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Next Gen Silk: Transcending Traditional Sericulture with Nanotechnological breakthroughs

Sowmiya Arumugam[1], Ravi Madhaiyan[1]*, Priyadharshini Pachiappan[2], Anand Ganesan[1], Kalaiselvi Periyasamy[1], Jegathambal Ramasamy[1]

[1]ICAR- Krishi Vigyan Kendra, Tamil Nadu Agricultural University, Sandhiyur - 636 203, Salem, Tamil Nadu, India
[2]Department of Sericulture, Forest College and Research Institute, Mettupalayam 641 301, Coimbatore, Tamil Nadu Agricultural University, Tamil Nadu, India

ABSTRACT

This comprehensive review provides an in-depth exploration of sericulture, delving into the biology of silkworms, the various species involved, and the cultivation techniques of mulberry plants, which are crucial for silk production. It underscores the transformative impact of nanotechnology in modernizing traditional sericulture by enhancing mulberry propagation, optimizing feeding efficiency, and promoting silkworm growth, development, and survival. The use of nanomaterials and nanofertilizers emerges as a promising strategy, significantly boosting mulberry leaf yield, aiding in tissue repair within silkworms, and improving cocoon characteristics, resulting in higher-quality silk fibers. Additionally, this study highlights the therapeutic benefits of nanomaterials in combating silkworm diseases, demonstrating their potential to strengthen immune responses and increase survival rates among infected populations. It also emphasizes the importance of carefully regulating nanomaterial dosages to prevent oxidative stress and potential cellular damage in silkworms. By championing green nanotechnology's sustainable and eco-friendly methods, the review illustrates its potential to reshape sericulture into a more environmentally responsible and economically feasible industry. This study addresses the critical challenge of leveraging nanotechnology's potential in sericulture by tackling its toxicity, environmental, and economic limitations. Lastly, the study calls for ongoing research to better understand the ecological effects of nanotechnology, aiming to enhance its advantages while minimizing any negative impact on the ecosystem and silk production methods.

Keywords: *Silkworm, Mulberry, Nanotechnology, Nanoparticles, Silk, Sericulture, Insect.*

Introduction

Sericulture is a science and an art, which mainly involves the cultivation of silkworms specifically for silk production. This practice, deeply rooted in agriculture, is a renowned cottage industry centered on rearing silkworms to produce raw silk (fine yarn), derived from cocoons spun by select insect species. The core activities of sericulture encompass cultivating food plants to nourish the silkworms and extracting silk filaments from their cocoons for further processing and weaving. India boasts a rich and intricate heritage in silk production, with its trade dating back to the 15th century. Today, sericulture provides employment opportunities to nearly 9 million individuals in rural and semi-urban areas across the country, highlighting its significance as a commercially viable and economically rewarding industry. Currently, it thrives primarily as a cottage and small-scale enterprise –[1].

Sericulture is divided into two primary sectors: mulberry and non-mulberry. Mulberry sericulture centers around the cultivation of silk from mulberries, while non-mulberry sericulture focuses on producing silk from species like eri, tasar, and muga. The silkworm, *Bombyx mori* L., is a monophagous insect that exclusively feeds on mulberry leaves due to the presence of the chemical compound "Morin" [2].

Corresponding Author: **Ravi Madhaiyan*

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Mulberry plants (*Morus sp.*) are essential for the nourishment and development of silkworms, directly influencing silk yield and productivity. The leaves of mulberry serve as the perfect food source, supplying a range of nutrients necessary for the physiological processes of *Bombyx mori* L., supporting its growth and promoting high-quality silk production. The nutritional content of mulberry leaves is crucial, as it significantly impacts the larvae's development, the quality of the cocoons, and ultimately the raw silk yield [3]. Notably, about 70% of the silk protein generated by mulberry silkworms derives from the high-quality protein present in mulberry leaves, which is essential for successful cocoon formation [4]. The quality of the cocoons depends largely on the nutritional intake of the silkworms, with adequate nutrition being key to their commercial traits [5]. Essential trace elements like iron, nickel, copper, manganese, potassium, zinc, and iodine are critical for the optimal growth and development of silkworms, playing a pivotal role in enhancing their performance in sericulture [3].

Nanotechnology, a relatively novel scientific discipline, has revolutionized agriculture by introducing nanomaterial-based formulations for managing pests and diseases [6]. Nanoparticles are ultrafine units characterized by their dimensions in the nanometer scale (1 nm = 10^{-9} m). These materials possess unique optical, electronic, and photocatalytic properties, making them valuable across diverse scientific fields, including agriculture, cosmetics, food, medicine, and pharmaceuticals [7-9]. The adoption of nanomaterials and nanotechnology-driven approaches is accelerating rapidly in nearly every scientific and technological domain.

While nanomaterials have made significant positive impacts, there are ongoing concerns regarding their safety, particularly in relation to their effects on the environment, human health, and experimental models. Research indicates that some nanomaterials may induce toxicity in the tissues and organs of *Bombyx mori*, the silkworm, while others have demonstrated therapeutic benefits, highlighting the dual nature of their impact.

Implementing sustainable agricultural practices that boost crop productivity without over-relying on fertilizers is crucial. In this context, nanomaterials have emerged as a promising alternative to traditional chemical or mineral fertilizers for enhancing plant nutrition. The application of nanomaterials in sericulture is relatively recent, making it essential to explore their potential impacts on silkworms and silk production, particularly in enhancing fecundity, survival rates, pest control, and disease management. This review highlights the significant influence of nanomaterials on the growth and development of silkworm larvae, as well as their role in improving silk quality. It also delves into the effects of nanomaterials on the tissues and organs of *Bombyx mori*, examining how these advanced materials can drive improvements in cocoon productivity and overall resistance to diseases in silkworms. Moreover, the review offers a comprehensive analysis of the impact of nanoparticles on the growth of mulberry plants and silkworms, presenting relevant data to illustrate their effects on larval development. Additionally, it synthesizes existing reports on the biological interactions between nanomaterials and silkworms, emphasizing how these innovations could revolutionize sericulture. The study also explores future opportunities within the fascinating field of nanotechnology in sericulture, paving the way for a more efficient and sustainable silk industry.

