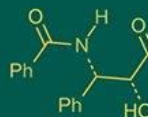


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Toxicological effects of insecticides on *Bombyx mori*: A review and future perspectives

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Abstract

Sericulture, a vital agro-based industry, depends heavily on the health and productivity of the silkworm *Bombyx mori*. However, pesticide drift and direct contamination of mulberry leaves pose a significant threat to silkworm survival, growth, cocoon quality, and silk yield. This review synthesizes findings from multiple studies on the impact of different insecticide groups—including diamides, neonicotinoids, pyrethroids, insect growth regulators (IGRs), organophosphates, carbamates, avermectins, spinosyns, fungicides, and biopesticides on silkworm biology, physiology, and silk productivity. It details how these chemicals induce neurotoxicity, oxidative stress, and impaired silk gland function, leading to reduced larval survival, defective cocoons, and significant yield losses. The review also discusses promising mitigation strategies, such as antioxidant supplementation and the adoption of eco-friendly alternatives. Ultimately, this review underscores the critical need for integrated pest management (IPM) strategies, strengthened pesticide regulation, and enhanced farmer awareness to ensure the long-term sustainability of sericulture.

Keywords: *Bombyx mori*, insecticide, IMP, sericulture

1. Introduction

Sericulture, the practice of raising silkworms for silk production, is a vital component of India's rural economy, providing employment opportunities to millions of livelihoods especially small and marginal farmers. Sericulture produces four types of silk—mulberry, muga, eri, and tasar, with mulberry silk being the most commercially significant, derived from *Bombyx mori*, which feeds exclusively on mulberry leaves (*Morus* spp.) (Annon. 2023) [2]. Currently, China and India are the leading producers of silk, jointly accounting for over 90% of the world's total output. India ranks second after China, contributing approximately 18% to global raw silk production (Roy and Mandal, 2024) [36]. Despite silk comprising less than 0.2% of the global textile market, it sustains livelihoods in over 60 countries, employing approximately 1 million workers in China and 7.9 million in India alone (International Sericulture Commission).

However, sericulture faces a range of mounting challenges that threaten its sustainability. The production of just 1 kilogram of raw silk requires approximately 4,500-6000 silkworm cocoons, highlighting the industry's inherently labor-intensive and resource-demanding nature (Annon. 2025) [3]. In addition to these operational constraints, climate variability and the recurrent incidence of silkworm diseases, such as pebrine, flacherie, grasserie, muscardine etc. poses significant threats to cocoon yield and overall productivity (Sisodia *et al.*, 2019) [45]. These biological and environmental stressors not only disrupt cocoon yield but also undermine the livelihoods of millions who depend on sericulture for income.

Mulberry (*Morus* spp.), the exclusive food source for *Bombyx mori*, is vulnerable to a complex array of pests. More than 300 insect and non-insect species infest mulberry crops, including sap-feeder (mealybugs, thrips, leafhoppers, whiteflies, jassids), defoliators (leaf rollers, hairy caterpillars), and borers (Das *et al.*, 2025) [10]. Among the most destructive insect pests affecting mulberry cultivation are defoliators such as noctuid moths, wasp moths, Bihar hairy caterpillars, cutworms, leaf webbers, moringa hairy caterpillars, tussock caterpillars, and various other lepidopteran species (Sakthivel *et al.*, 2019) [37]. These pests inflict damage primarily through biting and chewing, which significantly reduces the leaf surface area available for photosynthesis.

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The consequent decline in photosynthetic efficiency and leaf yield not only compromises overall plant health but also deteriorates the nutritional quality of mulberry leaves. Since mulberry foliage serves as the sole food source for silkworms, any reduction in leaf biomass or quality directly impairs larval development, ultimately affecting cocoon yield and silk production.

