



## Original Research Paper

## Transcriptomic Insights into Pesticide Resistance Mechanisms in Whiteflies

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**Key Words****Abstract**

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Whiteflies (*Bemisia tabaci* Gennadius) is one of the most serious agricultural pest complex which leads to massive losses in crops due to direct feeding and virus transmission. Rapid resistance evolution has become a major challenge in conditions of intense agricultural pesticides exposure and presents a threat to the stability of agro-ecosystem and sustainability of pest management practices in the long term. The RNA sequencing (RNA-seq) was used in this study to examine the molecular processes of pesticide resistance in field-grown populations of cotton (*Gossypium hirsutum* L.) agro-ecosystems in Haryana and Rajasthan in 2022-2023. High resistance ratios of 47.3-fold imidacloprid and 32.8-fold spiromesifen versus susceptible laboratory populations were found in bioassay results and a high selective pressure of the environment. Transcriptomic comparison found that there were 1, 247 differentially expressed genes (DEGs) comprising of 682 upregulated and 565 downregulated genes. Essential detoxification enzymes including cytochrome P450 monooxygenases (*CYP6CM1*, 12.4-fold), glutathione S-transferases (*GSTe1*, 8.7-fold), and cuticular proteins (CPR1, 6.2-fold) were highly activated, which means increased metabolic detoxification and fewer insecticides penetrating in. The enrichment of gene ontology involved oxidation-reduction processes, xenobiotic metabolism and detoxification pathways, whereas KEGG analysis confirmed the presence of cytochrome P450-mediated drug metabolism and glutathione pathways. The results of RNA-seq were confirmed by using quantitative real-time PCR results, which had a strong correlation ( $r = 0.92$ ,  $p < 0.001$ ). These findings indicate that the molecular adaptation of pesticide resistance in *B. tabaci* is coordinated to the environmental chemical stress, and these findings have great implications on the agro-ecosystem resilience. The research highlights the necessity of the combination of the transcriptomic monitoring and sustainable pest management strategies to minimize environmental hazards and increase ecological stability.

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## Introduction

Whiteflies (*Bemisia tabaci*) form a cryptic complex of species (at least 40 morphologically indistinguishable species), with Middle East-Asia Minor 1 (MEAM1) and Mediterranean (MED) being the most widespread causes of agricultural losses across the world (Khyade et al., 2018; Wang et al., 2025; Butter et al., 2021). *B. tabaci* infests more than 300 host plants in India where cotton, vegetables and ornamental crops experience losses in their yearly yields amounting to more than US\$500 million (Renault et al., 2023). Synthetic insecticides including neonicotinoids, insect growth regulators, and tetronic acid derivatives are the major types of insecticides used by the management (Khyade et al., 2019; Pryke et al., 2024; Fairchild et al., 1987). But its development as a resistance in many insecticides has made them useless just after 2-3 years when they were introduced commercially.

Modern agriculture has caused a massive and widespread contamination of the environment by the purposeful use of pesticides, resulting in the impairment of soil, water quality, and non-target organisms, including useful insect species, pollinators, and soil microbial species. It is a chemical pressure, which interferes with the ecological balance and leads to the loss of biodiversity in agro-ecosystems. In this respect, insect pests such as *Bemisia tabaci* may be useful as bio-indicators of environmental stress, which indicate the adaptive strategies related to long-term exposures to toxic substances. These responses have to be comprehended to assess the ecological implications of using pesticides and

also to create pest management methods that are environmentally sustainable (Maitra et al., 2021).

