

## Effect of silver and zinc oxide nanbiopesticides on the antioxidant enzymes in the gut of *Helicoverpa Armigera* larvae

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### Abstract

This study examines the toxicological effects of silver (Ag), zinc oxide (ZnO) nanbiopesticides (NBPs) on *Helicoverpa armigera* larvae, a significant pest of various crops worldwide. The nanoparticles were synthesized using a green method. This study investigates the effects of silver (Ag), zinc oxide (ZnO), and synergistic silver-zinc oxide (Ag-ZnO) nanbiopesticides on the activities of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the gut tissue of third instar larvae of *Helicoverpa armigera*. The larvae were fed on cotton leaves containing different concentrations of NBPs and enzyme activities were measured to evaluate oxidative stress responses. Results showed a dose-dependent increase in SOD, CAT and GPx activities across all NBPs treatments indicating oxidative stress caused by reactive oxygen species (ROS). The combined Ag-ZnO NBPs had the greatest effect on enzyme activity implying increased toxicity. These findings demonstrate the potential of NBPs as effective pest control agents by disrupting antioxidant defense mechanisms, offering insights into their application in integrated pest management strategies.

**Keywords:** Nanbiopesticides (NBPs), antioxidant enzymes, SOD, CAT, GPx

### Introduction

The increasing resistance of agricultural pests to conventional chemical pesticides has intensified the need for more sustainable and environmentally benign pest control strategies. Among the various alternatives nanbiopesticides (NBPs) derived from biological sources have emerged as promising candidates due to their enhanced bioavailability, targeted action and reduced ecological toxicity (Kah *et al.*, 2013; Khot *et al.*, 2012) [11, 12]. These nanoparticles often interact at the cellular and molecular levels potentially inducing oxidative stress and affecting the antioxidant defense systems of pest organisms (Barik *et al.*, 2008; Rai *et al.*, 2012; Gokulakrishnans *et al.*, 2024) [5, 10, 18]. *Helicoverpa armigera* (Lepidoptera: Noctuidae), commonly known as the cotton bollworm is a major polyphagous pest affecting a large range of economically important crops worldwide. The pest has demonstrated remarkable adaptability and resistance development against a variety of synthetic insecticides (Sharma, 2005; Arora *et al.*, 2017) [3, 20]. In recent years, NBPs synthesized using plant extracts or microbial agents, especially silver and zinc oxide nanoparticles, have been shown to exert significant larvicidal and growth-inhibitory effects on *H. armigera*, suggesting potential for integrated pest management (Kumar *et al.*, 2020; Ghosh *et al.*, 2013) [9, 13].

Antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) play pivotal roles in mitigating oxidative damage by scavenging generated reactive oxygen species (ROS) during stress conditions. The modulation of these enzymatic activities upon nanbiopesticide exposure can provide critical insights into the toxicological effects and underlying mechanisms affecting pest physiology and survival. Recent studies have

reported that exposure to metal-based nanoparticles can induce oxidative stress in insects, disrupting the balance of antioxidant enzymes and leading to cellular damage including lipid peroxidation and DNA impairment ultimately compromises insect viability and reproductive functions.

The insect midgut is the primary site for digestion and absorption, direct interface for ingested nanbiopesticides. Due to its high metabolic activity, the midgut is especially vulnerable to oxidative damage, making it a crucial target for studying nanoparticle-induced stress responses (Singh & Rizvi, 2011; Tripathi *et al.*, 2020) [21, 24]. Several studies have reported alterations in the antioxidant enzyme profile of insect pests following exposure to various nanoparticle-based formulations yet a detailed understanding of their mechanistic impact on specific antioxidant enzymes in *H. armigera* remains limited.

Thus, the present study aims to evaluate the effects of NBPs on the activity levels of key antioxidant enzymes (SOD, CAT, GPx) in the midgut tissue of 3<sup>rd</sup> instar larvae of *Helicoverpa armigera*. By elucidating the biochemical responses of this pest to nanbiopesticide exposure.

### Materials and Methods

#### Insect Collection

Third-instar larvae of *Helicoverpa armigera* were utilized in the present study. Following collection, the insects were maintained under laboratory conditions at ambient room temperature and reared on fresh cotton leaves to ensure optimal growth and development before experimentation.

