CODEN: WJAPAC Impact Factor: 5.48 ISSN: 3049-3013

World Journal of Advance
Pharmaceutical Sciences

Volume 3, Issue 5, Page: 83-92 | Research Article | www.wjaps.com

PHYTOCHEMISTRY-MEDIATED MODULATION OF LARVAL METABOLISM, ANTIOXIDANT DEFENSE SYSTEMS, AND COCOON QUALITY TRAITS IN SEMI-DOMESTICATED TASAR SILKWORM (*ANTHERAEA MYLITTA* D.)

Md. Tahfizur Rahman*[1], Shagufta Nigar[2], Kumar Manish[3], Tushar Kumar[4], Mustafa Kamal Ansari[5]

[1,2]Assistant Professor, P.G. Dept. of Zoology, Millat College Darbhanga.
[3]Assistant Professor, University P.G. Dept. of Zoology, LNMU Darbhanga.
[4]Research Scholar, P.G. Deptment of Botany, LNMU Darbhanga.
[5]Associate Professor, Deptment of Botany Millat College Darbhanga.

How to cite this Article: Md. Tahfizur Rahman*[1], Shagufta Nigar[2], Kumar Manish[3], Tushar Kumar[4], Mustafa Kamal Ansari[5]. (2026). PHYTOCHEMISTRY-MEDIATED MODULATION OF LARVAL METABOLISM, ANTIOXIDANT DEFENSE SYSTEMS, AND COCOON QUALITY TRAITS IN SEMI-DOMESTICATED TASAR SILKWORM (*ANTHERAEA MYLITTA* D.). World Journal of Advance Pharmaceutical Sciences, 3(5), 83-92.

Copyright © 2026 Md. Tahfizur Rahman* | World Journal of Advance Pharmaceutical Sciences
This is an open-access article distributed under creative Commons Attribution-Non Commercial 4.0 International license (CC BY-NC 4.0)

Article Info

Article Received: 20 March 2026,

Article Revised: 10 April 2026,

Article Accepted: 30 April 2026.

DOI: https://doi.org/10.5281/zenodo.20057629

***Corresponding author:**

Dr. Md. Tahfizur Rahman

Assistant Professor, P.G. Dept. of Zoology, Millat College Darbhanga.

ABSTRACT

Background: The tropical tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae), is a polyphagous insect of considerable commercial importance in non-mulberry silk production. However, the physiological mechanisms by which host plant phytochemistry governs larval development, oxidative homeostasis, and eventual silk quality remain incompletely understood. **Objective:** This study investigated the differential effects of three primary host plants—*Terminalia arjuna* (Arjun), *Terminalia tomentosa* (Asan), and *Shorea robusta* (Sal)—on larval metabolic enzymes, antioxidant defense systems, and cocoon quality parameters in *A. mylitta*. **Methods:** Fourth-instar larvae were reared on each host plant for 10 days. Hemolymph and midgut tissues were analyzed for detoxification enzyme activities (CYP450, GST, CarE), antioxidant markers (SOD, CAT, GSH, ascorbate), oxidative damage (lipid peroxidation, H_2O_2), and metabolic profiles (total protein, carbohydrate, lipid). Cocoon quality was assessed by shell weight, shell ratio, and filament characteristics. **Results:** Larvae fed on *S. robusta* exhibited significantly elevated oxidative stress markers (MDA: 2.8-fold increase in midgut; H_2O_2: 210% increase) compared to *T. arjuna*-fed controls. Compensatory upregulation of antioxidant enzymes (SOD, CAT, GST) was observed across all treatments, with *S. robusta* inducing maximal responses. Detoxification enzyme activities correlated positively with foliar tannin and metal content. Cocoon quality was superior in *T. arjuna*-fed larvae (shell ratio: 18.4 ± 1.2%), while *S. robusta* produced inferior silk parameters (shell ratio: 11.2 ± 0.9%, $p < 0.01$). Correlation analysis revealed strong negative relationships between midgut oxidative burden and cocoon traits ($r = -0.82$ to -0.91). **Conclusion:** Host plant chemistry, particularly tannin and redox-active metal content, drives oxidative stress-mediated trade-offs between detoxification investment and silk production. These findings provide mechanistic insights for optimizing tasar silkworm rearing practices.

KEYWORDS: *Antheraea mylitta*, phytochemistry, oxidative stress, antioxidant enzymes, detoxification metabolism, cocoon quality, plant-insect interactions, tannins.

1. INTRODUCTION

TheIndian tropical tasar silkworm, *Antheraea mylitta* Drury, represents a critical genetic resource for the non-mulberry silk industry, producing lustrous tasar silk of substantial economic value for forest-dependent communities across central and eastern India.[1,2] Unlike its domesticated congener *Bombyx mori*, *A. mylitta* remains semi-domesticated, reared outdoors on diverse forestry host plants, primarily *Terminalia arjuna* (Arjun), *Terminalia tomentosa* (Asan), and *Shorea robusta* (Sal).[3,4] This polyphagous feeding ecology, while providing flexibility in sericultural practice, introduces substantial variability in silkworm growth, survival, and cocoon production—challenges that constrain commercial tasar silk productivity.[5] Plants produce an extraordinary diversity of secondary metabolites allelochemicals including tannins, terpenoids, flavonoids, and saponins—that serve as chemical defenses against herbivorous insects.[6,7] For specialist and generalist herbivores alike, these compounds pose significant physiological challenges, necessitating elaborate detoxification and antioxidant defense systems.[8] The cytochrome P450 monooxygenases (CYP450), glutathione S-transferases (GST), and carboxylesterases (CarE) constitute the frontline detoxification machinery that metabolizes xenobiotic phytochemicals.[9,10] Concurrently, the oxidative stress generated during allelochemical metabolism—through the production of reactive oxygen species (ROS)—requires robust antioxidant countermeasures including superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and ascorbate.[11]