To manage pest infestations efficiently, farmers often rely on chemical insecticides due to their rapid action and ease of application. Insecticide application in mulberry pest management plays a critical role in safeguarding foliage essential for silkworm (*Bombyx mori* L.) nutrition and silk production. Chemical insecticides including organophosphates, carbamates, pyrethroids, and neonicotinoids etc. are commonly used to control pest populations and maintain crop productivity (Ashrith *et al.*, 2024) ^[4]. However, these treatments pose dual risks—contamination of mulberry leaves and direct toxicity to silkworms. Due to their exceptional sensitivity to chemical residues, silkworms often exhibit reduced feeding, slower growth, developmental delays, organ damage, cocoon deformities, and diminished silk quality. Moreover, their vulnerability to pesticide exposure has positioned them as effective bioindicators for assessing environmental contamination and chemical safety (Choudhary *et al.*, 2025) ^[9].

A comprehensive understanding of the physiological and biological effects of various insecticide groups on silkworms is crucial for promoting sustainable sericulture. Beyond immediate toxicity, pesticide exposure affects silk gland function, growth, reproduction, silk quality and even the viability of future generations. This review brings together findings from diverse studies examining the impact of major insecticide classes—including diamides, neonicotinoids, pyrethroids, insect growth regulators (IGRs), organophosphates, carbamates, avermectins, spinosyns, fungicides, and biopesticides on silkworm health and silk productivity. Additionally, mitigation strategies such as antioxidant supplementation are discussed, alongside broader implications for sericulture sustainability and recommendations for integrated pest management (IPM).

2. Effect of different group of insecticides on *Bombyx mori*

In sericulture, pest management is critical to ensuring the health and productivity of *Bombyx mori*, the domesticated silkworm. A wide array of pesticides is employed, broadly categorized into synthetic chemicals, botanicals, biopesticides, and essential oils.

2.1 Diamides

Diamide insecticides, such as chlorantraniliprole and flubendiamide, act by targeting ryanodine receptors and disrupting calcium regulation in insect muscle cells (Li *et al.*, 2019) ^[29]. Exposure to chlorantraniliprole has been shown to inhibit silkworm growth, decreased the body mass and caused the accumulation of reactive oxygen species (ROS). Structural damage to midgut cells, including disintegration, disappearance of microvilli, thinning of the stroma, and chromatin aggregation in nuclei has also been observed (Hu *et al.*, 2019) ^[19]. In *Bombyx mori* larvae, chlorantraniliprole exposure results in high mortality, feeding cessation, regurgitation, incomplete ecdysis, and the

formation of thin-shelled cocoons, which are unsuitable for silk reeling (Munhoz *et al.*, 2013) ^[33]. Research also highlights the oxidative stress, abnormal gland development, and suppressed immune defenses as further consequences of exposure of chlorantraniliprole (El Sherif & Soliman, 2024) ^[12]. These effects collectively decrease cocoon yield and silk quality. Long-term implications also include reduced reproductive fitness and impaired growth in subsequent generations, suggesting that pesticide residues can have intergenerational impacts (Jyothi *et al.*, 2019) ^[23].

2.2 Neonicotinoids

Neonicotinoid insecticides, including imidacloprid, thiamethoxam, clothianidin etc. act on nicotinic acetylcholine receptors (nAChRs), binding irreversibly and causing overstimulation that leads to paralysis. Unlike organophosphates, they do not directly inhibit acetylcholinesterase but instead disrupt cholinergic signaling pathways, particularly in insects with high receptor density (Houchat *et al.*, 2020) ^[21]. These insecticides are widely applied in an agricultural context and are especially effective with regard to sap-feeding insects, such as aphids, whiteflies, and planthoppers, as well as insects that chew on plants (Bas and Field, 2018) ^[6]. In sericulture, silkworm crop loss due to non-spinning of cocoons has been attributed to pesticide toxicity, with air-drift identified as a major route of contamination (Lokanath *et al.*, 2025) ^[28]. Neonicotinoids are highly water-soluble and persist in soil and plant tissues, making them prone to leaching into mulberry leaves via irrigation or drift from neighbouring crop fields (Bonmatin *et al.*, 2015) ^[7]. Even minimal exposure to neonicotinoid insecticides such as Actara and Confidor can cause acute toxicity in mulberry silkworms, as demonstrated by Avramova *et al.* (2012) ^[5]. Neonicotinoids were initially considered safe for beneficial insects, but later found to impair pollinators like bees through contaminated nectar and pollen. Low-level exposure to neonicotinoids may not cause immediate mortality in beneficial insects, but it impairs their normal physiological and behavioural functions (Shareefdeen & Elkamel, 2024) ^[42]. Sublethal exposure significantly prolonged larval development, decreased body weight, lowered pupation rates, and reduced cocoon quality (Chen *et al.*, 2023) ^[8]. Physiological effects included altered detoxification enzyme activity, oxidative stress, and DNA damage in hemocytes (Lokanath *et al.*, 2025) ^[28]. Sithara and Sadatulla (2024) ^[46] recorded that acetamiprid adversely affected the rearing performance of silkworm with residual toxicity symptoms like vomiting gut juice, rectal protrusion, stiffening of larval body and twisting of larval body in agony, hook shaped larvae with shrunken body, death of larvae without moulting, incomplete moulting and death of ripened larvae without spinning. Importantly, neonicotinoids do not always kill outright but disrupt normal metabolism and immune responses, leading to chronic sublethal stress that undermines silk productivity over time (Chen *et al.*, 2023) ^[8]. This raises concern for regions where neonicotinoids are widely used in crops adjacent to mulberry plantations.

