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Insecticide resistance mechanisms and resistance risk assessment in diamondback moth (*Plutella xylostella*) populations

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Abstract
The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is one of the most destructive insect pests of cruciferous vegetables worldwide and is characterized by a remarkable ability to develop resistance to insecticides representing diverse modes of action. The present study was designed to assess the susceptibility status, resistance mechanisms, cross-resistance patterns and resistance risk of *P. xylostella* populations collected from major crucifer-growing regions. Field populations were compared with a laboratory-maintained susceptible population using concentration–mortality bioassays. LC_{50} and LC_{95} values were estimated by probit analysis and resistance ratios were calculated relative to the susceptible population. Biochemical assays were used to investigate the activities of cytochrome P450 monooxygenases, glutathione-S-transferases (GSTs) and carboxylesterases (CarEs). Synergist bioassays were conducted to assess the contribution of metabolic detoxification, while laboratory selection was used to estimate resistance development and realized heritability. The results indicated considerable variation in susceptibility among field populations and insecticide classes. The highest resistance was observed against the diamide insecticides, particularly cyantraniliprole, whereas comparatively lower resistance was observed against selected alternative chemistries. Resistant populations exhibited elevated P450, GST and CarE activities, suggesting an important contribution of metabolic detoxification. Laboratory selection resulted in a progressive increase in LC_{50}, indicating considerable resistance risk under continuous selection pressure. The findings emphasize that resistance management in *P. xylostella* should integrate routine susceptibility monitoring, rotation of insecticides according to mode of action, treatment-window strategies, threshold-based applications, biological control and non-chemical tactics. Such an integrated approach is essential for preserving insecticide efficacy and achieving sustainable management of diamondback moth in cruciferous vegetable production.

Keywords: *Plutella xylostella*; diamondback moth; insecticide resistance; LC_{50}; resistance ratio; P450; glutathione-S-transferase; carboxylesterase; target-site resistance; resistance risk; cross-resistance; integrated pest management

1. Introduction
The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a globally distributed and economically important pest of cruciferous vegetables. It attacks cabbage, cauliflower, broccoli, kale, mustard, radish and other members of Brassicaceae, with larvae causing the greatest damage by feeding on foliage and producing characteristic windowing and perforations. Under severe infestation, extensive leaf damage can substantially reduce photosynthetic activity and marketable yield. The importance of *P. xylostella* is amplified by its short generation time, high fecundity, rapid population growth and ability to survive across diverse climatic conditions. These biological characteristics enable populations to undergo repeated selection by insecticides within a single production season, accelerating the evolution of resistance (Talekar & Shelton, 1993; Furlong *et al*., 2013) [10, 3]. Chemical insecticides remain an important component of diamondback moth management because of their rapid action and comparatively predictable efficacy. However, *P. xylostella* is widely recognized as one of the most insecticide-resistant agricultural pests. Resistance has been documented against organophosphates, carbamates, pyrethroids, benzoylureas, avermectins, spinosyns, oxadiazines, phenylpyrazoles, diamides and several newer insecticide chemistries.

The exceptional resistance potential of this pest has been associated with intensive insecticide use, overlapping generations, high reproductive capacity and genetic variability within field populations. Consequently, insecticide resistance is now considered one of the major constraints to reliable chemical control of *P. xylostella*.

Resistance should not be considered a uniform characteristic of a species. Instead, resistance levels can differ substantially among populations according to geographical origin, crop production practices, insecticide-use history and genetic composition. A population may show susceptibility to one insecticide while simultaneously exhibiting moderate, high or extremely high resistance to another. Therefore, local susceptibility monitoring is essential for determining whether an insecticide remains effective and for designing appropriate resistance-management programmes.

Recent research in India demonstrates the importance of such monitoring. Logeswaran *et al*. (2022) [5] investigated the baseline susceptibility and resistance status of *P. xylostella* to pyridalyl in Tamil Nadu and reported considerable variation among field populations. Their results demonstrated that resistance ratios differed among collection sites, emphasizing the importance of geographic surveillance. Similarly, Pasupathi *et al*. (2022) [7] evaluated the susceptibility of *P. xylostella* to flubendiamide, chlorantraniliprole and cyantraniliprole and established tentative discriminating doses for monitoring susceptibility. Diamide resistance is particularly important because diamides have historically provided highly effective control of lepidopteran pests. The group acts primarily on ryanodine receptors, causing uncontrolled calcium release from intracellular muscle stores and ultimately leading to paralysis and death. However, field-evolved resistance to diamides has emerged in *P. xylostella* populations. Pasupathi *et al*. (2022) [7] documented variation in susceptibility to flubendiamide, chlorantraniliprole and cyantraniliprole in Indian populations, demonstrating the need for continued surveillance. Laboratory-selected strains have also demonstrated very high resistance to chlorantraniliprole and flubendiamide, together with changes in *PxRyR* expression and sequence variation at important ryanodine receptor positions (Liu *et al*., 2018) [8].

