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Evaluation of antifeedant activity of nanobiopesticides against *Helicoverpa armigera* larvae using the no choice method

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Abstract
This study investigates the antifeedant activity of silver (Ag) and zinc oxide (ZnO) nanobiopesticides (NBPs) synthesized from *C. collinus* against third instar larvae of *Helicoverpa armigera*. Using a no-choice bioassay method, cotton leaf discs treated with different concentrations of Ag-NBPs and ZnO-NBPs were exposed to the larvae, and their feeding behavior was monitored. The results demonstrated a significant, concentration-dependent reduction in leaf consumption, indicating strong antifeedant effects of both NBPs. The antifeedant efficacy was higher at increased concentrations, suggesting that these NBPs effectively deter the pest from feeding, which could substantially reduce crop damage. These findings highlight the potential of Ag-NBPs and ZnO-NBPs as eco-friendly alternatives for pest management strategies targeting *H. armigera*.

Keywords: Ag-NBPs, ZnO-NBPs, antifeedant activity, no-choice method, *Helicoverpa armigera*

Introduction
The American bollworm, *Helicoverpa armigera*, is a notorious agricultural pest that poses significant threats to various crops, particularly in cotton-producing regions. Its widespread distribution and resistance to conventional insecticides necessitate the exploration of alternative pest management strategies (Mary *et al.*, 2014) [16, 17]. One promising approach is the use of natural plant extracts and their derivatives, which have been recognized for their potential insecticidal properties (Kranthi *et al.*, 2000; Ignacimuthu *et al.*, 2006; Jbilou *et al.*, 2008; Kamaraj *et al.*, 2008; Muthu *et al.*, 2010) [10, 12, 13, 14, 20].

In recent years, the synthesis of silver nanoparticles (Ag NPs) and zinc oxide (ZnO NPs) using plant extracts has gained attention due to their enhanced biological activities and eco-friendliness. *Cleistanthus collinus*, a plant known for its pesticidal properties has been identified as a suitable candidate for the green synthesis of nanobiopesticides (NBPs). This method not only provides a sustainable alternative to chemical synthesis but also leverages the inherent bioactive compounds present in the plant, which may contribute to the insecticidal efficacy of the nanoparticles (Mary *et al.*, 2014) [16, 17].

The antifeedant activity of a substance refers to its ability to deter herbivorous insects from feeding on treated plant material. This property is crucial in pest management, as it can significantly reduce crop damage by limiting the feeding behavior of pests (Bhaskar *et al.*, 2011). In this study, we aim to evaluate the antifeedant activity of Ag-NBPs and ZnO-NBPs, synthesized from the aqueous leaf extract of *C. collinus* against the third instar larvae of *Helicoverpa armigera*. By comparing the efficacy of the synthesized NBPs with that of the crude aqueous extract, we seek to comprehensively understand their potential as natural insecticides.

Through this investigation, we may contribute to the development of eco-friendly pest control strategies that can mitigate the impact of *H. armigera* on agricultural productivity while minimizing the reliance on synthetic chemicals. The findings from this study may pave the way for innovative applications of plant-derived NBPs in sustainable agriculture (Baskar *et al.*, 2010; Baskar *et al.*, 2011; AL-Asady *et al.*, 2012) [1, 3, 4].

Materials and methods
1. Preparation of NBPs
Leaves of *Cleistanthus collinus* (Roxb.) Benth. were collected and shade dried at room temperature, powdered by an electric blender. The preparation of the extract involved dissolving 25 g of powdered leaf material in 250 mL of distilled water at a ratio of 1:10. The mixture was then boiled at 80°C for 2 minutes and subsequently filtered. The resulting filtrate served as the basis for the synthesis of nanobioparticles. For the reduction of silver ions, a gradual addition of 50 mL of *C. collinus* leaf extract to 50 mL of 1 mM silver nitrate (HIMEDIA) solution was performed under continuous stirring. Similarly, in the case of zinc ions reduction, 50 mL of *C. collinus* leaf extract was slowly added to 50 mL of 1 mM zinc sulfate (HIMEDIA) solution with constant stirring. Upon the addition of the *C. collinus* extract (filtrate) to the aqueous solutions containing silver and zinc ions, the color of the reaction mixture transitioned from green to a yellowish hue within a timeframe of 20 minutes, indicating the successful synthesis of nanobioparticles.

