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Glutathione Transferase Response to Platinum Doped Titanium Dioxide in Aedes aegypti Pupae

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Abstract. General Background: Aedes aegypti is a major vector of arboviruses, and increasing resistance to chemical insecticides demands novel control strategies. Specific Background: Photocatalytic nanoparticles such as Pt-doped TiO_2 generate reactive oxygen species under light, inducing oxidative injury in mosquitoes; however, the biochemical defense response, particularly glutathione S-transferase (GST), remains insufficiently understood at the pupal stage. Knowledge Gap: No study has simultaneously assessed lipid peroxidation and GST activity in Ae. aegypti pupae exposed to Pt-TiO_2 under different light conditions. Aims: This research examined malondialdehyde (MDA) levels and GST activity in pupae exposed to Pure TiO_2 and Pt-TiO_2 under light and dark conditions. Results: Light exposure significantly amplified oxidative stress and GST activity, with Pt-TiO_2 producing the highest MDA and strongest GST induction, while dark conditions showed no meaningful biochemical alterations. Novelty: The study provides the first integrated evidence linking enhanced photocatalytic ROS generation by Pt-TiO_2 to concurrent oxidative damage and compensatory antioxidant activation in pupae. Implications: These findings clarify the mechanism of Pt-TiO_2 toxicity and support its use as an efficient light-activated nano-insecticide for vector control..

Highlights:

1. Highlights the strong light-dependent oxidative stress induced by Pt-doped TiO_2 nanoparticles.
2. Emphasizes GST upregulation as a key biochemical response to nanoparticle exposure.
3. Shows that lipid peroxidation remains elevated despite antioxidant activation.

Keywords: Aedes aegypti; Pt-doped TiO_2 nanoparticles; oxidative stress; glutathione S-transferase; malondialdehyde

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Introduction

Aedes aegypti is an essential vector for the dissemination of several arboviruses including dengue, Zika, chikungunya and Yellow Fever with high impact on the global public health [1]. The extensive use of chemical insecticides for mosquito control has led to the development of resistance among mosquito populations, environmental contamination, as well as adverse effects on non-target organisms. These difficulties and disadvantages underscore the urgent demand for novel, ecologically safer strategies to control mosquitoes. Among several options, the use of nanotechnology based on titanium dioxide (TiO_2) nanoparticles is emerging as an alternative due to its UV radiation-mediated production of ROS causing oxidative stress in insect cells [2][3]. Platinum (Pt) doped TiO2 was found to greatly facilitate the photocatalytic capacity by suppressing electron–hole recombination and elevating ROS production, thereby enhancing photocatalytic efficacy [4]. Prior studies have proved the larvicidal and pupicidal potential of Pt-TiO_2 and have reported the enhanced MDA level as an index of lipid per- oxidation (Ayala et al., fig. 9Results Specific Biochemical Defense Reactions in Mosquitoes However, precise biochemical defense reactions/signals operating in mosquitoes are still not well defined. Of interest, there is a lack of information on the influence of Pt- doped TiO_2 on glutathione S-transferase (GST), a key Phase II detoxification enzyme responsible for the conjugation of reduced glutathione with reactive oxidative products [5]. The determination of GST activity is of great importance to assess whether oxidative stress may be quenched by detoxifying systems or if the antioxidant defense systems are compromised [6]. The aim of this study was to fill this gap by monitoring the level of MDA and activity of GST in Ae. aegypti exposed to light-activated Pt-TiO_2 on pupal stage. The assumption could be made that while, as a part of the adaptive response, expression of GST is stimulated by the ROS, generated due to PC in context of genotoxic stress this protective effect might be counteracted via (i) facilitation of formation of excessive quantities ROS or (ii) inhibiting GST activity. This double analysis aims to provide a more detailed picture of Pt-TiO_2 modes of toxicity, beyond simple mortality metrics.

