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DEPLETION OF HAEMOLYMPH PROTEIN AND CARBOHYDRATE CONTENT IN *P. XYLOSTELLA* LARVAE INDUCED BY PLANT DERIVED SILVER NANOPARTICLES

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ABSTRACT

Phytogenic silver nanoparticles (AgNPs) synthesized from six botanicals (10–300 ppm) were assessed for biochemical toxicity against *Plutella xylostella* larvae. Untreated larvae maintained stable haemolymph protein (≈ 38 mg/g) and carbohydrate (≈ 58 mg/ml) levels. AgNP exposure elicited concentration-dependent macromolecular depletion, reflecting severe metabolic distress. *Acorus calamus* AgNPs caused the most pronounced protein (33.40 → 29.50 mg/g; 23.9%) and carbohydrate suppression (45.99 → 40.42 mg/ml; 22%), followed by *Melia azedarach*, *Strychnos nux-vomica*, *Azadirachta indica*, *Cymbopogon citratus* and *Eucalyptus globulus*. Positive correlations between residual biomolecule levels and LC_{50} (r = 0.70 for protein; r = 0.825 for carbohydrate) confirm that higher AgNP toxicity is tightly linked to nutritional stress, enzyme inhibition and impaired energy metabolism. These results establish haemolymph protein and carbohydrate depletion as precise biomarkers of AgNP-induced toxicodynamic impact in lepidopteran larvae.

Key words : AgNPs, Protein, Carbohydrate, *P. xylostella* larvae.

Introduction

Insect haemolymph serves as a vital physiological medium for nutrient transport, energy storage, hormonal regulation and immune responses, making it a sensitive indicator of toxicological stress (Nation, 2015). Disruption of haemolymph constituents such as total protein and carbohydrate content reflects impairment of metabolic homeostasis, often correlating with growth retardation, reduced fecundity and mortality in insect pests (Klowden, 2013). Recent advances in nanobiotechnology have facilitated the development of plant-derived silver nanoparticles (AgNPs) as promising eco-friendly alternatives to conventional chemical insecticides (Benelli, 2018). These phytosynthesized nanoparticles offer enhanced bioactivity due to their small size, high surface area and functionalization with bioactive phytochemicals, resulting in improved insecticidal efficacy, controlled release, and target specificity (Kumar *et al*., 2021). Although the toxic potential of botanical AgNPs has been well documented against various agricultural pests, their biochemical mode of action, particularly in relation to haemolymph macromolecule depletion, remains inadequately explored in *P. xylostella* (diamondback moth), a notorious pest of cruciferous crops with widespread resistance to synthetic insecticides (Furlong *et al*., 2013). Therefore, the present study evaluates the effect of AgNPs synthesized from six indigenous botanicals on haemolymph total protein and carbohydrate profiles of *P. xylostella* larvae, and examines the correlation between biochemical alterations and nanoparticle toxicity parameters (AI_{50}, LC_{50}, I_{50}), to establish these macromolecules as reliable biomarkers for nanoparticle-induced stress and to inform the development of metabolically disruptive nanobiopesticides for sustainable pest management.

Materials

Rearing of *Plutella xylostella*

A continuous laboratory colony of *P. xylostella* was established from field-collected larvae and pupae obtained from cabbage and cauliflower crops and maintained at 27 ± 1°C, 60 ± 5% RH, and a 14L:10D photoperiod. Adults were held in 27 × 21 × 21 cm oviposition cages, provided with 10% sugar solution on cotton swabs, and supplied with five-day-old mustard seedlings as oviposition substrates. Eggs were collected daily, and neonates were reared on cabbage leaves in plastic containers (25 × 20 cm) with fresh leaves provided to facilitate natural larval migration and minimize handling stress. Larval cohorts were maintained separately by oviposition date to ensure synchronized instar availability for bioassays.

