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BIO-SAFETY EVALUATION OF PLANT DERIVED SILVER NANOPARTICLES ON *BRACON HEBETOR*

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ABSTRACT

This study assessed the biosafety of silver nanoparticles (AgNPs) synthesized from six indigenous plant extracts *Acorus calamus*, *Melia azedarach*, *Strychnos nux-vomica*, *Azadirachta indica*, *Cymbopogon citratus* and *Eucalyptus globulus* on the larval parasitoid *Bracon hebetor*. AgNPs were prepared using aqueous extracts and tested at concentrations of 300, 200, 100, 50, 25, 10 and 5 ppm. Adult parasitoids were exposed via two methods: residual film and Potter's tower spray, while grubs were tested using only the spray method. Among the formulations, AgNPs synthesized from *S. nux-vomica* caused the highest adult mortality (6.67%) in both methods at 300 ppm, followed by *M. azedarach* and *A. calamus* (3.33%). AgNPs from *E. globulus*, *C. citratus* and *A. indica* were non-toxic to adults across all concentrations. Grub-stage larvae were more susceptible, with maximum mortality observed in *M. azedarach* (16.67%), *S. nux-vomica* (16.67%) and *A. calamus* (13.33%) at 300 ppm. In contrast, *C. citratus*, *E. globulus* and *A. indica* AgNPs caused minimal or no larval mortality. The results highlight the importance of stage-specific toxicity assessment and support the selective application of AgNPs to minimize non-target effects on beneficial parasitoids in IPM programs.

Key words : Biosafety, Silver nanoparticles, *Bracon hebetor*.

Introduction

As the drawbacks of conventional chemical pesticides become increasingly evident—ranging from environmental pollution to resistance development in pests—there has been a growing shift toward greener, more sustainable pest management solutions. Among these, silver nanoparticles (AgNPs) synthesized from botanical extracts have shown considerable promise. These green-synthesized nanoparticles combine the insecticidal potency of both silver and bioactive plant compounds, offering a dual mode of action that enhances pest control while reducing ecological harm.

Several indigenous plants such as *Acorus calamus*

(rhizomes), *Melia azedarach*, *Azadirachta indica* and *Strychnos nux-vomica* (seeds), along with leaves of *Cymbopogon citratus* and *Eucalyptus globulus*, have been successfully used to produce AgNPs through eco-friendly methods. These plants are known to contain phytochemicals like alkaloids, terpenoids and flavonoids, which not only aid in nanoparticle synthesis but also contribute to their biological activity. Studies have shown that these AgNPs are effective against a variety of insect pests, acting through mechanisms such as oxidative stress induction, cell membrane damage and inhibition of metabolic functions.

For any pest control strategy to be ecologically viable, it is important to consider its effects on non-target

organisms, particularly beneficial insects such as *Bracon hebetor*, a widely used larval parasitoid in biological control programs. Plant-based silver nanoparticles (AgNPs) have shown promising pest control potential, but their impact can vary depending on the formulation and the developmental stage of the exposed organism. While some AgNPs appear to be relatively harmless to adult parasitoids, others may affect more vulnerable stages such as larvae. Therefore, evaluating the biosafety of these nanoparticle formulations is crucial to ensure they can be used in ways that help protect *B. hebetor* and other natural enemies involved in integrated pest management systems.

Materials and Methods

Collection of plant materials

The rhizomes of *Acorus calamus*, seeds of *Melia azedarach*, *Azadirachta indica*, *Strychnos nux-vomica* and leaves of *Cymbopogon citratus* and *Eucalyptus globulus* were collected from tribal regions of Andhra Pradesh. Plant materials (leaves, rhizomes and seeds) were thoroughly washed with tap water and shade-dried in the laboratory for 6–7 days.

Preparation of silver nanoparticles

To prepare the plant extract, 5 g of dried powder was added to 100 mL of distilled water and boiled at 70/°C for 15–20 minutes. After cooling, the mixture was filtered through Whatman No.1 filter paper and the clear extract was stored at 4°C for further use (Iravani *et al.*, 2014). A 2 mM aqueous silver nitrate (AgNO₃) solution (340 ppm) was prepared by dissolving 340 mg of AgNO₃ in 1 L of distilled water (Song and Kim, 2009). For nanoparticle synthesis, 90 mL of AgNO₃ solution was mixed with 10 mL of plant extract (9:1 ratio) and incubated at room temperature with constant stirring. A gradual color change from pale yellow to brown indicated the formation of silver nanoparticles (AgNPs), attributed to surface plasmon resonance. The synthesis was confirmed by UV–Vis spectrophotometry, which showed a characteristic absorption peak between 420–450 nm (Ankanna *et al.*, 2010).