#### Synthesis of Ag-NBPs and ZnO-NBPs

Ag-NBPs and ZnO-NBPs were synthesized using a green synthesis method involving biological reducing agents derived from plant *C. collinus* leaf extracts. An aqueous

extract was prepared by boiling fresh plant material in distilled water, followed by filtration. A 1 mM silver nitrate ( $\text{AgNO}_3$ ) [HIMEDIA] and 1 mM Zinc sulphate ( $\text{ZnSO}_4$ ) [HIMEDIA] solution was prepared and mixed with the leaf extract in a 1:1 volume ratio, respectively. For the reduction of silver ions and zinc ions, 50 mL of *C. collinus* leaf extract was added dropwise to 50 mL of  $\text{AgNO}_3$  and  $\text{ZnSO}_4$  respectively, with constant stirring. Then, the reaction mixture was kept at room temperature in photoactivation, with periodic observation of color change from pale yellow to brown, indicating nanoparticle formation (Ahmed *et al.*, 2016) [2].

### Larval Treatment

To assess the oxidative stress response generated due to NBPs in *Helicoverpa armigera*, larvae were fed on cotton leaves under laboratory conditions. It contains varying concentrations of nanobiopesticides as per the LC50 calculation. The treatments included silver nanobiopesticides (Ag NBPs), zinc oxide nanobiopesticides (ZnO NBPs), and synergistic silver-zinc oxide nanobiopesticides (Ag-ZnO NBPs). For Ag NBPs, two concentrations were tested: a lower dose at 6  $\mu\text{L/g}$  of diet and a higher dose at 12  $\mu\text{L/g}$  of diet. Similarly, the ZnO NBP treatments included a lower dose of 8  $\mu\text{L/g}$  and a higher dose of 15  $\mu\text{L/g}$  of diet. In the case of the combined Ag-ZnO NBPs, larvae were also exposed to two concentrations: 8  $\mu\text{L/g}$  for the lower dose and 15  $\mu\text{L/g}$  for the higher dose. These treatments were designed to evaluate the co-dependent impact of individual and synergistic NBP formulations on the antioxidant defense

system in the larval gut.

### Antioxidant Enzyme Assay

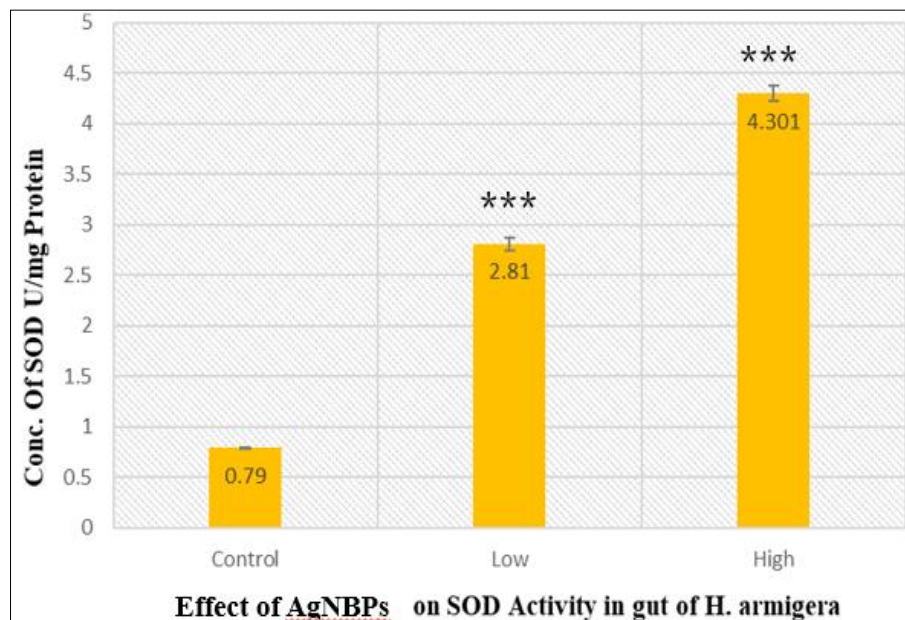
The activity of antioxidant enzymes in the larval gut was then analyzed. The total superoxide dismutase (SOD) activity was determined following the method described by Marklund and Marklund (1974) [14]. The catalase (CAT) activity was assessed by measuring the hydrolysis of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) according to the method of Aebi (1984) [1]. For the determination of glutathione peroxidase (GPx) activity, the process of Paglia and Valentine (1987) was employed, using  $\text{H}_2\text{O}_2$  as the substrate.