Dynamics of Sericulture

Sericulture, the cultivation of silkworms for the production of raw silk, is both an art and a scientific endeavor. This practice, which originated as an ancient innovation in China ––[10, 11], involves several key components, including the cultivation of mulberry plants, the development of silkworms, and the processes of collecting, reeling, and weaving silk [12, 13]. With a short gestation period of approximately 40 days, sericulture is resource-efficient, demands low investment, and offers high returns, making it an appealing agro-based industry that employs around 30 million individuals across more than 25 countries [14]. Its simplicity and minimal educational requirements render sericulture a powerful tool for alleviating poverty in rural regions '[15, 16].

Globally, sericulture is practiced extensively in countries such as China, India, Korea, Thailand, Vietnam, Brazil, and Bulgaria. India, the second-largest producer of silk after China, considers sericulture an essential part of rural life and a significant driver of economic development. Despite being an emerging economy, India's agricultural sector contributes only 8% to the global agricultural GDP while supporting 18% of the world's population. Sericulture provides a stable income source, particularly during lean agricultural seasons, thus enhancing economic stability for rural farmers and women [17]. With a domestic demand for silk at 25,000 metric tons and a production level of 18,475 metric tons, India bridges this gap through imports, primarily from China.

The economic potential of sericulture often exceeds that of many other cash crops, thanks to its favorable benefit-cost ratio. However, the industry faces challenges such as declining productivity, outdated technologies, insufficient state support, and infrastructural shortcomings '[18]. Addressing these issues is vital for revitalizing sericulture and enhancing its sustainability and profitability. In addition to producing silk fibers, sericulture generates valuable by-products such as moths, pupae, sericin, silk waste, and silkworm excreta. These by-products hold commercial value and find applications in various sectors, including cosmetics, pharmaceuticals, food products, animal feed, and ecological restoration efforts [19-22].

Legacy of Silk

Sericulture has played a significant role in shaping global cultures and economies for millennia. This practice originated in ancient China, where it was meticulously protected for nearly 3,000 years before gradually spreading to other regions [23]. Evidence suggests that sericulture was also practiced in the Indus Valley, known for its advanced textile craftsmanship. The expansion of the Silk Road around 139 BCE marked a pivotal moment in the history of silk, enabling cultural and economic exchanges across Asia, Europe, and North Africa, ultimately breaking China's monopoly on silk production. The Silk Road facilitated the connection of diverse civilizations, promoting the exchange of technologies and ideas that contributed to the establishment of silk industries around the world. This extensive trade network played a vital role in shaping global cultures and economies by linking distant areas and fostering innovations in textile manufacturing [23].

As silk production spread throughout Southeast Asia, techniques were refined, leading to the complete domestication of *Bombyx mori*, the silkworm species. This process rendered *Bombyx mori* entirely dependent on human care, stripping it of its ability to survive in the wild. Consequently, it became the only insect species that relies solely on humans for its survival and reproduction. Today, the global sericulture industry produces over 400 megatonnes of silk each year, encompassing both small-scale artisanal practices and large-scale industrial operations [24]. Recognized by UNESCO as an Intangible Cultural Heritage, sericulture involves essential activities such as mulberry cultivation, silkworm rearing, and silk harvesting, all of which emphasize sustainable and ethical practices to minimize environmental impacts. Even after 5,000 years, silk production remains a vital industry that continues to influence cultures, economies, and innovations around the globe.

Decoding Silkworms

Art of Domestication

Sericulture primarily depends on the domesticated silkworm, *Bombyx mori*, although other moths from the Saturniidae family, such as *Antheraea*, are responsible for producing wild silk varieties. *Bombyx mori*, the domesticated silkworm moth, is vital for silk production, primarily feeding on mulberry leaves that supply essential nutrients such as carbohydrates, proteins, lipids, and minerals [21, 25]. This species originated from its wild ancestor, *Bombyx mandarina*, in China approximately 4,600 years ago and can still interbreed with it [26]. Both species belong to the family Bombycidae and are related to the wild silk moths of the Saturniidae family, which includes notable species like the atlas and luna moths [27]. Environmental conditions play a crucial role in the health and silk output of these insects. For optimal growth and silk quality, *Bombyx mori* thrives within a temperature range of 25-28°C and at a relative humidity of 60-75%. In contrast, unfavorable conditions can diminish cocoon quality and disrupt spinning behavior and the properties of sericin [28]. Additionally, nutrient-rich mulberry leaves enhance disease resistance and productivity in silkworms [29].

Strain Variability

Silkworm strains have been selectively bred for desirable traits, including silk quality and disease resistance [30, 31]. These strains vary in their annual generation cycles: univoltine strains produce one generation per year, bivoltine strains yield two, and multivoltine strains can generate up to six [17]. Univoltine and bivoltine strains are adapted to seasonal climates and possess diapause mechanisms in their eggs, resulting in slower development but yielding higher-quality, white silk [32]. In contrast, multivoltine strains, prevalent in tropical regions, mature rapidly, exhibit disease resistance, and produce yellowish silk, albeit in lesser quantities [32, 33]. Hybrid varieties combine characteristics of both bivoltine and multivoltine strains, thereby improving silk quality and enhancing disease resistance [17].

Silkworm Life Stages

The life cycle of *Bombyx mori* spans approximately 6 to 8 weeks and is significantly affected by temperature. In warmer climates, the eggs typically hatch around 10 days after being laid, and the newly hatched larvae exclusively consume mulberry leaves. This life cycle undergoes complete metamorphosis, transitioning from the larval stage to adulthood, with the adult stage primarily dedicated to feeding and reproduction. *Bombyx mori* development consists of four distinct stages: egg, larva, pupa, and adult moth. Diapause, a phase of suspended development, occurs during the egg stage under cooler temperatures and shorter daylight conditions [34]. Eggs in diapause can be preserved at -2.5 °C for up to two years, allowing for long-term storage [35].

