PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): xx–xx
https://doi.org/10.14719/pst.7302

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RESEARCH ARTICLE

# Biochemical clues to insecticide resistance in mango hoppers of Bhubaneswar

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OPEN ACCESS

**ARTICLE HISTORY**
Received: 20 January 2025
Accepted: 18 February 2025
Available online
Version 1.0 : 24 February 2025

Check for updates

**Additional information**

**Peer review**: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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**CITE THIS ARTICLE**

Dash SS, Tripathy MK, Kar MR, Behera SK, Pradhan BK. Biochemical clues to insecticide resistance in mango hoppers of Bhubaneswar. Plant Science Today (Early Access). https:/doi.org/10.14719/pst.7302

## Abstract

Mango hoppers (*Amritodus atkinsoni, Idioscopus niveosparsus* and *Idioscopus clypealis*) are major pests affecting mango cultivation in Bhubaneswar, Odisha, where insecticide resistance has become a significant challenge. This study aimed to investigate the biochemical mechanisms of resistance, focusing on the activities of detoxifying enzymes Carboxylesterase (CE) and Glutathione-S-Transferase (GST). Seasonal variations in enzyme activity and kinetic properties ($K_m$ and $V_{max}$) were analysed alongside bioassays to assess insecticide efficacy and the role of enzyme inhibitors. CE and GST activities were measured using established biochemical assays, while inhibitors such as Diethyl Maleate (DEM) and Triphenyl Phosphate (TPP) were tested for their ability to reduce enzyme-mediated resistance. Results showed that CE activity peaked at 1.89 µmol/min/mg in July 2023, a 5.3-fold increase from March 2022, while GST activity reached 297.43 µmol of CDNB conjugated/min/mg. These trends correlated with higher $LC_{50}$ values for Acephate (4.442 ppm in October 2022, up from 1.471 ppm in March 2022) and Imidacloprid (1.313 ppm in July 2023, compared to 1.142 ppm in March 2022), indicating increased resistance. Kinetic analysis showed lower $K_m$ (11.33 µM for CE and 14.33 µM for GST in July 2023) and higher $V_{max}$ (1.32 µmol/min/mg for CE and 1.14 µmol/min/mg for GST), suggesting enhanced enzymatic efficiency. The use of DEM and TPP significantly reduced $LC_{50}$ values, with DEM lowering $LC_{50}$ by 45 % for Lambda-Cyhalothrin. These findings highlight the need for biochemical profiling and strategic enzyme inhibitor use to mitigate resistance. Seasonally tailored pest management strategies can enhance mango cultivation sustainability in Bhubaneswar.

## Keywords

Acephate resistance; biochemical adaptation; carboxylesterase activity; enzyme inhibition; glutathione-S-transferase; insecticide detoxification; mango pest management; metabolic resistance; seasonal enzyme variation; synergistic effect

## Introduction

Mango (*Mangifera indica* L.), revered as the "king of fruits," holds a prominent place in India's agricultural economy, contributing significantly to both domestic consumption and export revenue (1). Among the various challenges faced by mango cultivation, pest infestations pose the most significant threat, with mango hoppers (*Amritodus atkinsoni*, *Idioscopus niveosparsus* and

*Idioscopus clypealis*) being the primary culprits (2, 3). These phloem-feeding insects cause substantial economic losses by directly damaging mango crops and facilitating the spread of fungal infections such as sooty mold (1, 4). In Bhubaneswar, a key mango-growing region in Odisha, the problem is further exacerbated by the reliance on chemical insecticides for pest management.

Over time, the widespread and indiscriminate use of insecticides has led to the emergence of resistance in mango hopper populations (5). Metabolic resistance, one of the most common mechanisms, is primarily mediated by detoxifying enzymes such as Carboxylesterase (CE) and Glutathione-S-Transferase (GST). CE hydrolyses insecticides, reducing their toxicity, while GST conjugates harmful compounds with glutathione, aiding in their excretion. Elevated activities of these enzymes have been implicated in resistance, allowing pests to survive doses of insecticides that would otherwise be lethal (6). In addition to insecticide use, climatic factors such as temperature, humidity and seasonal variations play a significant role in shaping resistance dynamics. Higher temperatures can enhance enzyme activity and metabolic rates in pests, accelerating detoxification processes, while variations in humidity and rainfall can influence pest survival, reproduction and exposure to insecticides (7). These environmental factors, combined with prolonged insecticide exposure, create favourable conditions for the selection and propagation of resistant populations (7).Bhubaneswar, characterized by its unique agro-climatic conditions, presents an ideal case for studying the biochemical mechanisms of insecticide resistance. Seasonal variations in environmental factors, coupled with differing pest management practices, are likely to influence enzyme activity and resistance dynamics in this region. However, the specific role of CE and GST in mango hopper populations from Bhubaneswar remains underexplored.