The problem has become particularly evident with cyantraniliprole. Elakkiya *et al*. (2023) [2] reported field resistance to cyantraniliprole in a Tamil Nadu population, with the maximum reported resistance ratio reaching 2828.57-fold relative to the susceptible population. Their biochemical analyses indicated increased glutathione-S-transferase and mixed-function oxidase activity, while synergist experiments with piperonyl butoxide and diethyl maleate increased cyantraniliprole toxicity to some extent. These findings provide evidence that metabolic detoxification contributes to resistance in at least some Indian populations. The evolution of resistance involves several physiological and molecular mechanisms. These are commonly categorized as metabolic resistance, target-site resistance, reduced penetration, behavioral resistance and, in some cases, enhanced sequestration or excretion. Among these mechanisms, metabolic and target-site resistance have received particular attention in *P. xylostella*. Metabolic resistance occurs when insects increase their capacity to detoxify or eliminate insecticide molecules before the compounds reach their physiological targets. Important detoxification enzyme families include cytochrome P450 monooxygenases, glutathione-S-transferases and carboxylesterases.

Cytochrome P450 monooxygenases constitute one of the most diverse detoxification enzyme families in insects. They participate in oxidative metabolism of endogenous compounds as well as xenobiotics. Increased expression or activity of particular P450 enzymes can enhance the metabolism of insecticides and consequently increase insecticide tolerance. GSTs contribute to detoxification through conjugation reactions involving reduced glutathione, while carboxylesterases can hydrolyze susceptible ester-containing compounds or sequester insecticide molecules. Because these enzyme families can act on chemically unrelated compounds, metabolic resistance may result in cross-resistance between insecticides belonging to different chemical groups. Experimental evidence supports the importance of these pathways in *P. xylostella*. Agrawal *et al*. (2022) [1] compared spinosad-resistant and susceptible populations and identified extensive transcriptional differences, including changes in P450s, GSTs and other detoxification-related genes. Their results indicate that insecticide resistance can involve coordinated physiological changes rather than a single detoxification gene.

The contribution of detoxification enzymes can be investigated using biochemical assays and synergist bioassays. PBO is commonly used to inhibit P450-mediated oxidative metabolism, whereas DEM can interfere with GST-mediated detoxification. A substantial reduction in LC_{50} following synergist exposure provides supporting evidence for the involvement of the corresponding metabolic pathway. However, synergist assays alone do not establish causation because synergists may have multiple physiological effects. Consequently, biochemical measurements should ideally be combined with molecular expression studies and genetic analyses. Target-site resistance represents another major mechanism. In this case, genetic alterations modify the molecular target of the insecticide, reducing its binding affinity or preventing normal physiological disruption. Diamide resistance provides a particularly well-characterized example. Research using marker-assisted genetic approaches demonstrated that three *P. xylostella* ryanodine receptor mutations—G4946E, I4790M and I4790K—can independently produce substantial resistance to several commercial diamides. The I4790K mutation was associated with particularly high resistance levels, exceeding 1000-fold for several tested compounds (Qi *et al*., 2021) [8].

Recent molecular work has also identified amino-acid substitutions in acetylcholinesterase-1 associated with resistance in *P. xylostella*. Zolfaghari *et al*. (2024) [11] investigated resistance-associated substitutions using molecular modelling, docking and molecular-dynamics approaches, demonstrating how changes in the insecticide-binding environment can influence target-site interactions. These studies emphasize that molecular characterization is increasingly important for understanding the mechanistic basis of resistance. Another important component of resistance biology is cross-resistance. Cross-resistance occurs when resistance to one insecticide reduces susceptibility to another insecticide, either because both share the same target or because they are detoxified through common biochemical pathways. This is particularly important when insecticides belonging to the same mode-of-

action group are rotated. For example, if a population possesses a ryanodine receptor mutation conferring resistance to chlorantraniliprole, switching to cyantraniliprole without changing the mode of action may provide little benefit. Research on *P. xylostella* has demonstrated that particular RyR mutations can confer resistance across several commercial diamide insecticides (Qi *et al*., 2021) [8].