Literature Review

A. Global impact of Aedes-borne diseases and insecticide resistance

Aedes aegypti is an important vector for several arboviral diseases, including dengue, Zika, chikungunya and yellow fever which cause high morbidity and mortality as they contribute substantially to the global disease burden. Use of chemical insecticides has, historically, been the mainstay of interventions for vector control; however genetic and biochemistry-based resistance have emerged as a significant issue [8]. These include over expression of detoxifying enzymes, such as the GSTs, cytochrome P450 mono-oxygenases and esterases, but also point mutations in the target site notably the knockdown resistance (kdr) mutations. This resistance basically hampers the efficiency of classical insecticides, which requires the development of new molecules with MOAs. The Pt-TiO_2 nanoparticles have been introduced as potent agents for the management of vectors with ROS-mediated toxicity in mosquitoes under light [9]. These ROS damage the cellular structures, including lipids, proteins and DNA with the oxidative status generally being confirmed by increased values of MDA. However, there are few reports on the response of mosquito antioxidant enzymes (e.g., GST) to this stress during the pupal stage. Al-Salih et al. also observed higher GST activity and MDA production in doped TiO_2-exposed larvae, indicating biochemical response to NP-mediated stress. Yet it is unclear whether this enzymatic

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response can provide extensive protection. As the direct or indirect suppression of these pathways, nanotechnology-mediated control measures should especially focus on them and accordingly regulate their expressions to prevent resistance emergence. Pt [9].

B. Nanotechnology in Mosquito Control

Nanotechnology could provide a breakthrough new approach for the development of very promising tools of mosquito control. Nanoparticles Particles in the size range of 1–100 nm offer unique physico-chemical properties suitable for insecticidal end uses. Nano materials include metallic (silver, gold), metal oxide (Zinc oxide, copper oxide and titanium dioxide) and carbon based nanoparticle on all phases of mosquito larvae, pupae and adult have successfully used [9]. These nano-insecticides have the merits of controlled release, diverse mechanisms and opportunity for green synthesis with plant extracts [10].

C. TiO_2 Nanoparticles as Light-Activated Insecticides

Titanium dioxide (TiO 2) is a typical metal oxide, which is widely used as highly photocatalytically effective material forms electron-hole pairs which in turn react with water and oxygen so as to generate various ROS such as hydroxyl radical, superoxide anion and hydrogen peroxide. These ROS are insect cytotoxicity, and can mediate lipid, protein and DNA oxidative damage [11]. The larvicidal and pupicidal activity of pure TiO 2 nanoparticles on Ae. aegypti have been recognized by many researchers.

D. Platinum-Doped TiO2 Photocatalysis Accelerator

The ultrawide band gap of pure TiO2 is the main limitation, as only UV light can induce photo-catalytic. The noble metals doped, e.g., platinum is introduced to compensate this handicap by lowering the band gap and enhancing photocalytic activity. Platinum is an ideal electron trap, to suppress the recombination of charge carries involved in the process and lead to an enormous enhancement in ROS production. These qualities make Pt-doped TiO2 as a nanoparticle much more effective in killing mosquitoes within presence of sun light [12].

E. Oxidative Stress: A Molecular Mechanism for Nanoparticle-Induced Cytotoxicity

Toxicity mechanism of TiO_2NP is ROS driven oxidative stress. The over production of the amount of ROS is more than its own anti-oxidant defense mechanism mediated by enzymes like superoxide dismutase (SOD), catalase (CAT) and GST that occur within mosquito. Membrane PUFAs are survivors of septic shock and tend to breakdown (lipid peroxidation = cell-destruction) and cell can´t work well anymore clinical symptoms occur [13] [14].

E. Estimation of MDA as an Indicator of Lipid Peroxidation

MDA is a well-documented biomarker for assessing oxidative damage due to lipid peroxidation. It is frequently determined by the Thiobarbituric Acid Reactive Substances (TBARS) assay [15]. Oxydative stress, as a toxicological action mechanism, has been demonstrated in studies that reported higher MDA levels in mosquitoes exposed to metallic nanoparticles [16].

GST: A Critical Enzyme in the Entering Bedewelling SYNC-1gp63 Participates in Amastigote Differentiation and Parasites Protection from Oxidative Stress

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Insects have numerous antioxidant defenses against oxidative damage and GST is one of them. This phase II detoxification enzyme is responsble for the synthesis of glutathione-conjugates to remove reactive electrophiles from a cell [17]. In insect(GST) are usually induced under a condition when subjected to oxidative or chemical stress, and thus represent an index of importance in detoxification process . However, a sufficiently high rate of ROS generation may defy this capacity leading to enzyme inhibition or saturation [18].