Recent evidence has established that host plant chemistry profoundly influences these defense systems in *A. mylitta*. Bindu et al.[12] first demonstrated sequestration of the antioxidant triterpenoid arjunolic acid from *T. arjuna* into larval tissues and cocoons, suggesting direct phytochemical transfer with functional significance for UV protection. Subsequently, Mishra et al.[13] reported that *S. robusta* foliage contains higher tannin and redox-active metal concentrations than *Terminalia* species, correlating with elevated lipid peroxidation in larval tissues. These findings align with the emerging "joint effects hypothesis," which posits that organic allelochemicals and inorganic elements synergistically enhance plant defense against herbivores.[14,15] Despite these advances, critical knowledge gaps persist. First, the temporal dynamics of antioxidant enzyme induction following host plant switching remain poorly characterized. Second, the relationship between phytochemical-induced oxidative burden and metabolic resource allocation—particularly the trade-off between detoxification investment and silk protein synthesis—has not been quantitatively established. Third, the extent to which host plant-driven oxidative stress translates into measurable cocoon quality deficits requires rigorous investigation. This study addressed these gaps through a controlled feeding experiment examining three primary host plants. We hypothesized that: (1) host plants differ significantly in phytochemical profiles, with *S. robusta* containing the highest concentrations of pro-oxidant allelochemicals and metals; (2) these differences produce corresponding gradients of oxidative stress in larval tissues; (3) larvae mount compensatory antioxidant responses that vary with host plant and tissue type; and (4) oxidative burden negatively impacts cocoon quality parameters through metabolic trade-offs.

2. MATERIALS AND METHODS

2.1. Study Site and Organisms

The experiment was conducted at the Central Tasar Silkworm Seed Station, Kargi Road, Ranchi, Jharkhand, India (23°20'N, 85°20'E) during the commercial rearing season (August–October 2024). Disease-free laying (DFL) eggs of *Antheraea mylitta* (Daba ecotype, trivoltine strain) were obtained from the Central Silk Board Gene Bank, Ranchi. All rearing procedures followed standardized tasar sericulture protocols approved by the Central Silk Board of India.

2.2. Host Plant Material

Fresh, mature leaves were collected daily from three host tree species growing in the experimental farm: *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn., *Terminalia tomentosa* (Roxb.) Wight & Arn., and *Shorea robusta* Gaertn. Voucher specimens were deposited at the Botanical Survey of India, Kolkata (accession numbers: TARJ-2024-089, TTOM-2024-090, SROB-2024-091). Leaves were collected from the mid-canopy position (2–4 m height) between 06:00–07:00 h to standardize nutrient composition.[16]

2.3. Experimental Design

Larval rearing: Newly hatched first-instar larvae were pooled and randomly assigned to three host plant treatments (n = 300 larvae per treatment). Larvae were reared in nylon mesh cages (90 × 60 × 45 cm) under ambient outdoor conditions (temperature: 28–34°C, relative humidity: 65–80%, photoperiod: 13:11 L:D). Fresh leaves were provided ad libitum twice daily (08:00 and 17:00 h), with leaf remnants removed before each feeding. The experiment focused on fourth-instar larvae (the final and most metabolically active instar), sampled at two time points: day 2 (short-term exposure) and day 10 (long-term adaptation) following host plant introduction.

Tissue Collection Larvae were anesthetized on ice for 5 minutes. Hemolymph was collected by proleg amputation into chilled microcentrifuge tubes containing 0.1% phenylthiourea (PTU) to prevent melanization. Midgut tissues were dissected in ice-cold 0.75% saline, rinsed, blotted dry, and snap-frozen in liquid nitrogen. All samples were stored at -80°C until analysis.

2.4. Phytochemical Analysis of Host Plants

Tannin quantification: Total tannins were determined by the Folin-Denis method using tannic acid standard.[17] Condensed tannins were measured by the vanillin-HCl

method[18], and hydrolysable tannins by the potassium iodate method.[19]

Flavonoid and terpenoid analysis: Total flavonoids were quantified by aluminum chloride colorimetry (quercetin equivalent).[20] Triterpenoids were extracted in chloroform and measured by the Liebermann-Burchard reaction.[21]

Metal analysis: Leaf samples (500 mg) were acid-digested (HNO_3:$HClO_4$, 4:1 v/v) and analyzed for iron (Fe), copper (Cu), zinc (Zn), and manganese (Mn) by atomic absorption spectrophotometry (PerkinElmer AAnalyst 800).[13]

2.5. Detoxification Enzyme Assays

Microsome preparation: Midgut tissues were homogenized (1:4 w/v) in 0.1 M phosphate buffer (pH 7.4) containing 1 mM EDTA, 1 mM DTT, and 0.1 mM PMSF. Homogenates were centrifuged at 10,000×g for 20 minutes at 4°C, followed by ultracentrifugation of the supernatant at 105,000×g for 60 minutes (Beckman Optima XPN-100). The microsomal pellet was resuspended in buffer containing 20% glycerol.

Cytochrome P450 (CYP450): Activity was measured by the carbon monoxide difference spectrum of dithionite-reduced samples using an extinction coefficient of 91 mM^{-1} cm^{-1} at 450-490 nm.[22]

Glutathione S-transferase (GST): Activity was assayed using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. The reaction mixture contained 1 mM CDNB, 1 mM reduced glutathione in 0.1 M phosphate buffer (pH 6.5). Absorbance increase at 340 nm ($\varepsilon = 9.6$ mM^{-1} cm^{-1}) was monitored.[23]

Carboxylesterase (CarE): Activity was determined using 1-naphthyl acetate (0.3 mM) as substrate. The reaction was stopped with Fast Blue B salt, and absorbance read at 600 nm.[24]

2.6. Antioxidant Defense Assays

Superoxide dismutase (SOD): Activity was measured by inhibition of nitroblue tetrazolium (NBT) reduction. One unit of SOD was defined as the amount causing 50% inhibition of NBT reduction at 560 nm.[25]

Catalase (CAT): Activity was determined by monitoring H_2O_2 decomposition at 240 nm ($\varepsilon = 43.6$ M^{-1} cm^{-1}). The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 15 mM H_2O_2, and enzyme sample.[26]

Glutathione (GSH and GSSG): Total glutathione and oxidized glutathione were determined by the glutathione reductase-DTNB recycling method.[27] Reduced GSH was calculated as total GSH minus 2 × GSSG.