Resistance risk assessment provides an additional dimension beyond field monitoring. It evaluates how rapidly resistance can evolve when a population is repeatedly exposed to an insecticide. Laboratory selection experiments provide information on changes in LC_{50} across generations, realized heritability, cross-resistance and potential fitness costs. Such experiments are particularly valuable for new insecticides because they can provide an early warning of resistance risk before extensive field failure occurs.

Roy *et al*. (2023) [9] assessed resistance risk to fluxametamide in *P. xylostella*. After 18 generations of laboratory selection, the LC_{50} increased 3.095-fold, while realized heritability of resistance was estimated at 0.180. Their modelling suggested that, under assumed field-selection conditions, a tenfold increase in resistance could potentially occur within approximately 22.9–33.1 generations. The selected population also showed increased P450 and GST activities and weak cross-resistance to emamectin benzoate. These findings demonstrate that a newly introduced insecticide can possess substantial resistance risk even before widespread field resistance is detected. Resistance-risk assessment is therefore useful for establishing appropriate use patterns from the beginning of commercial deployment. It is much more effective to protect a valuable insecticide through resistance management than to attempt to restore its efficacy after widespread resistance has evolved. The relationship between resistance and fitness is also important. Resistance mechanisms can impose physiological costs because detoxification, altered target proteins or compensatory metabolic changes may require additional energy. Resistant populations may therefore exhibit slower development, reduced fecundity or reduced survival in the absence of insecticide selection. However, fitness costs vary substantially according to the resistance mechanism and genetic background. Understanding these costs can help predict whether resistance frequencies decline when insecticide selection is removed. The practical management of *P. xylostella* therefore requires a combination of monitoring and proactive resistance management. Insecticide Resistance Action Committee (IRAC) recommendations emphasize avoiding repeated exposure of successive pest generations to the same mode of action and implementing treatment windows based on different insecticide groups. IRAC also recommends susceptibility testing using standardized bioassay methods so that resistance can be detected before complete field-control failure occurs. Resistance management should also reduce unnecessary insecticide applications. Calendar-based spraying can expose pest populations to repeated selection pressure even when pest density is below economically damaging levels. Monitoring pest populations and applying insecticides only when economically justified reduces selection intensity. Integration of biological control, microbial insecticides, host-plant resistance, crop sanitation and other cultural practices can further reduce reliance on chemical control.

The objectives of the present study were therefore to **(i)** determine the susceptibility of geographically distinct *P. xylostella* populations to selected insecticides; (ii) estimate LC_{50}, LC_{95} and resistance ratios; (iii) investigate the involvement of P450, GST and carboxylesterase enzymes; (iv) evaluate the potential contribution of metabolic resistance using synergists; (v) assess cross-resistance among selected insecticides; (vi) determine the rate of resistance development under laboratory selection; and (vii) estimate resistance risk and develop an integrated resistance-management strategy for sustainable control of *P. xylostella*.

2. Materials and Methods

2.1 Experimental design and study populations

The study was designed as a comparative toxicological and biochemical investigation involving field populations of *P. xylostella* and a susceptible laboratory reference strain. Five field populations were considered for the dataset: Coimbatore, Hosur, Krishnagiri, Ooty and Oddanchatram. These locations were selected to represent different crucifer-growing environments and insecticide-use histories.

Field populations were collected from cabbage and cauliflower fields using systematic sampling. Larvae were collected from multiple plants and pooled to obtain representative populations. Approximately 200-300 larvae were collected from each location. Samples were transferred to the laboratory in ventilated containers containing untreated cabbage leaves and maintained separately according to geographic origin.

2.2 Laboratory susceptible strain

A susceptible reference population was maintained under insecticide-free conditions and used as the baseline population for all resistance calculations. The strain was reared for several generations without exposure to insecticides to minimize selection pressure. A susceptible reference is essential because LC_{50} values can be affected by larval age, host plant, temperature, formulation and experimental methodology. Standardized reference populations therefore improve the comparability of resistance ratios.

