

Sub-Lethal Agrochemical Stress in *Bombyx mori*: Monovalent Ionic Dyshomeostasis and Somatic Stunting Induced by Pyrethroid and Organophosphate Exposure

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ABSTRACT

The encroachment of synthetic agrochemicals into sericultural zones poses a critical threat to the domesticated silkworm, *Bombyx mori*. This study investigates the physiological and bio-economic impacts of sub-lethal pyrethroid (Lambda-cyhalothrin, Cypermethrin) and organophosphate (Chlorpyrifos) exposure on 5th instar larvae. Cohorts were exposed to concentration thresholds ranging from 0 to 200 ppm. Haemolymph extraction and biometric analyses revealed a severe, dose-dependent metabolic and neuro-ionic collapse. Toxicological stress induced profound monovalent ionic dyshomeostasis, characterized by a rapid depletion of extracellular sodium (Na^+) and a compensatory accumulation of potassium (K^+). Consequently, the neurophysiological K^+/Na^+ ratio escalated catastrophically from a pristine baseline of 2.70 in controls to a critical 13.30 at the 200 ppm threshold, indicating severe voltage-gated sodium channel disruption. This ionic crisis triggered "Metabolic Bankruptcy," forcing larvae to divert vital bioenergetic resources (ATP and amino acids) away from the silk gland to the fat body for xenobiotic detoxification. Phenotypically, this manifested as severe somatic stunting, with larval growth curves flattening abruptly post-Day 3. Furthermore, divalent cations (Ca^{2+} and Mg^{2+}) were depleted by approximately 50%, fundamentally crippling structural protein synthesis. The ultimate bio-economic consequence was a drastic collapse in commercial viability; the Cocoon Shell Ratio (SR%) plummeted from 19.85% to an unviable 8.45%, accompanied by an 85% rate of delayed pupal necrosis and malformation. These findings demonstrate that sub-lethal chemical trespassing destroys the commercial utility of *B. mori*, underscoring the urgent regulatory need for Sericulture-Specific Maximum Residue Limits (MRLs) calibrated to invertebrate neuro-physiological sensitivities.

Keywords: *Bombyx mori*, Lambda-cyhalothrin, Chlorpyrifos, Ionic Dyshomeostasis, Somatic Stunting, Cocoon Shell Ratio (SR%).

INTRODUCTION

For millennia, humans have selectively bred the domesticated silkworm, *Bombyx mori*, stripping away its wild survival mechanisms to create a highly efficient biological bioreactor for silk production. In return,

we provided a protected environment: the pristine mulberry garden. Today, this implicit contract is broken. Modern sericulture exists as fragile ecological "islands" surrounded by intensive agriculture. As adjacent cash crops are aggressively sprayed with synthetic neurotoxins like Chlorpyrifos and Lambda-cyhalothrin pesticide drift has made chemical trespassing a daily reality. The silkworm, having lost its evolutionary instinct to reject tainted food, obligatorily consumes these invisible poisons.

Once ingested, the silkworm's own biology turns against it. Evolved to rapidly digest mulberry leaves, its highly alkaline midgut inadvertently acts as a "physiological sink," rapidly funneling lipophilic pesticide molecules directly into the haemolymph. Because the domesticated silkworm lacks the robust detoxifying genetic diversity of wild agricultural pests, these neurotoxins circulate freely. They lock open voltage-gated sodium channels, triggering a catastrophic internal crisis of monovalent ionic dyshomeostasis.

Even at sub-lethal concentrations, this exposure traps the larva in a prolonged state of physiological torture and "Metabolic Bankruptcy." To survive, the insect must ruthlessly reallocate its internal resources. Massive reserves of ATP and free amino acids normally earmarked for the silk gland to synthesize fibroin are suddenly hijacked and diverted to the fat body to fuel emergency detoxification.

The physical price of this survival is the complete cessation of productive growth. The larvae suffer severe somatic stunting, prolonged instar durations, and the rapid depletion of structural minerals. When these metabolically exhausted insects finally pupate, the resulting cocoons are flimsy, paper-thin, and commercially unviable.

Despite the profound economic devastation this causes to rural sericulturists, global agricultural regulations remain blind to the crisis, relying entirely on human-centric safety standards that fail to protect exquisitely sensitive invertebrates. This study seeks to empirically map this cascade of failure in 5th instar larvae. By quantifying the dose-dependent escalation of the neurophysiological K^+/Na^+ ratio and the resulting collapse of the commercial Cocoon Shell Ratio (SR%), this research establishes the definitive bio-economic cost of chemical trespassing. Ultimately, these findings are intended to advocate for the urgent establishment of Sericulture-Specific Maximum Residue Limits (MRLs).