This study aims to bridge this knowledge gap by investigating the seasonal variations in CE and GST activities, their kinetic properties ($K_m$ and $V_{max}$) and the efficacy of enzyme inhibitors in mango hopper populations from Bhubaneswar. The findings will provide valuable insights into the biochemical basis of resistance, enabling the development of targeted pest management strategies for sustainable mango cultivation in the region.

## Materials and Methods

### *Study location and sampling*

The study was conducted in Bhubaneswar, a key mango-growing region in Odisha, India, characterized by tropical climatic conditions. Mango hoppers were collected from mango orchards during three distinct time points to capture seasonal variations March 2022, October 2022 and July 2023. The sampling was performed using sweep net to ensure the proper collection of live specimens. Collected insects were transported to the laboratory for further investigation. The samples were either processed immediately or stored at -20° C for further biochemical analyses.

### *Biochemical assays*

The biochemical activities of Carboxylesterase (CE) and Glutathione-S-Transferase (GST) were quantified using standard protocols. For CE, enzyme activity was measured using α- and β-Naphthyl acetate as substrates. The reaction mixture consisted of enzyme extract, substrate solution prepared in phosphate buffer and 0.1 M phosphate buffer (pH 7.0). After incubation for 15 minutes, the reaction was terminated by adding Fast Blue B salt and the absorbance of the resulting colour was measured at 660 nm. Enzyme activity was expressed in terms of the micromoles of naphthol released per minute per milligram of protein (8, 9). GST activity was assessed using 1-chloro-2, 4-dinitrobenzene (CDNB) as the substrate. The reaction involved enzyme extract, CDNB, reduced glutathione and phosphate buffer (pH 6.5). The change in absorbance at 340 nm was monitored and enzyme activity was expressed as micromoles of CDNB conjugated per minute per milligram of protein (10). Protein concentrations in all enzyme extracts were determined using the Bradford assay, with bovine serum albumin (BSA) as the standard (11).

### *Kinetic studies*

The kinetic properties of Carboxylesterase (CE) and Glutathione-S-Transferase (GST) were determined to evaluate enzyme efficiency and substrate affinity. The reaction rates were measured under optimal conditions by varying substrate concentrations from 0.01 to 5.00 mM (12, 13, 14). For CE activity, α-naphthyl acetate and β-naphthyl acetate were used as substrates. The reaction mixture contained 100 µL of enzyme extract, 200 µL of phosphate buffer (0.1 M, pH 7.0) and 100 µL of the substrate. The reaction was initiated by adding the substrate and incubating at 30°C for 15 min. To stop the reaction, 50 µL of Fast Blue B salt was added and the absorbance of the product was measured at 660 nm using a UV-Vis spectrophotometer. Enzyme activity was expressed as micromoles of naphthol released per minute per milligram of protein (8, 9). For GST activity, the reaction was carried out in a total volume of 200 µL, consisting of 100 µL of enzyme extract, 50 µL of phosphate buffer (0.1 M, pH 6.5), 25 µL of reduced glutathione (GSH) and 25 µL of 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate. The absorbance at 340 nm was recorded at 30-second intervals for 5 min to monitor enzyme activity. The enzyme activity was expressed as micromoles of CDNB conjugated per minute per milligram of protein (10). The kinetic parameters, including the Michaelis-Menten constant ($K_m$) and maximum velocity ($V_{max}$), were determined through nonlinear regression analysis using GraphPad Prism 9 software The seasonal variations in $K_m$ and $V_{max}$ values of carboxylesterase enzyme are illustrated in Fig. 1. The kinetic properties of Glutathione-S-Transferase (GST), including seasonal changes in $K_m$ and $V_{max}$, are presented in Fig. 2. Lower $K_m$ values indicated higher substrate affinity, while higher $V_{max}$ values suggested increased enzymatic efficiency (16, 17).