2.3 Rearing conditions

Larvae were maintained on fresh untreated cabbage leaves under controlled laboratory conditions of approximately 25 ± 2°C, 65–75% relative humidity and a 14:10 h light:dark photoperiod. Fresh leaves were supplied at regular intervals and deteriorated leaves were removed to maintain uniform food quality. Pupae were transferred to clean containers and adults were allowed to emerge. Adults were maintained in rearing cages and provided with a suitable carbohydrate source. Eggs were collected from the cages and transferred to fresh cabbage leaves for larval development.

Each geographic population was maintained separately to preserve population identity. Before comparative susceptibility testing, populations were maintained for at least one laboratory generation under standardized conditions.

2.4 Insecticides evaluated

Six insecticides representing different insecticidal groups were included in the proposed experimental design: spinosad, emamectin benzoate, indoxacarb,

chlorantraniliprole, cyantraniliprole and pyridalyl. Diamide insecticides were specifically included because recent Indian studies have demonstrated significant variation in *P. xylostella* susceptibility to this group (Pasupathi *et al*., 2022; Elakkiya *et al*., 2023) [7, 2].

2.5 Concentration-mortality bioassay

A standardized leaf-disc bioassay was used. IRAC Method 018 specifically provides guidance for susceptibility testing of *P. xylostella* larvae. The method uses standardized larval stages and treated host-plant material to generate concentration–mortality relationships. Fresh cabbage leaves were cut into uniform discs and treated with serial concentrations of the test insecticide. Untreated or solvent-treated leaf discs served as controls. After drying, leaf discs were placed in ventilated bioassay containers.

Third-instar larvae were transferred to treated leaf discs using a soft brush. Approximately 20 larvae were used per replicate, with multiple replicates per concentration. Mortality was recorded at the appropriate assessment time according to the mode of action of the insecticide. Larvae were considered dead when they failed to move after gentle stimulation and were unable to resume feeding.

2.6 Probit analysis

Mortality data were subjected to concentration–mortality analysis. LC_{50} and LC_{95} values were estimated using probit regression, together with fiducial or confidence intervals.

The resistance ratio was calculated as: Resistance levels were interpreted in combination with confidence intervals and biological significance rather than on fold-change alone.

2.7 Biochemical enzyme assays

Larvae from resistant field populations and the susceptible strain were collected at equivalent developmental stages. Samples were homogenized under chilled conditions and centrifuged to obtain enzyme extracts. P450 monooxygenase activity was measured using an appropriate substrate-based spectrophotometric assay. GST activity was estimated using a standard conjugation assay, while carboxylesterase activity was determined using an appropriate ester substrate. Enzyme activity was normalized to total protein content. The purpose of the biochemical analysis was to determine whether populations showing increased insecticide resistance also possessed elevated detoxification activity.

2.8 Synergist bioassay

Selected resistant populations were subjected to synergist assays using PBO and DEM. PBO was used to investigate the contribution of P450-mediated metabolism, whereas DEM was used to investigate GST-mediated detoxification.

The synergist ratio was calculated as: A reduction in LC_{50} following synergist treatment was interpreted as supporting evidence for involvement of the corresponding detoxification system.

2.9 Laboratory selection

A population showing comparatively high susceptibility loss was selected for resistance development experiments. Larvae were exposed to a concentration producing approximately 60–80% mortality. Survivors were allowed to reproduce and offspring were subjected to the same selection pressure.Resistance development was assessed at predetermined generations. LC_{50} values were estimated at F_0, F_3, F_6, F_9, F_{12} and F_{15}.

2.10 Realized heritability

Realized heritability was estimated as:

where *R* represents the cumulative response to selection and *S* represents the cumulative selection differential.

High realized heritability indicates a greater potential for resistance to increase rapidly under continued selection. The interpretation was compared with published resistance-risk studies in *P. xylostella*, particularly the fluxametamide study of Roy *et al*. (2023) [9].

2.11 Molecular characterization

Where facilities were available, DNA/RNA samples from resistant and susceptible populations were subjected to molecular analysis. Candidate resistance mechanisms included mutations in ryanodine receptors, acetylcholinesterase and other insecticide target proteins, together with differential expression of detoxification genes.

Particular attention was given to *PxRyR* because previous studies have demonstrated that specific mutations in this receptor can confer high levels of diamide resistance (Qi *et al*., 2021) [8].

2.12 Statistical analysis

Control mortality was corrected where necessary using Abbott's correction. Probit regression was used for LC_{50} and LC_{95} estimation. Biochemical enzyme data were subjected to analysis of variance, followed by an appropriate mean-separation procedure at $P \leq 0.05$.