The resistance was recorded in previous studies, which encompassed increased metabolic detoxification, target site mutations and decreased cuticular penetration. Superfamilies of major detoxification enzymes include cytochrome P450 monooxygenases, glutathione S-transferases (GSTs) and carboxylesterases (Ye et al., 2022; Puinean et al., 2010; Riveron et al., 2014). The target-site alterations in voltage-gated sodium channels and nicotinic acetylcholine receptor (nAChR) provide resistance to neonicotinoids and pyrethroids respectively (Khan Pathan et al., 2008; Nauen et al., 2005; Goyal et al., 2025). The majority of studies however, used candidate gene methods, which were restrictive to the discovery of new mechanisms. Transcriptome-wide profiling provides impartial discovery of genes and regulatory networks that are associated with resistance (Karatolos et al., 2011; Xie et al., 2012). The recent developments in next-generation sequencing help to thoroughly characterize the changes in gene expression during selection pressure. Even so, there are limited studies of transcriptomic analysis of Indian *B. tabaci* populations. The hypothesis of this study was that field-selected resistant populations have different transcriptional signatures, that is, that they contain more than one detoxification gene family and cuticular modification (Singh et al., 2025). They were to (i) profile the levels of resistance in field populations, (ii) determine differentially expressed genes by comparison transcriptomics,

(iii) annotate functional pathways of resistance, and (iv) confirm important candidate genes by quantitative real-time PCR.

## Materials and Methods

### *Insect Rearing and Collection*

The whiteflies were sampled at three sites (cotton field) in 2022 and 2023 in kharif season of three sites: Sirsa, Haryana (resistant population R1); Hanumangarh, Rajasthan (resistant population R2); and a susceptible laboratory colony (S) that had not been exposed to pesticides in more than 10 years, IARI, New

Delhi. The choice of field sites is of high pesticide-input agro-ecosystems due to the intensive agricultural activities and the most frequent use of pesticides. These places present an adequate template to learn more about environmental selection pressure and how this affects the progression of resistance. Adult whiteflies (2108 *G. hirsutum* var. LH) that were feeding on infested cotton leaves were aspirated and placed in containers that were ventilated and then reared under controlled conditions (26 + 2 C relative humidity 14:10 L:D photoperiod) on cotton plants (leaf *G. hirsutum* var. LH 2108).