The larval stage lasts between 24 to 33 days, during which the caterpillar undergoes four molts while intensively feeding on mulberry leaves. The subsequent pupal stage is marked by significant metamorphosis, where old larval tissues undergo programmed cell death, and new adult organs develop from imaginal discs [36]. During this phase, the pupa remains immobile, concentrating energy on internal reorganization [37]. In certain climates, this pupal phase can be prolonged to allow for overwintering through diapause, enabling the silkworms to survive until favorable conditions return [38].

Following this, the silkworm spins a protective cocoon using a single silk thread that can reach lengths of up to a mile over approximately 48 hours. The adult moth emerges after one to two weeks, with a lifespan of 7 to 14 days. To ensure the highest quality silk, cocoons are typically harvested before the moths emerge, as the enzyme secretion during their escape can damage the silk threads. Once harvested, the cocoons undergo treatment through boiling or steaming to unwind the silk thread in a process known as reeling, or they may be sun-dried in warmer climates. The pupae are often repurposed as food, animal feed, or compost after harvesting.

Mulberry as a driving force

The mulberry cultivation is integral to sericulture, as its leaves are the sole food source for the economically significant silkworm, *Bombyx mori*, renowned for its silk production. The nutritional content of mulberry leaves directly affects the quality of the cocoons and, consequently, the silk produced. Ensuring that these leaves are abundant in essential trace elements such as iron, nickel, copper, manganese, potassium, zinc, and iodine is crucial for optimizing silkworm growth and cocoon quality. These nutrients, along with vitamins found in the leaves, are essential to meet the dietary needs of silkworms, although their concentrations can fluctuate based on ecological conditions, fertilizer usage, and the specific mulberry variety cultivated. The ability of silkworms to convert leaf proteins into animal proteins in the form of silk fibers highlights the direct influence of leaf quality on cocoon development. Therefore, producing high-quality mulberry leaves is vital for achieving superior silk yields.

Research shows that cultivating one metric ton of mulberry leaves can yield approximately 25 to 30 kilograms of cocoons, emphasizing the importance of efficient and innovative mulberry cultivation techniques in sericulture [39]. Furthermore, intercropping mulberry with nitrogen-fixing plants such as alfalfa has proven highly advantageous. This practice not only enhances mulberry productivity but also improves soil health by increasing bacterial diversity and nitrogen availability, fostering a more sustainable agricultural ecosystem. In addition to its crucial role in silk production, mulberry trees provide additional agricultural benefits, yielding fruits, leaves, and wood with various applications. The domestication of mulberry has evolved alongside that of the silkworm, resulting in numerous cultivars developed for improved fruit and leaf quality. Currently, there are 10–16 recognized species of *Morus*, which include both temperate and tropical varieties distributed across nearly every continent [40-42]. This extensive genetic diversity not only enhances mulberry breeding programs but also contributes to the sustainability and resilience of silk production systems, ensuring the continuity of sericulture practices worldwide.

Unveiling Silk

Silk, primarily produced by *Bombyx mori*, is a natural fiber renowned for its strength, durability, and distinct tactile qualities, earning it the title of the "Queen of Textiles" [43]. With China and India leading in production, this fiber has a rich history in luxury textiles and is increasingly utilized in various industrial applications. The structural protein fibroin, which constitutes the core of the fiber, is enveloped by the adhesive protein sericin. This unique arrangement not only enhances strength but also provides elasticity through an organized beta-sheet structure [44].

Core Elements of Silk

Silk is a natural protein fiber produced by silkworms to construct their cocoons. The primary components of silk are two proteins: fibroin, which constitutes 65-85% of the fiber, and sericin, comprising 15-35%, alongside trace amounts of waxes, salts, and pigments [45]. Fibroin serves as the core of the silk fiber, contributing significantly to its strength, while sericin functions as an adhesive that binds the fibroin filaments together. These properties render silk a natural, biodegradable, and biocompatible material, leading to its increasing use in biomedical applications [46].

Fibroin (Core Protein)

Fibroin acts as the structural foundation of silk, whereas sericin is a gelatinous protein synthesized by *Bombyx mori* (silkworms), facilitating the bonding of two fibroin filaments within a silk fiber [47]. Silk fibroin is a hydrophobic protein primarily composed of glycine, alanine, and serine, responsible for the tensile strength and flexibility of silk —[48].

The fibroin structure consists of heavy (390-400 kDa) and light (25-26 kDa) chains linked by disulfide bonds, resulting in a stable configuration [49]. Its biodegradability is particularly advantageous for biomedical applications, as fibroin can be degraded by proteolytic enzymes in various environmental and biological conditions [50].

Sericin (Adhesive Protein)

Sericin is a globular protein that surrounds fibroin, enhancing the silk's adhesive properties and overall structural integrity. Composed of 18 amino acids, sericin prominently features glycine, serine, aspartic acid, and threonine. It possesses a high concentration of polar amino acids, such as serine, glutamic acid, and aspartic acid, allowing for interactions with other polymers, thereby broadening its utility in various sectors [51]. Although sericin is less biocompatible than fibroin, its hydrophilic nature and crucial role in maintaining silk fiber cohesion make it valuable for numerous industrial and biomedical applications.

Commercial Silk Varieties

Mulberry Silk

The most extensively produced silk worldwide, mulberry silk is derived from the *Bombyx mori* silkworm, which exclusively consumes mulberry leaves [52]. This silk is highly prized for its lustrous texture, durability, and excellent dye absorption, making it a fundamental material in the textile industry for crafting high-quality fabrics such as clothing, accessories, and bedding [53].

Eri Silk

Eri silk, often referred to as Ahimsa silk, is produced by the *Samia ricini* silkworm, which feeds on castor leaves. Unlike other silk varieties, harvesting eri silk does not involve killing the pupae, rendering it a more ethical option [54]. Eri silk features a warm, wool-like texture, making it ideal for winter garments, and is commonly used in scarves, shawls, and traditional attire.

Tasar Silk

Tasar silk is sourced from various *Antheraea* species in India and has a coarser texture than mulberry silk, lacking its distinctive shine [55]. This silk is often utilized for sarees, home furnishings, and blended textile products, appreciated for its rustic charm and durability [56]. Likewise, temperate tasar silk is mainly produced in China and Japan. It can be harvested from the cocoons of the *Antheraea pernyi* moth, which feeds on oak, cherry, and chestnut trees [57]. This silk is known for its thicker texture and natural golden-copper color, making it a preferred choice for a variety of textile applications [58].