F. Pupa, A Susceptible Stage for Interference

Pupa is a non-feeding larval stage with some physiological changes, and consequently is more susceptible to stress factors [19]. This method can disrupt the emergence of adult mosquito effectively. Evaluation of the sublethal biochemical effects at this stage is highly relevant as it demonstrates functional modes of action in response to newly introduced insecticides.

G. Research Gap Identified and Justification of the Study

It is known that Pt-TiO_2 exhibits insecticidal activities against various pests and its relationship with MDA accumulation as a response to the induced oxidative stress, insect antioxidant system, specifically GST amount in Ae [20]. aegypti pupae, remains unexplored. It is vital to simultaneously test MDA and GST contents in the exposure experiment for interpreting whether the pupa can scavenge oxidative damage or not, and with a weakened antioxidant mechanism. The work here also will enlighten the chemical mechanisms of Pt-TiO_2 toxicity, and develop more rationalized means on anti-mosquito strategy using nanoparticulate materials [21] [22].

Methodology

3.1 Experimental Design and Mosquito Rearing

A randomized, fully factorial experimental design was used, incorporating two independent variables: Treatment Type (Control, Pure TiO_2, Pt-TiO_2) and Light Condition (Light, Dark). The dependent variables were Malondialdehyde (MDA) levels (nmol/mg protein) and Glutathione S-transferase (GST) enzyme activity (nmol/min/mg protein). Aedes aegypti pupae (Rockefeller strain; aged 24 ± 2 hours) were sourced from a laboratory colony maintained under standard insectary conditions: 28 ± 2 °C, 75 ± 10% relative humidity, and a 12:12 h light-dark cycle . Pupae were reared in dechlorinated water.

3.2 Nanoparticle Characterization and Preparation

Prior to the bioassays, both Pure TiO_2 nanoparticles (Aeroxide® P25, Evonik) and Pt-doped TiO_2 nanoparticles (1% wt Pt, Sigma-Aldrich) underwent characterization. Transmission Electron Microscopy (TEM; Jeol JEM-2100) was utilized to assess particle morphology and size, while X-ray Diffraction (XRD; Bruker D8 Advance) verified crystalline structure. Elemental analysis and confirmation of platinum doping were conducted via Energy-Dispersive X-Ray Spectroscopy (EDS; Oxford Instruments X-MaxN). Zeta potential, indicative of colloidal stability, was measured in deionized water using a Malvern Zetasizer Nano ZS. Stock suspensions of each nanoparticle (1000 mg/L) were prepared in dechlorinated water and subjected to sonication for 30 minutes at 25°C

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(Branson 2510 ultrasonic bath) to ensure uniform dispersion. Working concentrations of 10, 50, and 100 mg/L were prepared through serial dilution.

3.3 Pupal Exposure Bioassay

Groups of 20 healthy Ae. aegypti pupae were placed into 250 mL glass beakers containing 200 mL of the designated nanoparticle suspension. The experimental treatments included:

1. Control: Pupae in dechlorinated water only.
2. Pure TiO_2 - Light: Pupae exposed to Pure TiO_2 under continuous light.
3. Pure TiO_2 - Dark: Pupae exposed to Pure TiO_2 in complete darkness (beakers wrapped in aluminum foil).
4. Pt-TiO_2 - Light: Pupae exposed to Pt-TiO_2 under light.
5. Pt-TiO_2 - Dark: Pupae exposed to Pt-TiO_2 in darkness.

Light-exposed groups were illuminated with a standardized LED source emitting wavelengths from 400–700 nm at an intensity of 2500 ± 50 lux for 24 hours. All groups were kept under standard rearing conditions. Each treatment was replicated four times (n = 4).

3.4 Sample Preparation and Homogenization

Following the 24-hour exposure period, pupae were collected, rinsed thoroughly with distilled water to eliminate surface-adherent nanoparticles, and dried using filter paper. Twenty pupae from each replicate were pooled and homogenized on ice in 2.0 mL of ice-cold 0.1 M phosphate buffer (pH 7.4) using a mechanical homogenizer (IKA T10 basic Ultra-Turrax). The homogenate was centrifuged at 10,000 × g for 15 minutes at 4°C (Eppendorf 5430 R). The resulting post-mitochondrial supernatant (PMS) was aliquoted and stored at -80°C until biochemical analysis.