MATERIALS AND METHODS

1. Silkworm Rearing and Environmental Standardization

Disease-Free Layings (DFLs) of a commercial *Bombyx mori* hybrid were procured from certified grainages to ensure genetic uniformity and pathogen-free baselines. The rearing process was conducted under meticulously standardized laboratory conditions, adhering strictly to established sericultural protocols.

The rearing room was environmentally calibrated to maintain a constant temperature of $25 \pm 1^\circ\text{C}$ and a relative humidity (RH) of $75 \pm 5\%$, ensuring optimal metabolic stability. Larvae were reared in pre-sterilized wooden trays and fed fresh, high-quality mulberry leaves (*Morus alba*) ad libitum, harvested twice daily to prevent nutritional degradation. The experimental timeline was strictly synchronized; all larvae were reared under identical, pristine conditions up to the fourth molt. The bioassay intervention commenced precisely on Day 1 of the 5th instar, the most critical phase for extreme hyperphagia and silk protein synthesis.

2. Agrochemical Selection and Stock Preparation

To accurately simulate the chemical trespassing of modern agricultural drift, three widely utilized commercial insecticides were selected for the bioassay:

- **Chlorpyrifos** (Organophosphate)

- **Cypermethrin** (Type II Synthetic Pyrethroid)
- **Lambda-cyhalothrin** (Type II Synthetic Pyrethroid)

Analytical grade or high-purity commercial formulations of these chemicals were procured. To ensure precise dosing, primary stock solutions were prepared by dissolving the active ingredients in a highly calibrated volume of distilled water. To mimic agricultural field sprays and ensure uniform dispersion of these lipophilic compounds across the waxy cuticle of the mulberry leaves, a non-toxic, non-ionic surfactant (0.1% Tween 80) was added to the diluent.

3. Formulation of Sub-Lethal Dosages

The core objective of this study was to map the physiological collapse rather than acute mortality. Therefore, the stock solutions were subjected to precise serial dilutions to generate a specific gradient of sub-lethal concentrations.

The experimental thresholds were established as follows:

- **Control (0 ppm):** Distilled water + 0.1% Tween 80 only (to rule out surfactant toxicity).
- **Low Stress (2.5 ppm):** Representing peripheral drift or highly degraded field residues.
- **Medium Stress (50 ppm and 100 ppm):** Representing moderate secondary exposure from adjacent field spraying.
- **Maximum Sub-Lethal Threshold (200 ppm):** Representing severe, direct drift just below the acute lethal dose (LD50) for a 5th instar larva.

4. The Leaf-Dip Bioassay and Exposure Protocol

Exposure was administered via a standardized leaf-dip bioassay to ensure that the toxins were ingested exactly as they would be in a contaminated agricultural environment.

Fresh, uniformly sized mulberry leaves were harvested and completely submerged in the respective pesticide dilutions for exactly 30 seconds to ensure a consistent, homogeneous chemical coating. The treated leaves were then air-dried at room temperature in the shade for 30 minutes; shaded drying is a critical methodological step to prevent the premature photolytic degradation (UV breakdown) of the synthetic pyrethroids.

Upon complete drying of the surface moisture, the treated foliage was introduced to the experimental cohorts. Each treatment group (including the control) consisted of 100 newly molted 5th instar larvae, with the experiment performed in biological triplicates (n=300 per concentration). The larvae were allowed to feed on the contaminated leaves obligatorily for a specified window, after which subsequent physiological extraction protocols (haemolymph sampling and weight tracking) were initiated.

5. Haemolymph Extraction and Electrolyte Quantification

• In Vivo Haemolymph Extraction Protocol

To accurately capture the physiological and ionic shifts during the xenobiotic stress phase, haemolymph extraction was strategically timed. Sampling was conducted on Day 3 and Day 5 of the 5th instar, capturing both the initial metabolic shock and the subsequent bioenergetic collapse observed in the 200 ppm high-dose cohorts.

Extracting haemolymph from chemically stressed, physically stunted larvae requires extreme precision to prevent artificial sample degradation. Larvae from each treatment group and the control were randomly selected and starved for two hours prior to extraction to clear the foregut and prevent contamination from regurgitated midgut fluids.

A localized incision was made by carefully snipping the first abdominal proleg using sterilized micro-scissors. The naturally exuding haemolymph was collected directly into pre-chilled 1.5 mL Eppendorf

microcentrifuge tubes. A critical methodological challenge in insect biochemistry is rapid melanization (the blackening of haemolymph upon exposure to air, driven by the enzyme phenoloxidase). To inhibit tyrosinase activity and prevent this cascade, a trace amount of phenylthiourea (PTU) crystals was pre-added to each collection tube.

The collected samples were immediately placed on ice and subsequently centrifuged at 3000 rpm for 10 minutes at 4°C. This centrifugation step precipitated the hemocytes (blood cells) and cellular debris into a pellet, leaving a clear, cell-free supernatant (blood plasma). This purified supernatant was meticulously transferred to fresh vials for immediate ionic analysis, ensuring the biochemical integrity of the sample.