Muga Silk

Hailing from Assam in northeast India, Muga silk is celebrated for its inherent golden-yellow hue, which requires no dyeing [59]. It is produced by the *Antheraea assamensis* silkworm, which primarily feeds on *Litsaea polyantha* and *Machilus bombycina* trees. Muga silk is employed in creating traditional Assamese clothing and luxury textile items due to its strength and luster [60].

The cultivation and processing of these silk varieties not only yield high-quality silk but also carry substantial cultural and economic significance within their respective regions, contributing to the diversity and sustainability of the textile industry.

The ongoing development of advanced sericulture techniques and hybrid strains continues to evolve the production of these silk types, aligning with global market demands while preserving traditional practices.

Unleashing Nanotechnology

Nanotechnology is a rapidly advancing field that offers innovative methods to enhance growth and production within the nutrition sector, promising transformative changes in agriculture. Often regarded as a new scientific revolution, nanotechnology addresses various challenges in agriculture, including plant protection, nutritional efficiency, and pesticide resistance [61-63]. In sericulture, the focus is not only on producing high-quality silk but also on generating biomass, silk proteins, and animal feed. The implementation of nanotechnology in sericulture significantly boosts the survival rates of silkworms, accelerates their growth and development, and enhances the overall quality of silk fibers.

Spotlight on Nanoparticles

Nanotechnology is advancing rapidly, creating new opportunities to enhance nutrition and revolutionize agriculture. It has garnered significant attention, focusing on the use of nanoparticles, which are tiny particles ranging from 1 to 100 nanometers in size, composed of materials like carbon, metals, metal oxides, or organic compounds [64]. Nanoparticles are generally described as particulate matter with at least one dimension under 100 nm, aligning them with ultrafine particles (airborne particulates) and positioning them as a subset of colloidal particles [65]. The International Organization for Standardization (ISO) defined a nanoparticle in 2008 as a distinct nano-object with all three Cartesian dimensions under 100 nm. Later, in 2011, the European Commission characterized nanoparticles as natural, incidental, or manufactured materials containing particles that are unbound, aggregated, or agglomerated, with 50% or more of the particles having at least one external dimension between 1 nm and 100 nm [66].

Nanoparticles are valued for their small size, low energy requirements, and minimal need for renewable inputs, all while maintaining an eco-friendly profile that avoids harm to the environment and human health [67]. Common components of nanoparticles include silica, iron, zinc oxide, titanium dioxide, cerium oxide, aluminum oxide, gold nanorods, and various core-shell structures like ZnCdSe/ZnS and Mn/ZnSe quantum dots. The effectiveness of nano-fertilizers in promoting plant growth is significantly influenced by factors such as particle size, composition, concentration, and chemical properties, with nutrient release being triggered when these nano-fertilizers interact with soil water [68]. Nanoparticles can be broadly classified into inorganic nanomaterials, such as metal oxides like AgO, MgO, ZnO, and TiO_2, and organic nanomaterials, including lipids, polymers, and carbon nanotubes (CNTs). Polymeric nanoparticles, such as chitosan, are seen as promising carriers for agrochemicals due to their biodegradable nature, agricultural safety, and ability to interact with negatively charged molecules or polymers [62]. Chitosan's cationic properties and its capability to bind with other molecules make it an ideal agent in the agricultural domain.

Nanoparticles possess unique physicochemical characteristics like high surface area, optical activity, mechanical strength, and chemical reactivity, making them highly versatile for various applications [69-72]. Different types of nanomaterials, including those made of copper, zinc, titanium, magnesium, gold, and silver, have shown significant antimicrobial activity against viruses, bacteria, and other microorganisms. These nanomaterials demonstrate antiviral, antibacterial, and antifungal properties, effectively combating pathogen-related diseases [73, 74]. Specifically, silver and magnesium nanoparticles have proven antibacterial and antiviral effects on both the silkworm and its host plant. Nanoparticles exhibit distinctive physical, chemical, and biological attributes compared to their larger-scale counterparts. This uniqueness is due to their high surface area-to-volume ratio, increased reactivity or stability in chemical reactions, enhanced mechanical strength, and other remarkable features. Given the novel use of nanomaterials in sericulture, understanding and leveraging their impact on mulberry silkworms and silk production is essential to maximize their potential in boosting silk quality and yield.

Cellular Uptake of Nanoparticles

The nutrient uptake process in plants involves a sequence of steps starting from the soil to the root surface, followed by the transport of ions through the membranes of root cells, their radial movement into the root xylem vessels, and their eventual translocation via the xylem to distribute ions to the aerial parts of the plant [75]. Recent research has concentrated on evaluating the cumulative nutrient uptake over time, as well as the specific uptake associated with individual roots and their development. From the soil, nanoparticles can enter plant tissues through two primary pathways: the apoplast or symplast. Apoplastic transport occurs via extracellular spaces, cell walls of neighboring cells, and xylem vessels, remaining outside the plasma membrane [76]. In contrast, symplastic transport involves the movement through plasmodesmata, specialized structures that link the cytoplasm of adjacent cells [77].

Within plant tissues, the radial movement of nanoparticles predominantly follows the apoplastic pathway, aiding their progress toward the root's central cylinder and vascular tissues [78-80]. Upon reaching the central cylinder, these nanoparticles are then transported upward to the aerial parts of the plant via the xylem [78, 81, 82]. When nanoparticles are applied foliarly, they must cross the plant's cuticular barrier, which can occur through either a lipophilic or hydrophilic pathway [83]. In the lipophilic pathway, diffusion takes place through cuticular waxes, while in the hydrophilic pathway, dispersion occurs through polar aqueous pores in the cuticle or the stomata –[84, 85]. These intricate processes of nanoparticle movement underscore the complexity of their interaction with plant tissues and their eventual integration into plant physiology.