Green synthesis of Silver nanoparticles

The indigenous plants *viz.*, rhizomes of *A. calamus*, fruits of *M. azedarach*, seeds of *S.nux-vomica* and *A. indica*, leaves of *C. citratus* and *E.s globulus* were collected from tribal areas of Andhra Pradesh. They were thoroughly washed and shade dried for 7 days then made into fine powder using grinder for further extraction. Plant-mediated silver nanoparticles (AgNPs) were synthesized using dried powders of indigenous species as bio reductants and stabilizing agents. Aqueous extracts were prepared by boiling 5 g of plant powders in 100 mL of distilled water at 70 °C for 15–20 min, followed by filtration through Whatman No.1 paper and storage at 4°C. A 4 mM $AgNO_3$ stock solution (786.8 mg L^{-1}) was prepared, and nanoparticle synthesis was initiated by mixing the $AgNO_3$ solution with plant extract in a 9:1 ratio under continuous stirring. Successful bio reduction of Ag^+ ions was evidenced by a distinct chromatic transition from pale yellow to dark brown, attributable to surface plasmon resonance (SPR), with UV–Vis spectrophotometry (Shimadzu UV-2450) showing a characteristic absorption band at 420–450 nm. Morphological features of the Phyto fabricated AgNPs were examined using FESEM (Zeiss Ultra 55) after sample immobilization on carbon-coated aluminum stubs and sputter-coating, while elemental confirmation was achieved through EDX microanalysis (Oxford INCAx-act), which verified the presence of metallic silver and trace phytoconstituent residues (Ankana *et al*., 2010).

Estimation of Protein Content (Lowry *et al.*, 1951)

Reagents

- **Reagent A**: 2% Na_2CO_3 in 0.1 N NaOH
- **Reagent B**: 1.35% sodium potassium tartrate + 5.5% $CuSO_4{\cdot}5H_2O$ (1:0.1 v/v)
- **Reagent C**: Alkaline copper solution (50 ml of Reagent A + 1 ml of Reagent B, freshly prepared)
- **Reagent D**: Folin–Ciocalteu's phenol reagent diluted 1:1 with distilled water
- **Standard**: Bovine Serum Albumin (BSA) working standards

Procedure

Five hundred milligrams of treated and untreated *P. xylostella* larvae were homogenized in 5 ml of 10% TCA, washed with chilled TCA, kept on ice (15 min) and centrifuged at 3500 rpm for 15 min. The precipitate was dissolved in 4 ml of 2 N NaOH and kept overnight, centrifuged again, and volume made up to 10 ml. Aliquots (0.1 ml) were mixed with 5 ml Reagent C, incubated 10 min, followed by addition of 0.5 ml Reagent D. Tubes were incubated for 30 min at room temperature in the dark and absorbance recorded at 660 nm. Protein concentrations were calculated from a BSA standard curve.

Estimation of Carbohydrate Content (Anthrone Method; Sadasivam and Manickam, 1992)

- Anthrone reagent (0.2% in 95% H_2SO_4)
- 80% ethanol
- 52% perchloric acid
- Glucose working standard (100 µg/ml)

Procedure

Five hundred milligrams of treated and control larvae were homogenized in hot 80% ethanol and centrifuged twice at 6500 rpm for 4 min to remove sugars. Residues were extracted with 6.5 ml of 52% perchloric acid on ice for 20 min twice, centrifuged (6500 rpm, 2 min) and pooled extracts diluted to 100 ml. A 0.1 ml aliquot was made up to 1 ml with distilled water, mixed with 4 ml anthrone reagent, heated in boiling water bath for 8 min, cooled, and absorbance was measured at 630 nm. Carbohydrate (%) was derived from the glucose standard curve.

Results and Discussion

Effect of silver nanoparticles on haemolymph total protein content of *P. xylostella*

Silver nanoparticles (AgNPs) synthesized from six botanical sources elicited marked suppression of haemolymph total protein content in *P. xylostella* larvae

Table 1 : Biochemical impact of silver nanoparticles of various indigenous plant extracts on haemolymph protein of *P. xylostella*.

Silver nanoparticles	Total protein content (mg/g)					
	300 ppm	**200 ppm**	**100 ppm**	**50 ppm**	**25 ppm**	**10 ppm**
A. calamus	29.5	29.68	30.35	30.62	33.38	33.40
M. azedarach	30.25	31.44	32.36	32.81	33.45	34.25
S. nux-vomica	30.50	33.55	34.82	34.76	35.21	35.38
E. globulus	35.25	34.21	35.25	35.28	35.68	35.91
C. citratus	32.96	34.56	35.65	35.33	36.00	36.36
A. indica	32.23	32.00	33.54	34.65	34.79	35.18
Control	38.76	38.77	37.98	38.42	37.95	38.54
SEm(±)	1.240	1.096	0.932	0.908	0.597	0.621
CD @ 0.05	3.034	2.683	2.280	2.222	1.461	1.520
CV%	5.01	4.67	3.19	3.95	4.49	4.62

Table 2 : Biochemical impact of silver nanoparticles of various indigenous plant extracts on haemolymph carbohydrate of *P. xylostella*.