### Observation and Result

#### Effect of AgNBPs on Antioxidant Enzyme Activities in the Gut of Larvae of *H. Armigera*

##### 1. SOD

Present study illustrates the effect of silver nanobiopesticides (AgNBPs) on the activity of the enzyme SOD in the gut of *Helicoverpa armigera* larvae. The control group, which was not exposed to AgNBPs, exhibited a baseline SOD activity of  $0.79 \pm 0.005$ . In contrast, larvae exposed to a low dose of AgNBPs showed a significant increase in SOD activity, with a mean value of  $2.81 \pm 0.063$  ( $p < 0.0001$ ). This activity further increased in the group treated with a high dose of AgNBPs, reaching  $4.301 \pm 0.08$ , also with a statistically significant p-value of  $< 0.0001$ . These results indicate a dose-dependent elevation in SOD activity following AgNBP exposure suggesting an induced oxidative stress response in the larval gut tissue.



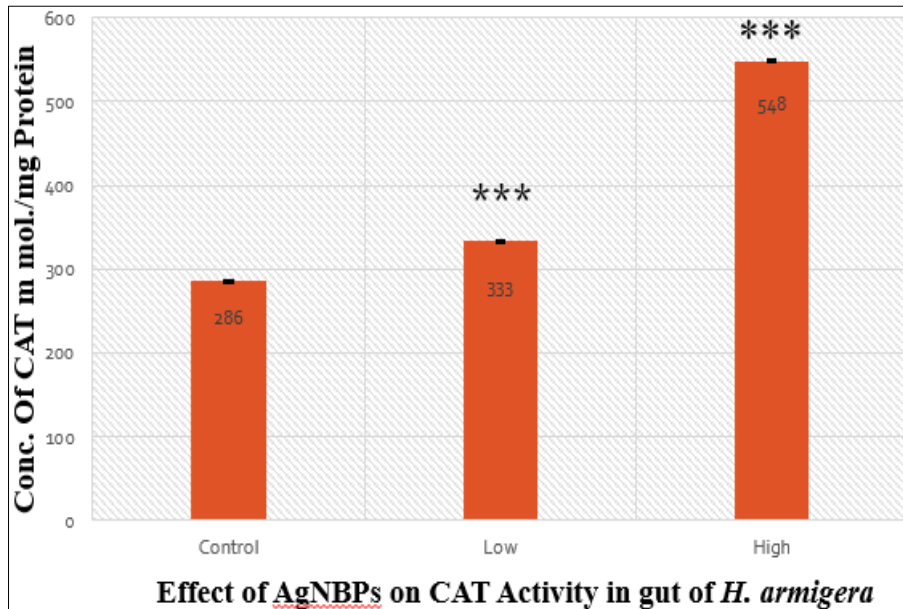
Values are presented as Mean $\pm$ SE; p-values:  $p < 0.05^*$ ,  $p < 0.001^{**}$ ,  $p < 0.0001^{***}$

**Fig 1:** Effect of AgNBPs on SOD enzyme in gut of 3rd instar of *H. armigera*

##### 2. Catalase (CAT)

According to the findings, CAT activity increased in a dose-dependent manner. A considerable increase in catalase activity (333 nmol/mg protein) was seen in the group treated with low dose of Ag NBPs compared to control group, which showed the lowest activity (286 nmol/mg protein). Furthermore, the group treated with a high dose of Ag NBPs

showed the highest catalase activity (548 nmol/mg protein). The difference between this increase and the control was statistically significant. According to these results, *H. armigera* experiences oxidative stress from Ag NBPs, which triggers an increased CAT enzyme activity as a defensive antioxidant response.



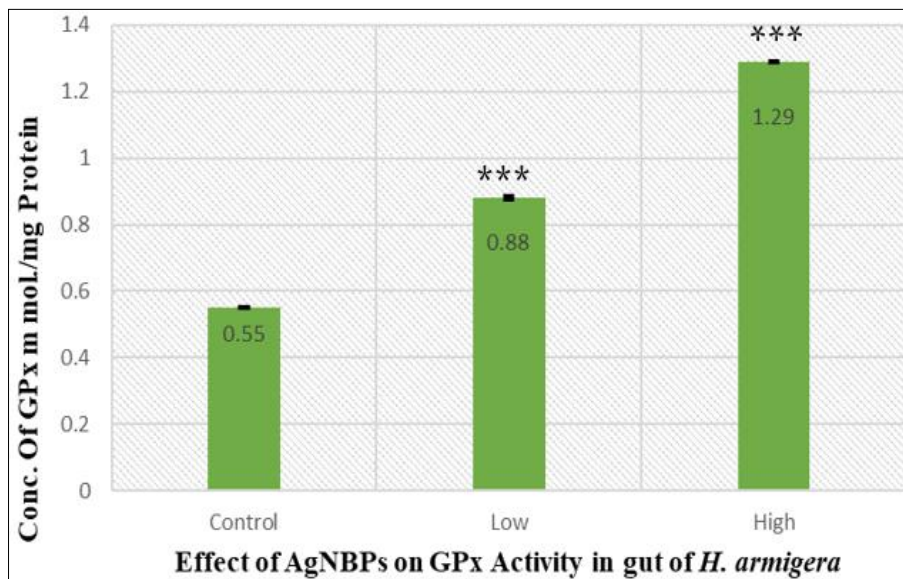
Values are presented as Mean±SE; p-values: p<0.05\*, p<0.001\*\*, p<0.0001\*\*\*