2.3 Pyrethroids

Pyrethroids, a common group of synthetic insecticides, are extensively used in agriculture due to their broad-spectrum efficacy and relatively low mammalian toxicity. These compounds act by modifying the gating kinetics of voltage-

gated sodium channels in nerve cells, resulting in prolonged depolarization (Hodosan *et al.*, 2023) ^[20]. This neurotoxic mechanism leads to hyperexcitation, tremors, paralysis, and ultimately death in target insects. However, pyrethroid insecticides frequently reach mulberry plantations through drift, inadvertently exposing non-target organisms such as *Bombyx mori* (Kumar *et al.*, 2017) ^[27]. In silkworms, exposure to pyrethroids like fenvalerate induces differential gene expression in the midgut, with over 1,500 genes affected in sensitive strains. These transcriptomic changes disrupt biological processes, cellular components, and molecular functions, indicating systemic toxicity (Xin *et al.*, 2023) ^[49]. Histopathological investigations further reveal severe damage to critical tissues including the midgut, silk glands, and gonads, with alterations attributed to oxidative stress, enzyme inhibition, and cellular necrosis (Sindhu *et al.*, 2025) ^[16]. Pyrethroids also inhibit acetylcholinesterase (AChE) activity, a key enzyme involved in neural transmission, leading to neurophysiological symptoms such as flaccid body, rectal protrusion, chain faeces, body shrinkage, partial or complete non-spinning, and increased larval mortality (Lokanath *et al.*, 2025) ^[28]. Toxicity studies have shown that even low concentrations of pyrethroids often in the parts-per-billion range can interfere with silkworm development across all life stages, compromising cocoon quality and yield.

2.4 Insect Growth Regulators (IGRs)

Insect growth regulators (IGRs), such as novaluron, diflubenzuron, and buprofezin, are widely used in pest management due to their targeted interference with insect development. These compounds disrupt chitin synthesis and moulting processes, rendering them highly effective against a broad spectrum of agricultural pests (Shewy *et al.*, 2024) ^[13]. However, in *Bombyx mori*, exposure to novaluron has been shown to cause cytotoxic damage across the anterior, middle, and posterior regions of the silk glands, leading to reduced protein secretion and defective cocoon formation (Santorum *et al.*, 2020) ^[40]. Supporting this, Sandhya *et al.* (2024) ^[47] reported that both chlorfenapyr and novaluron exhibited high toxicity toward silkworms and recommended that these compounds be strictly avoided near sericulture zones. IGRs like fenoxycarb, a juvenile hormone analogue, can prolong the fifth instar larval stage, resulting in increased feeding time and heavier silk glands. Application of 1 µg fenoxycarb significantly enhanced cocoon, pupal, and shell weights, thereby improving silk yield, although higher doses may negatively impact cocooning rates (Sisodia and Gaherwal, 2017) ^[44]. Additionally, a study involving growth regulators such as Serimore, Sampoorna, and folic acid revealed varied outcomes—Serimore (3.5 ml/L) and folic acid (200 ppm) promoted larval growth and cocoon quality, while Sampoorna (7.5 ml/L) shortened larval duration and reduced food intake, with adverse effects when applied prematurely (Yashodhara *et al.*, 2024) ^[50]. These findings underscore the importance of precise dosage and timing when using IGRs to ensure beneficial outcomes in sericulture without compromising silkworm health or productivity.