Silver nanoparticles	Total carbohydrate content (mg/ml)					
	300 ppm	**200 ppm**	**100 ppm**	**50 ppm**	**25 ppm**	**10 ppm**
A. calamus	40.42	43.75	43.94	46.30	47.26	50.25
M. azedarach	46.32	46.51	46.60	50.82	51.35	52.77
S. nux-vomica	50.86	52.56	52.68	53.48	55.24	55.64
E. globulus	51.74	52.64	53.48	54.56	55.38	57.12
C. citratus	53.42	53.75	55.25	56.00	56.69	57.26
A. indica	48.37	49.95	51.24	52.28	52.75	53.26
Control	58.47	57.98	58.24	58.63	58.68	58.79
SEm(±)	2.156	1.794	1.864	1.491	1.435	1.144
CD @ 0.05	5.275	4.391	4.561	3.648	3.512	2.799
CV%	4.68	4.69	4.63	6.54	6.52	5.09

in a dose-dependent manner (Table 1 and Fig. 1) when compared with untreated larvae, which exhibited stable protein levels ranging from 37.95–38.77 mg/g (mean ≈ 38.40 mg/g), indicating a healthy and uncompromised protein metabolism. Among the treatments, *A. calamus* AgNPs exerted the strongest inhibitory activity, reducing protein content from 33.40 mg/g at 10 ppm to 29.50 mg/g at 300 ppm corresponding to a 1.31-fold suppression (23.9%) relative to the control. *M. azedarach* AgNPs similarly recorded substantial reductions from 34.25 mg/g to 30.25 mg/g (1.28-fold; 21.9%), followed by *S. nux-vomica* AgNPs which decreased protein levels from 35.38 mg/g to 30.50 mg/g (1.27-fold; 21.3%). In contrast, *E. globulus* AgNPs had a comparatively milder impact, dropping only from 35.91 mg/g to 35.25 mg/g (1.10-fold; 9.0%). *C. citratus* AgNPs lowered protein content from 36.36 mg/g to 32.96 mg/g (1.17-fold; 15.0%), while *A. indica* AgNPs suppressed protein content from 35.18 mg/g to 32.23 mg/g (1.20-fold; 16.8%). In all cases, reduced nanoparticle concentration was associated with partial recovery in protein content, confirming distinct dose dependency.

Effect of silver nanoparticles on haemolymph total carbohydrate content of *P. xylostella*

Table 2 and Fig. 2 illustrates that biosynthesized AgNPs significantly disrupted carbohydrate content in *P. xylostella* larvae compared with untreated control (57.98–58.79 mg/ml; mean ≈ 58.63 mg/ml). Maximum suppression was detected in larvae treated with *A. calamus* AgNPs, where carbohydrate levels declined to 40.42 mg/ml at 300 ppm (average ≈ 45.99 mg/ml), indicating a 1.28-fold (22%) deficit. *A. indica* AgNPs ranked next, with carbohydrate content between 48.37-53.26 mg/ml (mean ≈ 51.48 mg/ml; 1.14-fold reduction). *M. azedarach* AgNPs resulted in moderate depletion (46.32-52.77 mg/ml; mean ≈ 49.90 mg/ml; 1.18-fold), while *S. nux-vomica* AgNPs showed marginal impact (average ≈ 53.58 mg/ml; 1.09-fold). Minimal effects were seen in larvae treated with *E. globulus* (mean ≈ 54.15 mg/ml; 1.08-fold) and *C. citratus* AgNPs (53.42-57.26 mg/ml; mean ≈ 55.73 mg/ml; 1.05-fold), indicating