Nano-fertilizers for Sustainable Farming

Mulberry (*Morus* spp.), a high-biomass-producing, fast-growing perennial woody plant, belongs to the family Moraceae. The quality of mulberry leaves is significantly affected by factors like planting density, irrigation, nitrogen application, seasonal changes, and exogenous supplementation through mulberry leaves [86]. Fertilizers play a crucial role in agricultural systems, and in the context of mulberry cultivation, approximately 40-50 metric tons of foliage are harvested annually per hectare. Mulberry plants can quickly rejuvenate this foliage by absorbing soil nutrients, which leads to nutrient depletion over time. To ensure sustainable production of high-quality mulberry leaves, it is essential to replenish these nutrients with the Recommended Dose of Fertilizer (RDF).

Effective nutrient management is vital for optimal root establishment, vigorous growth, and leaf production [87]. Micronutrients such as iron, copper, zinc, and manganese are required in trace amounts but are critical for the balanced growth and quality of mulberry leaves [88]. In cases of multi-micronutrient deficiencies, mulberry yields can decrease by up to 50%. To mitigate these deficiencies, chemical and chelated fertilizers are often applied, despite their higher cost and typical use on high-value crops [89]. Interestingly, nanoparticles synthesized from plant extracts have shown promise in enhancing seed germination, degrading pesticide residues, and improving soil quality [90, 91].

Nanotechnological advancements have introduced innovative approaches to enhance crop productivity and improve pesticide efficiency, addressing global agricultural challenges [92]. These techniques focus on increasing the effectiveness of both macro and micronutrients in plants. When compared to conventional fertilizers, various nanomaterials have demonstrated superior performance in agricultural applications. Maximizing nutrient-use efficiency is crucial for maintaining resource sustainability and ensuring long-term agricultural productivity. Nanoparticles, generally defined as particles with at least one dimension below 100 nm, offer an eco-friendly alternative to conventional fertilizers, significantly reducing environmental pollution [93]. Nano-fertilizers, developed through nanotechnology, represent an advanced version of traditional fertilizers. They are created by fortifying nutrients, either individually or in combination, onto nanoscale adsorbents. These fertilizers deliver nutrients to crops through one of three mechanisms: encapsulating the nutrients within nanomaterials like nanotubes or nanoporous materials, coating them with a thin protective polymer film, or providing them as nanoscale particles or emulsions to enhance agricultural output [94]. These findings underscore the transformative potential of nano-fertilizers in modern agriculture, paving the way for more sustainable and productive farming practices.

Advancing Mulberry Cultivation with Nanotechnology

Nano-particles have emerged as a promising solution for efficient nutrient management in agriculture, offering an eco-friendly alternative that helps minimize environmental pollution [93]. Due to their nanoscale size and enhanced surface area, these particles exhibit a greater ability to penetrate plant tissues when applied as a foliar spray, showing improved efficacy over traditional chemical micronutrient sprays [95]. The unique properties of nano-sized micronutrients facilitate better absorption and utilization by plants, leading to more effective nutrient delivery and uptake.

Impact of Nano-Fertilizers on Mulberry Soil Quality

Rohela et al. [96] discussed the latest advancements in bio-nanotechnology, emphasizing the development and applications of various nanomaterials. They highlighted that nano fertilizers have the potential to significantly enhance nutrient use efficiency while reducing the environmental impact caused by the excessive use of conventional fertilizers in agriculture. Their study also demonstrated that nano fertilizers release nitrogen at a controlled rate, continuing to supply nutrients evenly even on the 60th day, unlike traditional fertilizers, which tend to release large amounts initially and then taper off irregularly around the 30th day.

Impact of Nano-Fertilizers on Mulberry Growth and Yield

The application of iron oxide nanoparticles and EDTA-functionalized iron oxide nanoparticles to mulberry, through both soil and foliar methods, has been found to enhance overall growth metrics. Haydar et al. [97] demonstrated that soil application of iron oxide nanoparticles at a concentration of 10 mg/kg significantly increased the sprouting percentage (up to 82%), number of leaves (a 52.73% increase over control), plant biomass (with shoot and root biomass showing a 37.20% and 90.24% rise, respectively), and root length (by 34%), while also reducing the time to the first leaf appearance in *Morus* species.

Further, Nithya et al. [98] observed that the foliar application of nano zinc oxide at 20 ppm in the V-1 mulberry variety significantly enhanced growth parameters, including shoot height (96.63 cm), branch number per plant (8.47), leaf count per shoot (18.60), leaf number per plant (157.15), leaf area (96.90 cm^2), and leaf yield (0.46 kg per plant). They concluded that nano zinc fertilizer treatment was more cost-effective, offering a higher benefit-to-cost ratio (2.93) and greater net returns per hectare compared to traditional $ZnSO_4$ fertilizer. These studies collectively highlight the substantial impact of nanofertilizers on boosting growth and yield parameters in mulberry cultivation.

Similarly, Pooja et al. [99] reported improvements in growth parameters, such as shoot height, number of branches per plant, number of leaves per plant, and total leaf area, in mulberry plants treated with foliar application of nano nitrogen fertilizer. Their study found that a foliar spray of 0.4% nano nitrogen fertilizer on the 25th day after pruning, combined with soil application of 50% nitrogen and 100% phosphorus and potassium along with FYM, led to significant enhancements in shoot height (54.42 cm), branch count per plant (21.53), leaf count per plant (274.70), and total leaf area (25,733.54 cm^2/plant). This treatment also improved leaf yield (704.64 g and 928.53 g per plant on 45 and 60 days after pruning, respectively) and elevated quality parameters like carbohydrate content (22.79%), crude protein (28.44%), crude fiber (11.80%), chlorophyll 'a' (1.58 mg/g), chlorophyll 'b' (0.98 mg/g), and total chlorophyll (2.56 mg/g), along with better elemental composition in the V-1 mulberry variety.