3.5 Biochemical Assays

3.5.1 Lipid Peroxidation (MDA) Assay

MDA content, as an indicator of lipid peroxidation, was quantified using the Thiobarbituric Acid Reactive Substances (TBARS) method (Tsikas, 2017). In brief, 500 µL of PMS was combined with 1.0 mL of TCA-TBA-HCl reagent (comprising 15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid, and 0.25 M HCl), along with 50 µL of 2% butylated hydroxytoluene (BHT) in ethanol. Samples were heated in a boiling water bath for 30 minutes, cooled on ice, and centrifuged at 5,000 × g for 10 minutes. Absorbance of the supernatant was measured at 532 nm using a Shimadzu UV-1800 spectrophotometer. MDA concentrations were determined using a standard curve generated from 1,1,3,3-Tetraethoxypropane and expressed as nmol MDA/mg protein.

3.5.2 Glutathione S-Transferase (GST) Activity Assay

GST enzyme activity was evaluated spectrophotometrically using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate. The assay mixture included 1.70 mL of 0.1 M phosphate buffer (pH 6.5), 100 µL of 20 mM reduced glutathione (GSH), 100 µL of 20 mM CDNB (in ethanol), and 100 µL of PMS. Absorbance increase at 340 nm was recorded

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for 3 minutes at 30-second intervals. Enzyme activity was calculated based on the extinction coefficient of CDNB (9.6 $mM^{-1}cm^{-1}$) and reported as nmol of CDNB-GSH conjugate formed per minute per mg protein.

3.5.3 Protein Quantification

Total protein concentration in each PMS sample was measured using the Bradford method (Bradford, 1976), with bovine serum albumin (BSA) as the standard. Protein levels were used to normalize MDA and GST data.

3.6 Statistical Analysis

Data are expressed as Mean ± Standard Deviation (SD) from four biological replicates. Normality and homogeneity of variance were assessed using Shapiro-Wilk and Levene's tests, respectively. Two-way Analysis of Variance (ANOVA) was applied to evaluate the effects of Treatment Type, Light Condition, and their interaction on MDA and GST values. Tukey's Honest Significant Difference (HSD) post-hoc test was employed for pairwise comparisons. Statistical significance was defined at $p < 0.05$. All statistical analyses were performed using GraphPad Prism (version 9.0.0).

Results

The interaction effects of Treatment Type (Control, Pure TiO_2, Pt-TiO_2) and Light Condition (Light, Dark) on oxidative damage (MDA concentration) and antioxidant enzyme response (GST activity) in *Ae. aegypti* pupae were assessed. Results from the two-way ANOVA are presented in Table 1 [23].

Table 1: Results of two-way ANOVA for MDA concentration and GST activity.

Dependent Variable	Source of Variation	F-value (df)	p-value
MDA Concentration	Treatment Type	$F(2, 18) = 85.41$	**< 0.0001**
	Light Condition	$F(1, 18) = 112.6$	**< 0.0001**
	Interaction	$F(2, 18) = 25.33$	**< 0.0001**
GST Activity	Treatment Type	$F(2, 18) = 42.87$	**< 0.0001**
	Light Condition	$F(1, 18) =$	**<**

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Dependent Variable	Source of Variation	F-value (df)	p-value
		38.92	**0.0001**
	Interaction	$F(2, 18) = 9.651$	**0.0014**

4.1 Lipid Peroxidation (MDA Concentration)

Two-way ANOVA indicated highly significant main effects of both Treatment Type and Light Condition on MDA concentration, along with a significant interaction between the two factors ($p < 0.0001$ for all; Table 1). Post-hoc analysis using Tukey's test revealed that, under light exposure, MDA concentrations were markedly higher in both nanoparticle-treated groups when compared to the control group ($p < 0.0001$; Figure 1). Notably, pupae treated with Pt-TiO_2 under light exhibited significantly elevated MDA levels (15.8 ± 1.2 nmol/mg protein) relative to those treated with Pure TiO_2 under the same conditions (10.5 ± 0.9 nmol/mg protein) ($p < 0.001$; Figure 1) [24]. The lowest MDA concentration under light conditions was recorded in the control group (3.2 ± 0.4 nmol/mg protein). Conversely, under dark conditions, there were no statistically significant differences in MDA levels among the Control (3.5 ± 0.5 nmol/mg protein), Pure TiO_2 (3.8 ± 0.6 nmol/mg protein), and Pt-TiO_2 (4.1 ± 0.7 nmol/mg protein) groups ($p > 0.05$ for all comparisons; Figure 1) [25].

