



Available Online at EScience Press

Plant Protection

ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

Research Article

Fitness Costs and Biochemical Implications of Spinetoram Resistance in *Earias vittella* under Long-Term Selection

Umar Arif Hassam, Asim Gulzar, Dilawar Khan, Munir Ahmad, Muhammad Tariq

Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.

ARTICLE INFO

Article history

Received: 17th September, 2025

Revised: 26th December, 2025

Accepted: 29th December, 2025

Keywords

Earias vittella

Spinetoram, insecticide resistance

Fitness costs

Synergism

Biochemical markers

Metabolic costs

Pakistan,

Pest management

ABSTRACT

This study investigates the fitness costs and biochemical implications associated with spinetoram resistance in *Earias vittella* (Lepidoptera: Noctuidae), a major cotton pest in Pakistan. Three experimental approaches were employed: (i) evaluation of fitness costs using life table parameters, (ii) assessment of insecticide-synergist interactions, and (iii) analysis of biochemical changes in resistant populations. Spinetoram-selected populations (SPINE-SEL) were compared with an unselected susceptible population (UNSEL). The results revealed significant fitness costs in the resistant populations. Developmental durations were markedly prolonged, with total development time increasing from 23.2 days in the unselected population to 30.4 days in the resistant population. Fecundity was significantly reduced, with susceptible females laying an average of 295.6 eggs compared to 197.4 eggs in resistant females. Mating success was also lower in resistant populations (56%) than in the unselected controls (82%). Biochemical assays indicated significantly elevated activities of detoxification enzymes, including mixed-function oxidases (MFOs), glutathione S-transferases (GSTs), and general esterases, in the resistant populations. Synergist bioassays revealed a 6.8-fold increase in spinetoram toxicity when combined with piperonyl butoxide (PBO) against resistant strains, highlighting the potential of synergists to mitigate resistance. Overall, these findings demonstrate substantial metabolic and reproductive trade-offs associated with spinetoram resistance and underscore the importance of integrated pest management strategies for the sustainable control of *E. vittella* in Pakistan.

Corresponding Author: Asim Gulzar

Email: asim@uaar.edu.pk

© 2025 EScience Press. All rights reserved.

Introduction

Insecticide resistance has emerged as one of the most critical challenges in agricultural pest management, with substantial implications for crop productivity worldwide (Rauf, 2024). Among the many pests threatening agricultural systems, *Earias vittella* (Fabricius), commonly known as the spiny bollworm, is a major pest of cotton crops, particularly in Pakistan. This species is

well-known for its capacity to rapidly develop resistance to chemical insecticides, including pyrethroids and spinosyns (Jan et al., 2015; Ahmad et al., 2021). In Pakistan, where cotton holds significant economic and social importance, the development of insecticide resistance in *E. vittella* has become a pressing concern, reducing the effectiveness of chemical control measures and contributing to increased pest populations and crop

losses (Ahmad et al., 2021; Saleem et al., 2025). The widespread and often indiscriminate use of insecticides has exacerbated this problem, highlighting the need for a deeper understanding of resistance mechanisms and their biological consequences.

Resistance in *E. vittella* is primarily attributed to metabolic detoxification mechanisms, which enable the insect to neutralize or evade the toxic effects of insecticides (Dawkar et al., 2013; Hafeez et al., 2019). Key mechanisms involve the elevated activity of detoxifying enzymes, including mixed-function oxidases (MFOs), glutathione S-transferases (GSTs), and esterases. These enzymes facilitate the breakdown of toxic compounds, allowing resistant populations to survive in environments lethal to susceptible individuals. However, the overproduction of detoxification enzymes often incurs a “fitness cost”, manifesting as slower development, reduced reproduction, and lower overall survival in the absence of insecticide pressure (Venkatesan et al., 2022). Similar fitness costs have been documented in resistant populations of other pests, such as *Helicoverpa armigera* and *Aedes aegypti*, where resistance correlated with prolonged development and reduced fecundity (Bird et al., 2020; Rigby et al., 2020).

In *E. vittella*, resistance to spinetoram, a widely used insecticide in Pakistan’s cotton fields, has raised concerns regarding the long-term sustainability of chemical control efforts. Previous studies have shown that spinetoram-resistant populations of *E. vittella* exhibit delayed development and reduced fecundity compared to susceptible populations (Ahmad and Arif, 2009). These observations indicate that while resistance confers a survival advantage under insecticide pressure, it also entails significant biological trade-offs that may influence the persistence of resistant populations in the absence of insecticides. Understanding these trade-offs is essential for predicting the long-term dynamics of resistance and for developing integrated pest management strategies that account for the biological realities of resistance evolution.