Fig 2: Effect of AgNBPs on CAT enzyme in gut of 3<sup>rd</sup> instar of *H. armigera*

### 3. Glutathione Peroxidase (GPx)

In response to the oxidative stress caused by the NBPs, the GPx activity of the third-instar larvae treated with low dose of Ag NBPs increased significantly to 0.88 m mol/mg protein. Additionally, GPx activity was measured at 1.29 m

mol/mg protein in larvae treated with a high dose of Ag NBPs, the highest of all groups. This implies that GPx activity increases in a dose-dependent manner, emphasizing the increased oxidative stress at higher Ag NBP concentrations.



Values are presented as Mean±SE; p-values: p<0.05\*, p<0.001\*\*, p<0.0001\*\*\*

Fig 3: Effect of AgNBPs on GPx enzyme in gut of 3<sup>rd</sup> instar of *H. armigera*

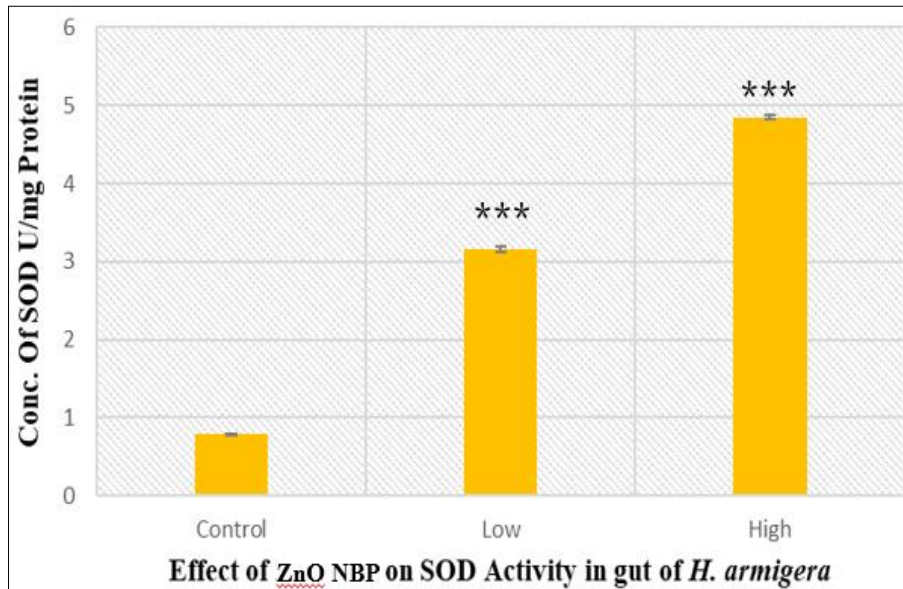
### Effect of ZnO Nbps on Antioxidant Enzyme Activities in the Gut of *H. Armigera* Larvae

#### 1. SOD

The effect of zinc oxide nanobiopesticides (ZnO-NBPs) on the activity of superoxide dismutase (SOD) in the gut of 3<sup>rd</sup> instar larvae of *Helicoverpa armigera*. Compared to the control

group, SOD activity was shown to be considerably greater in both the low and high treatment groups. The SOD activity of larvae exposed to low doses of ZnO-NBPs increased

significantly to 3.16 ± 0.042, whereas the control group's SOD activity was only 0.79 ± 0.006. At 4.85 ± 0.031, the group with the highest concentration had the highest activity. The statistical analysis revealed that there were significant and highly significant variations compared to the treated groups and control group. The rise in SOD activity indicates a significant oxidative stress response in the larval gut caused by ZnO-NBP exposure, indicating that ZnO-NBPs could be effective in causing toxicity by generating ROS.