### *Inhibition studies*

To assess the potential of enzyme inhibitors in mitigating resistance, inhibition studies were conducted using known

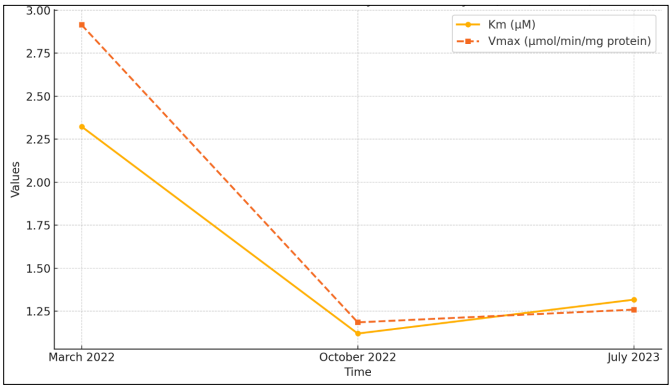


**Fig. 1.** Seasonal variations in Michaelis-Menten constant ($K_m$) and maximum velocity ($V_{max}$) of carboxylesterase enzyme in mango hoppers from Bhubaneswar (2022-23).

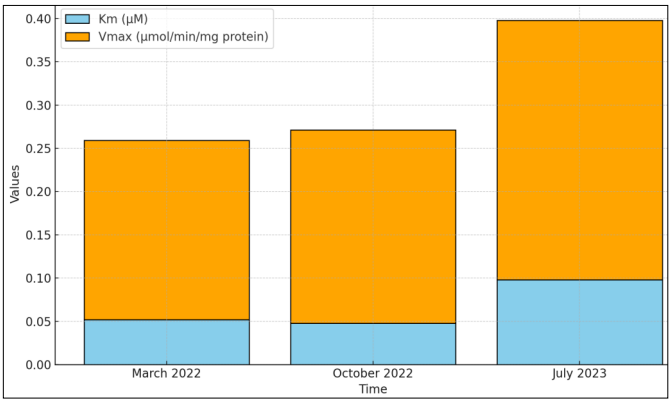


**Fig. 2.** Seasonal variations in Michaelis-Menten constant ($K_m$) and maximum velocity ($V_{max}$) of glutathione-s-transferase enzyme in mango hoppers from Bhubaneswar (2022-23).

inhibitors of detoxifying enzymes, including DEM for GST, TPP for CE and PBO for mixed-function oxidases (15). For CE inhibition, enzyme extracts were pre-incubated with increasing concentrations of TPP (0.01 to 10 mM) for 10 min at 30°C before adding the substrate (α- or β-naphthyl acetate). The reaction was then carried out as described in the kinetic study and enzyme activity was measured at 660 nm (15). For GST inhibition, enzyme extracts were pre-incubated with DEM at concentrations ranging from 0.01 to 10 mM for 10 min at 30°C before adding the CDNB substrate. The reaction was conducted as described earlier and absorbance was recorded at 340 nm. For oxidative enzyme inhibition, PBO was used as an inhibitor for mixed-function oxidases. The enzyme extract was pre-incubated with PBO (0.01 to 10 mM) at 30°C and the reaction was carried out using respective substrates. The inhibitory concentration ($I_{50}$), representing the concentration required to reduce enzyme activity by 50 %, was determined using dose-response curves. Additionally, the inhibition constant ($K_i$) and potency index ($PI_{50}$) were calculated using nonlinear regression analysis in GraphPad Prism 9 software (16, 17). The inhibitory effects of DEM, TPP and PBO on enzyme activity, as determined by $I_{50}$ values, are summarized in Fig. 3. The effectiveness of each inhibitor was compared based on its ability to reduce enzyme-mediated resistance. To assess the potential of enzyme inhibitors in mitigating resistance, inhibition studies were conducted using known inhibitors of detoxifying enzymes, including DEM for GST, TPP for CE and PBO for mixed-function oxidases (15). For CE inhibition, enzyme extracts were pre-incubated with increasing concentrations of TPP (0.01 to 10 mM) for 10 minutes at 30°C before adding the substrate (α- or β-naphthyl acetate). The reaction was then carried out as described in the kinetic study and enzyme activity was measured at 660 nm (15). For GST inhibition, enzyme extracts were pre-incubated with DEM at concentrations ranging from 0.01 to 10 mM for 10 min at 30°C before adding the CDNB substrate. The reaction was conducted as described earlier and absorbance was recorded at 340 nm. For oxidative enzyme inhibition, PBO was used as an inhibitor for mixed-function oxidases. The enzyme extract was pre-incubated with PBO (0.01 to 10 mM) at 30°C and the reaction was carried out using respective substrates. The inhibitory concentration ($I_{50}$), representing the concentration required to reduce enzyme activity by 50 %, was determined using dose-response curves. Additionally, the inhibition constant ($K_i$) and potency index ($PI_{50}$) were calculated using nonlinear regression analysis in GraphPad Prism 9 software (16, 17). The