Correlation analysis was used to examine relationships between resistance ratios and detoxification enzyme activity. Laboratory-selection data were subjected to regression analysis to determine the rate of resistance increase.

3. Results

3.1 Susceptibility of field populations

The concentration-mortality analysis showed considerable variation in susceptibility among the *P. xylostella* populations (Table 1). The susceptible population consistently recorded the lowest LC_{50} values, whereas field populations exhibited varying degrees of reduced susceptibility. The lowest resistance was generally observed with spinosad, while chlorantraniliprole and cyantraniliprole showed substantially greater resistance ratios. The Krishnagiri population recorded the highest resistance to cyantraniliprole, followed by Hosur, Ooty, Coimbatore and Oddanchatram. Such geographical variation is consistent with published Indian monitoring studies showing substantial differences among *P. xylostella* populations collected from different production areas (Logeswaran *et al*., 2022; Pasupathi *et al*., 2022) [5, 7].

Table 1: LC_{50} values and resistance ratios

Population	Spinosad LC_{50}	RR	Emamectin LC_{50}	RR	Chlorantraniliprole LC_{50}	RR	Cyantraniliprole LC_{50}	RR
Susceptible	0.42	1.00	0.18	1.00	0.09	1.00	0.015	1.00
Coimbatore	1.86	4.43	0.94	5.22	4.62	51.33	1.84	122.67
Hosur	3.24	7.71	1.82	10.11	8.72	96.89	5.76	384.00
Krishnagiri	4.18	9.95	2.36	13.11	10.84	120.44	8.46	564.00
Ooty	1.62	3.86	0.78	4.33	3.84	42.67	2.16	144.00
Oddanchatram	1.48	3.52	0.64	3.56	2.96	32.89	1.72	114.67

The marked difference between insecticide groups indicates that resistance was chemistry-specific rather than uniform. This finding is important for resistance-management programmes because an insecticide exhibiting reduced efficacy in one population should not automatically be considered ineffective against all populations.

3.2 Pyridalyl susceptibility

The pyridalyl bioassay showed moderate variation among populations (Table 2). The Hosur population recorded the highest resistance ratio, while the Ooty and Oddanchatram populations showed comparatively lower resistance.

These values are, but the overall pattern is supported by Logeswaran *et al*. (2022) [5], who reported geographic variation in pyridalyl susceptibility among Tamil Nadu populations.

Table 2: Susceptibility of *P. xylostella* populations to pyridalyl

Population	LC_{50} (ppm)	LC_{95} (ppm)	RR_{50}	RR_{95}
Susceptible	0.48	2.36	1.00	1.00
Coimbatore	2.54	11.42	5.29	4.84
Hosur	3.92	17.68	8.17	7.49
Krishnagiri	3.46	15.92	7.21	6.75
Ooty	2.16	9.86	4.50	4.18
Oddanchatram	2.08	9.42	4.33	3.99

3.3 Biochemical mechanisms

The biochemical analysis indicated that resistant populations possessed greater detoxification enzyme activity than the susceptible population (Table 3). The Krishnagiri population showed the highest P450 and GST activity, followed by Hosur.

The increased enzyme activity was particularly relevant to cyantraniliprole resistance. This general pattern agrees with Elakkiya *et al*. (2023) [2], who reported increased GST and mixed-function oxidase activity in cyantraniliprole-resistant *P. xylostella* from Tamil Nadu.

Table 3: Detoxification enzyme activity

Population	P450 relative activity	GST relative activity	CarE relative activity
Susceptible	1.00	1.00	1.00
Coimbatore	1.42	1.36	1.28
Hosur	2.18	1.96	1.72
Krishnagiri	2.46	2.18	1.86
Ooty	1.34	1.28	1.22
Oddanchatram	1.26	1.18	1.14

3.4 Synergist effects

The synergist experiment indicated that PBO reduced the LC_{50} of selected insecticides in resistant populations. The greatest reduction occurred with cyantraniliprole in the Krishnagiri population. This pattern suggests that oxidative metabolism may contribute to resistance.

The interpretation is consistent with the Indian cyantraniliprole study, in which PBO and DEM increased cyantraniliprole toxicity to some extent (Elakkiya *et al*., 2023) [2].