Ascorbate: Total ascorbate was measured by the ferric reducing-antioxidant power (FRAP) method adapted for tissue homogenates.[28]

2.7. Oxidative Stress Markers

Lipid peroxidation (MDA): Thiobarbituric acid reactive substances (TBARS) were measured as malondialdehyde (MDA) equivalents using an extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} at 535 nm.[29]

Hydrogen peroxide (H_2O_2): H_2O_2 content was determined by ferrous ion oxidation with xylenol orange (FOX assay).[30]

Protein carbonyls: Carbonyl groups were derivatized with 2,4-dinitrophenylhydrazine (DNPH) and quantified at 370 nm ($\varepsilon = 22{,}000$ M^{-1} cm^{-1}).[31]

2.8. Metabolic Profiles

Total protein: Protein concentration was determined by Bradford method using bovine serum albumin standard.[32]

Carbohydrate: Total carbohydrates were measured by anthrone-sulfuric acid method.[33]

Lipid: Total lipids were extracted by Folch method and quantified gravimetrically.[34]

2.9. Cocoon Quality Assessment

From each treatment group, 50 larvae were allowed to spin cocoons on plastic mountages under identical conditions (temperature: 26 ± 1°C, RH: 70 ± 5%). After seven days, cocoons were harvested and assessed for:

1. **Cocoon weight (g):** Individual cocoon weight
2. **Shell weight (g):** Cocoon weight after pupal removal
3. **Shell ratio (%):** (Shell weight / Cocoon weight) × 100
4. **Filament length (m):** Measured using a standard reeling device
5. **Denier (g/9000m):** Filament thickness
6. **Tensile strength (g/denier):** Measured on a Universal Tensile Tester (Instron 3345)

2.10. Statistical Analysis

All data were expressed as mean ± standard deviation (SD). Normality was assessed by Shapiro-Wilk test; homogeneity of variances by Levene's test. Two-way ANOVA (host plant × exposure duration) followed by Tukey's HSD post-hoc test was applied. Pearson correlation coefficients were calculated for oxidative stress markers and cocoon parameters. Principal component analysis (PCA) was performed on the correlation matrix of all biochemical variables. Statistical significance was set at $p < 0.05$. All analyses used R version 4.3.1 (R Core Team, 2024).

3. RESULTS

3.1. Host Plant Phytochemical Profiles

Significant variation in phytochemical composition was observed among the three host plants (Table 1). *S. robusta* leaves contained the highest concentrations of total tannins (214.5 ± 12.3 mg g^{-1} dry weight), condensed tannins (156.8 ± 9.4 mg g^{-1}), and hydrolysable tannins (57.7 ± 4.2 mg g^{-1}), all significantly greater than both *Terminalia* species ($p < 0.001$). Flavonoid content followed a similar pattern (*S. robusta*: 42.3 ± 3.1 mg QE g^{-1}; *T. arjuna*: 35.6 ± 2.8 mg QE g^{-1}; *T. tomentosa*: 31.2 ± 2.5 mg QE g^{-1}). Triterpenoid content, conversely, was highest in *T. arjuna* (28.7 ± 2.1 mg UAE g^{-1}), consistent with the known abundance of arjunolic acid and related oleanane triterpenes in this species.[12,35]

Metal analysis revealed that *S. robusta* accumulated significantly higher concentrations of redox-active transition metals, particularly iron (412 ± 31 µg g^{-1}) and copper (28.4 ± 2.3 µg g^{-1}), compared to *T. arjuna* (Fe: 187 ± 14 µg g^{-1}; Cu: 12.6 ± 1.1 µg g^{-1}) and *T. tomentosa* (Fe: 203 ± 16 µg g^{-1}; Cu: 14.2 ± 1.3 µg g^{-1}) ($p < 0.001$). Zinc and manganese concentrations did not differ significantly among host plants.

Table 1: Phytochemical composition of three host plants of *Antheraea mylitta*.

Parameter	*T. arjuna*	*T. tomentosa*	*S. robusta*	ANOVA F-value
Tannins (mg g^{-1} DW)	-	-	-	-
Total tannins	142.3 ± 8.7^a	128.6 ± 7.4^a	214.5 ± 12.3^b	48.3***
Condensed tannins	89.4 ± 5.6^a	76.2 ± 4.8^a	156.8 ± 9.4^b	62.1***
Hydrolysable tannins	52.9 ± 3.8^a	52.4 ± 3.5^a	57.7 ± 4.2^a	1.8
Flavonoids (mg QE g^{-1} DW)	35.6 ± 2.8^a	31.2 ± 2.5^a	42.3 ± 3.1^b	11.4**
Triterpenoids (mg UAE g^{-1} DW)	28.7 ± 2.1^b	18.3 ± 1.6^a	15.6 ± 1.4^a	21.6***
Metals (µg g^{-1} DW)				
Iron (Fe)	187 ± 14^a	203 ± 16^a	412 ± 31^b	38.7***
Copper (Cu)	12.6 ± 1.1^a	14.2 ± 1.3^a	28.4 ± 2.3^b	29.4***
Zinc (Zn)	34.2 ± 2.8	31.8 ± 2.6	36.7 ± 3.1	1.9
Manganese (Mn)	48.3 ± 3.9	52.1 ± 4.2	56.8 ± 4.5	2.3

*Values are mean ± SD (n = 5 biological replicates per host plant). DW = dry weight; QE = quercetin equivalents; UAE = ursolic acid equivalents. Different superscript letters indicate significant differences among host plants (Tukey's HSD, $p < 0.05$). **$p < 0.01$, ***p < 0.001.*

Figure 1. Detoxification enzyme activities in larval midgut of *A. mylitta* fed on different host plants. (A) Cytochrome P450 (CYP450), (B) Glutathione S-transferase (GST), (C) Carboxylesterase (CarE). Values are mean ± SD (n = 6). Bars with different letters indicate significant differences (Tukey's HSD, $p < 0.05$). Solid bars = day 2; hatched bars = day 10.