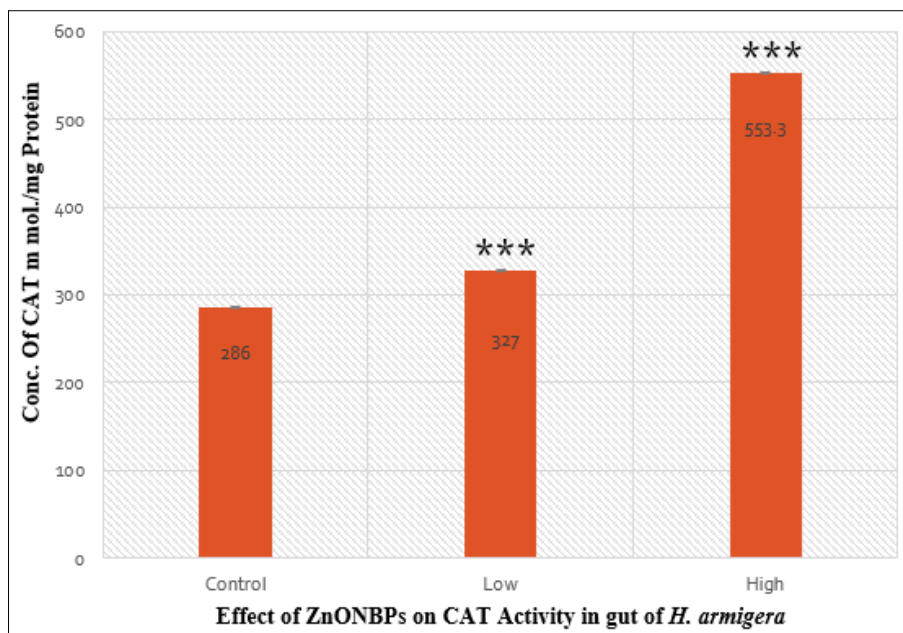
Values are presented as Mean±SE; p-values: p<0.05\*, p<0.001\*\*, p<0.0001\*\*\*

**Fig 4:** Effect of ZnO-NBPs on SOD enzyme in gut of 3<sup>rd</sup> instar of *H. armigera*

**2. Catalase (CAT)**

According to the findings, CAT activity increased in a dose-dependent manner. A considerable increase in catalase activity (327 mmol/mg protein) was seen in the group treated with low concentrations of ZnO-NBPs, whereas the control group showed the lowest activity (286 mmol/mg protein). Furthermore, the group treated with a high dose of AgNBPs

showed the highest catalase activity (553.3 mmol/mg protein). The difference between this increase and the control was statistically significant. According to these results, *H. armigera* experiences oxidative stress from ZnO-NBPs, which triggers an increased CAT enzyme activity as a defensive antioxidant response.



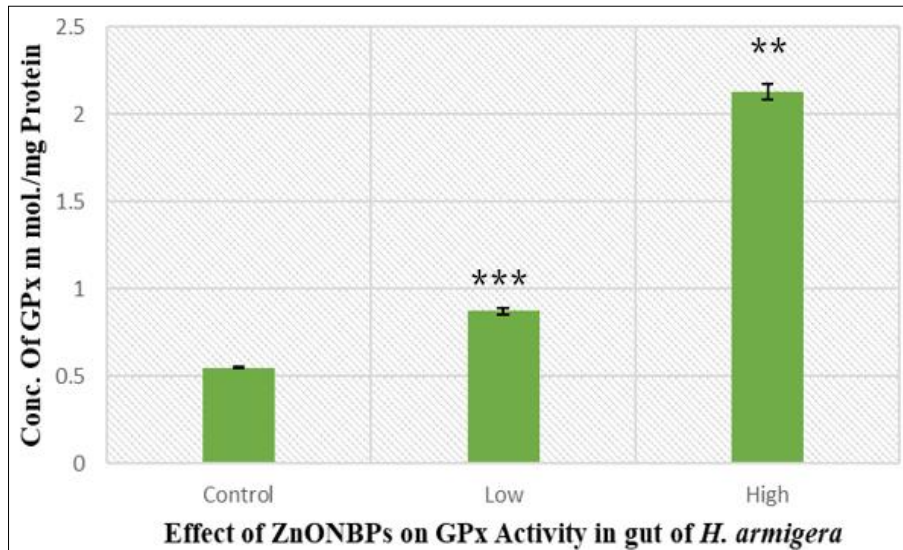
Values are presented as Mean±SE; p-values: p<0.05\*, p<0.001\*\*, p<0.0001\*\*\*

**Fig 5:** Effect of ZnO-NBPs on CAT enzyme in gut of 3<sup>rd</sup> instar of *H. armigera*

**3. Glutathione Peroxidase (GPx)**

In response to oxidative stress caused by the NBPs, the GPx activity of third-instar larvae treated with low concentrations of ZnO-NBPs increased significantly to 0.856 m mol/mg protein compared to the control group (0.55 m mol/mg). Additionally, GPx activity measured

at 1.848 m mol/mg protein in larvae treated with a high concentration of ZnO NBPs, the highest among all groups. This suggests that GPx activity increases in a dose-dependent manner, highlighting greater oxidative stress at higher ZnO NBP concentrations.