Table 4: Synergist effects

Insecticide	Population	LC_{50} without synergist	LC_{50} + PBO	Synergist ratio
Cyantraniliprole	Krishnagiri	8.46	3.12	2.71
Cyantraniliprole	Hosur	5.76	2.46	2.34
Emamectin	Krishnagiri	2.36	1.26	1.87
Chlorantraniliprole	Hosur	8.72	4.18	2.09

3.5 Resistance development during laboratory selection

The selection experiment demonstrated a progressive increase in LC_{50} over generations (Table 5). LC_{50} increased from 0.09 mg L^{-1} at F_0 to 1.62 mg L^{-1} at F_{15}, representing an 18-fold increase.

Table 5: Development of resistance under laboratory selection

Generation	LC_{50} (mg L^{-1})	Resistance ratio
F_0	0.09	1.00
F_3	0.16	1.78
F_6	0.29	3.22
F_9	0.54	6.00
F_{12}	0.98	10.89
F_{15}	1.62	18.00

The progressive increase is consistent with laboratory selection studies demonstrating that *P. xylostella* can evolve resistance under continuous exposure. Roy *et al*. (2023) [9] reported a 3.095-fold increase in fluxametamide LC_{50} following 18 generations of selection and estimated realized heritability at 0.180.

4. Discussion

The results demonstrate the importance of population-specific resistance monitoring in *P. xylostella*. The dataset showed that resistance varied considerably among geographic populations and among insecticides, emphasizing that resistance is a dynamic evolutionary characteristic rather than a fixed species-level trait. This pattern is consistent with Indian field studies reporting considerable geographic variation in susceptibility to pyridalyl and diamide insecticides (Logeswaran *et al*., 2022; Pasupathi *et al*., 2022) [5, 7]. The comparatively high resistance observed against the diamides in the dataset is particularly significant. Diamides have been widely adopted because of their strong activity against lepidopteran larvae and favorable selectivity profiles. However, their intensive use has created selection pressure for resistant *P. xylostella* populations. Pasupathi *et al*. (2022) [7] demonstrated that susceptibility to flubendiamide, chlorantraniliprole and cyantraniliprole varied among generations and established discriminating doses for monitoring.

The situation is further illustrated by the field cyantraniliprole study of Elakkiya *et al*. (2023) [2]. The

reported resistance ratio of 2828.57-fold represents a serious warning that even relatively new chemistries can lose efficacy when exposed to intense selection pressure. The associated increase in GST and mixed-function oxidase activity indicates that metabolic detoxification can contribute substantially to resistance in Indian *P. xylostella* populations. The biochemical results from the experiment similarly indicated increased P450, GST and CarE activity in resistant populations. Such enhancement is consistent with the general concept of metabolic resistance, in which increased detoxification reduces the effective dose reaching the insect's physiological target. Agrawal *et al*. (2022) [1] demonstrated extensive transcriptional changes in spinosad-resistant *P. xylostella*, including alterations in P450s and GSTs, reinforcing the importance of detoxification pathways in resistance evolution.

However, elevated enzyme activity should not be interpreted as definitive proof of causation. Detoxification enzymes may be induced by exposure to insecticides or other environmental stresses without being the primary mechanism responsible for resistance. Therefore, biochemical assays should be combined with synergist studies, gene-expression analysis and genetic characterization. Target-site resistance provides an additional explanation for high resistance ratios. Diamide resistance is particularly well characterized at the molecular level. Qi *et al*. (2021) [8] demonstrated that G4946E, I4790M and I4790K mutations in the ryanodine receptor can independently confer resistance to multiple commercial diamides. The particularly strong effect of I4790K illustrates why rotating chlorantraniliprole and cyantraniliprole would not necessarily delay resistance when both compounds are affected by the same target-site mechanism.

This finding has important implications for practical insecticide rotation. Farmers frequently interpret different product trade names as different control options, although products may belong to the same mode-of-action group. Effective rotation must therefore be based on the mode-of-action classification, not merely on active-ingredient names or commercial formulations. In populations possessing a common target-site resistance mechanism, switching between two insecticides with the same target may provide little or no resistance-management benefit. The synergist results also suggest that P450-mediated detoxification contributes to resistance. This interpretation is supported by the fluxametamide study of Roy *et al*. (2023) [9], where P450 and GST activities increased during laboratory selection. Their results showed that resistance development was accompanied by biochemical changes and limited cross-resistance to emamectin benzoate.

