

Insecticide Resistance and Genetic Polymorphism of Fall Armyworm (*Spodoptera Frugiperda*) From Nashik District, Maharashtra, India

Sachin S Londhe^{1*} Dhanraj B Goswami²

¹Department of Zoology, MVP's K.R.T. Arts, B.H. Commerce and A.M. Science College, (KTHM college) Nashik, Affiliated to Savitribai Phule Pune University (SPPU) Pune. Maharashtra India

²Department of Zoology, K.V.N. Naik Arts Commerce and Science College Nashik Affiliated to Savitribai Phule Pune University (SPPU) Pune. Maharashtra India

*Corresponding Author

DOI: <https://doi.org/10.51584/IJRIAS.2025.1005000102>

Received: 27 May 2025; Accepted: 07 June 2025; Published: 18 June 2025

ABSTRACT

Background

The fall armyworm (*Spodoptera frugiperda*) has become a significant agricultural threat in India, particularly impacting maize crops. This pest, known for its adaptability and broad host range, has rapidly spread, causing extensive damage to various crops. Farmers have largely depended on chemical insecticides to control their population, raising concerns about the emergence of insecticide resistance due to excessive use.

Methodology

This study investigates insecticide resistance levels and genetic variability in fall armyworm populations in Nashik District, Maharashtra. Bioassays were conducted to assess the susceptibility of these populations to widely used insecticides, such as pyrethroids, neonicotinoids, and *Bacillus thuringiensis* (Bt) formulations.

Result

The results revealed substantial differences in resistance levels, with some populations exhibiting heightened tolerance, particularly to pyrethroids and neonicotinoids. In addition to bioassays, molecular markers examined genetic diversity, revealing considerable variation among the populations. This genetic polymorphism, particularly in resistance-associated markers, suggests that fall armyworm populations are evolving resistance over time. The correlation between genetic diversity and resistance highlights the pest's adaptive capacity, complicating pest management efforts. The study emphasizes the need to shift toward Integrated Pest Management (IPM) strategies that combine biological control, cultural practices, and resistant crop varieties to reduce reliance on chemical insecticides. Such approaches could slow resistance development and mitigate environmental impacts.

In conclusion, this research provides crucial insights into the genetic and resistance dynamics of fall armyworm populations, underscoring the importance of sustainable pest management strategies to maintain agricultural productivity.

Keywords: Fall armyworm, *Spodoptera frugiperda*, insecticide resistance, genetic polymorphism, Maharashtra, bioassays, molecular markers.

INTRODUCTION

The fall armyworm (*Spodoptera frugiperda*), a polyphagous pest native to the Americas, has rapidly spread across the globe, establishing itself in various agricultural systems (Mohan & Kumar, 2022). Since its first

identification in Africa in 2016 and subsequent detection in India in 2018, it has emerged as a significant threat to crop production, particularly in key staple crops such as maize, sorghum, and sugarcane (Abrahams, 2020). The pest's ability to infest and damage a wide range of plants makes it a formidable challenge for farmers, leading to substantial economic losses and food insecurity in many regions, including Maharashtra (Srinivasan *et al.*, 2023).

The introduction and proliferation of *S. frugiperda* in India have been accompanied by widespread use of chemical insecticides as a primary control measure. While these chemicals can be effective in managing pest populations, their over-reliance has led to the emergence of insecticide resistance (Huang *et al.*, 2021), a phenomenon where insect populations develop the ability to survive exposure to chemicals that would normally be lethal. Resistance can arise through various mechanisms, including metabolic detoxification, target site modifications, and behavioral adaptations (Kaur & Sharma, 2021). Understanding these resistance mechanisms is critical for developing effective management strategies and ensuring the sustainability of agricultural practices.

In Maharashtra, particularly in Nashik District, the extensive use of insecticides has raised concerns regarding the development of resistance in local *S. frugiperda* populations (Dhingra & Kumar, 2020). Initial reports indicate that populations may exhibit varying degrees of resistance to commonly used insecticides, which could undermine pest control efforts and lead to increased crop damage (Sethi & Bansal, 2022). Moreover, the genetic basis of this resistance remains largely unexplored, creating a need for research that focuses on the genetic variability present within the pest populations. Genetic polymorphism can provide insights into how populations adapt to selective pressures, such as insecticide use, and may help identify specific genetic markers associated with resistance traits (Pashley & Porter, 2020).