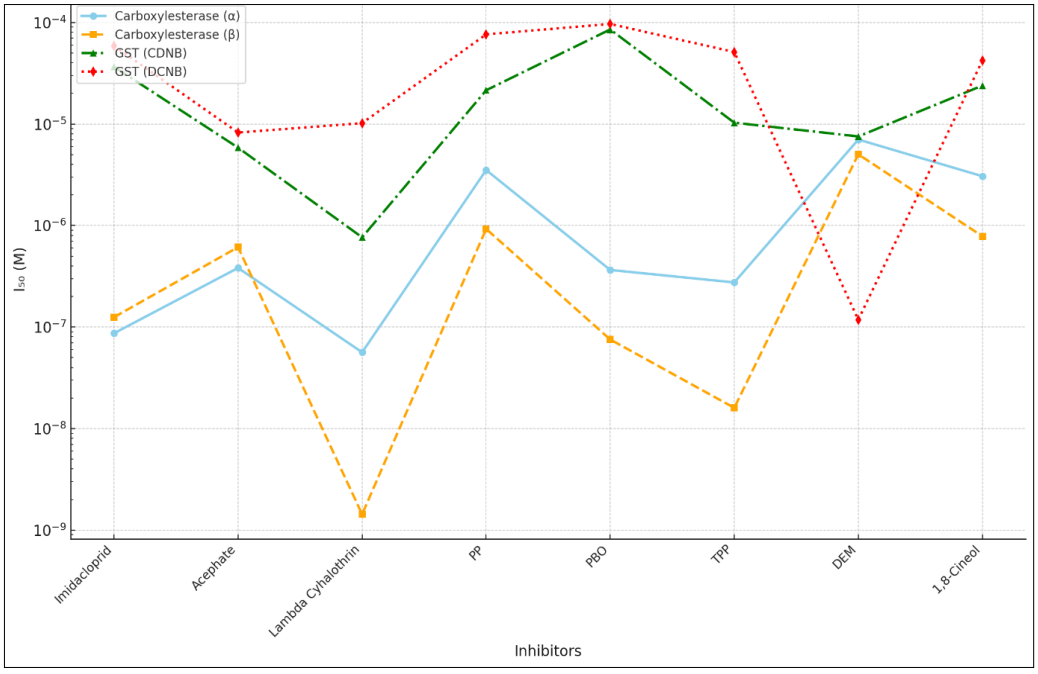


**Fig. 3.** $I_{50}$ values of various inhibitors across enzymes and substrates (2022-23).

inhibitory effects of DEM, TPP and PBO on enzyme activity, as determined by $I_{50}$ values, are summarized in Fig. 3. The effectiveness of each inhibitor was compared based on its ability to reduce enzyme-mediated resistance.

### *Statistical analysis*

All experiments were performed in triplicates and the results were expressed as mean ± standard deviation (SD). Seasonal variations in enzyme activities, kinetic parameters ($K_m$ and $V_{max}$) and inhibitor effects were statistically analysed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for pairwise comparisons. Statistical significance was defined as $p < 0.05$. All statistical analyses were conducted using GraphPad Prism 9 software to ensure precision and reliability in the results.