• **Quantification of Monovalent and Divalent Electrolytes**

Because the haemolymph contains high concentrations of diverse proteins that can coagulate and clog analytical instrumentation, the supernatant was first deproteinized. A measured aliquot of the cell-free haemolymph was treated with 10% trichloroacetic acid (TCA) to precipitate the proteins, followed by a second round of centrifugation. The resulting clear, deproteinized extract was diluted with deionized water to fall within the optimal detection range of the spectrophotometric instruments.

1. Monovalent Cations (Na^+ and K^+):

The concentrations of extracellular sodium (Na^+) and potassium (K^+) were quantified using a digital Flame Photometer. Standard calibration curves were prepared using high-purity NaCl and KCl solutions. The flame photometer measured the characteristic emission spectra of these alkali metals. The resulting data, expressed in mEq/L, was specifically utilized to calculate the critical neurophysiological K^+/Na^+ ratio the primary biochemical marker used in this study to determine the extent of voltage-gated sodium channel (VGSC) disruption caused by pyrethroid exposure.

2. Divalent Cations (Ca^{2+} and Mg^{2+}):

Because calcium and magnesium are present in fundamentally different physiological pools and require higher analytical sensitivity, their concentrations were determined using an Atomic Absorption Spectrophotometer (AAS). The deproteinized samples were atomized in an air-acetylene flame, and the specific light absorption for Ca^{2+} and Mg^{2+} was measured against strictly calibrated analytical standards. This highly precise quantification, also expressed in mEq/L, was essential to empirically validate the "Divalent Mineral Atrophy" and its direct correlation to the failure of structural silk protein synthesis observed in the stressed cohorts.

6. Biometric and Commercial Assessment

• **In Vivo Biometric Tracking (Somatic Weight Accumulation)**

To empirically quantify the onset and severity of "Metabolic Bankruptcy," daily somatic weight accumulation was meticulously tracked throughout the 5th instar. Because the physical handling of silkworms can induce mechanical stress and artificially alter feeding behavior, biometric tracking was conducted with extreme care.

A designated subset of thirty larvae ($n=30$) from each replicate group was randomly selected for continuous daily weighing. To prevent handling injuries, these larvae were gently transferred using soft camel-hair brushes onto a high-precision digital analytical microbalance (accurate to 0.001 g). Weights were recorded at the exact same hour each day (prior to the morning feeding) from Day 1 to Day 7 of the instar. This stringent daily tracking generated the data required to map the somatic growth curves, allowing us to pinpoint the exact physiological moment typically post-Day 3 in the high-dose cohorts when xenobiotic detoxification forced a total cessation of productive growth and led to the flattening of the weight trajectory.

• Post-Spinning Commercial Assessment (Cocoon Shell Ratio)

The ultimate objective of this study was to link internal physiological dyshomeostasis to tangible economic loss. Therefore, upon the completion of the spinning phase, a rigorous commercial assessment of the resulting cocoons was conducted.

Cocoons were harvested on the seventh day post-spinning, ensuring that the pupation process inside was complete. Before weighing, the harvested cocoons were phenotypically sorted, and any visible abnormalities such as "flimsy," stained, or malformed structures were documented.

To determine the bio-economic viability of the harvest, we calculated the **Cocoon Shell Ratio (SR%)**. This metric is the absolute gold standard in sericultural economics, as it represents the precise percentage of the cocoon's weight that is composed of commercially reelable silk protein, independent of the heavy pupa residing inside.

The assessment was conducted in three steps:

1. **Whole Cocoon Weight:** Each intact cocoon was individually weighed on the analytical balance to determine its total mass.
2. **Shell Extraction:** The cocoon was then carefully incised using a scalpel. The live pupa and the exuviae (the cast-off larval skin) were gently extracted, leaving only the empty silk shell.
3. **Shell Weight:** The empty cocoon shell was immediately weighed.

The commercial viability was then quantified using the following standard equation:

$$SR(\%) = \left(\frac{\text{Weight of Cocoon Shell (g)}}{\text{Weight of Whole Cocoon (g)}} \right) \times 100$$

By comparing the SR% of the pristine control group against the agrochemical-stressed cohorts, we could mathematically demonstrate the bioenergetic cost of survival. A drastic collapse in the SR% serves as the definitive economic expression of the larva diverting its amino acids and ATP away from the silk gland and toward the fat body for emergency detoxification.

RESULTS FOR THE GROWTH CURVES

1. Probit Log-Dose Response and Comparative Toxicity

To establish the physiological baselines of agrochemical stress and validate the experimental sub-lethal thresholds, acute toxicity was first determined using standard Probit regression analysis. The log-dose response curves generated for the 5th instar larvae revealed stark differences in the toxicological aggression of the three selected insecticides.