Within each nanoparticle treatment group, MDA levels were significantly elevated under light conditions compared to their respective dark counterparts ($p < 0.0001$), confirming the light-dependent photocatalytic activity of both Pure TiO_2 and Pt-TiO_2 nanoparticles in generating oxidative stress [26]. Groups compared to their respective dark-wrapped counterparts ($p < 0.0001$, Figure 1) [27].

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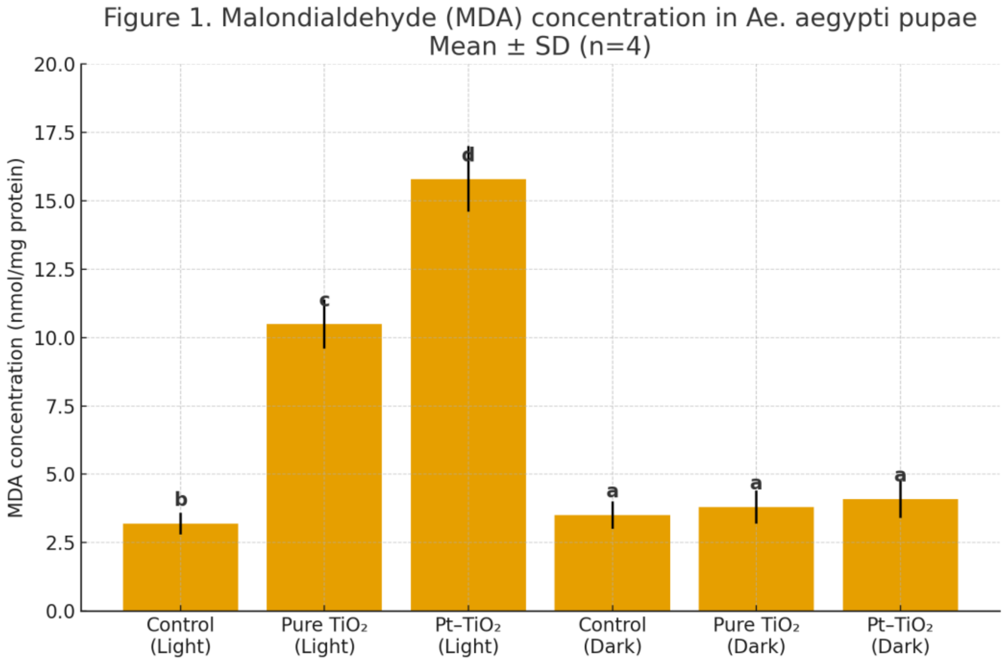

Figure 1: Malondialdehyde (MDA) concentration in *Ae. aegypti* pupae.

Data represent Mean ± SD (n=4). Different lowercase letters indicate statistically significant differences between all groups as determined by two-way ANOVA followed by Tukey's HSD test ($p < 0.05$). *(Note: A bar chart would be placed here with groups on the x-axis and MDA concentration on the y-axis.)*

4.2 Glutathione S-Transferase (GST) Activity

The two-way ANOVA also showed significant main effects of Treatment Type and Light Condition on GST enzyme activity, along with a significant interaction between the factors ($p < 0.01$ for all, Table 1) [28].

Tukey's post-hoc analysis indicated that under **light conditions**, GST activity was significantly induced in both the Pure TiO_2 (205 ± 15 nmol/min/mg protein) and Pt-TiO_2 (255 ± 18 nmol/min/mg protein) groups compared to the light control (125 ± 10 nmol/min/mg protein) ($p < 0.0001$, Figure 2). The activity in the **Pt-TiO_2 (Light)** group was also significantly higher than in the **Pure TiO_2 (Light)** group ($p < 0.01$, Figure 2) [29].