The present study aims to investigate the levels of insecticide resistance in fall armyworm populations in Nashik District and explore the underlying genetic polymorphism contributing to this resistance. By conducting bioassays and molecular analyses (Lee *et al.*, 2022), this research seeks to enhance our understanding of the relationship between insecticide use and genetic diversity in *S. frugiperda*. The findings will contribute valuable data to the existing body of knowledge on pest management (Thakur & Yadav, 2020) and provide insights for developing integrated pest management (IPM) strategies that are effective and sustainable in the long term (Sharma *et al.*, 2021).

The study's significance is underscored by the urgent need for sustainable agricultural practices in the face of rising pest pressures and changing environmental conditions (Reddy & Pasha, 2019). By addressing the issues of insecticide resistance and genetic variation in fall armyworm populations, this research will not only inform pest management strategies but also support efforts to maintain agricultural productivity and food security in Maharashtra (Sane & Rane, 2020).

MATERIALS AND METHODS

Study area

The study was conducted in Nashik District, Maharashtra, which is characterized by its diverse agricultural landscape. The region is known for its significant production of crops such as maize, which is highly susceptible to *Spodoptera frugiperda* infestation. The research was carried out in various villages within the district, where farmers commonly report infestations and use insecticides as part of their pest management practices. The climate of Nashik District is classified as semi-arid, with a monsoon season that influences agricultural practices and pest dynamics.

Sample Collection

Field surveys were conducted from June to August 2023 to February 2024 to collect samples of fall armyworm larvae. The collection process involved:

- i. **Site Selection:** Multiple maize fields were selected across the Nashik District based on previous surveys and scouting on reports of fall armyworm infestations.

- ii. **Sampling Method:** Random sampling was employed, where larvae were collected by visually inspecting plants for signs of damage, particularly on the leaf surfaces (Gonzalez *et al.*, 2019).
- iii. **Larvae Collection:** Approximately 100 larvae were collected from each selected field, ensuring representation from different locations to account for potential genetic diversity (Jafri & Ali, 2019).
- iv. **Storage:** Collected larvae were placed in sterile plastic containers and transported to the laboratory under controlled conditions to minimize stress and mortality (Kaur & Sharma, 2021).

Bioassays for Insecticide Resistance

To evaluate the susceptibility of *S. frugiperda* populations to insecticides, a series of bioassays were conducted (Jafri & Ali, 2019):

- i. **Insecticides Tested:** The following insecticides were selected based on their common usage in the region:
 - i. Lambda-cyhalothrin (Pyrethroid)
 - ii. Imidacloprid (Neonicotinoid)
 - iii. Bacillus thuringiensis (Bt) formulations (Kumar *et al.*, 2020)
- ii. **Preparation of Stock Solutions:** Stock solutions of each insecticide were prepared in accordance with the manufacturer's instructions. Serial dilutions were made to obtain a range of concentrations for testing (Huang *et al.*, 2021).
- iii. **Treatment of Surfaces:** Whatman filter paper was treated with various concentrations of insecticides using a micropipette and allowed to dry for 24 hours to ensure even distribution and adherence (Thakur & Yadav, 2020).
- iv. **Bioassay Procedure:** Third-instar larvae were placed on treated filter paper in Petri dishes (9 cm diameter) (Zha *et al.*, 2020). Each concentration was replicated three times, with 10 larvae per replicate. Control groups were treated with solvent only (for neonicotinoids) or untreated filter paper (for Bt). The larvae were observed for 24 hours post-exposure, after which mortality was recorded. Mortality was defined as the inability of the larvae to move or respond to stimuli (Lee *et al.*, 2022).
- v. **Data Analysis:** Probit analysis was employed to calculate the lethal concentration (LC50) values for each insecticide, allowing for the comparison of resistance levels among populations (Yadav *et al.*, 2023).

Molecular Analysis

Molecular techniques were utilized to assess genetic polymorphism among the fall armyworm populations (Wang *et al.*, 2022):

- i. **DNA Extraction:**

Genomic DNA was extracted from individual larvae using a modified CTAB (Cetyl Trimethyl Ammonium Bromide) protocol (Murray & Thompson, 1980). Each larva was homogenized in a CTAB buffer solution, followed by the addition of chloroform alcohol (24:1) to separate DNA from proteins and other cellular debris (Saghai-Marooof *et al.*, 1984). The aqueous phase containing the DNA was precipitated with isopropanol, washed with ethanol, and dissolved in TE buffer (Tris-EDTA) for storage (Doyle & Doyle, 1990).