Values are presented as Mean±SE; p-values: p<0.05\*, p<0.001\*\*, p<0.0001\*\*\*

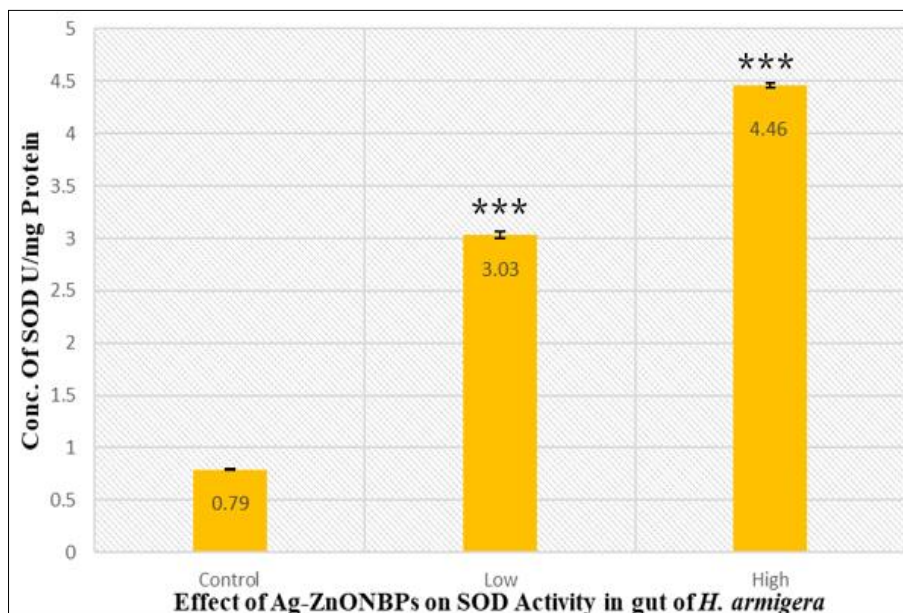
**Fig 6:** Effect of ZnO-NBPs on GPx enzyme in gut of 3<sup>rd</sup> instar of *H. armigera*

### Synergistic Effect of Ag-ZnO NBPs on Antioxidant Enzyme Activities in the Gut of *H. Armigera* Larvae

#### 1. SOD

The present study illustrates the synergistic effect of Ag-ZnO NBPs on the activity of the enzyme SOD in the gut of *Helicoverpa armigera* larvae. The control group, which was treated with normal water, exhibited a baseline SOD activity of  $0.79 \pm 0.005$ . In contrast, larvae exposed to a low concentration of Ag-ZnO NBPs showed a significant increase in SOD activity compared to the control, with a mean value

of  $2.81 \pm 0.063$  ( $p < 0.0001$ ). This activity further increased in the group treated with a high concentration of Ag-ZnO NBPs, reaching  $4.301 \pm 0.08$ , also with a statistically significant p-value of  $< 0.0001$ . These results indicate a dose-dependent elevation in SOD activity following synergistic Ag-ZnO NBP exposure, suggesting an induced oxidative stress response in the larval gut tissue.