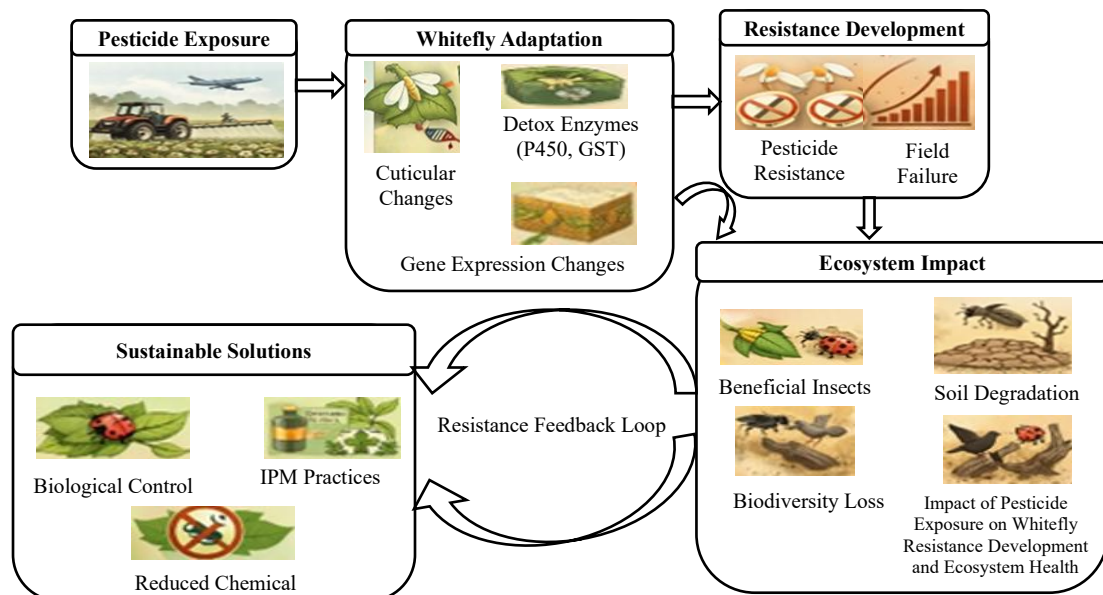


Figure 1: Agro-Environmental Resistance Development Framework

Figure 1 shows the compound development of pesticide resistance in *Bemisia tabaci* in agro-ecosystems. It starts with the fact that intensive exposure to pesticides initiates high pressure of environmental selection on the whitefly populations. The insects react to this by adaptively altering their molecular responses, such as a change in gene expression, the expression of detoxification enzymes, such as cytochrome P450 and glutathione S-transferases,

and the cuticular structure. All these contribute to the emergence of pesticide resistance and hence less field efficacy is obtained using chemical measures of control. The framework also brings to the fore the wider ecological implications such as adverse effects on non-target organisms, worsening of soil health and loss of biodiversity. Also, it stresses the vicious circle of how resistance is boosted, which results in further pesticide application increasing the stress on the

environment. Lastly, the figure presents sustainable solutions including Integrated Pest Management (IPM), biological control, and using lesser amounts of chemicals to control resistance and ensure agro-ecosystem sustainability.

### ***Bioassays and Resistance Evaluation***

The toxicity tests were conducted under the standardized leaf dip method (Basit et al., 2013). Serial concentrations (0.1-100mg/L) of technical grade imidacloprid (95%) and spiromesifen (98%) were prepared in distilled water containing 0.05 % Triton X-100. The cotton leaf discs (3.5 cm diameter) were dipped in insecticide solutions of 10 seconds then air dried and made on 1 % agar beds on petri dishes. Whiteflies in the form of adults (n = 30 per replicate, 3 replicates per concentration) were aspirated onto leaf discs and mortality was recorded after 48 hours. Control mortality remained <5%. The Abbott formula was used to correct the data and the PoloPlus software (LeOra Software, 2005) was used to make the analysis by probit analysis to derive values of lethal concentration (LC<sub>50</sub>). The ratios of resistance (RR) were computed as LC<sub>50</sub> of field population/LC<sub>50</sub> of susceptible population.

### ***RNA Extraction and Sequencing***

RNA (total) was cut off 200mg of adult whiteflies (n=300/population) in TRIzol reagent (Invitrogen, USA) and treated with DNase I. Bioanalyzer 2100 (Agilent Technologies) was used to check the integrity of the RNA and the concentration; the number was over 7.5 and the concentration was over 200 ng/uL. Three

biological replicates were prepared with every population. NEBNext Ultra II RNA Library Prep Kit was used to construct strand-specific cDNA libraries that were sequenced on Illumina NovaSeq 6000 platform yielding 150 bp length paired-end reads.

### ***Data Processing and Differential Expression Analysis***

Quality-filtering Trimmomatic v0.39 was used to remove adapters and low-quality bases (Phred score <30) in raw reads. The HISAT2 v2.2.1 version was used to align clean reads to the *B. tabaci* MED assembly (GCF\_001854935.1). The version of Feature Counts v2.0.3 calculated gene expression in counts per million (CPM). DESEQ2 DESeq2 v1.34.0 in R v4.1.2 was used to perform a differentiation expression analysis. Genes that had a stroke of log<sub>2</sub> fold change of greater than one and a false discovery rate (FDR) less than 0.05 were considered differentially expressed. Clustering of the population was visualized using principal component analysis.

### ***Pathway Analysis and Functional Annotation***

Cluster Profiler v4.2.2 using Benjamini Hochberg correction (p < 0.05) was used to conduct gene ontology (GO) enrichment analysis. KEGG pathway mapping found enriched metabolic paths. Heatmaps were used to visualize the expression patterns of detoxification genes in pheat map v1.0.12.