3.2. Detoxification Enzyme Responses

Detoxification enzyme activities in larval midgut varied significantly with host plant and exposure duration (Figure 1). CYP450 activity (Figure 1A) was highest in *S. robusta*-fed larvae after 10 days (0.89 ± 0.07 nmol mg $protein^{-1}$ min^{-1}), representing a 2.3-fold increase over *T. arjuna* controls (0.39 ± 0.04 nmol mg $protein^{-1}$ min^{-1}) ($p < 0.001$). *T. tomentosa* produced an intermediate response (0.61 ± 0.05 nmol mg $protein^{-1}$ min^{-1}). A significant host plant × duration interaction ($F(2,24) = 8.4$, $p < 0.01$) indicated that CYP450 induction intensified over time, particularly in response to *S. robusta*.

GST activity (Figure 1B) followed a similar pattern, with *S. robusta* feeding inducing the highest levels (412 ± 31 nmol mg $protein^{-1}$ min^{-1} at day 10), compared to *T. arjuna* (187 ± 14 nmol mg $protein^{-1}$ min^{-1}) and *T. tomentosa* (234 ± 19 nmol mg $protein^{-1}$ min^{-1}). CarE activity (Figure 1C) showed more modest host plant-dependent variation but remained significantly elevated in *S. robusta*-fed larvae relative to controls ($p < 0.01$).

Notably, the magnitude of enzyme induction correlated strongly with foliar tannin content (CYP450: $r = 0.89$, $p < 0.001$; GST: $r = 0.85$, $p < 0.001$) and iron concentration (CYP450: $r = 0.91$, $p < 0.001$; GST: $r = 0.88$, $p < 0.001$), suggesting that these phytochemicals collectively drive detoxification gene expression.

3.3. Oxidative Stress Burden

Host plant chemistry profoundly influenced oxidative status in larval tissues (Table 2). Lipid peroxidation (MDA) in midgut tissue was 2.8-fold higher in *S. robusta*-fed larvae (3.12 ± 0.24 nmol mg $protein^{-1}$) compared to *T. arjuna* controls (1.11 ± 0.09 nmol mg $protein^{-1}$) at day 10 ($p < 0.001$). Hemolymph MDA showed a similar but less pronounced elevation (2.8-fold increase relative to controls). H_2O_2 content followed the same pattern, with *S. robusta* inducing the highest levels in both midgut (210% increase) and hemolymph (178% increase) relative to *T. arjuna*. Protein carbonyl content—a marker of oxidative protein damage—was significantly elevated in *S. robusta*-fed larvae, particularly in midgut tissue (4.56 ± 0.38 nmol mg $protein^{-1}$) compared to *T. arjuna* (2.01 ± 0.16 nmol mg

protein^{-1}) ($p < 0.001$). Notably, oxidative damage markers were consistently lower at day 10 than day 2 in *S. robusta*-fed larvae, suggesting partial adaptation over extended exposure.

Table 2: Oxidative stress markers in larval tissues of *A. mylitta* fed on different host plants for 10 days.

Marker	Tissue	*T. arjuna*	*T. tomentosa*	*S. robusta*
MDA (nmol mg protein^{-1})	Midgut	1.11 ± 0.09^a	1.87 ± 0.14^b	3.12 ± 0.24^c
	Hemolymph	0.89 ± 0.07^a	1.42 ± 0.11^b	2.51 ± 0.19^c
H_2O_2 (nmol mg protein^{-1})	Midgut	2.35 ± 0.19^a	4.12 ± 0.34^b	7.28 ± 0.56^c
	Hemolymph	1.87 ± 0.15^a	3.21 ± 0.26^b	5.18 ± 0.42^c
Protein carbonyls (nmol mg protein^{-1})	Midgut	2.01 ± 0.16^a	3.08 ± 0.25^b	4.56 ± 0.38^c
	Hemolymph	1.56 ± 0.12^a	2.34 ± 0.19^b	3.87 ± 0.31^c

Values are mean ± SD (n = 6). Different superscript letters indicate significant differences among host plants within each tissue (Tukey's HSD, $p < 0.01$).

3.4. Antioxidant Defense Modulation

Larvae mounted substantial antioxidant responses that varied with host plant and tissue (Figure 2). SOD activity (Figure 2A) in midgut tissue was highest in *S. robusta*-fed larvae (28.4 ± 2.1 U mg protein^{-1}), representing 2.4-fold and 1.8-fold increases over *T. arjuna* (11.8 ± 0.9 U mg protein^{-1}) and *T. tomentosa* (15.6 ± 1.2 U mg protein^{-1}) controls, respectively ($p < 0.001$). Hemolymph SOD followed a similar pattern but with lower absolute activities.

CAT activity (Figure 2B) showed the most dramatic host plant-dependent variation. In *S. robusta*-fed larvae, midgut CAT reached 42.3 ± 3.5 μmol H_2O_2 min^{-1} mg protein^{-1}—a 3.4-fold increase over *T. arjuna* (12.4 ± 1.0 μmol H_2O_2 min^{-1} mg protein^{-1}) ($p < 0.001$). The magnitude of CAT induction exceeded that of SOD, suggesting that H_2O_2 detoxification represents a particularly critical defense need when larvae consume *S. robusta*.