The data demonstrated that the synthetic pyrethroids exhibited significantly higher acute toxicity and a more rapid onset of physiological disruption compared to the organophosphate. Lambda-cyhalothrin presented the steepest probit slope and the lowest median lethal dose (LD50), establishing it as the most highly potent neurotoxin among the tested chemicals. Cypermethrin followed closely, exhibiting a similarly aggressive Type II pyrethroid response. In contrast, Chlorpyrifos displayed a comparatively shallower slope and a higher LD50 threshold.

The steepness of the Lambda-cyhalothrin probit curve is a critical toxicological indicator. It signifies that once the buffering capacity of the silkworm's haemolymph is breached, even a marginal increase in the pesticide concentration triggers a rapid, cascading failure of the nervous system. This extreme potency is directly attributed to the highly lipophilic nature of the halogenated pyrethroid structure, which allows the molecule to partition rapidly across the midgut epithelium and directly penetrate the lipid sheaths of the central nervous system. Because the maximum experimental dose of 200 ppm was purposefully calibrated

to sit just below the acute LD50 threshold of Lambda-cyhalothrin, the surviving larvae in these high-dose cohorts were pushed to the absolute limits of their biochemical endurance, setting the stage for the chronic physiological collapse observed in the subsequent metrics.

2. Somatic Growth Atrophy and "Metabolic Bankruptcy"

The daily biometric tracking of the 5th instar larvae provided the first visible, phenotypic evidence of internal toxicological stress. In a healthy *Bombyx mori* larva, the 5th instar is characterized by aggressive hyperphagia, where the insect consumes over 80% of its total lifetime dietary intake to fuel the massive hypertrophy of the silk glands.

The somatic weight accumulation curve of the uncompromised Control (0 ppm) group perfectly modeled this biological imperative. From an initial weight on Day 1, the control larvae exhibited robust, near-exponential daily mass accumulation, peaking at an average maximum weight of approximately 4.85 g by Day 7, just prior to the spinning phase. The low-stress cohorts (2.5 ppm) displayed a marginal lag in early weight gain but were largely able to biochemically compensate, eventually mirroring the trajectory of the control group.

However, the growth curves for the medium (50 ppm and 100 ppm) and high-dose (200 ppm) cohorts revealed a profound and dose-dependent somatic stunting. In the 200 ppm Lambda-cyhalothrin group, the larvae maintained a highly compromised, slow rate of growth during Days 1 and 2. By Day 3, a critical physiological tipping point was reached. The somatic growth curve completely flattened, plateauing at a severely stunted maximum weight of just 2.60 g, representing a near 50% reduction in total body mass compared to the control.

This drastic flattening of the growth curve post-Day 3 empirically validates the "Metabolic Bankruptcy" hypothesis. By the third day of continuous obligatory exposure, the cumulative toxic load in the haemolymph overwhelms the basal defense mechanisms. To prevent immediate lethal neuro-motor paralysis, the larva is forced into a state of systemic emergency. Feeding behavior (hyperphagia) ceases almost entirely. The larva drastically shifts its internal resource allocation, diverting critical metabolic energy (ATP) and amino acid reserves away from the silk gland and somatic tissues. Instead, these resources are aggressively channeled to the fat body to fuel the hyper-activation of Phase I (Cytochrome P450) and Phase II (Glutathione S-transferase) detoxification enzymes, as well as the energy-intensive ion pumps attempting to stabilize the nervous system.

The physical expression of this invisible biochemical war is a complete cessation of productive growth. The larvae survive the acute toxicity, but their somatic development is violently arrested, rendering them structurally and metabolically ill-equipped for the subsequent demands of pupation and silk spinning.

3. Monovalent Ionic Dyshomeostasis: The Collapse of Neural Gradients

The most profound biochemical discovery of this study and the direct molecular link to the observed somatic stunting is the catastrophic disruption of monovalent electrolytes within the silkworm's circulatory fluid. Lepidopteran haemolymph is physiologically unique; to accommodate a highly alkaline, potassium-rich mulberry diet, *Bombyx mori* maintains an "inverted" ionic profile characterized by exceptionally high Potassium (K^+) and relatively low Sodium (Na^+) concentrations. Maintaining this steep, unconventional gradient against the forces of passive diffusion is an energy-intensive process that is absolutely critical for normal neuro-motor function. The spectrophotometric analysis of the haemolymph reveals that sub-lethal pyrethroid exposure completely dismantles this precarious homeostasis.

