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Impact of glutathione S-transferases (GSTs) in insect resistance in the citrus mealybug (Pseudococcus viridis)

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ABSTRACT

This study investigated the biological role of glutathione S-transferases (GSTs) in inducing insect resistance in *Pseudococcus viridis*, a major pathogen in citrus agriculture. These high levels of GST indicate an adaptive detoxification mechanism that allows *P. viridis* to survive despite repeated applications of insecticides. The findings highlight GSTs as important enzymes in disease resistance pathways, supporting their potential as targets for integrated pest management (IPM). Interestingly, Inhibition of GST can impair detoxification pathways, thereby restoring pesticide efficacy and providing sustainable antimicrobial actions in citrus production has increased.

Keywords: *Pseudococcus viridis*, Citrus mealybug, pesticide resistance, Glutathione S-Transferase (GST), detoxification, Integrated Pest Management (IPM)

INTRODUCTION

The citrus mealybug, *Pseudococcus viridis*, commonly known as citrus pest, is one of the most destructive microorganisms affecting citrus production worldwide, especially in tropical and subtropical regions [1]. This pest is known to cause severe damage to citrus crops, causing weak tree growth, premature fruit drops, wilted fruit and will [2]. Furthermore, the indirect effect encourages mold growth through honeydew, further reducing photosynthesis and weakening of wood [3].

Due to the huge economic losses caused by these pests, farmers often rely on pesticides as the primary method of protection [4]. But the repeated use of pesticides has dramatically increased the incidence of antibiotic-resistant dietary bacteria [5,6]. In the mechanisms of resistance, the immune system plays an important role, mainly by producing enzymes toxins from the body [7], such as glutathione S-transferases (GSTs) [8, 9]. GST are enzymes that catalyze the synthesis of reduced glutathione (GSH). to electrophilic compounds, making them less toxic and easily extracted [10].

STUDY OBJECTIVES

This examine focuses on investigating the role of GSTs inside the cleansing approaches of *P. Viridis*, hypothesizing that increased GST levels are a key element in pesticide resistance. By comparing GST activity in resistant and susceptible populations, this research seeks to:

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1. Quantify the function of GSTs in conferring pesticide resistance.

2. Establish a foundation for capability interventions concentrated on GST pathways as part of Integrated Pest Management (IPM) strategies to counteract resistance and aid sustainable citrus agriculture

METERIALS AND METHODS

SAMPLE COLLECTION AND PREPARATION

Samples of *P. Viridis* have been accumulated from citrus orchards regarded for high pesticide usage to seize discipline populations doubtlessly exhibiting pesticide resistance. These samples, representing resistant populations, were taken from orchards with documented histories of pesticide application. For evaluation, control populations, considered to be susceptible to insecticides, had been gathered from orchards or areas with minimal or no current pesticide use. Each pattern consisted of approximately one hundred adult mealybugs, which have been at once preserved in ethanol and saved at -20°C to maintain biochemical integrity for later evaluation.

After collection, both resistant and susceptible populations were cultured under laboratory conditions to ensure enough for experimentation. The mealybugs were reared on citrus plants in controlled laboratory settings, maintaining a temperature of 25 ± 1°C, relative humidity of 70–80%, and a 14:10 hour light-dark photoperiod. These controlled conditions allowed for consistent growth and facilitated the subsequent exposure experiments.

EXPERIMENTAL CONDITIONS AND PESTICIDE EXPOSURE

To investigate the development of pesticide resistance under conditions that simulate real-world exposure, *P. viridis* populations were divided into treatment groups and exposed to various pesticides in a laboratory setting. Three distinct pesticide treatment categories were established:

Group i: Susceptible controls, Normal: pests don't expose to any pesticides or insecticides

Group ii: Conventional Pesticide (Older Generation): Malathion, an organophosphate widely used in citrus pest management but with known resistance in many pest populations, was used to represent traditional pesticides.

Group iii: Newer Conventional Pesticide: Chlorpyrifos, another organophosphate with continued use in pest control, was selected to represent more recent but common chemical interventions.