2. Insects
The current investigation employed third-instar of *Helicoverpa armigera* larvae sourced from cotton fields in Junewani village, Hingna, Nagpur, Maharashtra. Following their collection, the larvae were reared in controlled laboratory conditions at room temperature and fed fresh cotton leaves to promote optimal growth and maturation in preparation for experimental procedures.

3. Antifeedant activity experiment
Different concentrations of AgNBPs and ZnONBPs with low and high doses against the 3rd instar larvae of *H.*

armigera and recorded the antifeedant activity by no choice method. Fresh cotton leaf (*Gossypium sp.*) discs were used, treated with low concentration (6µL/g) and high concentration (12µL/g) of Ag-NBPs, low concentration (8µL/g) and high concentration (15µL/g) of ZnO-NBPs. Cotton leaf discs sprayed with normal water served as control in the petridish. Treated and control leaf discs were placed separately inside the petridish having wet Whatman's No. 1 filter paper to avoid early drying of the leaf discs. In each petridish, single 3rd instar larvae of *H. armigera* was released individually for no-choice method, the experiment was repeated 10 times. Progressive leaf area consumption by the larva after 24 Hrs was recorded using a leaf area meter (Image J Software). The percentage of the antifeedant index was calculated using the formula given by Han *et al.*, 2006.

$$\text{Antifeedant Index} = \frac{\text{Area consumed in control leaf} - \text{Area consumed in the treated leaf}}{\text{Area consumed in control leaf} + \text{Area consumed in the treated leaf}} \times 100$$

Observation and Results

The feeding behavior of third instar of *Helicoverpa armigera* larvae was evaluated under the no-choice method to determine their preference and consumption rate when confined to a single leaf of the cotton plant. Under these conditions, each larva was provided with only one type of leaf material, and parameters such as the amount of leaf consumed were recorded. The results revealed distinct variations in consumption patterns recorded with the help of Image J software, indicating that even in the absence of choice, *H. armigera* exhibited measurable differences in feeding performance.

1. Feeding activity of *H. armigera*

Table 1: Showing the feeding behavior of *H. armigera* in no choice method exposed to normal water (control group) and treated groups (Ag-NBPs and ZnO-NBPs) with different concentrations

Groups	Leaf area Consumed (in %)	Leaf area consumed Mean and SE	p-value
Control	35.30 %	2.57 ±0.141	-
Ag-NBPs (Low)	22.37 %	1.079 ±0.052	0.0004
Ag-NBPs (High)	6.23 %	0.774 ±0.101	<0.0001
ZnO-NBPs (Low)	11.93 %	1.191 ±0.064	<0.0001
ZnO-NBPs (High)	5.11 %	0.853 ±0.028	<0.0001

The feeding behavior of *H. armigera* larvae under the no-choice method revealed a significant reduction in leaf area consumption following nanoparticle treatment compared to the control group. Larvae in the control group consumed 35.30% of the leaf area (2.57 ± 0.141 cm²), while those treated with Ag-NBPs showed a marked decrease in feeding, with 22.37% (1.079 ± 0.052 cm²; $p = 0.0004$) and 6.23% (0.774 ± 0.101 cm²; $p < 0.0001$) consumption at low and high concentrations, respectively. Similarly, ZnO-NBPs treatment resulted in 11.93% (1.191 ± 0.064 cm²; $p < 0.0001$) and 5.11% (0.853 ± 0.028 cm²; $p < 0.0001$) leaf area consumption at low and high doses, respectively. Overall, both NBPs exhibited a dose-dependent antifeedant effect, with maximum feeding inhibition observed at higher concentrations as compare to the lower concentration.

2. Assessment of antifeedant activity of NBPs

2.1 Antifeedant activity of Ag-NBPs

The antifedant activity of Ag-NBPs against *Helicoverpa armigera* larvae was evaluated using the no-choice feeding method. The degree of feeding deterrence was expressed as the Antifeedant Index (mean ± SE) for different concentrations of Ag-NBPs. Two treatment groups were tested—Low and High concentrations. At low concentration, the antifeedant index was 19.26 ± 3.947, indicating mild deterrence activity, with noticeable feeding damage on the leaf surface. While at high concentration, the antifeedant index significantly increased to 49.68 ± 5.97, suggesting a strong feeding deterrent effect, as larvae consumed a considerably smaller leaf area. ImageJ Photographs in Figure 2 (a) and Figure 2 (b) clearly illustrate this difference.