• The Depletion of Extracellular Sodium (Na^+)

The data demonstrated a severe, dose-dependent depletion of extracellular Na^+ in the haemolymph of the

stressed cohorts. To understand this depletion, one must look at the specific target site of synthetic pyrethroids like Lambda-cyhalothrin. These highly lipophilic neurotoxins partition across the neural sheaths and bind directly to the lipid-exposed cavities of the voltage-gated sodium channels (VGSCs) in their open state. By preventing the inactivation gate from closing, the pyrethroids lock the channels open. This causes a massive, unchecked influx of Na^+ ions *out* of the extracellular fluid (the haemolymph) and *into* the intracellular spaces of the central and peripheral nervous systems. Consequently, the spectrophotometric data captured a rapid draining of the extracellular sodium pool. As the concentration of the agrochemical increased, the measurable sodium in the haemolymph plummeted, signifying that the neural tissues were essentially flooding with Na^+ , obliterating their resting membrane potentials and triggering continuous, uncontrolled nerve discharges.

- **The Compensatory Accumulation of Potassium (K^+)**

Simultaneous to the sodium collapse, the analysis revealed a marked accumulation of Potassium (K^+) in the haemolymph. This accumulation is the biochemical footprint of a failing compensatory mechanism. As the neural cells flood with sodium, they attempt to violently repolarize and restore the resting potential. This triggers the hyper-activation of the Na^+/K^+ -ATPase pumps, which aggressively attempt to extrude the invading sodium while pumping potassium back in.

However, because the voltage-gated channels are mechanically locked open by the pyrethroids, this pumping effort is futile. The continuous depolarization of the neural membrane forces voltage-gated potassium channels to remain perpetually open, causing intracellular K^+ to bleed out into the extracellular space. In an insect that already possesses a naturally high baseline of haemolymph potassium, this unchecked efflux pushes the K^+ concentration to highly toxic levels, severely altering the osmotic pressure and viscosity of the blood plasma.

- **The Escalation of the Neurophysiological K^+/Na^+ Ratio**

The severity of this dual ionic crisis is best quantified by calculating the neurophysiological K^+/Na^+ ratio. This metric serves as the definitive biochemical signature of pyrethroid-induced electrical failure.

In the uncompromised Control (0 ppm) cohorts, the active ionic pumps maintained a stable, bio-inverted baseline ratio of 2.70 pm 0.15. However, as the toxic load increased, this regulatory capacity rapidly deteriorated. At the low-stress threshold of 50 ppm, the ratio escalated to 4.29 pm 0.32. At 100 ppm, as the sodium pools began to heavily deplete, the ratio spiked to 6.65 pm 0.55.

The true extent of the physiological collapse was realized at the maximum sub-lethal threshold of 200 ppm. In these high-dose cohorts, the simultaneous drain of Na^+ and accumulation of K^+ drove the neurophysiological ratio to an unprecedented 13.30 pm 0.85.

An ionic ratio of 13.30 is fundamentally incompatible with normal action potential propagation. It represents a complete electrical "short-circuit" of the insect's nervous system. This extreme ionic shift perfectly correlates with the phenotypic observations in Section 3.2: the exact moment this ratio spikes is the moment the larvae experience tetanic paralysis, mandibular spasms, and a total inability to feed. The escalation to a 13.30 ratio is the molecular trigger that forces the silkworm into Metabolic Bankruptcy, prioritizing cellular survival over commercial silk synthesis.

4. Divalent Mineral Atrophy: The Collapse of Structural Silk Synthesis

While the severe disruption of monovalent ions dictates the electrical and neurological failure of the silkworm, the systemic depletion of divalent cations specifically calcium (Ca^{2+}) and magnesium (Mg^{2+}) serves as the biochemical catalyst for the failure of the silk gland itself. In a healthy *Bombyx mori* larva,

these divalent minerals act as fundamental drivers of structural metabolism. Magnesium is the obligate enzymatic cofactor for cellular energy transfer, while calcium governs critical cellular signaling pathways and the unique rheological properties of liquid silk. Atomic absorption spectrophotometry of the stressed haemolymph revealed a catastrophic, dose-dependent atrophy of these vital mineral pools, directly linking the neurotoxic stress to the mechanical failure of silk protein synthesis.

The quantitative data demonstrated a severe systemic depletion under high-dose pesticide exposure. In the pristine Control (0 ppm) cohorts, the extracellular concentration of magnesium was maintained at a robust 18.20 mEq/L, and calcium at 4.85 mEq/L. However, as the larvae were subjected to escalating concentrations of synthetic pyrethroids and organophosphates, these mineral reservoirs rapidly collapsed. At the maximum sub-lethal threshold of 200 ppm, the circulating ionized magnesium plummeted to 9.30 mEq/L, while calcium dropped to an extreme low of 2.15 mEq/L. This represents an approximate 50% reduction in the total available pool of divalent cations, a state of profound mineral starvation.

This drastic mineral depletion is a secondary pathophysiological consequence of the "Metabolic Bankruptcy" detailed earlier. As the pyrethroid-induced sodium influx triggers extreme osmotic stress, the Malpighian tubules (the insect's primary excretory organs) are forced to aggressively filter the haemolymph to manage water retention and excrete the conjugated pesticide metabolites. During this high-volume diuresis, massive quantities of calcium and magnesium are inadvertently flushed from the circulatory fluid and lost via the frass. Additionally, the extreme oxidative stress generated by the hyper-activated Phase I detoxification enzymes triggers a cellular emergency; intracellular buffers rapidly sequester the remaining free Ca^{2+} from the haemolymph to prevent calcium-induced mitochondrial apoptosis in the fat body.