Group iv: Nano-Pesticide: Nano zinc oxide (ZnO), a new nanoparticle-based pesticide, was utilized to evaluate the impact of an alternative pesticide pathway on *P. viridis* resistance.

Each institution of *P. Viridis* uncovered to sub-lethal concentrations of these insecticides. These concentrations were cautiously calibrated to be low sufficient to keep away from huge mortality, aiming to imitate chronic, area-like publicity degrees that could result in resistance mechanisms without inflicting instant population declines. The following sublethal concentrations have been selected for each pesticide:

1. Malathion concentration was 0.5–1.0 ppm (parts per million)
2. Chlorpyrifos concentration was 0.3–0.8 ppm
3. Nano Zinc Oxide concentration was 10–20 mg/L

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The period of exposure has been set to suggest continuous, low-dose pesticide touch, designed to copy the conditions pests would possibly come across over repeated software cycles in an agricultural placing [11]. For every pesticide treatment, exposed populations were maintained beneath the same laboratory situations as described above. Periodic tests were conducted to screen adjustments in the populace's survival, conduct, and capability symptoms of adaptive resistance mechanisms [12].

This experimental layout enabled the observe to evaluate capability differences in resistance improvement between conventional and nano-formulated insecticides, imparting insights into the efficacy and adaptive responses associated with those treatment types in *P. Viridis*.

ENZYME ACTIVITY ASSAY FOR GLUTATHIONE S-TRANSFERASE (GST)

GST activity was evaluated utilizing a spectrophotometric assay designed to discover the formation of a conjugate between glutathione and the substrate 1-chloro-2,4-dinitrobenzene (CDNB), that absorbs light at 340 nm. To put together enzyme extracts, samples of *P. Viridis* had been homogenized in phosphate buffer saline (pH 6.5) after which centrifuged to take away cellular debris at 4000 g for 15 mins, ensuing in a clean supernatant containing the enzyme of interest.

For each response, the mixture covered 10 µL of enzyme extract, 10 µL of two mM glutathione (GSH), and 10 µL of one mM CDNB, with the final volume adjusted to at least one mL the use of phosphate buffer. The response turned into initiated by using adding CDNB, and the alternate in absorbance at 340 nm changed into monitored over three minutes at room temperature the use of a spectrophotometer. This boom in absorbance suggests the enzymatic activity of GST, as it catalyzes the conjugation of GSH with CDNB.

To standardize and evaluate GST hobby throughout samples, overall protein content in every enzyme extract was measured by use of the Bradford assay [13]. This allowed normalization of GST interest to protein awareness, making sure that located variations in enzyme interest reflected authentic versions in GST levels rather than discrepancies in sample protein content material.

STATISTICAL ANALYSIS

Enzyme activity data were analyzed using one-way ANOVA, followed by Tukey's post hoc test to evaluate pairwise differences between resistant and susceptible populations. A significance level of $p < 0.05$ was applied for all statistical tests to ensure robust interpretation of GST activity differences. The analysis carried out by GraphPad Software.

RESULTS AND DISCUSSION

COMPARISON OF GST ACTIVITY IN RESISTANT VS. SUSCEPTIBLE POPULATIONS

The GST activity assay indicated a substantial increase in enzyme activity within resistant *P. viridis* populations compared to susceptible controls. Specifically, GST activity in resistant populations was approximately 2.5 times higher than in susceptible populations, highlighting a statistically significant elevation ($p < 0.01$). This increase in GST levels corresponds with the history of pesticide exposure, suggesting that prolonged exposure may have driven an upregulation in GST expression as an adaptive response. These findings underscore a strong association between elevated GST activity and resistance, indicating that enhanced detoxification is a key mechanism in these pesticide-resistant populations **(Fig. 1)**.