## Results and Discussion

### *Bioassay of mango hoppers in Bhubaneswar*

The bioassay results revealed significant seasonal variations in the susceptibility of mango hopper population to three commonly used insecticides: Lambda cyhalothrin, Imidacloprid and Acephate. The $LC_{50}$ values for Lambda cyhalothrin ranged from 0.524 ppm in March 2022 to 0.612 ppm in July 2023, indicating its high efficacy across seasons. In contrast, Acephate exhibited the highest $LC_{50}$ values, ranging from 3.217 ppm to 4.112 ppm during the same period, suggesting substantial resistance in the mango hopper population. Imidacloprid showed intermediate efficacy, with $LC_{50}$ values varying between 1.142 ppm and 1.313 ppm. These findings highlight Lambda cyhalothrin as the most effective insecticide, while Acephate was the least effective against mango hoppers in Bhubaneswar. The bioassay results revealed significant seasonal variations in the susceptibility of mango hopper populations to three commonly used insecticides (Table 1).

The bioassay results revealed that Lambda-Cyhalothrin remained the most effective insecticide across all seasons, while Acephate exhibited the highest $LC_{50}$ values, indicating substantial resistance. Seasonal variations in insecticide efficacy were evident, with higher $LC_{50}$ values recorded during the monsoon season (July 2023). Climatic factors such as increased humidity and temperature during the monsoon are known to enhance pest metabolism and detoxification, contributing to reduced susceptibility. Lambda cyhalothrin's superior mode of action, reduced susceptibility to enzymatic detoxification, environmental stability and faster knockdown effect make it more effective than Acephate for managing mango hoppers (18, 19). These findings underscore the influence of environmental conditions on resistance and highlight the need for seasonally tailored pest management strategies. Correlation between bioassay and detoxifying enzyme activities

A strong positive correlation was observed between $LC_{50}$ values and detoxifying enzyme activities in the mango hopper population. For example, higher $LC_{50}$ values for Acephate coincided with elevated CE and GST activities, particularly during the monsoon season (July 2023), when CE activity reached 1.31 µmol/min/mg protein and GST activity peaked at 252.72 µmol/min/mg protein. This correlation suggests that increased detoxification capacity, mediated by CE and GST, contributes to reduced insecticide efficacy and heightened resistance. The seasonal variations in Carboxylesterase (CE) activity revealed a peak in July 2023, correlating with increased resistance levels (Table 2).

**Table 2.** Seasonal dynamics of Carboxylesterase (CE) activity in mango hoppers from Bhubaneswar (2022-23)

| Time | α-Naphthol Hydrolysed (µmol/ min/mg protein) | β-Naphthol Hydrolysed (µmol/ min/mg protein) |
|---|---|---|
| March 2022 | $0.53^{ab} \pm 0.009$ | $0.31^{ab} \pm 0.012$ |
| October 2022 | $0.23^{b} \pm 0.001$ | $0.08^{c} \pm 0.004$ |
| July 2023 | $0.79^{a} \pm 0.009$ | $0.40^{a} \pm 0.012$ |

Elevated activities of Carboxylesterase (CE) and Glutathione-S-Transferase (GST) during the monsoon season suggest that detoxifying enzymes play a critical role in metabolic resistance. The elevated CE and GST activities during the monsoon season (July) can be attributed to high temperature and humidity, which enhance enzymatic activity and metabolic rates, facilitating detoxification (20). Frequent insecticide application, often without rotation between chemicals with different modes of action, exerts strong selection pressure on mango hopper populations, favoring individuals with enhanced detoxification mechanisms (21). Furthermore, the secondary metabolites present in mango leaves, such as flavonoids and terpenoids, may act as natural inducers of detoxifying enzyme activity (22, 23).

### *Kinetic parameters of detoxifying enzymes*

The kinetic analysis supported the bioassay findings by demonstrating enhanced enzyme efficiency during periods of higher resistance. For CE, the lowest $K_m$ value (11.33 µM) and the highest $V_{max}$ value (1.32 µmol/min/mg protein) were observed during July 2023, indicating increased substrate affinity and enzymatic efficiency. Similar trends were noted for GST, with $K_m$ decreasing from 18.42 µM in March 2022 to