2.5 Organophosphates and Carbamates

Organophosphate and carbamate pesticides exert their toxic effects on *Bombyx mori* primarily by inhibiting acetylcholinesterase (AChE), a critical enzyme for neural

function (Kaleem Ullah *et al.*, 2023) ^[24]. This inhibition leads to the accumulation of acetylcholine at synaptic junctions, triggering neurotoxic symptoms such as vomiting, tremors, body shrinkage, rectal protrusion, and ultimately mortality in silkworms (Lokanath *et al.*, 2025) ^[28]. Sublethal exposure has also been linked to non-spinning syndrome, where larvae fail to spin cocoons, severely compromising silk production. Sandhya *et al.* (2024) ^[47] reported that while pesticides like chlorfenapyr and novaluron were highly toxic, even after recommended waiting periods carbofuran (a carbamate) and dimethoate (an organophosphate) were comparatively safer, with silkworms fed on treated mulberry leaves showing improved larval growth, cocoon weight, and biochemical stability. Lokanath *et al.*, 2025 ^[28] further validated AChE inhibition as a reliable biomarker for pesticide toxicity, using spectrophotometric analysis to demonstrate significant enzyme suppression in silkworm heads exposed to various compounds. Sithara *et al.* (2024) ^[47] recorded the residual effects of Acetamiprid and Acephate caused significant reduction in cocoon weight, shell weight, pupal weight, filament length, increased incidence of malformed cocoons, lowered silk quality metrics such as denier and filament strength.

2.6 Avermectins

Avermectins, including abamectin and emamectin benzoate, are widely used insecticides in agricultural pest control that act by targeting glutamate-gated chloride channels, increasing chloride ion influx and ultimately causing paralysis in insects (Salman *et al.*, 2022) ^[38]. While effective against pests, these compounds have been shown to exert significant molecular and physiological effects on *Bombyx mori*, the domesticated silkworm. Recent genomic studies revealed that exposure to avermectin-treated mulberry leaves alters chromatin architecture and gene expression in the midgut tissues of silkworms. Notably, silkworms exhibited a more relaxed chromatin configuration and intensified short-range DNA interactions, particularly in detoxification-related genes such as those encoding ABC transporters (Guo *et al.*, 2025) ^[18]. These epigenetic changes were accompanied by elevated expression of stress response and metabolic genes, suggesting a systemic adaptation to chemical stress. Complementary research by Shen *et al.* (2011) ^[43] demonstrated that even low doses of avermectin induced DNA damage in silkworm hemocytes, with a dose-dependent increase in tail DNA percentage observed through single-cell gel electrophoresis. Furthermore, 15 differentially expressed genes were identified, many of which were associated with DNA repair and cellular stress pathways. Kumar and Naika (2019) ^[26] reported that emamectin benzoate is highly toxic to *Bombyx mori*, causing 100% mortality even 30 days after spraying on mulberry plants and make it unsuitable for use in sericulture. Collectively, these findings underscore the genotoxic and epigenetic risks posed by avermectins to non-target organisms like *Bombyx mori*, emphasizing the importance of cautious pesticide application and residue management in sericulture zones to safeguard silkworm health and silk productivity. El-Ashram (2022) ^[11] investigated the impact of emamectin benzoate and lemon oil on *Bombyx mori*, revealing that emamectin benzoate significantly prolonged the duration of the 5th instar larval stage. At higher concentrations, it proved lethal—causing larval death either before cocoon formation or within the cocoon itself, ultimately preventing pupation.