The consequences of this 50% systemic depletion are utterly devastating to the posterior and middle silk glands. During the 5th instar, the silk gland requires astronomical quantities of adenosine triphosphate (ATP) to fuel the continuous transcription and translation of fibroin and sericin proteins. However, ATP cannot function biologically on its own; it must bind with a magnesium ion to form a bioactive Mg-ATP complex. The 50% collapse in available Mg^{2+} effectively starves the silk gland's enzymatic machinery. Even if the larva possesses residual energy reserves, the lack of magnesium renders the ATP unusable, completely halting the formation of peptide bonds required to build the silk polymer.

Simultaneously, the depletion of calcium destroys the physical properties of the silk filament itself. Within the lumen of the silk gland, the massive fibroin proteins are stored in a highly concentrated, liquid-crystalline state. Calcium ions act as crucial rheological regulators; they bind specifically to the carboxylate groups of the fibroin molecules, preventing premature crystallization and maintaining the correct viscosity. When the larva begins spinning, a highly calibrated exchange of calcium and hydrogen ions triggers the liquid-to-solid phase transition. Because the stressed larvae have exhausted their calcium reserves, this intricate biochemical cross-linking fails. The liquid silk lacks the necessary structural cohesion, resulting in a fractured, discontinuous phase transition. This dual mineral starvation fundamentally cripples the silk gland, ensuring that whatever silk the surviving larva manages to extrude will be structurally defective and commercially useless.

5. Bio-Economic Impact and Phenotypic Sorting: The Final Commercial Collapse

The overarching objective of this study was to demonstrate that molecular damage inside the silkworm does not remain confined to biochemistry; it invariably translates into severe phenotypic defects and catastrophic economic losses for the sericulturist. Section 3.5 presents the final bio-economic data,

proving that the ionic dyshomeostasis and mineral atrophy documented in the previous sections culminate in the total collapse of the commercial cocoon.

- **The Collapse of the Cocoon Shell Ratio (SR%)**

The primary metric of agricultural success in sericulture is the Cocoon Shell Ratio (SR%), which isolates the weight of the harvestable silk protein from the weight of the living pupa. The phenotypic sorting and subsequent biometric analysis of the spun cocoons revealed a staggering economic deterioration that perfectly mirrored the dose-dependent severity of the chemical exposure.

The uncompromised Control (0 ppm) group yielded structurally pristine, highly compact cocoons, achieving a robust and commercially viable baseline SR% of $19.85 \pm 0.65\%$. These cocoons possessed the dense architectural integrity required for high-grade commercial reeling.

However, as the neurotoxic load increased, the commercial viability rapidly disintegrated. In the low-stress cohorts (2.5 ppm to 50 ppm), the SR% began a steady decline, dropping to $15.20 \pm 0.85\%$ as the larvae diverted initial amino acid pools away from the silk gland. The true bio-economic collapse materialized at the medium and high-dose thresholds. In the 100 ppm cohorts, the SR% fell to a marginal $13.25 \pm 1.10\%$, a yield barely sufficient to offset rearing costs.

At the maximum sub-lethal threshold of 200 ppm, the economic failure was absolute. The surviving larvae produced severely malformed, "flimsy," and paper-thin cocoons, resulting in a dismal SR% of just $8.45 \pm 1.35\%$. This represents a nearly 60% destruction of total harvestable silk weight. This catastrophic drop from 19.85% to 8.45% is the ultimate physical manifestation of "Metabolic Bankruptcy." Because the 200 ppm larvae were forced to aggressively consume their own structural amino acids and deplete their divalent minerals ($\text{Ca}^{2+}/\text{Mg}^{2+}$) to survive the neuro-ionic short circuit, they entered the spinning phase entirely bankrupt of the raw materials required to construct a viable shell.

- **Delayed Lethality and High Pupal Mortality**

The economic devastation is compounded by a high rate of delayed ontogenetic lethality. The assumption that a larva that survives pesticide exposure and successfully spins a cocoon represents a "recovery" is physiologically false. Phenotypic sorting of the pupae seven days post-spinning revealed severe internal mortality rates.

In the Control group, the robust larvae exhibited a near-total metamorphic success rate, with 98% developing into healthy, structurally sound pupae. Conversely, the 200 ppm cohorts exhibited massive delayed lethality. Despite surviving the 5th instar and managing to spin a rudimentary, flimsy cocoon, 85% of these stressed populations failed metamorphosis entirely inside the shell.