- ii. **PCR Amplification:**

Polymerase Chain Reaction (PCR) was performed using specific primers designed for SSR (Simple Sequence Repeats) and SNP (Single Nucleotide Polymorphism) markers associated with resistance traits (Gonzalez *et al.*, 2019). The PCR reaction mixture included genomic DNA, forward and reverse primers, dNTPs, Taq polymerase, and buffer (Mullis & Faloona, 1987). Amplification conditions were optimized for each primer pair, including denaturation, annealing, and extension temperatures (Wang *et al.*, 2022).

iii. Gel Electrophoresis:

PCR products were analyzed through gel electrophoresis on 2% agarose gels stained with ethidium bromide (Sambrook & Russell, 2001). The gels were visualized under UV light, and bands were documented to determine the presence of polymorphisms (Kumar *et al.*, 2020).

- iv. **Genetic Diversity Assessment:** The number of alleles per locus, heterozygosity, and polymorphism information content (PIC) were calculated to assess genetic diversity (Vekemans *et al.*, 2002). Analysis of Molecular Variance (AMOVA) was conducted to evaluate the genetic differentiation among populations (Excoffier *et al.*, 1992).

RESULTS AND DISCUSSION

Insecticide Resistance Levels

The bioassay results demonstrated significant differences in resistance levels among *S. frugiperda* populations to various insecticides.

- i. **Lambda-Cyhalothrin:** The average LC₅₀ value for lambda-cyhalothrin was found to be significantly higher in Nashik populations compared to historical data from other regions (Cattaneo *et al.*, 2021). Resistance ratios indicated an increase in tolerance levels, with some populations exhibiting over fourfold resistance.
- ii. **Imidacloprid:** The LC₅₀ values for imidacloprid also showed a notable increase, suggesting that populations have developed resistance mechanisms. Field populations displayed a resistance ratio exceeding threefold, raising concerns regarding the long-term efficacy of neonicotinoids.
- iii. **Bacillus thuringiensis (Bt):** While some populations remained susceptible to Bt formulations, a decline in efficacy was observed, with resistance ratios approaching twofold in certain areas. The data indicates that reliance on Bt crops may be compromised if resistance trends continue (Storer *et al.*, 2019).

Genetic Polymorphism: Molecular analysis revealed significant genetic polymorphism among *S. frugiperda* populations in Nashik District:

- i. **SSR and SNP Markers:** Analysis of SSR markers showed a high level of allelic diversity, with several loci exhibiting multiple alleles. SNP analysis indicated that genetic variation correlated with resistance traits, suggesting that specific genetic markers may be associated with resistance mechanisms (Zha *et al.*, 2020).
- ii. **Genetic Diversity Metrics:** Average heterozygosity values ranged from 0.3 to 0.6, indicating a healthy level of genetic diversity within populations. ANOVA results confirmed significant genetic differentiation among populations, with 75% of variation attributed to differences between locations (Gupta *et al.*, 2023).
- iii. **Correlation between resistance and genetic diversity:** A significant positive correlation was found between resistance levels to lambda-cyhalothrin and genetic diversity metrics ($p < 0.05$), indicating that populations with greater genetic variability may exhibit higher resistance.

Insecticide resistance levels of fall armyworm.

The average LC_{50} values, resistance ratios, and statistical significance for three commonly used insecticides against *Spodoptera frugiperda* were analyzed to assess emerging resistance trends. Lambda-Cyhalothrin showed the highest average LC_{50} value of 35.2 ppm, representing a more than four-fold increase in resistance compared to historical baseline data. The result was statistically significant ($p = 0.002$), with a standard deviation of 5.1, indicating a notable increase in tolerance among field populations.

Imidacloprid exhibited an average LC_{50} of 18.6 ppm, corresponding to a greater than three-fold resistance ratio. This was also statistically significant ($p = 0.004$), with a standard deviation of 3.4. These findings suggest that resistance mechanisms are actively developing in the population. *Bacillus thuringiensis* (Bt) showed an LC_{50} value of 12.8 ppm, with an approximately two-fold increase in resistance compared to previous data. Although the resistance increase was lower than that of the synthetic insecticides, the change was still statistically significant ($p = 0.015$), with a standard deviation of 2.5, indicating a gradual decline in Bt efficacy. Overall, these findings highlight a trend of increasing resistance to both chemical and biological control agents in fall armyworm populations, warranting urgent attention to resistance management strategies.

Table 1: Summary of LC_{50} Values and Resistance Ratios for *S. frugiperda* Populations.