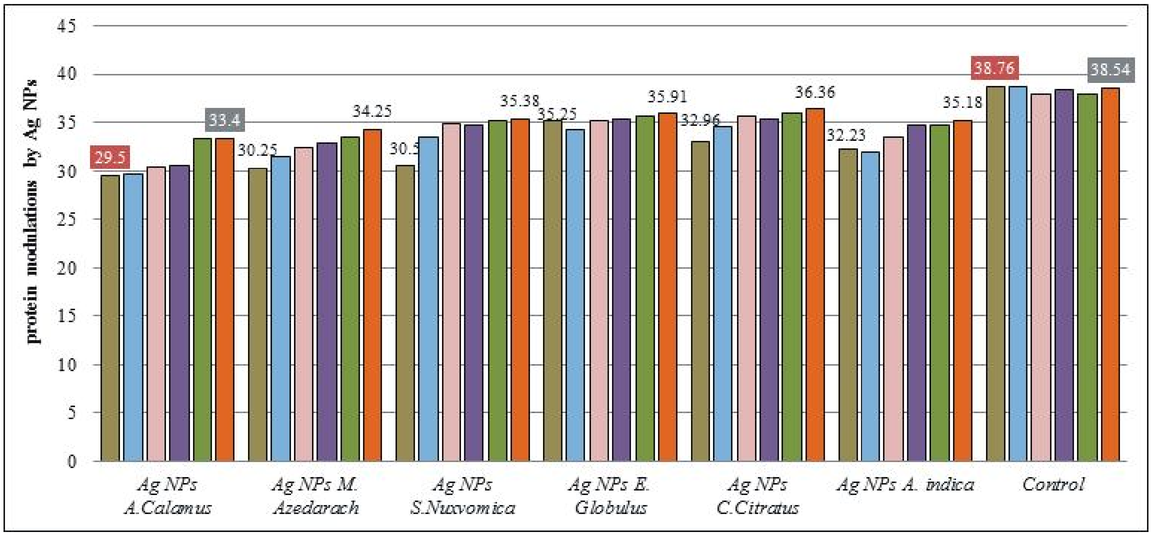

Fig. 1 : Haemolymph protein modulation in *P. xylostella* by AgNPs of plant extracts.

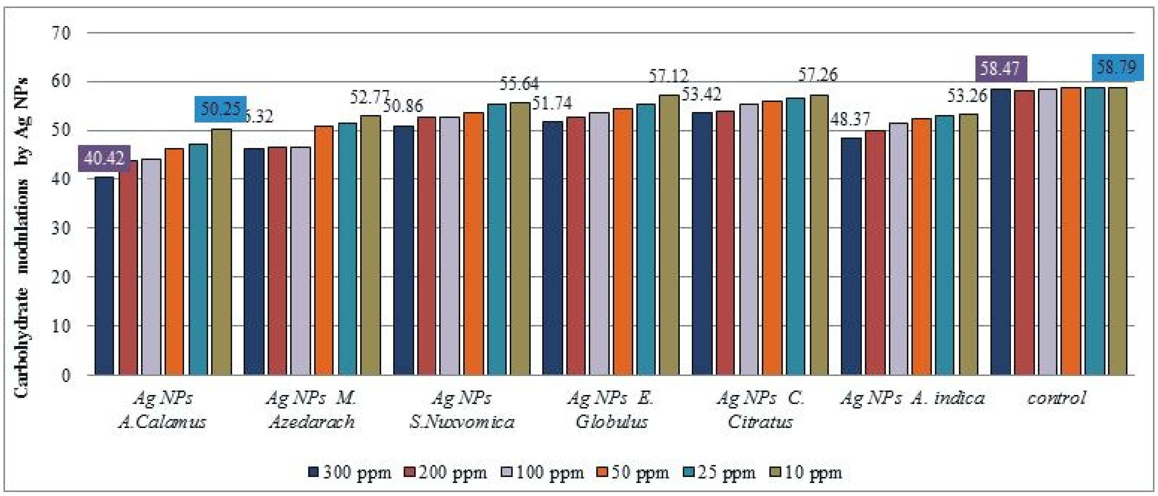

Fig. 2 : Haemolymph carbohydrate modulation in *Plutella xylostella* by AgNPs of plant extracts.

comparatively mild metabolic stress. The toxicity gradient followed the trend *A. calamus* > *A. indica* > *M. azedarach* > *S. nux-vomica* > *E. globulus* > *C. citratus*.

Correlation between nanoparticle toxicity and protein–carbohydrate suppression in *P. xylostella*

A strong relationship was observed between the toxicological efficacy of botanical silver nanoparticles (AgNPs) against *P. xylostella* and their capacity to suppress key metabolic biomolecules, namely total protein and carbohydrate content as represented in Tables 3 and 4 Residual protein levels exhibited significant positive correlations with AI_{50} (r = +0.57), LC_{50} (r = +0.70), and I_{50} (r = +0.69), while carbohydrate levels showed even stronger positive correlations with AI_{50} (r = +0.784), LC_{50} (r = +0.825) and I_{50} (r = +0.797); indicating that as AgNP toxicity increased, both protein and carbohydrate levels were progressively depleted. *A. calamus* AgNPs, which recorded the lowest toxicity thresholds (AI… € = 5.486 ppm, LC_{50} = 21.385 ppm, I_{50} = 54.183 ppm), caused the maximum biochemical suppression, whereas *C. citratus* AgNPs with the highest toxicity thresholds retained comparatively higher levels of both biomolecules, suggesting milder physiological impairment. These significant correlations confirm that alterations in total protein and carbohydrate content serve as reliable biochemical biomarkers for rapid prediction of nanoparticle-induced mortality and sub-lethal stress, thereby aiding in the optimization of botanical nanoinsecticides for eco-friendly pest management.