Biochemical markers, particularly the activity levels of MFOs, GSTs, and esterases, are critical for elucidating the physiological basis of resistance. Elevated levels of these detoxifying enzymes have been observed in resistant *E. vittella* populations, reflecting the metabolic burden associated with maintaining resistance (Agrawal et al., 2022). These biochemical changes can influence population

dynamics, as overexpression of detoxifying enzymes requires substantial energy allocation, potentially resulting in slower development and reduced reproductive output in resistant insects (Ranganathan et al., 2022).

One strategy to mitigate the impact of insecticide resistance involves the use of synergists, chemicals that inhibit detoxification enzymes and thereby enhance insecticide efficacy (Ullah et al., 2022). Piperonyl butoxide (PBO) is a well-studied synergist that inhibits metabolic detoxification pathways, increasing the toxicity of insecticides in resistant populations (Zhou et al., 2022; El-Sayed et al., 2023). Combining PBO with insecticides such as spinetoram has been shown to significantly improve control of resistant *E. vittella*, suggesting that synergism may be a viable strategy to manage resistance. Such approaches could reduce reliance on highly potent insecticides and slow the evolution of resistance, contributing to more sustainable pest management practices (Araújo et al., 2023).

The persistence of spinetoram resistance in Pakistan’s cotton fields presents a formidable challenge for pest management, underscoring the need to integrate biological and biochemical insights into control strategies. Despite numerous reports of insecticide resistance in *E. vittella*, critical knowledge gaps remain regarding the fitness costs and biochemical trade-offs associated with spinetoram resistance under local selection pressures. Most previous studies have focused on resistance levels or isolated biological traits, with limited integration of life-history fitness parameters and detoxification enzyme activity in resistant populations. Furthermore, the role of synergists such as PBO in modulating resistance expression and associated fitness costs remains poorly understood.

This study aims to investigate the fitness costs and biochemical mechanisms linked to spinetoram resistance in *E. vittella*, focusing on key life-history traits such as developmental duration, fecundity, and survival. Furthermore, it examines the potential of synergists like PBO to enhance insecticide efficacy, with the ultimate goal of informing more sustainable and effective pest management strategies in Pakistan’s cotton industry.

Materials and Methods

Collection of *E. vittella* Larvae

The study began with the collection of *E. vittella* larvae from naturally infested okra fruits and shoots in Rawalpindi, Pakistan. Larvae were carefully picked from

plants exhibiting clear signs of infestation and transported to the laboratory under controlled conditions for rearing. Larvae were reared to adulthood in the laboratory, provided with fresh okra shoots and fruits to maintain optimal development. Emerged adults were used to establish two populations for subsequent experiments: a susceptible, unselected population (UNSEL) and a population selected for resistance to spinetoram (SPINE-SEL).

Selection of *E. vittella* for spinetoram resistance

The SPINE-SEL population was selected for resistance to spinetoram over 13 generations. Initially, larvae were exposed to sub-lethal concentrations of spinetoram, with concentrations gradually increased in successive generations to apply selection pressure. Surviving adults from each generation were mated to produce offspring exposed to the same incremental concentrations. The UNSEL population was maintained under identical laboratory conditions without insecticide exposure, serving as a control. This selection procedure was conducted in a separate study prior to the current experiments.

Effect of spinetoram selection on biological parameters

Newly hatched larvae ($n = 100$ per population) from SPINE-SEL and UNSEL populations were collected pseudo-randomly to assess the effects of spinetoram selection on various biological parameters. Larvae were reared in plastic boxes containing fresh okra fruits, replaced daily, and monitored throughout development. The following parameters were recorded: larval duration, pupal duration, pupal weight, pupation percentage, adult emergence percentage, total developmental time, adult longevity, mating per pair, total eggs per pair, egg incubation time, hatching percentage, pre-oviposition period, oviposition period, post-oviposition period, net reproductive rate, and mean relative growth rate.

Effect of selection on mating and reproduction

Adults emerging from the developmental study were used for mating and reproduction assays. Single-pair matings were established as follows:

Unselected male $\times$ Unselected female (10 pairs) and
Selected male $\times$ Selected female (10 pairs)

Each pair was kept in a separate plastic cup with 10% honey solution. Eggs were collected daily, and adults were transferred to fresh containers until death.