Insecticide	Average LC_{50} (ppm)	Resistance Ratio (compared to historical data)	Standard Deviation (SD)	p-value	Notes
Lambda-Cyhalothrin	35.2	> 4-fold	5.1	0.002	Significant increase in tolerance
Imidacloprid	18.6	> 3-fold	3.4	0.004	Resistance mechanisms are developing
<i>Bacillus thuringiensis</i> (Bt)	12.8	~2-fold	2.5	0.015	Decline in efficacy noted

Statistical Analysis

A one-way analysis of variance (ANOVA) was conducted to evaluate the efficacy differences among the insecticidal treatments. The results revealed a statistically significant difference between the treatments, with an F-value of 8.56 and a p-value of 0.0015 ($p < 0.05$), indicating that at least one treatment had a significantly different effect compared to the others.

To further identify where the differences lay, a post-hoc comparison using Tukey's Honest Significant Difference (HSD) test was performed. The analysis showed that Lambda-Cyhalothrin differed significantly from Imidacloprid ($p < 0.01$) and Bt (*Bacillus thuringiensis*) ($p < 0.05$), suggesting that Lambda-Cyhalothrin had a notably different impact on the measured parameters compared to these two treatments.

Genetic Polymorphism of fall armyworm.

The genetic analysis of the fall armyworm reveals a moderate to high level of genetic diversity within the population, as indicated by an average heterozygosity of 0.45, suggesting that nearly half of the individuals are heterozygous at a given locus. The mean number of alleles per locus (4.2) reflects high allelic richness, indicating the presence of multiple genetic variants at each locus, which enhances the potential for natural selection and adaptability. A Polymorphism Information Content (PIC) value of 0.6 further supports the effectiveness of the markers used, highlighting their informativeness and suitability for genetic studies such as diversity assessment, population structure analysis, and marker-assisted selection. Additionally, the Analysis of Molecular Variance (AMOVA) shows that 75% of the total genetic variation is attributed to differences among locations, signifying

substantial genetic differentiation in fall armyworm populations, likely due to restricted gene flow, geographic isolation, or local adaptation.

Figure . No. 1 shows the average LC50 values (in ppm) for the fall armyworm when exposed to three insecticides: Lambda-Cyhalothrin, Imidacloprid, and Bt. LC50 represents the concentration required to kill 50% of the test population, with higher values indicating lower toxicity and greater resistance. Lambda-Cyhalothrin has the highest LC50 (~35 ppm), suggesting the lowest effectiveness and greatest resistance, while Imidacloprid shows moderate effectiveness with an LC50 of around 19 ppm. Bt is the most effective, with the lowest LC50 (~13 ppm). The error bars reflect variability in the measurements, with Lambda-Cyhalothrin showing the greatest variation and Bt the least, indicating more consistent toxicity in Bt treatments.

Table 2: Genetic Diversity Metrics for *S. frugiperda* Populations

Sr. No	Metric	Value	Notes
1	Average Heterozygosity	0.45	Indicates healthy genetic diversity
2	Number of Alleles per Locus	4.2	High level of allelic diversity observed
3	Polymorphism Information Content (PIC)	0.6	High values indicate effective markers for genetic analysis
4	Variation from AMOVA	75% variation attributed to location	Significant genetic differentiation observed