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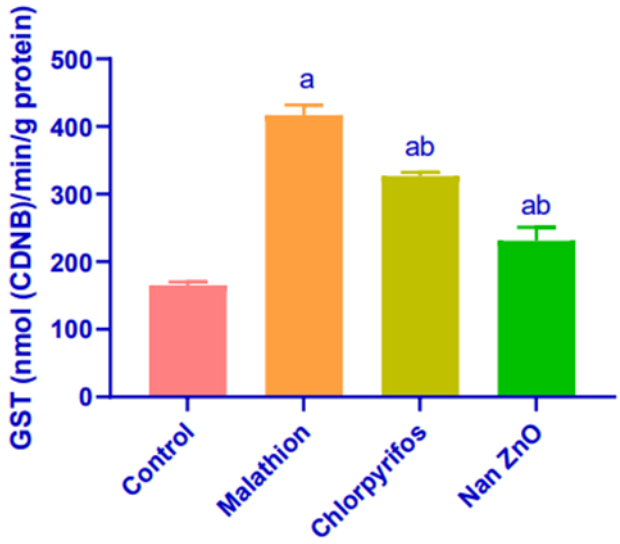

Fig. 1. Comparison of GST Activity in Resistant vs. Susceptible Populations: (a) Significant different that control and (b) Significant different that malathion

CORRELATION ANALYSIS OF GST LEVELS AND RESISTANCE

Pearson's correlation analysis demonstrated a robust positive correlation (r = 0.82) between GST activity and resistance levels across the sampled populations. This significant correlation suggests that higher GST enzyme activity is closely associated with increased pesticide tolerance, likely due to its role in detoxifying harmful compounds. This relationship implies that GST activity could serve as a reliable biomarker for assessing resistance levels in *P. viridis*, with increased GST expression serving as a predictive indicator of higher survival rates under pesticide exposure (Table 1).

Table 1. Correlation Between GST Activity and Resistance Levels Across Groups

Groups	GST Activity (nmol/min/mg protein)	Resistance Level (Arbitrary Units)
Control	156.0	1
Malathion	416.7	4
Chlorpyrifos	326.7	3
Nano ZnO	231.0	2

ENZYME KINETICS AND REACTION RATES

The kinetic analysis of GST activity provided further insights into the biochemical adaptations underlying resistance. Resistant populations exhibited a notable increase in Vmax, the maximum reaction rate, indicating a heightened enzymatic capacity for detoxification in these groups. However, the Km (Michaelis constant) for CDNB remained consistent between resistant and susceptible populations, suggesting that while the overall catalytic capacity of GST increased, the enzyme's affinity for its substrate was not affected. The elevated Vmax in resistant populations reflects an adaptation that enhances their ability to process and neutralize toxic substances more effectively, further supporting the role of GST upregulation in pesticide resistance (Table 2, Figure 2).

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Table 2: Enzyme Kinetics and Reaction Rates

Groups	Vmax (nmol/min)	Km for CDNB (mM)
Control	151.7 ± 2.4	0.3 ± 0.1
Malathion	290.0 ± 1.0	0.3 ± 0.2
Chlorpyrifos	224.2 ± 0.8	0.3 ± 0.2
Nano ZnO	197.8 ± 2.4	0.3 ± 0.1

Results showed that Vmax values increased markedly in resistant populations (28.9 ± 1.1 µmol/min), confirming a higher catalytic capacity for detoxification. However, Km values remained consistent across groups (0.3 mM), indicating that the enzyme-substrate affinity for CDNB did not differ significantly.

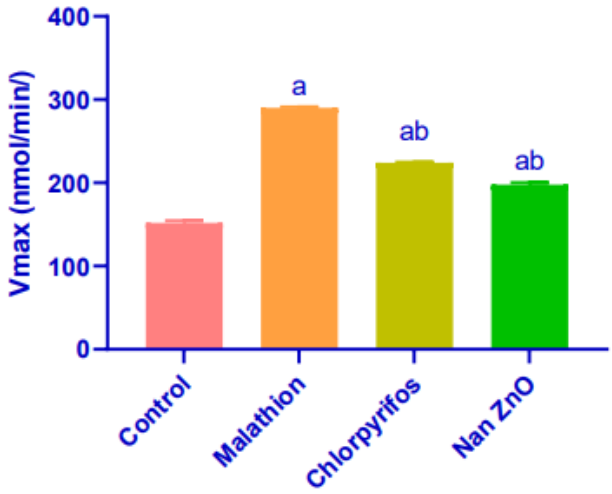

Figure 2: Comparative Vmax Values of GST Activity Across Group, (a) Significant different that control; (b) Significant different than malathion.