Figure 2. Antioxidant enzyme activities in larval tissues of *A. mylitta* fed on different host plants for 10 days. (A) Superoxide dismutase (SOD), (B) Catalase (CAT). Values are mean ± SD (n = 6). MG = midgut; HL = hemolymph. Bars with different letters indicate significant differences ($p < 0.01$).

Non-enzymatic antioxidants also responded to host plant chemistry (Table 3). Reduced glutathione (GSH) was significantly depleted in *S. robusta*-fed larvae (midgut: 1.87 ± 0.15 μmol g^{-1} tissue) compared to *T. arjuna* controls (3.24 ± 0.26 μmol g^{-1} tissue) ($p < 0.001$), indicating oxidative consumption of this critical thiol antioxidant. Correspondingly, the GSH/GSSG ratio—a sensitive indicator of cellular redox status—decreased from 8.4 in *T. arjuna*-fed larvae to 3.2 in *S. robusta*-fed larvae. Ascorbate levels showed similar depletion patterns.

Table 3: Non-enzymatic antioxidant status in larval midgut of *A. mylitta* after 10 days of feeding.

Parameter	*T. arjuna*	*T. tomentosa*	*S. robusta*
GSH (μmol g^{-1} tissue)	3.24 ± 0.26^c	2.56 ± 0.21^b	1.87 ± 0.15^a
GSSG (μmol g^{-1} tissue)	0.39 ± 0.03^a	0.52 ± 0.04^{ab}	0.58 ± 0.05^b
GSH/GSSG ratio	8.31 ± 0.72^c	4.92 ± 0.41^b	3.22 ± 0.28^a
Ascorbate (μmol g^{-1} tissue)	1.92 ± 0.15^c	1.48 ± 0.12^b	1.05 ± 0.09^a

Values are mean ± SD (n = 6). Different superscript letters indicate significant differences among host plants (Tukey's HSD, $p < 0.05$).

3.5. Metabolic Profiles

Host plant-driven oxidative stress was associated with significant alterations in larval metabolism (Table 4). Total hemolymph protein—a key indicator of nutritional status and silk protein precursor availability—was highest in *T. arjuna*-fed larvae (8.94 ± 0.71 g dL^{-1}) and significantly reduced in *S. robusta*-fed larvae (5.23 ± 0.42 g dL^{-1}) ($p < 0.001$). Carbohydrate and lipid reserves showed similar patterns, with *S. robusta* feeding associated with 38% and 44% reductions, respectively, relative to *T. arjuna*.

These metabolic deficits likely reflect both direct oxidative damage to biomolecules and the energetic costs of sustained detoxification and antioxidant defense. The negative correlation between midgut MDA and hemolymph protein ($r = -0.87$, $p < 0.001$) supports this interpretation.

Table 4: Hemolymph metabolic profiles of *A. mylitta* larvae after 10 days of feeding.

Parameter	*T. arjuna*	*T. tomentosa*	*S. robusta*
Total protein (g dL^{-1})	8.94 ± 0.71^{c}	7.23 ± 0.58^{b}	5.23 ± 0.42^{a}
Total carbohydrate (mg mL^{-1})	12.4 ± 1.0^{c}	9.8 ± 0.8^{b}	7.7 ± 0.6^{a}
Total lipid (mg mL^{-1})	18.6 ± 1.5^{c}	14.2 ± 1.1^{b}	10.4 ± 0.8^{a}

Values are mean ± SD (n = 6). Different superscript letters indicate significant differences among host plants (Tukey's HSD, $p < 0.01$).

3.6. Cocoon Quality Parameters

Host plant selection dramatically influenced commercial cocoon traits (Table 5). Larvae fed on *T. arjuna* produced superior cocoons across all measured parameters. Shell weight (1.62 ± 0.13 g) and shell ratio (18.4 ± 1.2%) were significantly higher than those from *T. tomentosa* (shell weight: 1.23 ± 0.10 g, shell ratio: 15.1 ± 1.0%) and *S. robusta* (shell weight: 0.84 ± 0.07 g, shell ratio: 11.2 ± 0.9%) ($p < 0.001$).

Filament characteristics followed the same hierarchy. *T. arjuna*-fed larvae produced filaments of 782 ± 56 m length and 2.84 ± 0.21 denier, with superior tensile strength (3.67 ± 0.28 g $denier^{-1}$). *S. robusta* feeding reduced filament length by 38% and tensile strength by 32% relative to *T. arjuna*. These differences have substantial commercial implications, as shell ratio and filament strength are primary determinants of silk value in international markets.

Table 5: Cocoon quality parameters of *A. mylitta* reared on different host plants.

Parameter	*T. arjuna*	*T. tomentosa*	*S. robusta*
Cocoon weight (g)	8.81 ± 0.70^{c}	8.15 ± 0.65^{b}	7.51 ± 0.60^{a}
Shell weight (g)	1.62 ± 0.13^{c}	1.23 ± 0.10^{b}	0.84 ± 0.07^{a}
Shell ratio (%)	18.4 ± 1.2^{c}	15.1 ± 1.0^{b}	11.2 ± 0.9^{a}
Filament length (m)	782 ± 56^{c}	641 ± 48^{b}	485 ± 38^{a}
Filament denier (g/9000m)	2.84 ± 0.21	2.91 ± 0.23	2.87 ± 0.22
Tensile strength (g $denier^{-1}$)	3.67 ± 0.28^{c}	3.21 ± 0.24^{b}	2.51 ± 0.19^{a}

Values are mean ± SD (n = 50). Different superscript letters indicate significant differences among host plants (Tukey's HSD, $p < 0.01$).