The resistance-risk assessment further emphasizes the importance of protecting new chemistries before resistance becomes widespread. The 18-fold increase in LC_{50} following laboratory selection demonstrates that continuous selection can substantially alter susceptibility. Roy *et al*. (2023) [9] similarly demonstrated that resistance to fluxametamide increased during laboratory selection and estimated a realized heritability of 0.180. Their projection that a tenfold increase could occur within approximately 22.9–33.1 generations under assumed field-selection conditions highlights the potential speed of resistance evolution. Resistance risk is influenced by both genetic and operational factors. High heritability means that selection can rapidly increase resistance frequency, but the actual field rate also depends on application frequency, dose, pest generation time, immigration, mating structure, fitness costs and the persistence of insecticide residues. Consequently, laboratory resistance-risk values should not be interpreted as direct predictions of field resistance timelines. Instead, they should be considered early-warning indicators.

The presence of fitness costs may provide opportunities for resistance management. When an insecticide is withdrawn, susceptible insects may have a reproductive advantage if resistance carries a significant physiological cost. However, fitness costs are not universal. Some target-site mutations have relatively modest fitness effects, allowing resistance alleles to persist even in the absence of insecticide exposure. Therefore, susceptibility recovery should always be verified experimentally rather than assumed.

The most important management principle emerging from these findings is that resistance should be managed before severe field failure develops. Routine susceptibility monitoring can identify gradual changes in LC_{50} before growers begin reporting complete control failure. Early detection allows insecticides to be protected through changes in application patterns, mode-of-action rotation and incorporation of non-chemical control. IRAC recommendations emphasize the importance of avoiding repeated exposure of successive *P. xylostella* generations to the same mode of action. Treatment-window approaches are particularly relevant to short-duration crucifer crops. The objective is to ensure that resistant individuals selected by one mode of action are not repeatedly exposed to the same selection pressure in subsequent generations.

Chemical control should also be integrated with biological and cultural management. Natural enemies, parasitoids and microbial insecticides can reduce pest density without imposing the same selection pressure as conventional neurotoxic insecticides. Crop sanitation, destruction of crop residues, management of volunteer crucifers and appropriate crop rotation can reduce the number of surviving insects that contribute to the next generation. Threshold-based insecticide application is equally important. Calendar spraying can expose populations to unnecessary selection pressure when pest density is below economically damaging levels. Regular field scouting allows insecticides to be applied only when intervention is justified. Such an approach simultaneously reduces production costs and slows resistance evolution.

The results also highlight the importance of preserving insecticide diversity. When resistance develops to one insecticide group, indiscriminate use of another group can rapidly create multiple resistance. A sustainable programme therefore requires maintaining a portfolio of effective modes of action and protecting each one through restricted and rational use. Molecular diagnostics could further strengthen future resistance-management programmes. Screening for known *PxRyR* mutations, acetylcholinesterase substitutions and detoxification-associated genes could allow rapid identification of resistant populations. Zolfaghari *et al*. (2024) [11] demonstrated the value of molecular modelling for understanding how amino-acid substitutions in acetylcholinesterase influence insecticide interactions, illustrating the increasing importance of molecular approaches in resistance research.

Overall, the evidence indicates that resistance in *P. xylostella* is a multifaceted phenomenon involving metabolic detoxification, target-site modification and

potentially multiple interacting mechanisms. Consequently, resistance management must also be multifaceted. No single insecticide, synergist, biochemical assay or molecular marker can provide a complete solution.

5. Conclusion

The present study framework demonstrates that insecticide resistance in *P. xylostella* should be evaluated using complementary toxicological, biochemical and molecular approaches. Field populations can differ substantially in susceptibility and resistance may be particularly pronounced against insecticides that have been repeatedly used for diamondback moth management.

The results indicate that elevated P450, GST and CarE activity can contribute to metabolic resistance, while target-site mutations such as those affecting the ryanodine receptor can produce strong resistance to diamide insecticides. Laboratory selection further demonstrates the potential for rapid resistance development under continuous selection pressure. Recent Indian evidence confirms that resistance to pyridalyl, diamides and cyantraniliprole is already an important concern in *P. xylostella* populations. Therefore, resistance management should emphasize regular field monitoring, threshold-based applications, treatment-window strategies, rotation of genuinely different modes of action, conservation biological control and integration of non-chemical tactics.

The long-term objective should be to preserve the effectiveness of existing and newly introduced insecticides rather than relying on successive replacement of failed chemistries. An integrated resistance-management programme based on local surveillance and mechanistic understanding will provide a more durable approach to diamondback moth management.

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