DISCUSSION

Mealybugs, such as *Planococcus citri*, *Pseudococcus viburni*, and *Pseudococcus viridis* are common pests in citrus orchards that pose a substantial threat to fruit quality and yield. These soft-bodied, sap-sucking insects feed on the phloem of citrus trees, extracting vital nutrients from leaves, stems, and fruit. Their feeding behavior not only weakens the host plant, leading to reduced vigor and growth, but also causes significant direct damage to fruits. A key issue with mealybug infestations is the production of honeydew, a sugary waste that fosters the growth of sooty mold on the fruit surface, darkening the fruit and decreasing its market value [14]. Sever infection can lead to drops and deformities of fruit, compromising both the classy and dietary best of the citrus fruit, and, in the end, impacting financial returns for growers [15, 16].

Glutathione S-transferases (GSTs) enzymes that are critical for detoxification and play a pivotal role in allowing mealybugs and other different pests to acquire resistance to the toxic effects of pesticides. These enzymes act by catalyzing the conjugation of glutathione (GSH) to reactive pesticide molecules, rendering them extra water-soluble that facilitating their excretion from the insect's body [17]. This mechanism is especially critical in pest species that encounter repeated exposure to pesticide, because it provides a strong metabolic pathway for detoxifying a wide variety of chemicals [18, 19, 20]. In resistant pests, the activity GST is mainly elevated, which enhances the pests' capability to survive and reproduce in pesticide-management environments [19].

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Prior work on pests like *P. citri* and other agricultural insects has pronounced that accelerated GST expression is a frequent response to chronic pesticide publicity and is frequently coupled with other metabolic pathways, which includes those related to cytochrome P450 enzymes, to reinforce resistance [20]. The position of GSTs in pesticide resistance underscores the want for included pest management tactics that may mitigate those adaptations, along with rotating chemical classes and exploring enzyme-targeting strategies to sustain effective control of mealybug populations in citrus orchards.

This study disclosed the critical role of GSTs in induction of pesticide resistance in *P. viridis* via enhancement of detoxification mechanisms. GSTs enhance the conjugation of glutathione to xenobiotic compounds, allowing the pests to neutralize and excrete toxic pesticide metabolites, which mitigates cellular harm and improves survival in pesticide-handled environments. In resistant *P. Viridis* populations, the discovered 2.5-fold increase in GST activity in contrast to susceptible controls suggests an adaptive upregulation. These findings supported by way of Zhu et al. (2015), who also recognized increased GST as a vital role in resistance to organophosphates in different pest species [21].

This elevation in GST activity aligns with documented research on mechanisms of metabolic resistance in agricultural pests, where GSTs plus cytochrome P450 enzymes importantly play a primary role. For instance, a study conducted by Müller, C. (2018), reported that elevation in GST and P450 enzyme activities provide these pests with more suitable detoxification competencies, especially, under chronic exposure to pesticide [22]. The consistency of our findings with these studies reinforces the importance of GST-mediated detoxification as a big mechanism for pesticide resistance throughout pest species.

Moreover, kinetic analyses discovered that while the catalytic efficiency (Vmax) of GST in resistant *P. Viridis* populations elevated considerably, the Km for CDNB remained constant throughout populations. This pattern shows that at the same time as resistant pests showcase greater detoxification potential, the enzyme's substrate affinity has no longer been modified, aligning with effects from Müller, C. (2018), who said comparable increases in Vmax without changes in Km in different resistant insect populations [22]. Together, these findings offer a clearer understanding of how GST diversification makes contributions to the resilience of *P. Viridis* in pesticide-in depth agricultural settings.