Preparation of test solutions

The silver nanoparticles obtained from the above method was dissolved in their respective solvents and then diluted with water containing Triton X-100, emulsifier (0.5g in 100ml of distilled water).

Test insect

Bracon hebetor was reared in the Entomology Laboratory, College of Horticulture, Venkataramannagudem, following the procedure

described by Sujatha *et al.* (2003). Mass multiplication was carried out using an alternate laboratory host, *Corcyra cephalonica*. Fully grown larvae of *C. cephalonica* were placed on a stretched muslin cloth secured over the mouth of wide-mouthed glass jars, with a glass plate placed above to restrict larval movement. Adult *B. hebetor* were introduced into the jars, where gravid females paralyzed the host larvae and laid eggs externally. Upon hatching, the parasitoid larvae fed ectoparasitically on the immobilized hosts. Mature grubs were transferred onto clean rectangular paper strips (2" × 1") to facilitate cocoon formation. Adult emergence occurred approximately seven days after parasitization, marking the beginning of the next generation.

Bioassay studies of *B. hebetor* grubs

Two-day-old larvae of *Bracon hebetor* were placed in sterilized petri dishes (8 cm diameter), ten per dish, with three replicates per treatment. Grubs were exposed to silver nanoparticles (AgNPs) synthesized from indigenous plant extracts at concentrations of 300, 200, 100, 50, 25, 10 and 5 ppm using a Potter's spray tower (340 g/cm²). Post-treatment, larvae were air-dried for 10–15 minutes, and a honey–water solution was provided to support adult emergence. Controls were treated with emulsified distilled water. Larval mortality was recorded at 24, 48-, 72-, 96- and 120-hours post-exposure to assess the dose-dependent acute toxicity of the AgNP formulations.

Results

Bioassay studies of *B. hebetor* adults

Biosafety of silver nanoparticles (AgNPs) synthesized from indigenous plant extracts on *Bracon hebetor*, two exposure techniques were employed: the residual film method and the Potter's tower spray technique. In the residual film method, clean glass test tubes were uniformly coated with AgNP solutions at concentrations of 300, 200, 100, 50, 25, 10 and 5 ppm, then shade-dried to allow nanoparticle adherence. In the Potter's tower method, the same concentrations were atomized onto the inner surfaces of Petri dishes for uniform deposition. Following treatment, ten adult parasitoids were released into each test unit and a honey–water solution was provided as a nutritional source. Each treatment was replicated three times and untreated controls were exposed to emulsified water under identical conditions. Mortality and behavioral responses were assessed at 12- and 24-hours post-exposure to determine any acute or sublethal effects induced by the nanoparticle formulations.