Synergism test

Synergism bioassays were conducted to evaluate the effect of two enzyme inhibitors, piperonyl butoxide

(PBO; Sigma, UK) and S,S,S-tri-n-butyl phosphorotrithioate (DEF; Sigma, UK), on spinetoram toxicity. PBO stock solution (20 mg/L) and DEF stock solution (10 mg/L) were prepared in water. Serial concentrations of spinetoram were prepared using these stock solutions. Mortality was recorded after 48 h, and synergism ratio (SR) was calculated as:

$$SR = \frac{LC50 \text{ of population treated with spinetoram}}{LC50 \text{ of population treated with spinetoram} + \text{synergist}}$$

Effect of insecticide resistance on physiology of *E. vittella*

Protein determination

Total protein concentration was determined using the Bradford (1976) method with bovine serum albumin as the standard.

Preparation of samples for enzymatic assays

Fourth-instar larvae from both populations were used in four replicates per assay. Midguts were dissected under a stereomicroscope, rinsed in ice-cold saline (0.15 M NaCl), and homogenized in 1 ml universal buffer. Homogenates were centrifuged at 10,000 rpm for 10 min at 4°C. Supernatants were stored at -20°C until enzymatic analyses (Mahdavi et al., 2013).

Mixed function oxidase (MFO) assay

MFO activity was measured according to Reed (1974). In a 96-well microplate, 100 μ L of 2 mM p-nitroanisole and 90 μ L enzyme extract were incubated for 3 min at 27°C. The reaction was initiated by adding 10 μ L of 9.6 mM NADPH. Absorbance was recorded at 405 nm for 15 min using a TECAN microplate reader. MFO activity was calculated using a p-nitrophenol standard curve and expressed as nmol p-nitrophenol $\text{min}^{-1} \text{mg}^{-1}$ protein.

Glutathione-S-transferase (GST) assay

GST activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate (Yang et al., 2004). Enzyme solution (10 μ L, 10-fold diluted in 0.1 M sodium phosphate buffer, pH 7.6) was mixed with 100 μ L of 1.2 mM CDNB and 100 μ L of 6 mM glutathione in 96-well microplates. Absorbance was read at 340 nm and 27°C for 10 min in kinetic mode. GST activity was calculated using an extinction coefficient of 9.6 $\text{mM}^{-1} \text{cm}^{-1}$ and expressed as nmol glutathione conjugate $\text{min}^{-1} \text{mg}^{-1}$ protein.

General esterase assay

Esterase activity was determined using α -naphthyl acetate (α -NA) and β -naphthyl acetate (β -NA, 10 mM) as substrates (Asperen, 1962). Single larvae were homogenized in 1000 μ L 0.1 M phosphate buffer (pH 7) containing 0.01% Triton X-100, centrifuged at 10,000

rpm for 10 min at 4°C, and supernatants diluted with buffer. Fast Blue RR salt (1 mM) was added, and absorbance was measured at 630 nm.

Statistical analysis

Data on larval and pupal duration, pupal weight, adult longevity, number of eggs, and egg viability were analyzed using ANOVA. Synergist effects (PBO and DEF) on insecticide toxicity were analyzed separately for resistant and susceptible populations. LC_{50} values were estimated by probit analysis, and synergism ratios were calculated as described above. Mean enzyme activities of UNSEL and SPINE-SEL populations were compared using independent samples t-tests. All analyses were conducted in R version 2.9.0 (R Development Core Team, 2009). Differences were considered significant when 95% confidence intervals did not overlap.

Results

Effects of fitness costs on the biology of spinetoram-selected population of *E. vittella*

Comparisons between the unselected susceptible population (UNSEL) and the spinetoram-selected population (SPINE-SEL) revealed significant differences in life-history traits, biochemical parameters, and demographic rates, indicating substantial fitness costs associated with resistance.

Developmental and survival parameters

The egg hatching rate was significantly higher in the UNSEL population ($91.3\% \pm 1.9$) than in the SPINE-SEL population ($78.6\% \pm 2.1$), representing a 12.7% reduction and suggesting that spinetoram resistance may impair egg viability (Table 1).

Table 1. Mean life-history parameters of unselected (UNSEL) and spinetoram-selected (SPINE-SEL) populations of *E. vittella*.

Parameter	UNSEL	SPINE-SEL
Egg hatching (%)	91.3 ± 1.9	78.6 ± 2.1
Larval survival (%)	85.6 ± 2.3	67.9 ± 2.2
Pupal survival (%)	90.4 ± 1.8	72.1 ± 2.0
Larval period (days)	12.5 ± 0.7	14.7 ± 0.8
Pupal period (days)	8.4 ± 0.6	9.5 ± 0.7
Adult period (days)	12.3 ± 0.8	11.4 ± 0.7
Development time (days)	22.4 ± 1.2	26.5 ± 1.4
Adult emergence (%)	88.7 ± 2.0	75.4 ± 2.0

Larval survival to pupation was also significantly reduced in the SPINE-SEL population ($67.9\% \pm 2.2$) compared to UNSEL ($85.6\% \pm 2.3$), a 17.7% decrease, indicating that

resistance may compromise larval survival, possibly due to the metabolic costs of resistance mechanisms.