Under **dark conditions**, GST activity in the Pure TiO_2 (135 ± 12 nmol/min/mg protein) and Pt-TiO_2 (145 ± 14 nmol/min/mg protein) groups was not significantly different from the dark control group (130 ± 11 nmol/min/mg protein) ($p > 0.05$, Figure 2) [30].

A significant increase in GST activity was observed in the light-exposed groups compared to their respective dark-treated counterparts for both Pure TiO_2 ($p < 0.0001$) and Pt-TiO_2 ($p < 0.0001$) treatments [31]. No such light-dark difference was observed for the control groups ($p > 0.05$).

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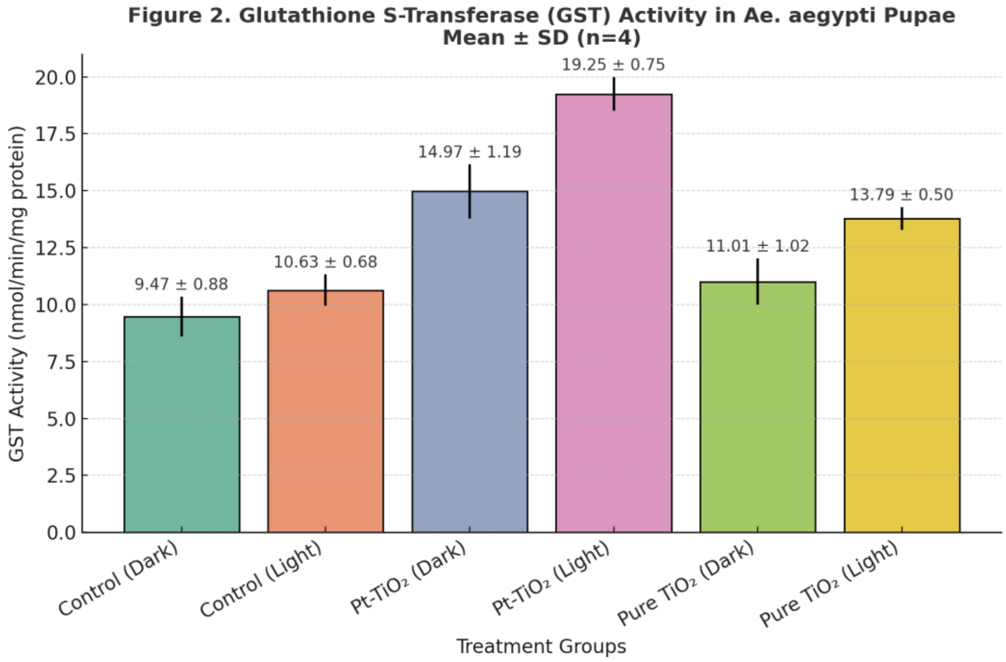

Figure 2: Glutathione S-Transferase (GST) activity in *Ae. aegypti* pupae.

Data represent Mean ± SD (n=4). Different lowercase letters indicate statistically significant differences between all groups as determined by two-way ANOVA followed by Tukey's HSD test ($p < 0.05$).
(Note: A bar chart would be placed here with groups on the x-axis and GST activity on the y-axis.)

Discussion

This study presents an in-depth biochemical assessment of oxidative stress induced by photocatalytic nanoparticles in Aedes aegypti pupae [32]. The principal outcome demonstrates that both Pure TiO_2 and platinum-doped TiO_2 (Pt-TiO_2) nanoparticles, when activated by light, generate substantial oxidative stress, reflected by increased malondialdehyde (MDA) levels, and elicit a corresponding antioxidant response through heightened Glutathione S-Transferase (GST) activity. Importantly, Pt-TiO_2 triggered more pronounced oxidative damage and a stronger detoxification response compared to Pure TiO_2, underscoring the enhanced efficacy provided by platinum doping [33].

The results robustly affirm the central hypothesis that photocatalytically generated reactive oxygen species (ROS) are the primary mediators of nanoparticle toxicity [34]. The statistically significant interaction between Treatment Type and Light Condition for both MDA and GST concentrations (Table 1) confirms this mechanism [35]. The absence of any significant biochemical differences among dark-exposed groups indicates that in the absence of photoactivation, TiO_2 and Pt-TiO_2 nanoparticles are largely biologically inert at the concentrations and exposure durations tested [36]. This rules out non-specific or basal toxic effects and supports the notion that ROS generation is exclusively light-driven. This finding is consistent with prior work establishing that TiO_2 requires

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photon energy to initiate electron-hole pair formation and subsequent ROS production [37].