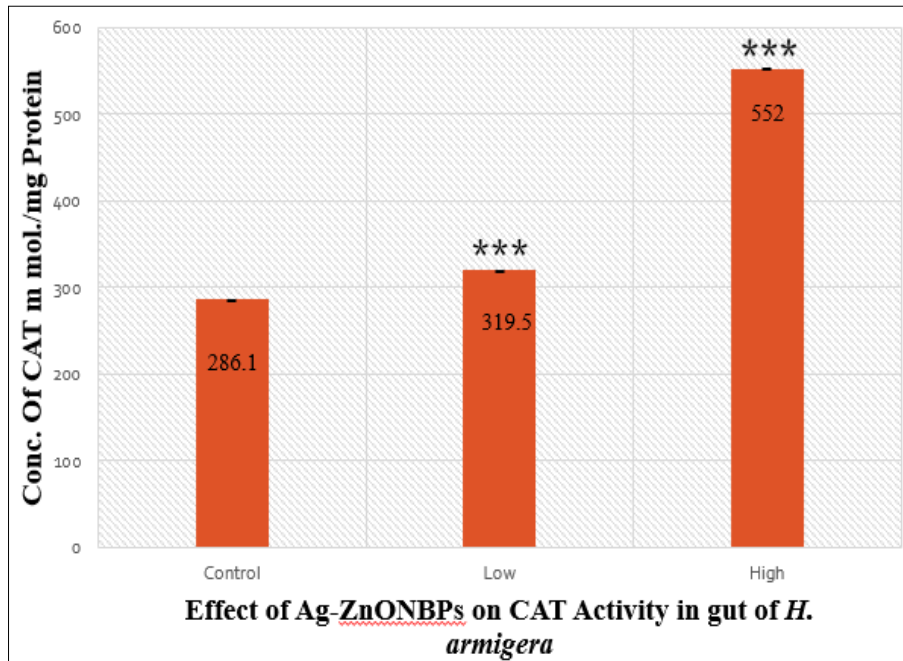
Values are presented as Mean±SE; p-values: p<0.05\*, p<0.001\*\*, p<0.0001\*\*\*

**Fig 7:** Effect of Ag-ZnONBPs on SOD enzyme in gut of 3<sup>rd</sup> instar of *H. armigera*

#### 2. Catalase (CAT)

The catalase (CAT) enzyme activity was found to increase in a dose-dependent manner following treatment with Ag-ZnO NBPs. Specifically, the group exposed to low doses of Ag-ZnO NBPs exhibited a marked rise in CAT activity ( $319.5 \text{ nmol/mg protein}$ ) compared to the control group, which demonstrated the lowest activity ( $286.1 \text{ nmol/mg protein}$ ).

Moreover, the high dose group of Ag-ZnO NBPs showed a significantly high CAT activity ( $552 \text{ m mol/mg protein}$ ) than both the control and low-dose groups. These statistically significant increases in CAT activity indicate that exposure to synergistic Ag-ZnO NBPs induces oxidative stress in *H. armigera*, which in turn stimulates an upregulation of catalase as a protective antioxidant response.



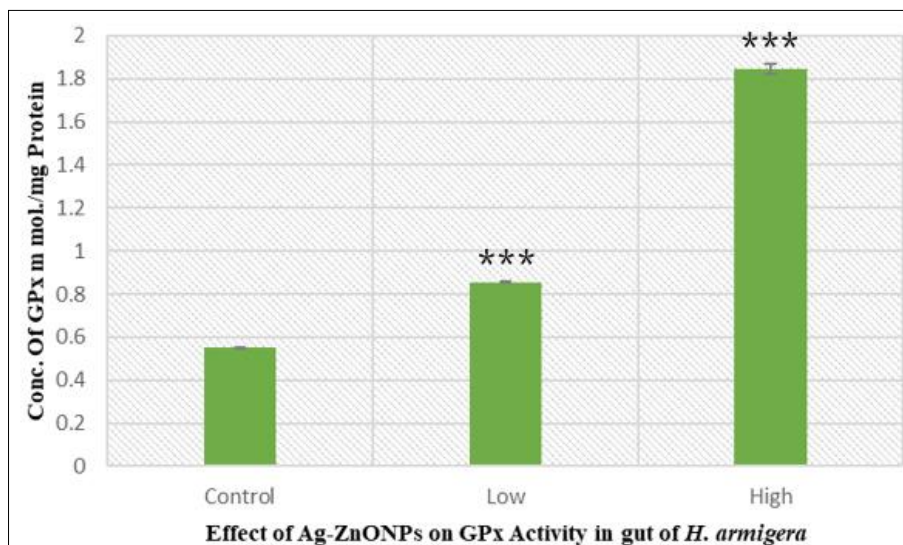
Values are presented as Mean±SE; p-values: p<0.05\*, p<0.001\*\*, p<0.0001\*\*\*

**Fig 8:** Effect of Ag-ZnO NBPs on CAT enzyme in gut of 3<sup>rd</sup> instar of *H. armigera*

### 3. Glutathione Peroxidase (GPx)

In response to oxidative stress caused by the NBPs, the GPx activity of third-instar larvae treated with low concentrations of Ag-ZnO NBPs increased significantly to 0.856 m mol/mg protein compared to the control group (0.55 m mol/mg). Additionally, GPx activity measured at 1.848 m mol/mg

protein in larvae treated with a high concentration of Ag-ZnO NBPs, the highest among all groups. This suggests that GPx activity increases in a dose-dependent manner, highlighting greater oxidative stress at higher Ag-ZnO NBP concentrations.