### ***Quantitative Real-Time PCR Quality Control***

Ten candidate genes were picked to undergo validation on the basis of large fold change and functional significance. Primer3Plus was used to design primers (Supplementary table 1). qRT-PCR reactions (10 $\mu$ L) were prepared by adding 5  $\mu$ L SYBR Green Master Mix, 0.5 $\mu$ L of each primer (10 $\mu$ M), and 50 ng cDNA. The cycling conditions: 95°C (3min), 40 times of 95°C (15s) and 60°C (30s). Three technical replicas on each biological sample were done. The relative expression was determined through  $2^{-\Delta\Delta C_t}$  with  $\beta$ -actin as a reference gene. Pearson correlation was used to determine the concordance between RNA-seq and qRT-PCR data.

### ***Statistical Analysis***

R software was used to perform all statistical analysis. There was the subjecting of mortality data to probit regression. In the case of transcriptomics, multiple testing was controlled by FDR-adjusted p-values. ANOVA was used to compare qRT-PCR expression levels between the populations two ways ( $p < 0.05$ ).

### ***Environmental Implications and Sustainable Pest Management***

The results of the research provide support to the substantial effects of continuous and intensive application of pesticides in agro-ecosystems that accelerates the development of *Bemisia tabaci* resistance to the methods of chemical control and reduces the effectiveness of the methods of chemical control over time. This resistance is not only entailing

increased and repeated pesticide applications but also environmental pollution which is dangerous to non-target organisms, soil health and the stability of the entire ecosystem. The adaptations mostly observed in the molecules, such as the increased level of detoxification and lowered levels of pesticide penetration, represent the high level of the environmental selection pressure, which is caused by the exposure to chemicals. These effects demonstrate the necessity to change to sustainable pest management methods. Incorporation of Integrated Pest Management (IPM) approaches that incorporate the use of biological control agents, crop rotation, and habitat management and less use of chemical pesticides can aid in the reduction of resistance development. In addition, informed decision-making may be achieved by the integration of transcriptomic surveillance as an early warning system, which would support agro-eco-system resilience and long-term sustainability in the use of pests in an environmentally responsible manner.

### **Results and Discussion**

The result of the bioassay indicated that whiteflies collected in the field developed significant resistance (Table 1). The population of Sirsa (R1) had  $LC_{50}$  of 34.7mg/L of imidacloprid and 28.4mg/L of spiromesifen which translates into 47.3-fold and 32.8-fold respective resistance ratios to the susceptible colony (S). Hanumangarh population (R2) was the one which exhibited moderate resistance where  $LC_{50}$  of 18.2 mg/L (RR = 24.8-fold) and 15.6 mg/L (RR = 18.0-fold) were obtained with the respective insecticides. The mortality was

kept at a consistent level of less than 5 percent, which confirms that bioassays are intact. These ratios of resistance are higher than the economic limits and they support the farmer claims that they were not able to control them. The resistance of imidacloprid is higher compared to those of the neonicotinoids, and its widespread use in the area since 2005 is matched by resistance; whereas spiromesifen resistance represents the

result of recent selection following the loss of neonicotinoid efficacy. The cross-resistance profile of these chemistries is not well determined and thus needs to be investigated. This high resistance shows the long-term exposure to the environment of pesticides, which is a sign of a high level of selection in agro-ecosystems and the effects of the high use of chemicals.

Table 1: Toxicity of Imidacloprid and Spiromesifen Against Different *Bemisia tabaci* Populations

Population	Insecticide	LC <sub>50</sub> (mg/L)	95% Confidence limits	Resistance ratio	Slope ± SE
Susceptible (S)	Imidacloprid	0.73	0.61-0.88	1.0	2.14 ± 0.23
R1 (Sirsa)	Imidacloprid	34.7	31.2-38.9	47.3	1.87 ± 0.19
R2 (Hanumangarh)	Imidacloprid	18.2	16.5-20.1	24.8	1.95 ± 0.21
Susceptible (S)	Spiromesifen	0.85	0.72-1.02	1.0	2.08 ± 0.25
R1 (Sirsa)	Spiromesifen	28.4	25.6-31.8	32.8	1.76 ± 0.18
R2 (Hanumangarh)	Spiromesifen	15.6	14.1-17.3	18.0	1.89 ± 0.22