Impact of Nano-Fertilizers on Mulberry Nutrient Profiles

Applying nano nitrogen fertilizer as a foliar treatment on mulberry plants has been shown to substantially enhance leaf yield and elevate key quality attributes, such as total carbohydrate levels, crude protein, fiber content, chlorophyll concentration, and essential leaf nutrients. According to Das, and Mandal [100], nano-silver solutions act as effective preservatives that boost both enzymatic and non-enzymatic antioxidant activities, thereby mitigating the adverse effects of reactive oxygen species (ROS). By preventing ROS formation, these solutions help maintain chlorophyll stability and extend leaf longevity. Furthermore, they reported that total chlorophyll, protein, sugar, reducing sugar, proline, phenol, ascorbic acid, carotenoid, and flavonoid contents were significantly higher after 7 days of leaf preservation when treated with biosynthesized silver nanoparticles. These findings collectively suggest that the use of nanomaterials can significantly enhance both the nutritional value and shelf life of mulberry leaves.

Advancing Silkworm Rearing with Nanotechnology

Impact of Nano Particles on Larval Feeding Efficiency

The feeding efficiency of silkworm larvae plays a crucial role in their growth and development. Research indicates that low concentrations of nanoparticles (NPs) can enhance both larval body growth and feeding efficiency. For instance, silkworms fed with TiO_2 NPs at concentrations of 5 or 10 mg/L exhibited improved ingestion and digestibility of mulberry leaves, which led to significant increases in body weight gain. Specifically, silkworms consuming mulberry leaves treated with 10 mg/L TiO_2 nanoparticles showed the highest feed efficiency compared to other treatments, demonstrating that TiO_2 NP treatments can enhance feed efficiency in *Bombyx mori* at lower concentrations [101]. Moreover, feeding silkworms low concentrations of TiO_2 NPs significantly increased the amount of ingested food (g/larvae), the amount digested (g/larvae), and the percentage of food consumed (%) [101, 102].

Furthermore, exposure to biosynthesized silver nanoparticles (AgNPs) also led to improvements in body weight, shell weight, and feeding efficiency in *Bombyx mori* larvae, pupae, and cocoons [103]. In this study, MR2 mulberry leaves were dipped in a 25% silver nanoparticle solution at varying dilutions (25%, 50%, 75%, and 100%), dried, and then fed to fifth instar larvae of the CSR2 × CSR4 breed. The results indicated that silkworms fed leaves treated with silver nanoparticles at a 25% concentration had significantly higher food consumption (52.76 g), food utilization (49.48 g), approximate digestibility (90.41%), consumption index (43.58%), and coefficient of food utilization (91.29%) (Mathivanan, 2011).

Additionally, riboflavin NPs have been found to enhance feed efficacy in mulberry leaves, resulting in higher production and metabolism rates in silkworms [104]. When newly emerged larvae were fed mulberry leaves treated with riboflavin nanoparticles at concentrations of 20, 40, and 60 μg/mL, significant improvements were observed in consumption rate (190.923 mg/day), assimilation rate (157.201 mg/day), production rate (70.107 mg/day), metabolism rate (87.093 mg/day), and approximate digestibility (82.337%) when the concentration was 60 μg/mL. The supplementation of riboflavin nanoparticles enhances the absorption of nutrients across gut tissue, optimizing nutrient utilization rather than allowing food to pass through the digestive system unabsorbed [104].

Collectively, the existing literature highlights the effective role of nanofertilizers in enhancing silkworm feeding efficiency. However, further exclusive studies are needed to investigate the feeding efficiencies of silkworms raised on nano fertilizer-treated mulberry leaves under both septic and aseptic conditions.

Impact Nano Particles on Larval Growth and Development

During their larval stages, silkworms (*Bombyx mori*) primarily consume mulberry leaves, which provide essential nutrients for their growth and development. The application of silver nanoparticles, often in combination with spirulina, has been shown to significantly enhance the length and weight of fifth-instar silkworm larvae due to improved nutritional efficiency [105, 106]. Similarly, Prabu et al. [107] reported increases in the length, width, and weight of third, fourth, and fifth instar larvae when fed silver nanoparticles. The fat body of the silkworm also plays a crucial role in storing, utilizing, and transferring nutrients necessary for larval growth [108]. Notably, riboflavin nanoparticles at 60 μg/mL significantly increased larval weight (1.63 g) and silk gland weight (1.67 g) [104].

Tian et al. [109] investigated the effects of 5 mg/L titanium dioxide nanoparticles (TiO_2 NPs) on the nutrient metabolism of the silkworm fat body, finding that TiO_2 NPs activated the insulin signaling pathway, thereby enhancing the metabolism of carbohydrates, proteins, and fats compared to the control group. Additionally, Li et al. [101] demonstrated that low concentrations of nanoparticles enhance larval body growth and feeding efficiency. Silkworms fed with 5 or 10 mg/L TiO_2NPs (with particle sizes between 5–6 nm) showed improved ingestion and digestibility of mulberry leaves, which significantly accelerated their weight gain relative to both the control group and larvae treated with higher concentrations of TiO_2 NPs (40, 80, and 160 mg/L) after 168 hours. *Bombyx mori* larvae benefit from the nutrient-rich mulberry leaves during their growth stages [110]. Nithya et al. [98] also reported increased larval weight and effective rearing rate (ERR) when silkworms consumed mulberry leaves treated with nano zinc oxide. Furthermore, reduced molting duration and shorter fifth instar larval duration were observed with this treatment. In a separate study, mulberry leaves soaked in TiO_2 nanoparticles at concentrations of 100, 200, 400, 800, 1600, and 3200 mg/L were dried and then fed to *Bombyx mori* larvae from the fourth instar. The larvae fed with leaves treated at 400 mg/L exhibited significantly higher body weights during both the fourth and fifth instars.

Additionally, it was found that silkworms fed ≥800 mg/L of Ag NPs exhibited increased mortality rates compared to the control group and those receiving lower concentrations. Conversely, growth-promoting effects were observed at lower silver nanoparticle (AgNPs) concentrations (≤ 200 mg/L), while higher concentrations (≥ 800 mg/L) diminished these effects [106]. Pandiarajan et al. [111] noted that exposing silkworm larvae to 1 ppm of Ag NPs improved their growth rate and cocoon weight compared to those exposed to higher concentrations (10 and 100 ppm), with the latter resulting in the highest mortality rates. Feeding silkworms (CSR2 × CSR4) mulberry leaves treated with 25% silver nanoparticles resulted in enhanced third instar larval length (1.93 cm), width (0.38 cm), and weight (0.12 g), as well as improved measurements for the fourth and fifth instars. AgNPs are believed to function similarly to vitamins, stimulating feeding activity and improving food digestibility, thereby promoting increased larval dimensions[107].