**Table 1.** Seasonal variations in insecticide toxicity against mango hoppers in Bhubaneswar (2022-23)

| Time | Insecticide Used | $LC_{50}$ of Insecticide (ppm) | Chi-Square Value | Slope ± SE | Fiducial Limit (ppm) | Fiducial Limit (ppm) |
|---|---|---|---|---|---|---|
| | | | | | Lower | Upper |
| Mar-22 | Lambda Cyhalothrin | 0.524 | 0.980 | 0.773 ± 0.226 | 0.189 | 1.453 |
| | Imidacloprid | 0.797 | 0.773 | 0.767 ± 0.220 | 0.295 | 2.155 |
| | Acephate | 0.890 | 0.968 | 1.421 ± 0.124 | 0.507 | 1.561 |
| Oct-22 | Lambda Cyhalothrin | 0.982 | 0.991 | 1.300 ± 0.139 | 0.525 | 1.836 |
| | Imidacloprid | 1.053 | 0.980 | 1.375 ± 0.132 | 0.581 | 1.909 |
| | Acephate | 1.011 | 0.986 | 1.415 ± 0.129 | 0.564 | 1.812 |
| Jul-23 | Lambda Cyhalothrin | 1.335 | 0.999 | 1.602 ± 0.114 | 0.796 | 2.237 |
| | Imidacloprid | 1.650 | 0.970 | 1.988 ± 0.093 | 1.087 | 2.506 |
| | Acephate | 1.873 | 0.993 | 2.223 ± 0.083 | 1.287 | 2.725 |

14.33 µM in July 2023 and $V_{max}$ increasing from 0.74 µmol/min/mg protein to 1.14 µmol/min/mg protein. These kinetic adaptations likely play a critical role in the mango hopper's ability to detoxify insecticides and survive exposure. The activity of Glutathione-S-Transferase (GST) varied significantly across seasons, with the highest levels recorded during the monsoon period (Table 3).

**Table 3.** Seasonal dynamics of Glutathione-S-Transferase (GST) activity in mango hoppers from Bhubaneswar (2022-23)

| Time | CDNB Conjugated (µmol/min/mg protein) | DCNB Conjugated (µmol/min/mg protein) |
|---|---|---|
| March 2022 | $170.50^{c} \pm 0.014$ | $87.34^{c} \pm 0.007$ |
| October 2022 | $140.83^{c} \pm 0.014$ | $49.61^{c} \pm 0.015$ |
| July 2023 | $454.06^{a} \pm 0.052$ | $182.34^{a} \pm 0.034$ |

CDNB – 1-Chloro-2,4-Dinitrobenzene, DCNB – 1,2-Dichloro-4-Nitrobenzene

The kinetic analysis of CE and GST revealed lower $K_m$ values and higher $V_{max}$ values during the monsoon season, indicating increased substrate affinity and enhanced catalytic efficiency. Lower $K_m$ values during the monsoon indicate increased substrate affinity, enabling CE and GST to bind and process insecticides more efficiently, even at lower concentrations (24, 25). Higher $V_{max}$ reflects enhanced catalytic efficiency due to elevated enzyme expression and activity, likely driven by environmental factors (temperature, humidity), increased insecticide exposure and dietary inducers from mango foliage (26). These adaptations provide a biochemical advantage for detoxification, contributing to heightened resistance during this period. Such kinetic adaptations allow resistant populations to rapidly detoxify insecticides, conferring a survival advantage. Climatic variations, combined with frequent insecticide applications, create favourable conditions for these biochemical adaptations to evolve (27, 28). The findings emphasize the need for monitoring enzymatic adaptations as part of resistance management programs.

### *Inhibitory effects and their implications*

The inclusion of inhibitors such as Diethyl Maleate (DEM), Triphenyl Phosphate (TPP) and Piperonyl Butoxide (PBO) significantly altered the bioassay results. For Lambda cyhalothrin, the addition of DEM reduced the $LC_{50}$ value by 45 %, highlighting its potential as a synergist in enhancing insecticide efficacy. Similarly, TPP and PBO improved the performance of Imidacloprid and Acephate by lowering their respective $LC_{50}$ values. These findings indicate that enzyme inhibitors can play a pivotal role in overcoming resistance and improving pest management strategies.