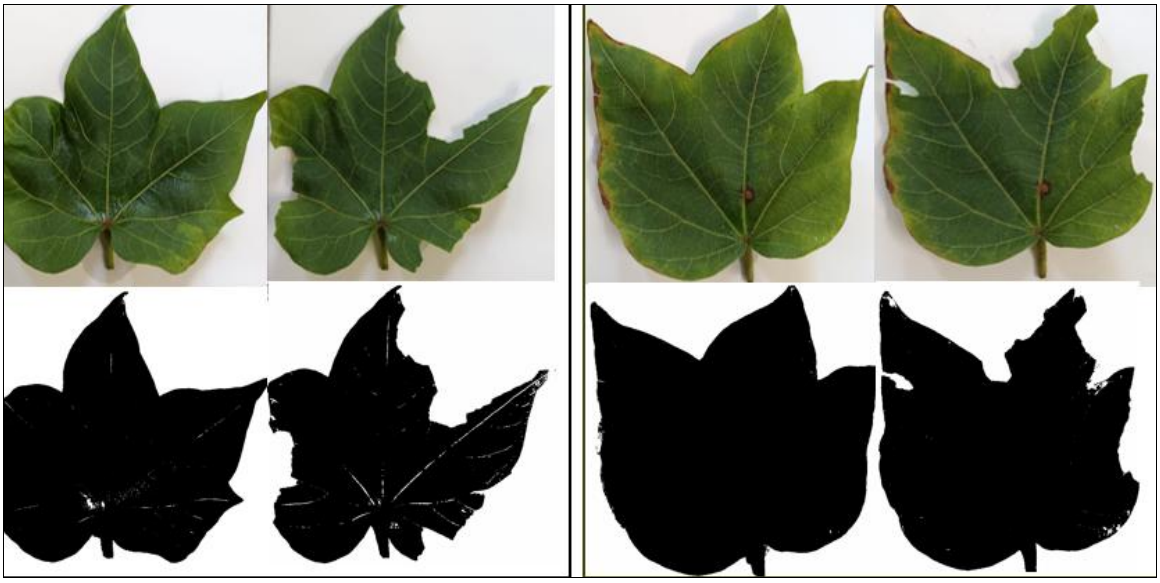

Fig 2 a: Antifeedant activity after spraying Ag-NBPs (Low) **Fig 2 b:** Antifeedant activity after spraying Ag-NBPs (High)

In the low concentration group (Fig. 2a), more leaf area was consumed by the larvae. In contrast, the high concentration group (Fig. 2b) shows minimal feeding, demonstrating higher efficacy of Ag-NBPs in deterring larval feeding behavior. The results reveal a dose-dependent increase in the antifeedant index, confirming that higher concentrations of Ag-NBPs are more effective in suppressing the feeding activity of *H. armigera*. This suggests that Ag-NBPs have potential as a biocompatible pest management agent.

2.2 Antifeedant activity of ZnO-NBPs

The antifedant activity of zinc oxide nanoparticle-based products (ZnO-NBPs) against *Helicoverpa armigera* larvae was evaluated using the no-choice leaf disc method. The antifedant index, expressed as mean ± standard error (SE), was calculated for two different concentrations of ZnO-NBPs (low and high). The results showed that the antifeedant index increased with concentration, indicating a concentration-dependent deterrent effect on larval feeding. At low concentration, the antifedant index was 36.51 ± 3.45, showing moderate feeding deterrence. The leaves displayed visible feeding marks, suggesting partial repellence to larvae. While, at high concentration, the antifedant index increased to 48.96 ± 2.67, signifying a strong antifeedant effect. The treated leaves exhibited minimal feeding damage, demonstrating a higher efficacy of ZnO-NBPs at elevated concentrations. As illustrated in Figure 3 (a) and Figure 3(b), the leaf images show a clear difference between low and high concentration treatments. Leaves treated with a higher concentration had significantly less consumed area, confirming reduced palatability to *H. armigera* larvae.