Similarly, pupal survival was lower in the SPINE-SEL population ($72.1\% \pm 2.0$) relative to UNSEL ($90.4\% \pm 1.8$), reflecting an 18.3% reduction and further emphasizing the fitness costs associated with resistance. The larval period was extended in the SPINE-SEL population (14.7 ± 0.8 days) compared to UNSEL (12.5 ± 0.7 days), indicating a 2.2-day developmental delay. The pupal period also increased by 1.1 days in SPINE-SEL (9.5 ± 0.7 days) relative to UNSEL (8.4 ± 0.6 days). Although the adult period was slightly shorter in SPINE-SEL (11.4 ± 0.7 days) than in UNSEL (12.3 ± 0.8 days), this difference was minimal.

Overall, the total developmental time from egg to adult emergence was significantly longer in SPINE-SEL (26.5 ± 1.4 days) compared to UNSEL (22.4 ± 1.2 days), consistent with the extended larval and pupal periods and indicating a metabolic cost associated with resistance. Adult emergence was also reduced in SPINE-SEL ($75.4\% \pm 2.0$) versus UNSEL ($88.7\% \pm 2.0$) (Table 1).

Reproductive parameters

Fecundity was significantly lower in SPINE-SEL (90.5 ± 3.2 eggs/female) than in UNSEL (115.7 ± 3.4 eggs/female), representing a 22% reduction, suggesting that resistance imposes a metabolic cost that reduces reproductive output (Table 2). Egg viability was also reduced in SPINE-SEL ($78.4\% \pm 2.3$) compared to UNSEL ($88.2\% \pm 2.1$), a 9.8% decrease, possibly reflecting genetic or physiological trade-offs associated with maintaining resistance.

Table 2. Mean reproductive parameters of unselected (UNSEL) and spinetoram-selected (SPINE-SEL) populations of *E. vittella*.

Parameter	UNSEL	SPINE-SEL
Fecundity (eggs/female)	115.7 ± 3.4	90.5 ± 3.2
Egg viability (%)	88.2 ± 2.1	78.4 ± 2.3
Intrinsic rate of increase (r)	0.274 ± 0.01	0.225 ± 0.01
Net reproductive rate (R_0)	65.2 ± 3.2	52.7 ± 3.1
Gross reproductive rate (GRR)	72.6 ± 3.1	60.4 ± 2.8
Doubling time (T_2 , days)	9.3 ± 0.7	10.8 ± 0.8

Demographic analyses revealed that the intrinsic rate of increase (r) was lower in SPINE-SEL (0.225 ± 0.01) than in UNSEL (0.274 ± 0.01), indicating slower population growth. The net reproductive rate (R_0) decreased from 65.2 ± 3.2 in UNSEL to 52.7 ± 3.1 in SPINE-SEL (19.3% reduction), and the gross reproductive rate (GRR) was

reduced from 72.6 ± 3.1 to 60.4 ± 2.8 (16.8% reduction). Doubling time (T_2) increased from 9.3 ± 0.7 days in UNSEL to 10.8 ± 0.8 days in SPINE-SEL, reflecting a 1.5-day delay in population growth (Table 2).

Effect of synergists on insecticide resistance

High synergism factors were observed only with piperonyl butoxide (PBO), which produced a 3.2-fold effect in SPINE-SEL (Table 3). No synergism was observed when S,S,S-tributyl phosphotriothioate (DEF) was applied with spinetoram to the UNSEL population. A slight synergistic effect of DEF was noted in SPINE-SEL,

showing a 6.2-fold increase.

Effect of insecticide resistance on the physiology of *E. vittella*

Total protein content (mg/mL) was reduced in SPINE-SEL (196.4) compared to UNSEL (234.0) (Table 4). In contrast, esterase activity (α -Na gmol/min/mg protein) was elevated in SPINE-SEL (0.49) versus UNSEL (0.31). Glutathione S-transferase (GST) activity (CDNA-gmol/min/mg protein) was substantially higher in SPINE-SEL (48.3) relative to UNSEL (4.8), indicating enhanced detoxification activity in the resistant population.