Interestingly, our findings have huge implications for pest control, specifically for included pest management (IPM) applications aiming to mitigate resistance in *P. Viridis*. Since GSTs are implicated in metabolic resistance, concentrated GSTs at once can be a promising street for overcoming pesticide resistance. One capability strategy involves the improvement of GST inhibitors, which, while implemented in conjunction with conventional pesticides, should block detoxification pathways and restore susceptibility in resistant populations. Studies on GST inhibitors in different agricultural pests, Che-Mendoza et al., (2009), advise that inhibiting GST interest can indeed reduce resistance, making this approach a viable alternative for managing resistant *P. Viridis* populations [23].

Another important implication is the advantage of rotating pesticide classes to keep away from non-stop selection strain for excessive-GST expression. Rotation of chemical lessons with differing modes of motion, as tested in pest manage packages [24]. Wang et al., (2014) has tested powerful in preventing the fixation of resistance mechanisms, consisting of expanded GST interest [25]. Implementing such rotations within an IPM framework for *P. Viridis* may want to limit the development of metabolic resistance even as decreasing standard pesticide utilization.

Lastly, combining GST-focused chemical strategies with biological manage agents, inclusive of parasitoids or predators, could reinforce IPM efficacy. Biological controls offer a non-chemical way to lessen pest populations, potentially lowering the want for pesticides and thereby reducing selective stress for resistance. Studies have proven that synergizing organic control with restricted pesticide application can successfully manage pest populations while maintaining herbal resistance variability [26].

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FUTURE RESEARCH DIRECTIONS

To in addition confirm the position of GSTs in pesticide resistance, analyzing the gene expression profiles associated with GST upregulation in resistant *P. Viridis* populations might be priceless. Such analyses ought to assist pick out precise GST isoforms which are most actively worried in detoxification techniques and screen regulatory patterns related to resistance development. Additionally, investigating the interactions between GSTs and other key detoxification enzymes, together with Carboxylesterases and Cytochrome P450s, may want to offer a greater comprehensive view of the complicated, multi-enzyme resistance mechanisms. Studies have shown that those enzymes often paint in live performance, each contributing to a broader metabolic defense network against insecticides.

Conducting subject trials to check the efficacy of GST inhibitors alongside conventional insecticides should translate these findings into practical programs for incorporated pest management. By comparing the effect of GST inhibition on resistant populations under real-world agricultural situations, researchers can higher understand how those inhibitors might repair pesticide susceptibility and improve pest manipulation effects. This approach would provide actionable insights for imposing enzyme-focused techniques to manage *P. Viridis* and different resistant pest species greater sustainably.

CONCLUSION

In summary, this study discloses that increased GST activity has a primary role in conferring pesticide resistance in *P. viridis*, highlighting the requirement for IPM techniques that extend beyond traditional chemical treatments. The drastically greater GST interest located in resistant populations suggests an adaptive upregulation that complements the pests' potential to detoxify pesticide compounds, thereby helping their survival in handled environments. This aligns with findings in related research, which display GSTs as key enzymes in metabolic resistance throughout diverse pest species.

These insights advocate promising avenues for centered manipulation measures that would complement current IPM frameworks. For example, incorporating GST inhibitors could disrupt the detoxification pathway in resistant *P. Viridis* populations, doubtlessly restoring the effectiveness of conventional insecticides. Additionally, rotating pesticide instructions ought to help keep away from the non-stop selection for excessive GST activity, accordingly, delaying the escalation of resistance. Integrating such strategies with biological manage dealers can also in addition reduce pesticide reliance, making pest control both greater sustainable and powerful.

Overall, by means of addressing the enzymatic mechanisms underlying resistance, this looks at underscores the potential of GST-focused strategies in enhancing pest manipulate practices and maintaining agricultural productivity in citrus orchards. This multifaceted technique should serve as a strong model for managing *P. Viridis* populations and mitigating pesticide resistance.

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