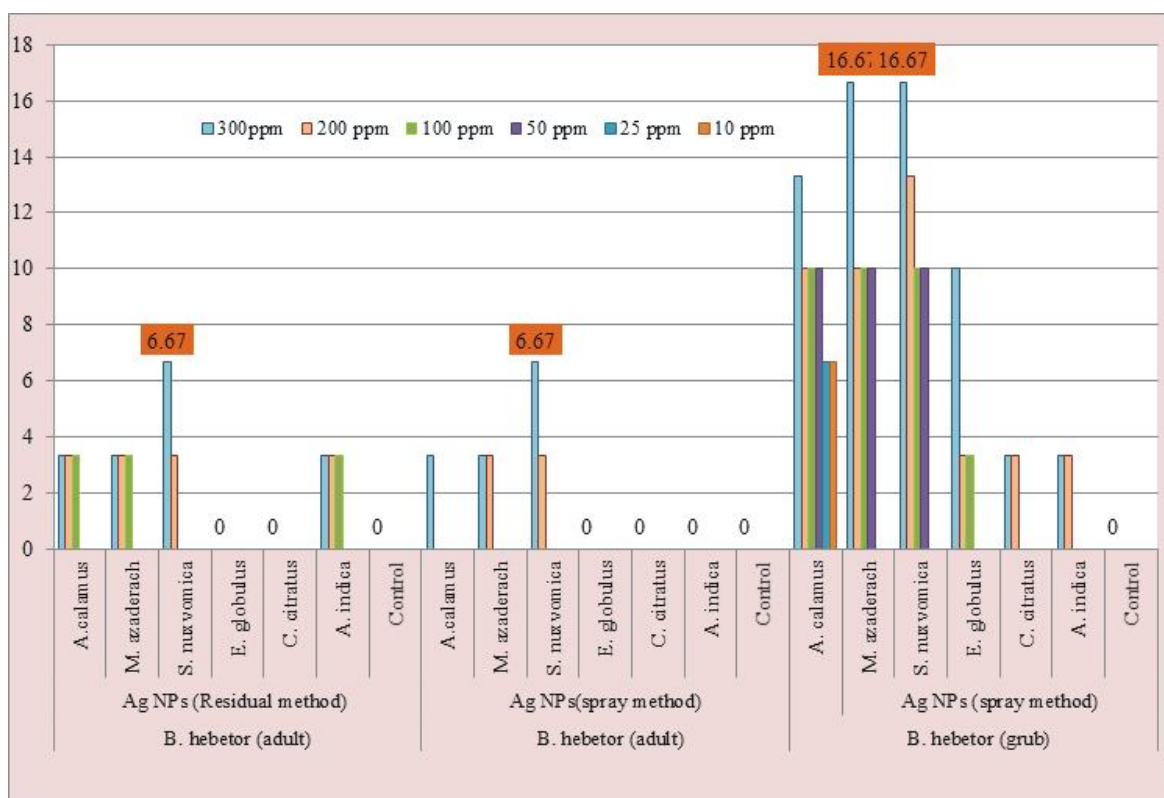


Fig. 1 : Biosafety evaluation of Plant derived AgNPs on *Bracon hebetor*.

Adult Toxicity via Residue Film method

The mean data of the residual contact toxicity of silver nanoparticles (AgNPs) synthesized from various indigenous plant extracts against *Bracon hebetor* adults evaluated using the residue film method are presented in Table 1 and Fig. 1. Results revealed that *Acorus calamus*, *Melia azedarach*, *Strychnos nux-vomica* and *Azadirachta indica* AgNPs caused sublethal adult mortality (3.33%–6.67%) at higher concentrations (100–300 ppm), while lower concentrations (≤ 50 ppm) were non-toxic. Notably, *S. nux-vomica* exhibited the highest adult mortality (6.67%) at 300 ppm, suggesting a relatively higher contact toxicity among the tested extracts. Conversely, AgNPs derived from *Eucalyptus globulus* and *Cymbopogon citratus* exhibited no adult toxicity across all tested concentrations, indicating poor residual toxicity and suggesting their relative safety to this parasitoid.

Adult Toxicity via Direct Spray exposure

Direct spray application of AgNPs showed a more acute effect at the highest dose but diminished rapidly at lower concentrations. *S. nux-vomica* AgNPs again displayed the highest adult mortality (6.67%) at 300 ppm, with a dose-dependent decline observed at 200 ppm (3.33%) and no mortality at lower concentrations. *A. calamus* and *M. azedarach* showed mild toxicity

(3.33%) only at 300 ppm, while *E. globulus*, *C. citratus* and *A. indica* showed no mortality at any tested level. This highlights the relatively narrow toxic threshold of certain plant-derived AgNPs and underscores the importance of dose optimization for preserving beneficial parasitoids during field application (Table 1, Fig. 1).

Grub Toxicity of AgNPs

Grub-stage larvae of *B. hebetor* exhibited significantly higher susceptibility to AgNPs compared to adults, suggesting heightened vulnerability during developmental stages which can be seen in Table 1 and Fig. 1. AgNPs of *M. azedarach*, *S. nux-vomica* and *A. calamus* caused pronounced mortality, with *M. azedarach* reaching 16.67% at 300 ppm and maintaining $\geq 10\%$ mortality down to 100 ppm. *S. nux-vomica* and *A. calamus* also showed consistent toxicity across concentrations, with measurable mortality down to 25 ppm. *E. globulus* exhibited moderate toxicity at 300 ppm (10.0%), declining sharply at lower doses. In contrast, *C. citratus* and *A. indica* caused negligible larval mortality ($\leq 3.33\%$) and were comparable to the untreated control. These findings emphasize the stage-specific toxicodynamics of AgNPs and the necessity for selective bioformulations to minimize non-target effects on beneficial parasitoids.