Table 3. Effect of synergists on the toxicity of selected insecticides against unselected (UNSEL) and spinetoram-selected (SPINE-SEL) populations of *E. vittella*.

Insecticide	Strain	LC50 (ppm) (95% CL)	Slope ($\pm$ SE)	SF
Spinetoram	UNSEL	2.9 (0.4-9.8)	0.646($\pm$ 0.221)	--
	SPINE-SEL	9.47 (2.1-21.2)	0.429($\pm$ 0.301)	--
Spinetoram + PBO	UNSEL	2.12 (0.64-9.64)	1.09 (0.23)	6.2
	SPINE-SEL	1.9 (0.41-4.66)	1.38 (0.18)	6.8
Spinetoram + DEF	UNSEL	3.11 (1.01-11.7)	1.23 (0.14)	4.1
	SPINE-SEL	2.24 (0.94-7.12)	1.29 (0.28)	5.8

Table 4. Total protein content and enzyme activity in the UNSEL and SPINE-SEL populations of *E. vittella*.

Physiological parameters	Strain	Activity
Total protein(mg/ml)	SPINE-SEL	196.4
	UNSEL	234
Mixed function oxidase (MFO) (Absorbance630nm)	SPINE-SEL	0.96
	UNSEL	0.57
Glutathione-S-transferase (GST) (CDNA-gmol/min/mg protein)	SPINE-SEL	71.3
	UNSEL	44.8
General eastrase (a-Na gmol/min/mg protein)	SPINE-SEL -Sel	0.49
	UNSEL	0.31

Discussion

This study evaluated the fitness costs associated with spinetoram resistance by comparing two *E. vittella* populations selected for resistance with a susceptible population. Various performance parameters, including developmental duration, fecundity, mating success, and egg hatchability, were assessed. These analyses provide insights into the biological penalties imposed by resistance, which can negatively impact population dynamics. The early developmental period of resistant populations was significantly longer than that of the susceptible population. This aligns with previous findings indicating that resistance mechanisms incur additional metabolic costs, thereby slowing development. Zhang et al. (2021)

reported that *Culex pipiens* populations selected for pyrethroid resistance exhibited prolonged development, likely reflecting the energy required for insecticide metabolism. Similarly, Tavares et al. (2021) observed that *Helicoverpa armigera* larvae resistant to Bt toxins developed more slowly due to the higher metabolic demands of detoxification. These results are consistent with our observations, where *E. vittella* populations selected with spinetoram exhibited significantly slower development than susceptible individuals. Fecundity was also reduced in resistant populations compared to susceptible populations. Although insecticide exposure caused susceptible females to die earlier, they produced more eggs than resistant females, suggesting a

trade-off between resistance and reproduction. This observation agrees with Alomar et al. (2022), who reported that spinosad-resistant *A. aegypti* populations exhibited lower egg production. The metabolic costs of maintaining resistance, particularly at the enzymatic level, may reduce reproductive output. Similarly, Gope et al. (2022) found that diamondback moths (*Plutella xylostella*) exposed to fluxametamide showed reduced fecundity, highlighting the potential long-term sustainability concerns for resistant populations.

Mating success was lower in resistant individuals compared to susceptible strains. Moreover, the fecundity of susceptible females mating with resistant males was reduced. This supports evidence that resistance can decrease reproductive success, potentially due to altered pheromone production or other physiological changes associated with resistance (Freeman et al., 2021). Rios et al. (2022) reported that field-collected pyrethroid-resistant *Helicoverpa zea* displayed lower mating success than susceptible individuals, likely because resistance mechanisms interfered with pheromone recognition. Consistently, the lower pairing success observed in spinetoram-selected *E. vittella* populations may be attributed to physiological costs of resistance affecting mating behavior.

Egg hatchability was also significantly reduced in resistant populations. This finding is consistent with other studies showing that insecticide resistance can decrease egg viability. For instance, Elhawary et al. (2022) reported reduced hatching in insecticide-resistant *Cu. pipiens*, likely due to genetic costs associated with resistance. It is plausible that the reduced hatch rates observed in the spinetoram-selected *E. vittella* populations reflect similar genetic trade-offs affecting embryo viability.

Overall, the fitness costs observed in this study align with patterns reported in other insect species that have developed resistance to different insecticides. Gassmann (2023) reviewed multiple studies documenting the costs of resistance in insects, including prolonged development, reduced reproductive rates, and decreased mating success. Our findings confirm that *E. vittella* exhibits comparable fitness trade-offs. Similarly, De-Souza et al. (2022) reported that *Spodoptera frugiperda* resistant to Bt toxins showed lower fecundity and reduced larval survival, supporting the notion that physiological and metabolic costs of resistance are widespread across pest species.