In a study involving MR2 mulberry leaves soaked in silver nanoparticles and spirulina, significant improvements were seen in bivoltine double hybrid silkworms fed treated leaves from the third instar. The fifth instar larvae showed a length of 6.2 cm, a weight of 2.4 g, and a silk gland weight of 0.87 g, attributed to the nutritional benefits conferred by the silver nanoparticles [105]. When fifth instar larvae (PM x CSR2) were fed mulberry leaves treated with gold nanoparticles (GNPs) at various concentrations (50, 100, 200, and 300 ppm), they exhibited superior growth parameters, including an increased total larval duration (695 h) and silk gland weight (3.31 g) at the highest concentration (300 ppm). This effect may result from the small size of GNPs directly stimulating specific receptors in the posterior part of the silk gland[112].

The impact of nano zinc and nano copper nutrition on larval traits was studied by spraying V-1 mulberry with nano zinc and nano copper at intervals post-pruning. Mulberry leaves treated with each at 500 ppm resulted in significantly enhanced full-grown larval weight (40.68 g/10 larvae) and ERR (93.98%), alongside decreased total larval duration (581.61 h) and fifth instar larval duration (180.29 h). This improvement is attributed to the nanoparticles stimulating metabolic activities in silkworms, fostering better growth and development [95]. Furthermore, mulberry leaves of the V-1 variety treated with nano zinc oxide at 5, 10, 20, and 50 ppm demonstrated significantly higher larval weight (25.78 g/10 larvae) and ERR (94.00%) while also reducing molting duration (91.25 h) and fifth instar larval duration (7.22 days) when silkworms were fed leaves treated with 20 ppm of nano zinc oxide. This enhancement likely stems from the adequate supply of zinc nanoparticles, which accelerates enzyme activity and auxin metabolism, thus improving larval parameters[98].

Pooja et al. [99] reported a significant enhancement in the larval traits of silkworms fed with mulberry leaves treated with nitrogen nano-fertilizers. These included shorter durations for the fourth (4.73 days) and fifth instar (8.69 days), as well as improved overall larval duration (24.42 days), fifth instar larval weight (36.66 g/10 larvae), better ERR (97.78%), and enhanced cocoon yield (26.36 kg/10,000 worms). Furthermore, parameters such as average filament length (1510.19 m) and filament weight (0.46 g) also benefitted when *Bombyx mori* silkworms (FC1 x FC2) were fed on mulberry leaves raised with a 0.4% foliar application of nano nitrogen fertilizer on the 25th day after pruning. Although existing studies highlight the significant impacts of nanofertilizers on silkworm rearing, further research is needed to standardize the use of different nano fertilizers at varying concentrations within the field of sericulture.

Impact Nano Particles on Silkworm Organs

The integration of nanotechnology into various scientific fields is relatively novel, and assessing its impacts on the tissues and organs of model organisms is crucial. The silk gland, predominantly present during the larval stage of the silkworm (*Bombyx mori*), plays a vital role in storing and synthesizing silk proteins, including sericin and fibroin [19, 113]. However, the application of fungicides and pesticides in agriculture can contaminate mulberry leaves, which, when ingested by silkworms, may lead to detrimental effects on essential tissues and organs [114]. For instance, Li et al. [114] investigated the effects of feeding titanium dioxide nanoparticles (TiO2 NPs) to silkworms exposed to phoxim, a pesticide. They found that the silk glands of larvae consuming contaminated mulberry leaves exhibited significant damage, characterized by extensive vacuolization within the gland lumen and a reduction in epithelial cell density. Importantly, the administration of TiO_2 NPs was shown to mitigate the damage caused by phoxim exposure, suggesting a protective effect. Furthermore, phoxim exposure was associated with decreased concentrations of fibroin and a downregulation of sericin genes (ser2 and ser3), elucidating the observed vacuolation in the silk gland and the reduced cocooning rate[114].

Innate immunity serves as the first line of defense against pathogens, thwarting bacterial and viral infections through mechanisms such as phagocytosis of pathogens and dead cells [115, 116]. Hemocytes, the blood cells of invertebrates, are valuable models for studying the effects of drugs and nanomaterials on innate immune responses and DNA damage [117]. Rajasekharreddy et al. [118] explored the effects of flavonoids (FLV), biosynthesized flavonoid silver nanoparticles (FLV-Ag NPs), and silver nanoparticles (Ag NPs) on silkworms infected with *Staphylococcus aureus*. Their findings indicated that the administration of FLV and FLV-Ag NPs resulted in an increased hemocyte density, enhancing bactericidal activity against *S. aureus*.

Consequently, bacterial growth was significantly higher in control groups compared to those treated with FLV-Ag NPs, which effectively inhibited bacterial proliferation. Ag NPs are recognized for their favorable physicochemical properties and antimicrobial applications; numerous studies have demonstrated their efficacy in combating bacteria and other pathogens [119-121]. Beyond their antimicrobial properties, Govindaraju et al. [120] revealed that an antiviral assay utilizing Ag NPs synthesized with *Spirulina platensis* effectively controlled *Bombyx mori* nuclear polyhedrosis virus (BmNPV) infections by promoting an increase in hemocyte density. An elevated hemocyte population in invertebrates signifies an active defense mechanism responding to foreign invaders.

Moreover, quantum dots are characterized by unique optical properties that lend themselves to biological imaging applications [122-125]. However, they also exhibit size-dependent cytotoxicity [126, 127]. Li et al. [58] exposed silkworms to varying concentrations of cadmium telluride quantum dots (CdTe QDs), citric acid–nitrogen-doped carbon dots (C-NCDs), and silicon nanoparticles (Si NPs) to investigate the differential immune responses and programmed cell death in hemocytes. Their results indicated that exposure to Si NPs induced autophagy and apoptosis, which could be reversed, whereas C-NCDs and CdTe QDs triggered autophagy, apoptosis, and necrosis in hemocytes. Thus, this research underscores the importance of understanding the potential impacts of nanomaterials on the immune responses of silkworms, paving the way for future studies in this burgeoning field.