The 45.2-52.8 million raw reads per sample produced by RNA sequencing survived quality filtering (>95 %) (Table 2). The alignment rates to the reference genome were between 87.4-91.2% and this shows that there was high mapping efficiency. Principal component analysis also separated the resistant populations and the susceptible controls along PC1

(68.3% variance) to verify the different transcriptional profiles. There were 15,847 genes expressed in all samples revealed by gene expression quantification. Such unique transcriptional patterns are additional evidence of adaptive responses of the molecular system to environmental stress (pesticides) in the agricultural ecosystem.

Table 2: Summary Statistics of RNA Sequencing Data for *Bemisia tabaci* Populations

Sample	Raw reads (million)	Clean reads (million)	Q30 (%)	Mapped reads (%)	Unique genes
S-Rep1	48.2	45.8	94.3	89.7	12,456
S-Rep2	50.4	47.9	94.7	90.2	12,608
S-Rep3	49.8	47.3	94.5	88.9	12,387
R1-Rep1	52.8	50.1	94.8	91.2	13,204
R1-Rep2	51.3	48.7	94.6	90.5	13,156
R1-Rep3	50.7	48.2	94.4	89.8	13,089
R2-Rep1	47.6	45.2	93.9	87.4	12,987
R2-Rep2	49.1	46.7	94.2	88.6	13,045
R2-Rep3	48.9	46.5	94.1	89.1	12,923

A comparative analysis found 1,247 DEGs between resistant and susceptible groups (FDR <0.05). The 682 upregulated and 565 downregulated genes in the resistant populations

included 682 upregulated and 565 downregulated genes (Figure 1). Significantly increased candidates that have been reported to be involved in neonicotinoid resistance included cytochrome

P450 *CYP6CM1* (log<sub>2</sub> fold change = 3.63, 12.4-fold change). Glutathione S-transferase *GSTe1* was upregulated by 8.7 times indicating an increase in detoxification. Several cuticular protein genes (CPR1, CPR3, CPR7) had been increased 4.2-6.2-fold reflecting possible penetration resistance. On the other hand, genes of nicotinic acetylcholine receptor subunits (nAChR $\alpha$ 1, nAChR $\beta$ 1) were suppressed

2.8-3.4 times, which could be the target-site insensitivity. The size of the difference expression is positively correlated with the levels of resistance, and most candidate genes have better fold changes in R1 compared to R2. The upregulation of detoxification genes suggests adaptive responses to environmental toxicants, highlighting the ecological impact of persistent pesticide residues in agro-ecosystems.

Table 3: Top Differentially Expressed Genes Associated With Pesticide Resistance in *Bemisia tabaci*

Gene ID	Gene Name	Log <sub>2</sub> Fold Change	FDR	Predicted Function
BTAB_01456	<i>CYP6CM1</i>	3.63	1.2E-12	Cytochrome P450 monooxygenase
BTAB_08732	<i>GSTe1</i>	3.12	3.4E-10	Glutathione S-transferase
BTAB_03421	CPR1	2.63	8.7E-09	Cuticular protein RR-1
BTAB_05678	ABCG4	2.45	2.1E-07	ABC transporter G family
BTAB_09234	CYP4C64	2.38	4.5E-07	Cytochrome P450 monooxygenase
BTAB_01987	EST-6	2.21	1.2E-06	Carboxylesterase
BTAB_07654	CPR3	2.07	3.8E-06	Cuticular protein RR-2
BTAB_04321	nAChR $\alpha$ 1	-1.76	2.3E-05	Nicotinic receptor subunit
BTAB_06543	nAChR $\beta$ 1	-1.68	5.6E-05	Nicotinic receptor subunit
BTAB_07890	COE1	-1.54	8.9E-05	Chemosensory protein