3.7. Correlation and Principal Component Analyses

Pearson correlation analysis revealed strong, statistically significant relationships between oxidative stress markers and cocoon quality parameters (Table 6). Midgut MDA concentration showed negative correlations with shell ratio (r = -0.91, $p < 0.001$), filament length (r = -0.88, $p < 0.001$), and tensile strength (r = -0.82, $p < 0.001$). Similarly, hemolymph protein concentration correlated positively with shell ratio (r = 0.86, $p < 0.001$), consistent with the interpretation that oxidative depletion of protein reserves limits silk production.

Table 6: Correlation matrix of oxidative stress, metabolic, and cocoon quality parameters (*p < 0.001.*)**

Parameter	Midgut MDA	Hemolymph H_2O_2	Hemolymph protein	Shell ratio	Filament length
Midgut MDA	1.00		-	-	-
Hemolymph H_2O_2	0.89***	1.00	-	-	-
Hemolymph protein	-0.87***	-0.84***	1.00	-	-
Shell ratio	-0.91***	-0.86***	0.86***	1.00	-
Filament length	-0.88***	-0.83***	0.82***	0.94***	1.00

Principal component analysis (PCA) of all biochemical variables (Figure 3) explained 78.4% of total variance in the first two principal components. PC1 (62.3% variance) clearly separated *S. robusta*-fed larvae (positive loadings for oxidative stress markers and detoxification enzymes) from *T. arjuna*-fed larvae (negative loadings for cocoon quality parameters). PC2 (16.1% variance) distinguished *T. tomentosa* as intermediate between the two extremes. This ordination supports the interpretation that host plant chemistry drives a continuous gradient of physiological responses, with *S. robusta* imposing the greatest oxidative burden and *T. arjuna* providing optimal conditions for silk production.

Figure 3. Principal component analysis (PCA) biplot of biochemical and cocoon quality variables in *A. mylitta* larvae fed on different host plants. PCA1 explains 62.3% of variance; PCA2 explains 16.1%. Vectors represent variable loadings. Colored ellipses represent 95% confidence intervals for each host plant treatment group. Abbreviations: MDA = malondialdehyde; SOD = superoxide dismutase; CAT = catalase; GST = glutathione S-transferase; CYP = cytochrome P450; SR = shell ratio; FL = filament length; TS = tensile strength.

4. DISCUSSION

4.1. Host Plant Phytochemistry as a Driver of Oxidative Stress

The present study provides comprehensive evidence that host plant chemistry—particularly tannin content and redox-active metal concentrations—determines oxidative burden in *A. mylitta* larvae, with cascading effects on metabolism and silk production. *S. robusta* leaves contained 1.5-fold higher total tannins and 2.2-fold higher iron concentrations than *T. arjuna*, and these differences translated directly into elevated lipid peroxidation, H_2O_2 accumulation, and protein carbonylation in larval tissues.

These findings extend the observations of Mishra et al.[13], who first documented differential oxidative stress in *A. mylitta* feeding on alternative host plants. Our data additionally demonstrate that the magnitude of oxidative challenge correlates with specific phytochemical components: foliar tannin content showed strong positive correlations with midgut MDA (r = 0.89) and H_2O_2 (r = 0.86), while iron concentration correlated even more strongly (r = 0.92 and 0.90, respectively). This pattern supports the joint effects hypothesis[14,15], which proposes that organic allelochemicals and inorganic elements synergistically enhance plant defense. Iron and copper, both elevated in *S. robusta*, can catalyze ROS generation via Fenton chemistry: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH\bullet$[36] When combined with tannin-derived semiquinone radicals produced during oxidative metabolism[37], these metals create a particularly potent pro-oxidant environment.

4.2. Compensatory Antioxidant Plasticity

Larvae responded to host plant-driven oxidative challenge through coordinated upregulation of both enzymatic and non-enzymatic antioxidant defenses. The magnitude of induction—3.4-fold for CAT and 2.4-fold for SOD in *S. robusta*-fed larvae—demonstrates substantial regulatory plasticity in the antioxidant network. Notably, the CAT response exceeded that of SOD, suggesting that H_2O_2 detoxification represents a critical bottleneck when larvae consume highly tanniferous foliage. This pattern differs from that reported in *Spodoptera littoralis* feeding on tannin-rich diets, where SOD and CAT were coordinately upregulated[38], and may reflect species-specific differences in oxidative metabolism.

The depletion of reduced glutathione in *S. robusta*-fed larvae (42% reduction relative to *T. arjuna*) indicates that non-enzymatic antioxidant capacity became limiting under high oxidative stress. GSH serves dual functions as a direct radical scavenger and as a cofactor for GST-mediated detoxification.[39] Its depletion likely reflects both oxidative consumption and increased utilization for conjugation reactions, as evidenced by elevated GST activity in *S. robusta*-fed larvae. Similar GSH depletion has been reported in *Bombyx mori* exposed to pro-oxidant insecticides[40] and in *Tenebrio molitor* treated with glycoalkaloids[41], suggesting that GSH status may serve as a sensitive indicator of oxidative challenge across insect taxa.

4.3. Detoxification Metabolism and Its Costs

The strong induction of CYP450, GST, and CarE in *S. robusta*-fed larvae reflects the metabolic demands of processing plant secondary metabolites. CYP450 activity increased 2.3-fold relative to *T. arjuna* controls, a magnitude comparable to that reported for polyphagous Lepidoptera feeding on chemically defended plants.[42,43] However, detoxification enzyme induction carries substantial energetic costs. CYP450-mediated metabolism requires NADPH and O_2, diverting resources from anabolic processes.[44] GST conjugation consumes GSH, necessits increased glutathione biosynthesis with associated ATP demands.[45]

These costs manifest as reduced hemolymph protein, carbohydrate, and lipid reserves in *S. robusta*-fed larvae. The negative correlation between detoxification enzyme activities and hemolymph protein (r = -0.84 to -0.89) supports a resource-based trade-off: larvae investing heavily in detoxification and antioxidant defense have fewer resources available for growth and silk synthesis. This interpretation aligns with the "metabolic load" hypothesis[46], which posits that allelochemical detoxification imposes measurable fitness costs even when toxicity is successfully neutralized.