Table 1 : Bio- Safety evaluation of plant-based AgNPs on the parasitoid *Bracon hebetor*.

Natural enemy	Silver nanoparticles		Concentration (ppm)					
			300ppm	200 ppm	100 ppm	50 ppm	25 ppm	10 ppm
<i>B. hebetor</i> Adult	Ag nanoparticles (Residue film method)	<i>A. calamus</i>	3.33	3.33	3.33	0.00	0.00	0.00
		<i>M. azaderach</i>	3.33	3.33	3.33	0.00	0.00	0.00
		<i>S. nuxvomica</i>	6.67	3.33	0.00	0.00	0.00	0.00
		<i>E. globulus</i>	0.00	0.00	0.00	0.00	0.00	0.00
		<i>C. citratus</i>	0.00	0.00	0.00	0.00	0.00	0.00
		<i>A. indica</i>	3.33	3.33	3.33	0.00	0.00	0.00
		Control	0.00	0.00	0.00	0.00	0.00	0.00
<i>B. hebetor</i> Adult	Ag nanoparticles (spray method)	<i>A. calamus</i>	3.33	0.00	0.00	0.00	0.00	0.00
		<i>M. azaderach</i>	3.33	3.33	0.00	0.00	0.00	0.00
		<i>S. nuxvomica</i>	6.67	3.33	0.00	0.00	0.00	0.00
		<i>E. globulus</i>	0.00	0.00	0.00	0.00	0.00	0.00
		<i>C. citratus</i>	0.00	0.00	0.00	0.00	0.00	0.00
		<i>A. indica</i>	0.00	0.00	0.00	0.00	0.00	0.00
		Control	0.00	0.00	0.00	0.00	0.00	0.00
<i>B. hebetor</i> Grub	Ag nanoparticles	<i>A. calamus</i>	13.33	10.00	10.00	10.00	6.67	6.67
		<i>M. azaderach</i>	16.67	10.00	10.00	10.00	0.00	0.00
		<i>S. nuxvomica</i>	16.67	13.33	10.00	10.00	0.00	0.00
		<i>E. globulus</i>	10.00	3.33	3.33	0.00	0.00	0.00
		<i>C. citratus</i>	3.33	3.33	0.00	0.00	0.00	0.00
		<i>A. indica</i>	3.33	3.33	0.00	0.00	0.00	0.00
		Control	0.00	0.00	0.00	0.00	0.00	0.00

Discussion

Nanoparticle-mediated plant extracts have emerged as promising tools in pest management due to their enhanced efficacy and selective toxicity. Murugan *et al.* (2015) reported that silver nanoparticles (AgNPs) synthesized from *Datura metel* exhibited strong larvicidal activity against *Anopheles stephensi*, while simultaneously enhancing the predatory efficiency of *Anax immaculifrons*, indicating a dual-action benefit. Hala *et al.* (2016) demonstrated potent acaricidal and ovicidal activity of silica nanoparticles (SiNPs) against *Tetranychus* spp., though varying levels of toxicity were observed among beneficial predators, with *Stethorus punctillum* highly susceptible, while *Orius insidiosus* and *Phytoseiulus persimilis* showed lower sensitivity. Similarly, Al-Azzazy *et al.* (2019) confirmed the selective toxicity of AgNPs, achieving high mortality in phytophagous mites while maintaining relative safety for predatory species like *Euseius scutalis* and *Neosius cucumeris*.

These studies highlight the potential of nanoparticle-based formulations for targeted pest suppression with reduced non-target impacts, supporting their integration into sustainable pest management strategies.

Conclusion

The study revealed significant differential toxicity of biosynthesized silver nanoparticles (AgNPs) from indigenous plants against *Bracon hebetor*. AgNPs from *Strychnos nux-vomica* and *Melia azedarach* exhibited pronounced insecticidal activity, inducing high adult and larval mortality, likely due to alkaloid-mediated nanoparticle bioactivity. In contrast, *Eucalyptus globulus*, *Cymbopogon citratus* and *Azadirachta indica* AgNPs showed negligible toxicity, even at 300 ppm, indicating high selectivity and environmental compatibility. Notably, *E. globulus* and *C. citratus* AgNPs demonstrated strong safety margins for non-target parasitoids. These results support their use in IPM programs prioritizing natural enemy conservation.

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