2.7 Spinosyns

Spinosyns, particularly spinosad, are bio-insecticides derived from *Saccharopolyspora spinosa* are widely used in agriculture due to their selective toxicity and favourable environmental profile. Despite their targeted action, spinosad has been shown to exert significant adverse effects on non-target organisms such as *Bombyx mori*, the domesticated silkworm. It acts on the insect nervous system by binding to nicotinic acetylcholine receptors, causing hyperexcitation, which leads to symptoms like convulsions, reduced feeding, paralysis, and ultimately high larval mortality (Santos and Pereira, 2020) ^[41]. A study conducted by Soliman and Sherif (2019) ^[48] demonstrated that Spinosad (Tracer), when applied to mulberry trees, adversely affects the productivity of *Bombyx mori*, leading to increased larval mortality and diminished cocoon quality. However, its impact was comparatively less severe than that of Cymbush, a synthetic pyrethroid. In a recent study, Mohamed *et al.* (2023) ^[32] assessed the impact of spinosad on fifth instar larvae of *Bombyx mori* through mulberry leaf dipping bioassays and reported a striking mortality rate of 88.9% at just 0.1 ppm, indicating acute toxicity. Molecular analysis further revealed altered expression of the acetylcholinesterase (Ace) gene along with several apoptosis-related genes, suggesting that spinosad not only impairs neural function but also triggers cellular stress and programmed cell death.

2.8 Fungicides and Other Chemicals

The application of fungicides in sericulture has raised concerns due to their potential impact on the health and productivity of the silkworm, *Bombyx mori*. A study by Abdallah and Abdelmegeed (2021) ^[1] investigated the effects of the fungicide Actamyl on silkworm larvae infected with *Aspergillus* species and found that Actamyl at 6% concentration significantly reduced larval mortality and improved survival rates compared to untreated controls. Carbendazim, generally used to control muscardine and powdery mildew of mulberry, showed no significant effect on larval or pupal mortality of *Bombyx mori* at 1-2 g/L concentrations, but caused up to 37% reduction in larval weight and slight declines in economic traits, with no

concentration-dependent impact (Etebari and Bizhannia, 2005) ^[14]. In contrast, Manjunatha *et al.*, (2017) ^[31] found that carbendazim 50% WP and wettable sulphur 80% WP at 0.1% concentration caused zero larval mortality and significantly enhanced larval growth, cocoon yield, and silk quality evidenced by increased filament length, denier, and cocoon parameters. Supporting these findings, Fayaz *et al.* (2024) ^[15] also observed no larval mortality in silkworms treated with varying concentrations of carbendazim and Dithane M-45, reinforcing the safety of these fungicides when used appropriately in sericulture.

2.9 Biopesticides and Botanicals

Botanicals and biopesticides are increasingly recognized as eco-friendly alternatives to synthetic pesticides in sericulture, offering pest control with minimal harm to *Bombyx mori* (Manda *et al.*, 2020) ^[30]. Neem-based products containing azadirachtin have been shown to effectively reduce pest infestation while causing negligible adverse effects on silkworm health when used at recommended concentrations. However, excessive doses may lead to reduced feeding and delayed moulting, indicating the importance of dosage control (Gaurav and Hassan, 2023) ^[17]. Essential oils such as garlic and clove oil exhibit mild deterrent effects but are significantly less toxic than conventional chemical pesticides (Nuryanti and Budiarti, 2022) ^[35]. Microbial formulations like *Bacillus thuringiensis* also present a safe option for pest management, as they target specific pests without affecting silkworm physiology (Zou *et al.*, 2024) ^[51]. Moreover, botanical extracts such as *Psoralea corylifolia* and *Phyllanthus niruri* have demonstrated antiviral properties against grasserie disease, improving cocoon traits and filament length (Kumar *et al.*, 2022) ^[25]. Supporting these findings, Naik and Naik (2015) ^[34] reported that silkworms fed with extracts of *Azadirachta indica*, *Ocimum sanctum*, and *Parthenium hysterophorus* maintained normal tissue structure, whereas those fed on tukra-infected leaves exhibited mild cellular degeneration. Collectively, these studies highlight the potential of botanicals and biopesticides to improve silkworm health and silk quality when applied judiciously in pest management strategies.