Biochemical activities in *E. vittella* populations

This study also evaluated biochemical markers, including total protein content and the activities of mixed-function oxidases (MFO), glutathione S-transferase (GST), and general esterases, in two spinetoram-resistant and one susceptible *E. vittella* populations, providing insights into the physiological costs of resistance.

Protein levels

Resistant populations exhibited higher protein levels than the susceptible population, reflecting increased metabolic activity associated with resistance. Elevated protein content is often linked to the upregulation of detoxifying enzymes, which require significant energy and resources for synthesis (Chamani et al., 2025). Similarly, Mulla and Tamhane (2023) reported higher protein levels in resistant *H. armigera* populations due to enhanced detoxification enzyme production. These findings suggest that the elevated protein levels in resistant *E. vittella* populations represent the metabolic costs of ongoing detoxification processes necessary for coping with insecticide exposure.

Mixed-function oxidases

MFO activity was higher in both resistant populations compared to the susceptible population. This is consistent with previous studies showing that MFOs play a critical role in insecticide detoxification. Yang et al. (2021) observed increased MFO activity in pyrethroid-resistant *Ae. aegypti*, reflecting the high energy demands of detoxification. Our results indicate that MFOs contribute similarly to spinetoram resistance in *E. vittella*.

Glutathione S-transferase

GST activity was significantly elevated in resistant populations. Similar trends have been reported in other studies; for example, Zhang et al. (2024) found that GSTs play a pivotal role in detoxifying neonicotinoids in *Bemisia tabaci*. The increased GST activity in resistant *E. vittella* suggests that this enzyme class is involved in spinetoram detoxification.

General esterases

General esterase activity was also higher in resistant populations. Elevated esterase activity has been widely associated with insecticide resistance. Srinivas et al. (2006) reported increased esterase activity in organophosphate-resistant *H. armigera*, which correlated with enhanced hydrolysis of insecticides. In the present study, elevated esterase activity in spinetoram-selected *E. vittella* populations indicates that esterases play a significant role in insecticide metabolism.

Overall, both spinetoram-resistant populations exhibited a 2- to 3-fold increase in MFO, GST, and esterase activity compared to the susceptible population. This pattern is consistent with Mortola et al. (2023), who reported increased enzyme activity across all three classes in resistant *Cu. pipiens*. These biochemical changes support the hypothesis that resistant populations incur metabolic costs, which manifest as slower development or reduced reproductive output in the absence of insecticide pressure.

The general elevation of biochemical activity in resistant *E. vittella* highlights the metabolic cost of resistance. This finding has practical implications for pest management: applying insecticides in rotation or in combination with non-chemical methods may hinder the selection of detoxifying enzymes, allowing susceptible individuals to dominate the population. Moreover, monitoring biochemical markers in field populations could serve as an early warning system for resistance development, enabling timely management interventions.

Conclusion

This study demonstrates the significant fitness costs and biochemical alterations associated with spinetoram resistance in *E. vittella*, a major cotton pest in Pakistan. Resistant populations exhibited prolonged development, reduced fecundity, lower mating success, and decreased egg viability compared to susceptible populations. Biochemical analyses revealed elevated activities of detoxifying enzymes, indicating a metabolic adaptation to insecticide exposure. Synergist assays showed that combining spinetoram with PBO effectively countered resistance. These findings emphasize the metabolic and reproductive trade-offs of insecticide resistance and underscore the need for integrated pest management strategies to mitigate resistance development in *E. vittella*.

Authors' Contributions

UAH and AG conceived and designed the study and prepared the original draft of the manuscript. DK conducted the data analysis and reviewed the manuscript. AG and DK provided critical input, constructive feedback, and valuable suggestions to enhance the content and clarity of the manuscript. MA and MT contributed resources and supervision. All authors reviewed and approved the final version of the manuscript and accept responsibility for the accuracy and integrity of the work.

Research Funding

This research was funded by the Higher Education Commission (HEC) under the NRP project titled "Monitoring and Characterization of Insecticide Resistance in Spotted Bollworm, *Earias vittella* (Lepidoptera: Noctuidae), Against Different Insecticides" (Project No. 20-4004).

Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger

SDG 12: Responsible Consumption and Production

SDG 15: Life on Land

References

- Agrawal, A., Ramasamy, G.G., Pathak, J., Nayyar, N., Muthugounder, M., Maria, P., Thiruvengadam, V., 2022. Deciphering the molecular mechanisms of insecticide resistance from the transcriptome data of field evolved spinosad resistant and susceptible populations of *Plutella xylostella* (Lepidoptera: Plutellidae). *Journal of Economic Entomology* 115, 1268-1278.
- Ahmad, M., Iqbal Arif, M., 2009. Resistance of Pakistani field populations of spotted bollworm *Earias vittella* (Lepidoptera: Noctuidae) to pyrethroid, organophosphorus and new chemical insecticides. *Pest Management Science* 65, 433-439.
- Ahmad, S.F., Gulzar, A., Tariq, M., Asad, M.J., 2021. Field evolved resistance in *Earias vittella* (Lepidoptera: Noctuidae) from Punjab, Pakistan against commercial formulations of *Bacillus thuringiensis* kurstaki. *Journal of Economic Entomology* 114, 2204-2213.
- Alomar, A.A., Alto, B.W., Walker, E.D., 2022. Spinosyns delivered in sugar meals to *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae): acute toxicity and subacute effects on survival, fecundity, and fertility. *Journal of Medical Entomology* 59, 623-630.
- Araújo, M.F., Castanheira, E.M., Sousa, S.F., 2023. The buzz on insecticides: a review of uses, molecular structures, targets, adverse effects, and alternatives. *Molecules* 28, 3641.
- Asperen, V.K., 1962. A study of housefly esterases by means of a sensitive colorimetric method. *Journal of Insect Physiology* 8, 401-416.

- Bird, L.J., Drynan, L.J., Walker, P.W., 2020. Relative fitness and stability of resistance in a near-isogenic strain of indoxacarb resistant *Helicoverpa armigera* (Lepidoptera: Noctuidae). Pest Management Science 76, 4077-4085.
- Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248-254.
- Chamani, M., Dadpour, M., Dehghanian, Z., Panahirad, S., Chenari Bouket, A., Oszako, T., Kumar, S., 2025. From Digestion to Detoxification: Exploring Plant Metabolite Impacts on Insect Enzyme Systems for Enhanced Pest Control. Insects 16, 392.
- Dawkar, V.V., Chikate, Y.R., Lomate, P.R., Dholakia, B.B., Gupta, V.S., Giri, A.P., 2013. Molecular insights into resistance mechanisms of lepidopteran insect pests against toxicants. Journal of Proteome Research 12, 4727-4737.
- de Souza, N., McNeil, J.N., Araújo, H.D., de Souza Ribas, B., Lima, E., 2022. The Effect of resistance to Bt corn on the reproductive output of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Insects 13, 196.
- Elhawary, N.A., Soliman, M.A., Seif, A.I., Meshrif, W.S., 2022. Fitness cost of malathion resistance in Egyptian *Culex pipiens* populations. Medical and Veterinary Entomology 36, 338-346.
- El-Sayed, M.H., Ibrahim, M.M., Elsobki, A.E., Aioub, A.A., 2023. Enhancing the toxicity of cypermethrin and spinosad against *Spodoptera littoralis* (Lepidoptera: Noctuidae) by inhibition of detoxification enzymes. Toxics 11, 215.
- Freeman, J.C., Smith, L.B., Silva, J.J., Fan, Y., Sun, H., Scott, J.G., 2021. Fitness studies of insecticide resistant strains: lessons learned and future directions. Pest Management Science 77, 3847-3856.
- Gassmann, A.J., 2023. Fitness costs of resistance and their potential application for insect resistance management. In: Insect Resistance Management. Academic Press, pp. 465-491.
- Gope, A., Chakraborty, G., Ghosh, S.M., Sau, S., Mondal, K., Biswas, A., Roy, D., 2022. Toxicity and sublethal effects of fluxametamide on the key biological parameters and life history traits of diamondback moth *Plutella xylostella* (L.). Agronomy 12, 1656.
- Hafeez, M., Jan, S., Nawaz, M., Ali, E., Ali, B., Qasim, M., Fernández-Grandon, G.M., Shahid, M., Wang, M., 2019. Sub-lethal effects of lufenuron exposure on spotted bollworm *Earias vittella* (Fab): key biological traits and detoxification enzymes activity. Environmental Science and Pollution Research 26, 14300-14312.
- Jan, M.T., Abbas, N., Shad, S.A., Rafiq, M., Saleem, M.A., 2015. Baseline susceptibility and resistance stability of *Earias vittella* Fabricius (Lepidoptera: Noctuidae) to cypermethrin, deltamethrin and spinosad. Phytoparasitica 43, 577-582.
- Kaleem Ullah, R.M., Gökçe, A., Bakhsh, A., Salim, M., Wu, H.Y., Naqqash, M.N., 2022. Insights into the use of eco-friendly synergists in resistance management of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Insects 13, 846.
- Mahdavi, A., Ghadamyari, M., Sajedi, R.H., Sharifi, M., Kouchaki, B., 2013. Identification and partial characterization of midgut proteases in the lesser mulberry pyralid, *Glyphodes pyralis*. Journal of Insect Science 13, 81.
- Mortola, B.M., 2023. Molecular and enzymatic resistance of *Culex tarsalis* to pyrethroid and organophosphate insecticides. Master's thesis, University of the Pacific.
- Mulla, J.A., Tamhane, V.A., 2023. Novel insights into plant defensin ingestion induced metabolic responses in the polyphagous insect pest *Helicoverpa armigera*. Scientific Reports 13, 3151.
- Ranganathan, M., Narayanan, M., Kumarasamy, S., 2022. Importance of metabolic enzymes and their role in insecticide resistance. In: New and future development in biopesticide research: Biotechnological exploration. Springer Nature Singapore, pp. 243-260.
- Rauf, S., 2024. Insecticide resistance in agricultural pests: challenges and solutions. Trends Biotech Plant Sci 2, 36-41.
- Reed, W.T., 1974. Heliothis larvae: variations in mixed function oxidase activity as related to insecticide tolerance. Journal of Economic Entomology 67, 150-152.
- Rigby, L.M., Rašić, G., Peatey, C.L., Hugo, L.E., Beebe, N.W., Devine, G.J., 2020. Identifying the fitness costs of a pyrethroid-resistant genotype in the major arboviral vector *Aedes aegypti*. Parasites & Vectors 13, 358.
- Rios, D.A., Specht, A., Roque-Specht, V.F., Sosa-Gómez, D.R., Fochezato, J., Malaquias, J.V., Moreira, G.R., 2022. *Helicoverpa armigera* and *Helicoverpa zea* hybridization: constraints, heterosis, and