This failure presented primarily as necrotic pupae (50% mortality rate) and severely deformed "half-pupae" (35% malformation rate) trapped mid-ecdysis. This delayed mortality occurs because the larva completely exhausted its finite fat body reserves to fuel the emergency detoxification process during the larval stage. When it transitioned to the non-feeding pupal stage, it simply lacked the residual ATP necessary to execute the highly energy-intensive physiological tissue reorganization required for metamorphosis. The surviving silkworms do not recover; they merely delay their mortality until they are inside the cocoon.

Ultimately, this data confirms that chemical trespassing in mulberry gardens destroys the bio-economic utility of the silkworm at every level. The farmer is left with a drastically reduced volume of harvestable silk (the 8.45% SR%) and a massive rate of pupal necrosis, effectively wiping out the next generation's reproductive potential and crippling the rural economy.

DISCUSSION

The empirical data presented in this study provides a definitive molecular mapping of how sub-lethal agrochemical exposure fundamentally dismantles the physiological and commercial utility of *Bombyx mori*. While previous toxicological assessments in sericulture have largely focused on acute mortality rates or observational reductions in cocoon weight, this investigation bridges the critical gap between invisible ionic disruptions and the macroscopic phenotypic collapse. By synthesizing the dynamics of monovalent dyshomeostasis, divalent mineral atrophy, and bioenergetic reallocation, we can construct a comprehensive mechanistic model of pyrethroid and organophosphate toxicity in domesticated lepidopterans.

1. Mechanisms of Neuro-Ionic Collapse and the Arrest of Hyperphagia

The most immediate and catastrophic consequence of xenobiotic trespassing in the 5th instar larva is the complete disruption of monovalent electrolyte gradients. The escalation of the neurophysiological K^+/Na^+ ratio from a stable baseline of 2.70 to an unsustainable 13.30 at the 200 ppm threshold is not merely a symptom of toxicity; it is the molecular engine of the larva's physical collapse.

This extreme ionic shift mechanistically confirms the targeted action of synthetic pyrethroids (Lambda-cyhalothrin and Cypermethrin) on the voltage-gated sodium channels (VGSCs) within the insect's nervous system. By holding the activation gates perpetually open, the lipophilic neurotoxins force an influx of extracellular sodium (Na^+) into the neural tissues, rapidly draining the haemolymph sodium pool. The larva's compensatory response hyper-activating Na^+/K^+ ATPase pumps to extrude the invading sodium ultimately fails, leading to the massive leakage and accumulation of potassium (K^+) into the circulating plasma.

This 13.30 ratio effectively acts as an electrical short-circuit, obliterating the resting membrane potentials required for coordinated muscle contraction. Phenotypically, this translates directly into mandibular paralysis and the immediate cessation of feeding. Because the 5th instar is biologically programmed for aggressive hyperphagia to fuel silk gland hypertrophy, this chemically induced starvation serves as the primary physical catalyst for the severe somatic stunting observed in the biometric growth curves post-Day 3.

2. The "Metabolic Bankruptcy" Hypothesis and Mineral Atrophy

The cessation of feeding is only the first phase of the crisis. To survive the circulating neurotoxins, the larva is forced into a state of "Metabolic Bankruptcy." The organism must ruthlessly reallocate its finite internal resources to prioritize immediate survival over ontogenetic development and commercial silk synthesis.

This reallocation explains the profound systemic depletion of divalent cations (Ca^{2+} and Mg^{2+}) and the ultimate collapse of the Cocoon Shell Ratio (SR%). Detoxification is an astronomically expensive bioenergetic process. The hyper-activation of Phase I Cytochrome P450 monooxygenases and Phase II Glutathione S-transferases in the fat body, combined with the desperate overdrive of ionic pumps, completely drains the insect's adenosine triphosphate (ATP) reserves. Because magnesium (Mg^{2+}) is the obligate enzymatic cofactor for ATP, the 50% depletion of the haemolymph magnesium pool effectively cripples any remaining cellular energy transfer mechanisms. The silk gland is thus starved of bioactive Mg-ATP, halting the translation of fibroin proteins.

Simultaneously, the larva cannibalizes its own amino acid reserves. Glycine, the primary structural building block of the silk fibroin polymer, is aggressively redirected to synthesize glutathione for Phase II xenobiotic conjugation and to mitigate extreme oxidative stress (lipid peroxidation). Furthermore, the

50% depletion of calcium (Ca^{2+}) a critical rheological regulator that prevents premature crystallization of liquid silk ensures that the phase transition during spinning fails. The combination of ATP starvation, amino acid hijacking, and calcium depletion mechanistically guarantees the production of the flimsy, paper-thin cocoons observed in the 200 ppm cohorts, driving the SR% down to a commercially unviable 8.45%.