Table 1: Comparative analysis table on mode of action of different insecticides on *Bombyx mori*

Insecticide group	Mode of action	Key effect on silkworm	Impact on Cocoon/Silk Yield
Diamides	Target ryanodine receptors, disrupt Ca ²⁺ regulation	High mortality, feeding cessation, thin cocoons, oxidative stress	Reduced cocoon weight and poor quality
Neonicotinoids	Bind to nicotinic acetylcholine receptors	Prolonged larval duration, oxidative stress, DNA damage	Reduced pupation, cocoon weight and silk quality
Pyrethroids	Prolong sodium channel opening	Tremors, vomiting, paralysis, high mortality	Lower cocoon yield, prolonged development
Insect Growth Regulators	Disrupt chitin synthesis, moulting	Silk gland cytotoxicity, non-spinning syndrome	Defective cocoons, reduced thread quality
Organophosphates and carbamates	Inhibit acetylcholinesterase	Vomiting, body shrinkage, nervous system failure	Partial/non-spinning, cocoon loss
Avermectins	Stimulate glutamate-gated chloride channels	Paralysis, feeding inhibition, mortality	Reduced cocoon weight, incomplete spinning
Spinosyns	Activate nicotinic acetylcholine receptors	Hyperexcitation, convulsions, feeding reduction	Defective cocoons, short silk filaments
Fungicides	Varied biochemical interference	Physiological stress, feeding impairment	Cocoon deformities and yield reduction
Biopesticides and botanicals	Natural compounds disrupt growth and feeding	Mild deterrence, delayed moulting at high doses	Minimal impact at recommended levels

3. Preventive Measures and Mitigation Strategies

- **Antioxidants:** Supplementation with ascorbic acid reduces oxidative damage and improves silk productivity under pesticide stress (El Sherif and Soliman, 2024) ^[12]. Other antioxidants, such as glutathione and vitamin E, have shown potential in protecting silkworms against pesticide-induced oxidative stress.
- **Detection Methods:** Recent advances include acetylcholinesterase-based paper-strip assays for rapid pesticide detection on mulberry leaves, enabling early intervention (Lokanath *et al.*, 2025) ^[28]. Such field-ready technologies empower farmers to monitor pesticide contamination before feeding leaves to silkworms.
- **Regulatory Measures:** Restricting pesticide use near mulberry plantations and promoting integrated pest management (IPM) practices are essential to safeguard sericulture (Munhoz *et al.*, 2013) ^[33]. Awareness programs and training for farmers can reduce indiscriminate pesticide use, while promoting alternatives such as biological control of mulberry pests.
- **Research and Breeding:** Developing silkworm strains with enhanced tolerance to environmental stressors, including pesticide residues, could provide long-term resilience for the sericulture industry. Moreover, continuous monitoring of pesticide drift and its correlation with cocoon yield losses should inform regional agricultural policies.

4. Future Perspectives

The integration of biopesticides and safer pest management practices into large-scale sericulture offers a sustainable pathway forward. Future programs should focus on incorporating neem-based products, microbial formulations, and other botanicals into Integrated Pest Management (IPM) strategies tailored for mulberry cultivation. Research on optimal dosage, compatibility with silkworm biology, and cost-effectiveness will be critical. Additionally, farmer education and policy support are needed to encourage widespread adoption of eco-friendly alternatives and to reduce dependence on hazardous synthetic insecticides.

5. Conclusion

Different classes of insecticides adversely affect silkworms through various mechanisms, including neurotoxicity, disruption of calcium signaling, oxidative stress, impaired silk gland function, and interference with moulting. These effects translate into reduced larval survival, defective cocoons, and significant losses in silk yield. Mitigation strategies, including the application of antioxidants and adoption of eco-friendly pest management practices, are crucial for sustainable sericulture. Strengthening pesticide regulation and farmer awareness programs is necessary to minimize pesticide-induced damage to silkworms. Future research should also focus on intergenerational impacts, genetic resilience, and environmentally safe pesticide alternatives that balance agricultural productivity with sericulture sustainability.

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