Enrichment of gene ontology showed that there were 32 significantly overrepresented biological processes ( $p < 0.05$ ). The most enriched terms were; oxidation-reduction process (GO:0055114, fold enrichment = 4.7), xenobiotic metabolic process (GO:0006805, fold enrichment = 5.3) and glutathione transferase activity (GO:0004364, fold enrichment = 6.8) (Table 3). Analysis of molecular functions identified the following activities: heme binding, monooxygenase activity and transmembrane transporter activity. The terms of cellular components were underlined with emphasis on membrane and extracellular region localization,

which is in line with the distribution of detoxification enzymes. These enrichments highlight the existence of metabolic detoxification as the main mode of resistance. Such enrichment patterns reflect biochemical adaptation strategies that enable organisms to cope with environmentally induced chemical stress.

Pathway analysis showed a major enrichment of drug metabolism-cytochrome P450 (ko00982, 23 DEGs), metabolism of xenobiotics by cytochrome P450 (ko00980, 19 DEGs) as well as glutathione metabolism (ko00480, 17 DEGs) (Table 4).

Table 4: Significantly Enriched Gene Ontology Terms Among Differentially Expressed Genes

GO term	Category	Description	Fold enrichment	FDR
GO:0006805	Biological process	Xenobiotic metabolic process	5.3	2.3E-08
GO:0055114	Biological process	Oxidation-reduction process	4.7	4.1E-07
GO:0016705	Molecular function	Oxidoreductase activity	4.2	8.9E-06
GO:0004364	Molecular function	Glutathione transferase activity	6.8	1.2E-09
GO:0005783	Cellular component	Endoplasmic reticulum	3.1	3.4E-04
GO:0042302	Cellular component	Structural constituent of cuticle	4.9	5.6E-07

The ABC transporter pathways (ko02010) were also enriched, which implies active efflux insecticid molecules. Significantly, biosynthesis of insect hormone was impaired and this may have an impact on the regulation of development during chemical stress. These pathway-scale modifications are evidence that there were

coordinated responses of several genes, as opposed to individual gene responses. These pathways indicate biochemical adaptation mechanisms that enable survival in chemically stressed environments and reflect the broader ecological consequences of sustained pesticide exposure.

Table 5: Enriched KEGG Pathways Associated With Pesticide Resistance

KEGG pathway	Pathway ID	DEGs in pathway	P-value	Pathway description
Drug metabolism-cytochrome P450	ko00982	23	3.2E-09	Phase I detoxification
Metabolism of xenobiotics	ko00980	19	8.7E-08	Xenobiotic degradation
Glutathione metabolism	ko00480	17	2.1E-06	Phase II conjugation
ABC transporters	ko02010	14	4.5E-05	Efflux transport
Insect hormone biosynthesis	ko00981	8	1.2E-03	Juvenile hormone synthesis

Ten randomly selected genes were validated in quantitative real-time PCR and showed a high level of concordance with RNA-seq ( $r = 0.92$ ,  $p < 0.001$ ) (Figure 1). The trend of RNA-seq was validated because *CYP6CMI* expression was increased 11.8-fold in R1 and 7.2-fold in R2. *GSTe1* increased by 8.4 and 5.6-fold respectively. CPR1 cuticular protein displayed upregulation of 5.9 and 4.1-fold. The level of expression was significantly related to  $LC_{50}$  values ( $r = 0.88$ ,  $p < 0.01$ ) and could be considered as a good resistance biomarker. This agreement of transcriptomic findings by different populations makes them robust. This strong correlation

reinforces the reliability of transcriptomic markers for monitoring environmentally driven resistance development in field populations (Table 5).