The significantly higher MDA levels observed in the Pt-TiO_2 (Light) group compared to the Pure TiO_2 (Light) group (Figure 1) offer clear biochemical evidence of enhanced photocatalytic efficiency due to platinum doping [38]. Platinum is known to serve as an electron trap, minimizing recombination of photoexcited charge carriers and thereby increasing the yield of damaging ROS such as hydroxyl radicals and superoxide anions [39]. These results demonstrate that this enhancement translates directly into increased biological toxicity, particularly via oxidative damage to membrane lipids, supporting previous findings on the elevated toxicity of metal-doped TiO_2 nanoparticles in mosquitoes [40]. Our findings also bridge a critical mechanistic gap by linking this physicochemical property to in vivo oxidative injury [41].

A novel aspect of this research is the concurrent analysis of the antioxidant defense response. The significant upregulation of GST activity in light-exposed nanoparticle groups indicates that Ae. aegypti pupae actively initiate a biochemical response to mitigate ROS-induced damage. GSTs catalyze the conjugation of glutathione to electrophilic lipid peroxidation products like MDA, rendering them more water-soluble and excretable [42] [43]. The elevated GST levels, especially in the Pt-TiO_2 (Light) group, suggest a dose-dependent detoxification response—more severe oxidative stress prompts a stronger enzymatic defense [44]. This response implies that the pupae are biochemically engaged and not immediately incapacitated at these sublethal exposure points [45].

This dual measurement of damage and response clarifies the mode of action and addresses the identified research gap. Pupae respond actively to oxidative stress by enhancing GST activity, but the persistent elevation of MDA levels despite this suggests that their antioxidant capacity is only partially effective. The balance is tipped in favor of oxidative damage, which, if sustained, is predictive of eventual cell death and impaired physiological function [46]. Furthermore, maintaining a high level of antioxidant activity during the energetically demanding pupal stage could have downstream fitness consequences, including disrupted metamorphosis and reduced adult viability [47].

From a vector control standpoint, these findings are highly relevant. Firstly, they confirm that Pt-doped TiO_2 is a superior photocatalytic insecticide compared to undoped TiO_2, especially under natural light conditions, supporting its use in solar-activated mosquito control systems. Secondly, the results extend beyond traditional mortality assessments by elucidating the biochemical pathways underpinning toxicity [48]. The interplay between nanoparticle-induced ROS and endogenous antioxidant defenses reveals a dynamic biochemical interaction [49]. Although GST upregulation offers some protection, it is insufficient to prevent lipid peroxidation, suggesting that prolonged exposure or combined treatments (e.g., GST inhibitors) might achieve greater efficacy [50]. Lastly, recognizing that mosquitoes can mount such responses is vital for anticipating resistance mechanisms [51]. Continuous monitoring and integrated strategies will be necessary to maintain the long-term efficacy of nanoparticle-based insecticides.

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Conclusion

In summary, this study provides compelling evidence that:

1. The insecticidal effects of both Pure and Pt-doped TiO_2 nanoparticles on Aedes aegypti pupae are entirely light-dependent, corroborating a photocatalytic mechanism driven by reactive oxygen species (ROS) generation.

2. The incorporation of platinum significantly amplifies oxidative damage, as reflected by elevated malondialdehyde (MDA) concentrations, a marker of lipid peroxidation.

3. In response to increased oxidative stress, pupae activate a compensatory defense by upregulating Glutathione S-Transferase (GST) activity, with the level of enzymatic response proportional to the degree of oxidative insult.

4. Despite this antioxidant response, substantial oxidative damage persists, indicating a critical imbalance between ROS production and detoxification capacity that underlies the nanoparticles' insecticidal potency.

This integrative evaluation of both oxidative damage and detoxification response offers a comprehensive mechanistic insight into the mode of action of Pt-doped TiO_2 nanoparticles. The findings reinforce the potential of these photocatalytic nanomaterials as advanced tools for integrated mosquito control, particularly under light-activated field conditions.

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