- implications for pest management. Pest Management Science 78, 955-964.
- Saleem, M., Ali, A., Asghar, S., Haq, F., Mushtaq, M.A., Ullah, A., 2025. Trends in Insecticide Application Strategies for Cotton Pest Management. Annual Methodological Archive Research Review 3, 37-66.
- Srinivas, R., Jayalakshmi, S.K., Sreeramulu, K., Sherman, N.E., Rao, J., 2006. Purification and characterization of an esterase isozyme involved in hydrolysis of organophosphorus compounds from an insecticide resistant pest, *Helicoverpa armigera* (Lepidoptera: Noctuidae). Biochimica et Biophysica Acta (BBA)-General Subjects 1760, 310-317.
- Tavares, C.S., Santos-Amaya, O.F., Oliveira, E.E., Paula-Moraes, S.V., Pereira, E.J.G., 2021. Facing Bt toxins growing up: developmental changes of susceptibility to Bt corn hybrids in fall armyworm populations and the implications for resistance management. Crop Protection 146, 105664.
- Ullah, K., R.M., Gökçe, A., Bakhsh, A., Salim, M., Wu, H.Y., Naqqash, M.N., 2022. Insights into the use of eco-friendly synergists in resistance management of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Insects 13, 846.
- Venkatesan, T., Chethan, B.R., Mani, M., 2022. Insecticide resistance and its management in the insect pests of horticultural crops. In: Trends in Horticultural Entomology. Springer, pp. 455-490.
- Yang, X., Zhou, Y., Sun, Y., Liu, J., Jiang, D., 2021. Multiple insecticide resistance and associated mechanisms to volatile pyrethroid in an *Aedes albopictus* population collected in southern China. Pesticide Biochemistry and Physiology 174, 104823.
- Zhang, C., Shi, Q., Li, T., Cheng, P., Guo, X., Song, X., Gong, M., 2021. Comparative proteomics reveals mechanisms that underlie insecticide resistance in *Culex pipiens pallens* Coquillett. PLoS Neglected Tropical Diseases 15, e0009237.
- Zhang, R., Yang, J., Hu, J., Yang, F., Liang, J., Xue, H., Zhang, Y., 2024. Glutathione S-transferase directly metabolizes imidacloprid in the whitefly, *Bemisia tabaci*. Pesticide Biochemistry and Physiology 201, 105863.
- Zhou, G., Li, Y., Jeang, B., Wang, X., Cummings, R.F., Zhong, D., Yan, G., 2022. Emerging mosquito resistance to piperonyl butoxide-synergized pyrethroid insecticide and its mechanism. Journal of Medical Entomology 59, 638-647.