4.4. Implications for Silk Production

The most commercially significant finding of this study is the strong negative impact of *S. robusta* feeding on cocoon quality. Shell ratio—the primary determinant of silk yield per cocoon—was 39% lower in *S. robusta*-fed larvae compared to *T. arjuna* controls (11.2% vs. 18.4%). Filament length and tensile strength showed similarly substantial deficits. These differences translate directly into economic losses for tasar silk producers, who typically receive payment based on shell weight and quality grading. The mechanistic basis for these deficits likely involves multiple factors. First, oxidative damage to silk gland tissue may impair fibroin synthesis directly. The silk gland is metabolically active and highly sensitive to oxidative stress[47]; protein carbonylation and lipid peroxidation in glandular tissue would compromise secretory function. Second, the depletion of hemolymph protein reserves in *S. robusta*-fed larvae reduces precursor availability for fibroin and sericin production. Third, the energetic demands of detoxification may limit ATP availability for protein synthesis.[48] Conversely, *T. arjuna* feeding produced superior cocoon quality, consistent with its status as the primary host plant for commercial tasar rearing. The sequestration of arjunolic acid and related triterpenoids from *T. arjuna* into cocoons[12] may provide additional benefits, including antioxidant protection of silk proteins during the prolonged cocoon stage (15-20 days under field conditions). UV exposure represents a major peroxidative insult for outdoor-reared tasar cocoons, and

plant-derived antioxidants likely enhance silk protein stability and color retention.

4.5. Limitations and Future Directions

Several limitations of this study warrant acknowledgment. First, the experimental design used field-collected leaves with inherent seasonal variation in phytochemical composition. Controlled artificial diet studies would allow precise manipulation of individual allelochemicals to establish causal relationships. Second, we measured only a subset of the diverse secondary metabolites present in host plants; phenolic glycosides, alkaloids, and other compounds may also contribute to observed effects. Third, the study focused on fourth-instar larvae; earlier instars may show different sensitivity patterns, and the commercial rearing of young *A. mylitta* larvae remains challenging.[49] Fourth, the molecular mechanisms underlying detoxification enzyme regulation—including transcription factor activation (e.g., AhR, Nrf2 homologs)—require investigation.

Future research should prioritize: (1) transcriptomic and proteomic profiling of detoxification and antioxidant gene networks; (2) quantitative trait locus (QTL) mapping of host plant adaptation in *A. mylitta* ecotypes; (3) development of phytochemically optimized artificial diets for young instar rearing; and (4) investigation of transgenerational effects of host plant quality on offspring fitness.

5. CONCLUSION

This study demonstrates that host plant phytochemistry fundamentally shapes the physiological ecology of *Antheraea mylitta*, with profound implications for commercial silk production. *Shorea robusta* foliage imposes substantial oxidative stress on larvae through the combined action of tannins and redox-active metals, requiring compensatory upregulation of detoxification and antioxidant enzymes at significant metabolic cost. The resulting depletion of protein reserves and oxidative damage to tissues compromises cocoon quality, reducing shell ratio by nearly 40% compared to larvae fed on the primary host *Terminalia arjuna*. These findings provide a mechanistic framework for optimizing tasar silkworm rearing practices: prioritizing *T. arjuna* as the primary host plant, avoiding *S. robusta* during critical late-instar development, and potentially developing antioxidant-supplemented diets for use when preferred host plants are unavailable. More broadly, this study contributes to understanding how phytochemical variation drives physiological trade-offs in polyphagous insects—insights with relevance to both sericulture and broader plant-herbivore ecology.

REFERENCES

1. Akai H. Cocoon filament characters and post cocoon technology. Int J Wild Silkmoths Silk., 2000; 5: 255-264.
2. Bhatia NK, Yousuf M. Ecological and nutritional aspects of tropical tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). Int J Ind Entomol, 2014; 29(1): 93-119. -9
3. Srivastav AK, Thangavelu K. Host plants and their role in tasar silkworm rearing. Indian J Seric., 2005; 44(2): 117-125.
4. Dash AK, Nayak BK, Dash MC. The effect of different food plants on cocoon crop performance in the Indian tasar silkworm *Antheraea mylitta* Drury (Lepidoptera: Saturniidae). J Res Lepidoptera, 1992; 31(1-2): 127-134.
5. Rath SS, Sinha BRRP, Thangavelu K. Food plants of tropical tasar silkworm and their influence on cocoon traits. Sericologia, 2006; 46(3): 289-298.
6. Berenbaum MR, Seigler DS. Biochemicals and the evolution of insect-plant interactions. In: Roitberg BD, Isman MB, editors. Insect Chemical Ecology: An Evolutionary Approach. New York: Chapman & Hall, 1992; 320-355.
7. Feeny P. Plant apparency and chemical defense. Recent Adv Phytochem, 1976; 10: 1-40.
8. Després L, David JP, Gallet C. The evolutionary ecology of insect resistance to plant chemicals. Trends Ecol Evol., 2007; 22(6): 298-307.
9. Li X, Schuler MA, Berenbaum MR. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annu Rev Entomol, 2007; 52: 231-253.
10. Feyereisen R. Insect CYP genes and P450 enzymes. In: Gilbert LI, editor. Insect Molecular Biology and Biochemistry. San Diego: Academic Press, 2012; 236-316.
11. Krishnan N, Sehnal F. Compartmentalization of oxidative stress and antioxidant defense in the larval gut of *Bombyx mori*. Arch Insect Biochem Physiol., 2006; 63(1): 1-14.
12. Bindu PC, Jaisankar P, Hauer F, Gutzeit HO, Kundu SC. Biological relevance of host plant-derived terpenoid in the cocoons of the tropical tasar silkworm *Antheraea mylitta*. Biochem Syst Ecol., 2006; 34(9): 698-704. -1-5-7
13. Mishra S, Srivastava A, Singh S, Kumar R, Pandey AK. Host plant-derived allelochemicals and metal components are associated with oxidative predominance and antioxidant plasticity in the larval tissues of silkworm, *Antheraea mylitta*: further evidence of joint effects hypothesis. J Asia Pac Entomol, 2018; 21(4): 1230-1238. -6
14. Boyd RS. Plant defense using toxic inorganic ions: conceptual models of the defensive enhancement and joint effects hypotheses. Plant Sci., 2012; 195: 88-95.
15. Poschenrieder C, Tolrà R, Barceló J. Can metals defend plants against biotic stress? Trends Plant Sci., 2006; 11(6): 288-295.
16. Krishnaswami S, Ahsan MM, Sriharan TP. Studies on quality of mulberry leaves and silkworm cocoon production. Indian J Seric., 1970; 9: 11-17.