3. Delayed Lethality: The Cost of Metamorphosis

The study also shatters the sericultural assumption that a larva successfully spinning a cocoon equates to a physiological recovery from chemical stress. The 85% post-spinning mortality rate documented in the high-dose cohorts highlights the chronic, inescapable nature of the bioenergetic deficit.

Pupation and metamorphosis are non-feeding stages characterized by massive tissue histolysis and physiological reorganization, demanding immense reserves of energy stored in the fat body. Because the stressed larvae functionally exhausted their fat body reserves to survive the chemical assault during the larval stage, they enter the pupal casing entirely devoid of the ATP required to execute ecdysis. The manifestation of necrotic pupae and deformed "half-pupae" is the ultimate biological expression of this deficit. The insect does not recover from sub-lethal toxicity; it merely delays its systemic failure until the energetic demands of metamorphosis exceed its bankrupt reserves.

4. Ecological and Agricultural Implications

The synthesis of these biochemical and phenotypic failures presents a stark warning regarding the current trajectory of modern agricultural governance. The data definitively proves that the domesticated silkworm functions as an exquisitely sensitive "physiological sink." Its inability to biochemically adapt to synthetic neurotoxins unlike polyphagous agricultural pests renders it fundamentally incompatible with the chemical realities of the Green Revolution.

The catastrophic bio-economic collapse triggered at exposures as low as 50 ppm to 100 ppm demonstrates that peripheral pesticide drift is sufficient to wipe out the profit margins of rural sericulturalists. Relying on generalized, mammalian-centric Maximum Residue Limits (MRLs) provides zero ecological or economic protection for this highly vulnerable invertebrate. To secure the future of sericulture as an engine for rural economic stability and a source of pristine biomaterials for modern medicine, environmental policy must evolve. The establishment of strict, Sericulture-Specific MRLs, calibrated directly to the neuro-ionic thresholds of *Bombyx mori*, is no longer just an entomological recommendation; it is an urgent socio-economic imperative.

CONCLUSION

The findings of this investigation provide a definitive, mechanistic mapping of how sub-lethal agrochemical drift fundamentally dismantles the biological and commercial integrity of the domesticated silkworm, *Bombyx mori*. By tracing the toxicological pathways of pyrethroids and organophosphates from initial cellular contact to ultimate phenotypic failure, this study establishes that chemical trespassing in sericultural zones triggers a catastrophic and inescapable cascade of physiological collapse. The domesticated silkworm, entirely devoid of the robust detoxifying plasticity found in wild agricultural pests, acts as an exquisitely vulnerable physiological sink, absorbing the lethal externalities of the modern Green Revolution.

At the molecular level, this collapse is driven by profound monovalent ionic dyshomeostasis. Sub-lethal exposure to highly lipophilic neurotoxins, particularly Lambda-cyhalothrin, locks open the voltage-gated sodium channels, inducing a massive influx of extracellular sodium and a corresponding leakage of

intracellular potassium. The resulting escalation of the neurophysiological K^+/Na^+ ratio from a pristine baseline of 2.70 to a paralyzing 13.30 at high-dose thresholds functions as a systemic electrical short-circuit. This ionic crisis immediately arrests the critical hyperphagia of the 5th instar larva, initiating a severe bioenergetic deficit.

To survive this neurotoxic assault, the insect is forced into a state of "Metabolic Bankruptcy." The larva ruthlessly reallocates its finite internal resources, hijacking massive reserves of ATP and structural amino acids away from the silk gland and channeling them toward the fat body to fuel emergency Phase I and Phase II detoxification pathways. This metabolic diversion, coupled with a nearly 50% systemic depletion of essential divalent cations (Ca^{2+} and Mg^{2+}), completely starves the silk gland's enzymatic machinery. The physical manifestation of this invisible biochemical war is severe somatic stunting and the utter failure of liquid-to-solid phase transitions during spinning.

Consequently, the molecular damage translates directly into profound bio-economic devastation. The harvestable commercial yield, quantified by the Cocoon Shell Ratio (SR%), plummets from a viable 19.85% to a disastrous 8.45%. Furthermore, the staggering 85% rate of delayed pupal mortality demonstrates that larvae do not recover from sub-lethal exposure; they simply defer their systemic failure until the energetic demands of metamorphosis exceed their bankrupt reserves.

Ultimately, this study exposes the fatal inadequacy of current global agricultural governance. Regulating pesticide application based solely on mammalian toxicology and human food safety standards leaves beneficial, highly sensitive invertebrates entirely unprotected. The environmental and economic survival of sericulture both as a vital socio-economic stabilizer for rural agrarian economies and as a future source of pristine biomaterials for modern medicine demands immediate regulatory evolution. There is an urgent, evidence-based imperative for policymakers to establish strict, Sericulture-Specific Maximum Residue Limits (MRLs). Only by calibrating environmental protections to the specific neuro-ionic vulnerabilities of *Bombyx mori* can we secure the future of this ancient, indispensable biological triad.

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