Values are presented as Mean±SE; p-values: p<0.05\*, p<0.001\*\*, p<0.0001\*\*\*

**Fig 9:** Effect of Ag-ZnO NBPs on GPx enzyme in gut of 3<sup>rd</sup> instar of *H. armigera*

### Discussion

The findings of this study provide critical insights into the oxidative stress responses induced by silver (Ag), zinc oxide (ZnO), and synergistic silver-zinc oxide (Ag-ZnO) nanobiopesticides (NBPs) in the midgut tissue of 3<sup>rd</sup> instar larvae of *Helicoverpa armigera*. The dose-dependent increase in the activities of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase observed across all the treated groups emphasizes the physiological impact of NBPs on the midgut of *H. armigera*, which is the primary site for digestion and nanoparticle

interaction (Manimegalai *et al.*, 2022). The study also contributes to understanding the sublethal physiological effects of nano-based biocontrol agents, potentially guiding their safe and effective application in pest management. The significant elevation in SOD activity across all NBP treatments suggests a defense mechanism against oxidative stress brought on by reactive oxygen species (ROS). SOD plays a vital role in converting superoxide radicals into hydrogen peroxide (Aucoin *et al.*, 1991; Wang *et al.*, 2001; Weirich *et al.*, 2002) [4, 25, 26] which further detoxified by CAT and GPx. The observed increase in CAT activity indicates the

breakdown of hydrogen peroxide into water and oxygen highlighting the larvae's defensive mechanism against oxidative damage (Diaz-Albiter *et al.*, 2011) [7]. The dose-dependent rise in GPx activity reflects the enhanced detoxification of hydrogen peroxide and lipid peroxides further emphasizing the oxidative stress induced by NBPs (Costa *et al.*, 2025) [6].

Oxidative stress produced by NBPs may result in the production of free radicals and changes to antioxidants or ROS-scavenging enzymes and may also play a major role in pesticides toxicity processing (Bagchi *et al.*, 1995; Gultekin *et al.*, 2000). This study demonstrated that applying low and high dosages of AgNBPs, ZnO-NBPs, and Ag-ZnO NBPs altered the levels of SOD, CAT, and GPx activity in midgut tissue. This suggested that by generating ROS in the midgut tissue, these NBPs cause oxidative damage in *H. armigera*. The levels of CAT, SOD, and GPx were elevated in the treated groups compared to the control, to cope with oxidative damage. Since oxidative stress leads to starvation, it affects the feeding behavior of larvae.

Among the treatments, the synergistic Ag-ZnO NBPs demonstrated the most notable impact on antioxidant enzyme activities suggesting a co-dependent mechanism that amplifies ROS generation and toxicity. This aligns with previous studies indicating that metal-based nanoparticles can disrupt cellular homeostasis by inducing oxidative stress leading to DNA damage, lipid peroxidation and impaired physiological functions (Subramanayan *et al.*, 2012) [22]. The elevated oxidative stress observed at higher doses of NBPs suggests that these formulations could effectively compromise pest viability and reproductive functions, making them promising formulation for integrated pest management.

The study also highlights the potential of NBPs to overcome limitations associated with conventional pesticides such as resistance development and environmental toxicity (Raliya *et al.*, 2017) [19]. By targeting the antioxidant defense systems of pests, NBPs offer a novel mechanism of action that could reduce reliance on synthetic chemicals while minimizing ecological risks. However, the oxidative stress induced by NBPs raises concerns about their potential non-target effects, necessitating further research to evaluate their safety and environmental impact (Barik *et al.*, 2008; Rai *et al.*, 2012) [5, 18]. The study may provide a foundation for developing effective and sustainable pest control strategies. Future studies should focus on optimizing NBPs formulations assessing their long-term ecological effects and exploring their application in diverse agricultural systems.

## Conclusion

Ag NBPs and ZnO NBPs have shown promise as nanobiopesticides against *H. armigera* larvae, and their effect on antioxidant enzymes in the gut of larvae could effectively compromise pest viability and reproduction, making them environmentally sustainable potential tools for integrated pest management. Effective pest management of *H. armigera* larvae using NBPs can lead to improved crop yields and reduced economic losses. The application of NBPs suggests that these formulations could effectively compromise pest viability and reproductive functions, making them promising candidates for sustainable and integrated pest management.

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