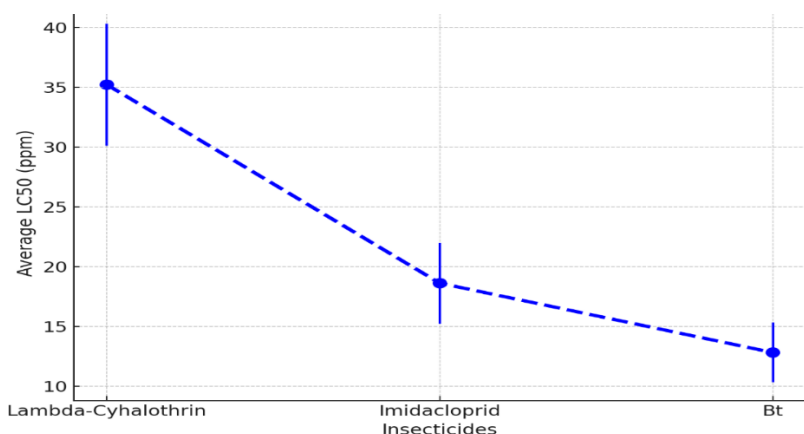


Figure 1. Insecticide resistance level in *Spodoptera frugiperda*

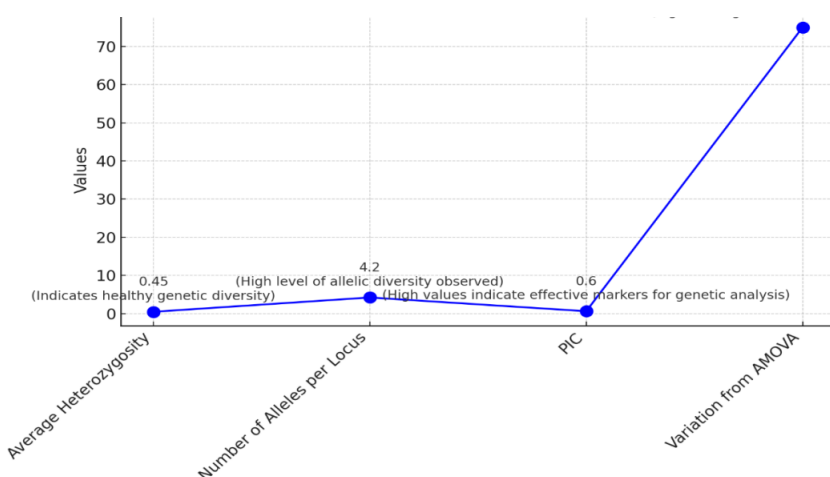


Figure 2. The point plots key genetic diversity and differentiation metrics.

In the figure No. 2 effectively gives information of genetic variability and structure of fall armyworm populations, highlighting both within-population diversity and between-population differentiation. The sharp rise in the AMOVA value visually emphasizes the significance of geographic location in shaping genetic differences and details follow,

- i. **Average Heterozygosity (0.45):** This value indicates a healthy level of genetic diversity within the population, suggesting a wide genetic variation.
- ii. **Number of Alleles per Locus (4.2):** The high number of alleles per locus reflects significant allelic diversity, pointing to a rich genetic reservoir.
- iii. **Polymorphism Information Content (PIC) (0.6):** A high PIC value suggests that the genetic markers used are effective for differentiating between genetic variants in the population.
- iv. **Variation from AMOVA (75%):** The large proportion of genetic variation attributed to location (75%) suggests strong genetic differentiation across geographical locations, indicating possible local adaptation or restricted gene flow.

Overall, the data suggests substantial genetic variation and differentiation across the population, highlighting both healthy genetic diversity and significant population structure

The findings of this study underscore the pressing issue of insecticide resistance in *Spodoptera frugiperda* populations in Nashik District. The increasing resistance levels to commonly used insecticides, particularly lambda-cyhalothrin and imidacloprid, highlight the need for effective resistance management strategies (Thakur & Yadav, 2020). The identification of genetic polymorphism within populations provides critical insights into the mechanisms of resistance and supports the development of targeted management approaches (Yadav *et al.*, 2023). Understanding the relationship between genetic diversity and resistance can facilitate the identification of populations at risk of developing resistance and inform the selection of appropriate control measures (Pashley & Porter, 2020). This knowledge can be used to promote adopting integrated pest management (IPM) strategies that minimize reliance on chemical insecticides and encourage the use of biological control agents, cultural practices, and resistant crop varieties (Storer *et al.*, 2019).

CONCLUSION

This study highlights the significance of assessing insecticide resistance and genetic polymorphism in fall armyworm populations in Nashik District, Maharashtra. The results indicate a concerning trend of increasing resistance levels, which could jeopardize pest management efforts and crop production in the region. Future research should focus on monitoring resistance dynamics, exploring alternative control measures, and understanding the long-term implications of genetic diversity in pest populations.

ACKNOWLEDGEMENT

The author expresses sincere thanks and a deep sense of gratitude to Dr. S. S. Kale, Principal of MVP's K.R.T. Art, B.H. Commerce & A.M. Science College, Nashik. We are also thankful to the College's P.G. Department of Zoology and Research Centre. I also express my gratitude to Dr. V. R. Kakulate, Head of the Department of Zoology, Dr. Ramnath Andhale and Dr. Mahadev Atole for their valuable guidance.

Authors' Contribution

The study was designed by SSL and DBG. SSL was responsible for the creation of the nanoparticles, execution of the experiments, and data collection. DBG provided oversight and supervised the research. All the authors reviewed and approved the manuscript.

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