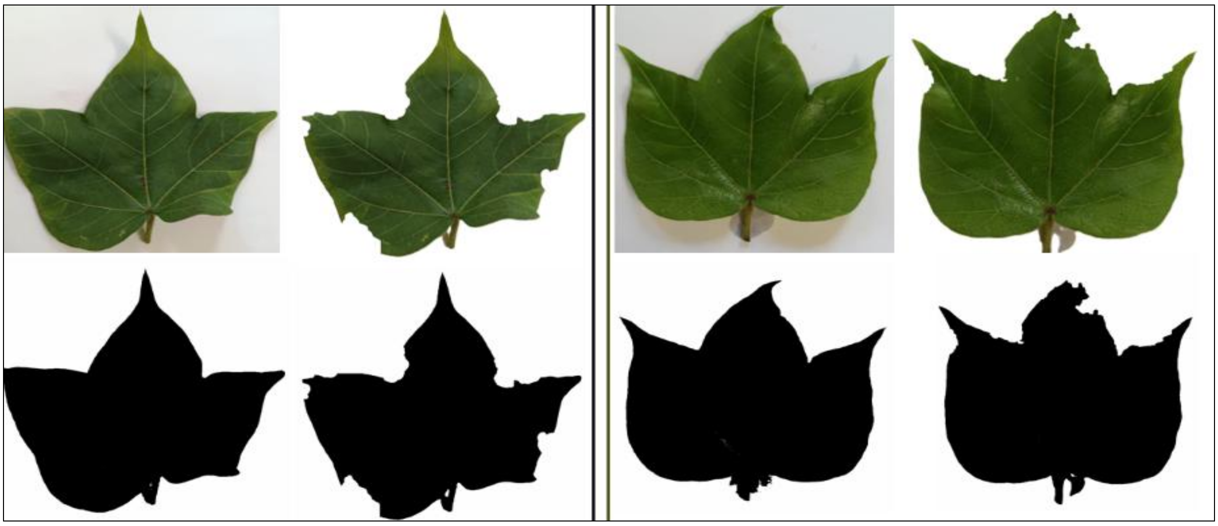

Fig 3 a: Antifeedant activity after spraying ZnO-NBP (Low) **Fig 3 b:** Antifeedant activity after spraying ZnO-NBP (High)

The results indicate that ZnO-NBPs possess effective antifeedant properties, with activity increasing in a dose-dependent manner. The nanoparticles likely interfere with the feeding behavior or gustatory perception of *H. armigera*, thereby reducing leaf consumption. These findings suggest that ZnO-NBPs could serve as a promising eco-friendly nanobiopesticide for managing *Lepidopteran* pests.

Discussion

The present study demonstrates the potent antifeedant activity of silver (Ag-NBPs) and zinc oxide nanobiopesticides (ZnO-NBPs) against third instar larvae of *Helicoverpa armigera*, highlighting their potential for sustainable pest management. The superior efficacy of NBPs compared to crude extracts suggests that nanoparticle formulations enhance bioactivity and crop protection by reducing larval feeding. Similar findings using phytofabricated silver nanoparticles have shown strong insecticidal effects against *Lepidopteran* pests (Rani *et al.*, 2016) [23].

The enhanced reactivity and bioavailability of nanoparticles enable better penetration through insect cuticle and gut barriers, increasing biological impact (Santhoshkumar *et al*., 2011; Melanie *et al.,* 2022) [18, 24]. This nano-scale advantage supports improved delivery of plant-derived compounds and sustained release at target sites, amplifying their antifeedant potential (Mary *et al*., 2014) [16, 17]. The mechanisms may possibly involve disruption of sensory or digestive physiology (Ayilara *et al.,* 2023) [2]. Ag-NBPs and ZnO-NBPs act through multiple pathways such as ROS generation, enzyme inhibition, and membrane damage, leading to oxidative stress and feeding inhibition (Sirelkhatim *et al.,* 2015) [25]. Their concentration-dependent activity aligns with prior nanoparticle-based pest deterrence studies (Hazafa *et al.,* 2022) [9]. Additionally, nanobiopesticides offer advantages over conventional pesticides by providing longer residual activity, reduced application frequency, and minimal non-target toxicity (Jamplek *et al.,* 2015; Mossa, 2016) [19].

While results affirm the promise of Ag-NBPs and ZnO-NBPs as eco-friendly pest deterrents, future studies should focus on molecular action, field-scale efficacy, and ecological safety (Ferreira *et al.,* 2019; Chowdhury *et al.,* 2023) [6, 7]. Overall, this work supports the integration of nanobiopesticides into next-generation, sustainable pest management systems (Lade, 2017) [15].

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