The use of enzyme inhibitors, such as Diethyl Maleate (DEM) and Triphenyl Phosphate (TPP), showed significant potential in mitigating resistance by suppressing detoxifying enzyme activity. DEM is particularly effective as a synergist because it specifically inhibits Glutathione-S-Transferase (GST), a key enzyme in detoxification pathways (29). By depleting intracellular glutathione, DEM reduces GST-mediated insecticide conjugation and excretion, thereby enhancing insecticide toxicity (29). Its superior performance in reducing $LC_{50}$ values for Lambda Cyhalothrin highlights its strong inhibitory effect on resistance mechanisms. This makes DEM highly suitable for counteracting metabolic resistance in regions like Bhubaneswar, where elevated GST activity is prevalent.

### *Correlation between bioassay and enzymatic activity*

The strong correlation between $LC_{50}$ values and CE/GST activities confirms the critical role of these detoxifying enzymes in resistance development. Elevated $LC_{50}$ values for Acephate and Imidacloprid during the monsoon season correspond with increased enzymatic activity, demonstrating that enhanced detoxification capacity reduces insecticide efficacy. This relationship underscores the value of biochemical markers, such as enzyme activity levels, in predicting resistance trends. Regular enzymatic profiling can identify resistance hotspots, allowing for precise timing and tailored application of insecticides to optimize pest management strategies.

The correlation between $LC_{50}$ values and detoxifying enzyme activities highlights the role of metabolic resistance in mango hoppers. Increased $LC_{50}$ values for Acephate and Imidacloprid during the monsoon season coincided with higher CE and GST activities, suggesting enhanced detoxification capacity reduces insecticide efficacy (16, 17). Seasonal variations in enzyme activity indicate that environmental factors such as temperature and humidity influence metabolic resistance, making pest control more challenging (20). Higher CE and GST levels allow mango hoppers to survive insecticide exposure, demonstrating the importance of enzymatic profiling in resistance monitoring (21). Identifying resistance hotspots through biochemical markers can help optimize insecticide application and rotation strategies (22). The use of enzyme inhibitors like DEM and TPP may enhance insecticide efficacy by suppressing detoxification mechanisms (23, 24).

Regular biochemical monitoring, combined with field validation of resistance patterns, is essential for developing effective pest management strategies (25). Integrating enzyme inhibition and seasonally tailored insecticide application can improve control measures, ensuring sustainable mango pest management in Bhubaneswar and similar regions (26, 27).

## Conclusion

This study reveals critical insights into the biochemical mechanisms driving insecticide resistance in mango hopper populations from Bhubaneswar. The findings demonstrate significant seasonal variations in resistance levels, with heightened detoxifying enzyme activities and kinetic adaptations observed during the monsoon season. Increased Carboxylesterase (CE) and Glutathione-S-Transferase (GST) activities were strongly associated with reduced insecticide efficacy, particularly for Acephate, which exhibited the highest $LC_{50}$ value of 4.442 ppm in October 2022. The inclusion of enzyme inhibitors significantly enhanced insecticide effectiveness, with Diethyl Maleate (DEM) reducing the $LC_{50}$ of Lambda-Cyhalothrin by 45 % and Triphenyl Phosphate (TPP) lowering the $LC_{50}$ of Imidacloprid by 32 %, demonstrating their strong inhibitory effects.

These results highlight the need for seasonally tailored pest management strategies and regular enzymatic profiling to monitor resistance development. Furthermore, integrating enzyme inhibitors into insecticide formulations and exploring additional detoxifying enzymes can improve resistance management. By adopting these approaches, sustainable pest control strategies can be developed to protect mango crops, ensuring economic and environmental benefits.

## Acknowledgements

We gratefully acknowledge the Department of Entomology, College of Agriculture, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar, for providing laboratory facilities and technical support for this research.

## Authors' contributions

SSD designed the study, conducted biochemical analyses and drafted the manuscript. SSD and MKT contributed to data analysis, manuscript editing and coordination. SSD and MRK provided technical support in substrate kinetics and experimental methodology. SKB assisted in the study and reviewed the statistical framework. SSD and BKP contributed to the conceptualization and supervision of the research. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** The authors declare no conflicts of interest in this study.

**Ethical issues:** None. The research complies with ethical standards.

### Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used Perplexity solely for language refinement to improve readability and clarity. The author(s) reviewed and edited the content as needed and take(s) full responsibility for the accuracy and integrity of the publication.

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