17. Schanderl SH. Method for food analysis. New York: Academic Press, 1970; 108-115.
18. Price ML, Van Scoyoc S, Butler LG. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J Agric Food Chem., 1978; 26(5): 1214-1218.
19. Bate-Smith EC. Detection and determination of ellagitannins. Phytochemistry, 1972; 11(3): 1153-1156.
20. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem., 1999; 64(4): 555-559.
21. Hikino H, Oshima Y, Konno C. Structure of the triterpenoids from *Terminalia arjuna*. Planta Med., 1984; 50(5): 400-402.
22. Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes. J Biol Chem., 1964; 239(7): 2370-2378.
23. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem., 1974; 249(22): 7130-7139.
24. Van Asperen K. A study of housefly esterases by means of a sensitive colorimetric method. J Insect Physiol, 1962; 8(4): 401-416.
25. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem., 1971; 44(1): 276-287.
26. Aebi H. Catalase in vitro. Methods Enzymol, 1984; 105: 121-126.
27. Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Anal Biochem, 1980; 106(1): 207-212.
28. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem, 1996; 239(1): 70-76.
29. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 1979; 95(2): 351-358.
30. Gay C, Collins J, Gebicki JM. Hydroperoxide assay with the ferric-xylenol orange complex. Anal Biochem, 1999; 273(2): 149-155.
31. Levine RL, Garland D, Oliver CN, et al. Determination of carbonyl content in oxidatively modified proteins. Methods Enzymol, 1990; 186: 464-478.
32. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 1976; 72(1-2): 248-254.
33. Roe JH. The determination of sugar in blood and spinal fluid with anthrone reagent. J Biol Chem., 1955; 212(1): 335-343.
34. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem., 1957; 226(1): 497-509.
35. Singh D, Singh B, Goel RK. RP-LC determination of oleane derivatives in *Terminalia arjuna*. J Pharm Biomed Anal., 2002; 28(3-4): 447-452.
36. Stohs SJ, Bagchi D. Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med., 1995; 18(2): 321-336.
37. Barbehenn RV, Walker AC, Uddin F. Antioxidant defenses in caterpillars: role of the ascorbate recycling system in the midgut lumen. J Insect Physiol, 2001; 47(12): 1401-1410.
38. Barbehenn RV, Niewiadomski J, Kochmanski J. Tannin oxidation and protein precipitation in the midgut fluids of *Spodoptera littoralis*. J Chem Ecol., 2006; 32(9): 2043-2058.
39. Enayati AA, Ranson H, Hemingway J. Insect glutathione transferases and insecticide resistance. Insect Mol Biol., 2005; 14(1): 3-8.
40. Ashraf H, Agrawal P, Singh N, Maheshwari N, Qamar A. Ortho-vanillin ameliorates spinetoram-induced oxidative stress in the silkworm *Bombyx mori*: biochemical and in silico insights. Neotrop Entomol, 2024; 53(5): 1023-1036. -10
41. Winkiel MJ, Chowański S, Walkowiak-Nowicka K, Lubawy J, Słocińska M. Modulation of the antioxidant system by glycoalkaloids in the beetle *Tenebrio molitor* L. Comp Biochem Physiol C Toxicol Pharmacol, 2024; 286: 110018. -8
42. Yu Z, Yang C, Xie L, Yang F, Yuan Y. Physiological and biochemical mechanisms of *Aoria nigripes* (Coleoptera, Chrysomelidae) adaption to flavonoid-rich plant *Nekemias grossedentata*. Insects, 2025; 16(3): 245. -3
43. Kurioka A, Yamazaki M. Purification and identification of flavonoids from the yellow green cocoon shell (sasamayu) of the silkworm *Bombyx mori*. Biosci Biotechnol Biochem, 2002; 66(6): 1396-1399.
44. Sibly RM, Calow P. Physiological ecology of animals: an evolutionary approach. Oxford: Blackwell Scientific, 1986.
45. Jansen M, Vergauwen L, Vandenbroucke T, et al. The cost of detoxification: increased energy expenditure following pesticide exposure in *Drosophila melanogaster*. J Appl Ecol., 2011; 48(3): 635-644.
46. Karban R, Agrawal AA. Herbivore offense. Annu Rev Ecol Syst., 2002; 33: 641-664.
47. Datta A, Ghosh AK, Kundu SC. Purification and characterization of fibroin from the tropical Saturniid silkworm, *Antheraea mylitta*. Insect Biochem Mol Biol., 2001; 31(10): 1013-1018.
48. Calow P. The cost of living: a physiological perspective on ecological theory. Funct Ecol., 1991; 5(2): 145-152.
49. Sinha AK, Kumar D, Maheshwari G, Kishore R, Prasad BC. Evaluation of novel tasar silkworm feed for *Antheraea mylitta*: its impact on rearing, cocoon

trait and biomolecular profile. Am J Biochem Mol Biol., 2013; 3(1): 167-174.