The dramatic suppression of total protein and carbohydrate content in *P. xylostella* larvae upon exposure to phytogenic silver nanoparticles underscores a potent disruption of both macromolecular biosynthesis and energy metabolism. The protein decline reflects oxidative stress-induced denaturation of structural proteins, ribosomal inhibition, and interference with amino

Table 3 : Correlation of haemolymph protein reduction with toxicity in *P. xylostella* by botanical silver nanoparticles.

Botanical AgNPs	Strong protein inhibition value (mg/g)	AI_{50} (ppm)	LC_{50} (ppm)	I_{50} (ppm)
A. calamus	29.50	5.486	21.385	54.183
M. azedarach	30.25	7.070	34.796	83.243
S. nuxvomica	29.68	13.605	72.172	154.131
E. globulus	33.35	15.128	93.178	175.606
C. citratus	32.96	20.630	110.570	260.987
A. indica	30.20	9.482	60.517	126.969
Pearson r value	—	+0.57	+0.70	+0.69

Table 4 : Correlation between toxicity and carbohydrate suppression in *P. xylostella* exposed to botanical silver nanoparticles.

Botanical AgNPs	Strong carbohydrate inhibition value (mg/g)	AI_{50} (ppm)	LC_{50} (ppm)	I_{50} (ppm)
A. calamus	40.42	5.486	21.385	54.183
M. azedarach	46.32	7.070	34.796	83.243
S. nuxvomica	50.86	13.605	72.172	154.131
E. globulus	51.74	15.128	93.178	175.606
C. citratus	53.42	20.630	110.570	260.987
A. indica	48.37	9.482	60.517	126.969
Pearson r value	—	+0.78	+0.82	+0.79

acid metabolism, a mode of action widely reported for AgNPs (Mariappan *et al.*, 2022; Abdel-Meguid, 2022; Madasamy *et al.*, 2023). Higher toxicity in *A. calamus*, *M. azedarach* and *S. nux-vomica* AgNPs is likely attributed to phytochemical capping agents like α-asarone, limonoids and indole alkaloids enhancing nanoparticle reactivity, while weaker effects in *E. globulus* and *C. citratus* are associated with reduced cellular penetration and pro-oxidant activity. Likewise, substantial carbohydrate depletion indicates disruption of enzymes such as amylase, invertase, trehalase and glycogen phosphorylase, leading to exhaustion of glycogen reserves and impaired ATP production consistent with starvation-like effects, reduced locomotion and delayed development as described by Parthiban *et al.* (2019), Abdou and Zyaan (2023) and Osman *et al.* (2025). Collectively, these findings consolidate earlier reports highlighting AgNP-mediated enzymatic inhibition, metabolic suppression and physiological stress in insect pests, affirming protein and carbohydrate levels as reliable biomarkers for predicting nanoparticle toxicodynamics

Summary and Conclusion

Botanical silver nanoparticles (AgNPs) imposed stronger biochemical toxicity than crude extracts, sharply reducing larval protein and carbohydrate levels compared to controls. *A. calamus* AgNPs caused the greatest protein decline from 33.40 to 29.50/ mg/g (23.9%), while *M. azedarach* (34.25–30.25 mg/g, 21.9%), *S. nux-vomica* (35.38–30.50 mg/g, 21.3%), *A. indica* (35.18–32.23 mg/g, 16.8%), *C. citratus* (36.36–32.96 mg/g, 15.0%) and *E. globulus* (35.91–35.25 mg/g, 9.0%) showed varying toxicity. Carbohydrates dropped most with *A. calamus* AgNPs, averaging 45.99 mg/ml and falling to 40.42/ mg/ml at 300 ppm (22% decrease). *A. indica* averaged 51.48 mg/ml (12–18% decrease), *M. azedarach* 49.90 mg/ml, while *S. nux-vomica* (53.58 mg/ml), *E. globulus* (54.15 mg/ml) and *C. citratus* (55.73 mg/ml) showed milder reductions. These outcomes confirm AgNPs as potent nanotoxicants, causing severe metabolic disruptions beyond those induced by crude plant extracts.

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