Its identification as *CYP6CMI* being upregulated by a large margin (12.4-fold) supports its participation in detoxification of neonicotinoids by hydroxylating them. This P450 enzyme has been functionally confirmed in various researches though our results indicate increased induction in Indian individuals. The increase of *GSTe1* indicates a possibility of greater conjugation of insecticide metabolites and glutathione to allow excretion. The

simultaneous increase in a set of cuticular protein genes incriminates decreased cuticular penetration as an adjunctive mechanism. Reduction of nAChR subunits can be a sign of receptor insensitivity that decreases the binding efficiency of imidacloprid. Active sequestration and excretion of xenobiotics is also indicated by the coordinated upregulation of the ABC transporters. These processes are probably synergistic and that is why there are high ratios of resistance. These combined mechanisms illustrate how organisms evolve multifaceted defenses in response to prolonged environmental chemical exposure, emphasizing the adaptive resilience of pest populations (Singh et al., 2024).

Our results are consistent with transcriptomic results of the Chinese and American *B. tabaci* populations, with varying magnitudes of expression. Under imidacloprid selection of Chinese MEAM1 populations, *CYP6CM1* was upregulated 8.3-fold, compared to our 12.4-fold, perhaps due to regional selection strength. GST induction on the other hand was similar in the studies. The downregulation of the nAChR genes in our study is unique and was less pronounced in Mediterranean populations, indicating that the populations have population-specific adaptations. These geographic differences highlight the necessity of monitoring resistance regionally. These geographic variations highlight the influence of local environmental conditions and pesticide usage patterns on resistance evolution, underscoring the need for region-specific monitoring and management strategies.

## Conclusion

The current work gives detailed information about the molecular processes involved in the mechanism of pesticide resistance in *Bemisia tabaci* that involve the significant role of detoxification enzymes, such as cytochrome P450 monooxygenases and glutathione S-transferases, as well as cuticular changes. The high upregulation of *CYP6CM1* and *GSTe1* is an indication that they can be useful molecular biomarkers used in the early detection of resistance. This type of molecular diagnostics can be used to provide advanced resistance management of neonicotinoids by anticipating failure of this control at the field level before it is visibly apparent. The implications of the discoveries in addition to the molecular understanding have significant environmental implications. The high speed of development of resistance can be explained by the long-term and high-intensity exposure to pesticides in agriculture, which is a factor in the increase of the chemical load and ecological imbalance. Ongoing use of the chemical control does not only contribute to the rapid development of resistance but also negatively impacts on the non-target organisms, health of soil, and biodiversity. Hence, there is a need to shift towards sustainable pest management practices in order to sustain agro-ecosystem stability and productivity. Transcriptomic surveillance is a potent instrument to be deployed in the context of biomonitoring because it can be used to monitor the resistance evolution in real-time. These methods may assist in evidence-based decision-making and allow timely interventions.

Moreover, the study of the relationship between pesticide resistance and climate change should be carried out in the future, as the increased temperature and the shifts in weather conditions can additionally impact the pest adaptation, distribution, and resistance processes. Exploration of gene-environment interaction at different climatic conditions will improve the predictability of pest attack. Policywise, the paper stresses the necessity of controlled use of pesticides, popularization of Integrated Pest Management (IPM), and the use of biological control methods in order to minimize risks to the environment. Enhanced recommendations on the use of pesticides and promoting the use of sustainable agricultural techniques will play an important role in managing resistance in the long-run. All in all, an efficient and environmentally friendly management of *B. tabaci* by incorporating the molecular tools, ecological background, and policy-related strategies can be able to provide resilience and sustainability in agricultural systems.

#### ***Authors' contribution***

##### **Conceptualization of Research:**

N.K. Thakre.

##### **Designing of the Experiments:**

Dr. Swoyam Singh.

##### **Contribution of Experimental Materials:**

Ashok Kumar Sharma

##### **Execution of Field/Lab Experiments and Data Collection:**

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##### **Analysis of Data and Interpretation:**

Dr.D. Neelamegam

#### **Preparation of the Manuscript:**

Shilpy Singh

#### ***Declaration***

The authors declare that they do not have any conflict of interest.

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