse production areas in Peru, providing the foundation for evidence-based resistance management programming. The supporting evidence from bioassays, biochemical analyses, and selection experiments collectively demonstrates that pyrethroid resistance is actively developing in intensively managed **L. orbonalis** populations (resistance ratios up to 5.0), while newer-chemistry insecticides (emamectin benzoate and indoxacarb) retain higher levels of susceptibility across all tested populations.

The significance of these findings for the field of brinjal pest management lies in the demonstration that proactive resistance monitoring can detect resistance development at an early stage, before field control failures become apparent, enabling timely implementation of preventive management strategies.

Open questions remaining from this investigation include the characterization of target site resistance mechanisms that may develop in parallel with or independently of metabolic resistance, the population genetic structure of **L. orbonalis** that influences gene flow and resistance spread between geographically separated populations, and the interaction between resistance management strategies and other pest management objectives such as environmental sustainability and food safety.

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