Impact of Nano Particles on Silkworm ROS

Reactive oxygen species (ROS) play a pivotal role in cellular signaling and homeostasis, representing a common byproduct of oxygen metabolism [128]. However, elevated levels of ROS can lead to oxidative stress, which inflicts damage on DNA, proteins, and lipids [129]. This oxidative imbalance results from an excess of free radicals within the organism [130]. Notably, ROS generation is crucial for activating cellular defense mechanisms and stimulating antioxidant enzymes [131, 132]. In a study by Xu et al. [133], silkworm larvae exposed to 10–70 μg/mL of zinc oxide nanoparticles (ZnO NPs) through subcutaneous injection exhibited increased activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-PX), all of which are essential for neutralizing excess ROS. The activation of these enzymes was linked to the expression of apoptosis-related genes in the midgut of the silkworm, such as Dronc, Caspase, and Trt. Moreover, the expression levels of these antioxidant enzymes were significantly higher in the ZnO NPs-exposed groups compared to the controls, indicating time-dependent toxicity; antioxidant activity remained elevated for 12 hours post-injection but declined by 36 hours. Higher concentrations and sublethal doses of certain nanomaterials were associated with increased mortality rates, reduced cocoon quality, and lower body weights compared to control groups.

In another investigation, silkworms fed 100 ppm of silver nanoparticles (Ag NPs) showed elevated ROS production, leading to cell apoptosis, necrosis, and DNA damage [134]. The mortality rate in this group was higher than that in silkworms exposed to 1 and 10 ppm of Ag NPs. These findings align with those of Meng et al. [106], who reported that while concentrations of Ag NPs exceeding 800 mg/L promoted silkworm growth, they also resulted in increased mortality. Similarly, Abdelli et al. [135] indicated that although higher concentrations of Ag NPs had a positive impact on silkworm body weight, they caused detrimental effects on tissue health and potentially harmed primary organs. Chen et al. [136] explored the toxic effects of varying concentrations (100, 200, and 400 mg/L) of Ag NPs on the midgut tissues of silkworms, revealing that exposure to 400 mg/L led to symptoms of poisoning and significant tissue damage. Additionally, increasing Ag NPs concentrations downregulated the expression of digestive enzymes, which further harmed silkworm tissues and suppressed SOD activity and heat shock protein 1 (HSP 1). Consequently, this downregulation activated oxidative stress pathways, resulting in heightened ROS production that adversely affected the silkworm digestive system.

Xing et al. [137] examined the effects of introducing silicon nanoparticles (Si NPs) into the hemolymph of silkworms. They found that a dose of 3.9 μg of Si NPs was toxic to hemocytes compared to groups exposed to lower concentrations (0.39 and 0.039 μg). Interestingly, this higher dose did not elevate ROS production, suggesting that the hematopoietic damage was limited and capable of self-repair. Liu et al. [138] studied the impact of sublethal doses (0.08 and 0.32 nM) of cadmium telluride quantum dots (CdTe QDs) with sizes of 530 and 720 nm on fifth-instar silkworm larvae over 48 hours. Their results indicated that larvae exposed to the quantum dots exhibited reduced body mass compared to the control group, with significantly higher mortality rates observed in the 530 nm CdTe QD group. They concluded that smaller-sized CdTe QDs (530 nm) induced greater hemocyte apoptosis compared to larger-sized (720 nm) CdTe QDs, primarily due to their inhibitory effects on hematopoiesis.

Impact of Nano Particles on Silkworm Diseases

Viral diseases

Silkworm farmers experience significant economic losses, estimated at 15–20% of their larvae, due to diseases [139]. In severe cases, losses can be so substantial that farmers are compelled to completely revamp their larvae-rearing systems. A notable disease affecting *Bombyx mori*, caused by the *Bombyx mori* Nuclear Polyhedrosis Virus (BmNPV), primarily targets fully grown early fifth instar larvae and is transmitted via the oral route. This highly lethal viral infection can kill larvae within 24 to 30 hours, a condition commonly referred to as "Grasserie." Currently, no antidotes are available on the market to combat this disease; however, nano-silica has demonstrated efficacy in managing Grasserie at a concentration of 7 μL [140]. The mechanism of action involves the physical interaction of nano-silica with the viral polyhedron, facilitated by the high free surface energy of amorphous nano-silica. The lipophilic capping moieties on the silica surface stabilize its inherent instability and interact with the environment, redistributing excess free energy. This interaction allows the amorphous nano-silica to serve as an activation site, disrupting the three-dimensional structure of the viral polyhedron into a thinner, two-dimensional form. Consequently, the high surface free energy of the amorphous nano-silica leads to significant distortion of the viral polyhedral surface, ultimately resulting in the death of the viruses within the silkworm hemolymph [141]. Similarly, Kaman et al. [142] noted that nanoparticles with proper surface functionalization could effectively prevent BmNPV infections by targeting the viral polyhedron.

For instance, titanium dioxide nanoparticles (TiO_2 NPs) have shown promise in inhibiting BmNPV proliferation in silkworm larvae while enhancing larval survival rates and cocoon quality following BmNPV infection [143-145].

In one study, mulberry leaves were soaked in TiO_2 nanoparticles at a concentration of 5 mg/L, dried, and subsequently fed to fourth-instar silkworms (*Jingsong × Haoyue*). Afterward, these fifth instar larvae were fed mulberry leaves treated with a BmNPV solution (5.6 x 10^6 polyhedra/mL). After 120 hours, silkworms in the control group showed disease symptoms, whereas those in the treated group remained healthy and exhibited significantly improved survival rates (99.44%), cocooning rates (49.13%), cocoon weights (1.73 g), cocoon shell weights (0.41 g), and cocoon shell ratios (23.82%). These findings indicated that TiO_2 NPs substantially inhibited BmNPV proliferation in silkworm larvae [145]. In another experiment, mulberry leaves soaked in BmCPV solution (1.1 x 10^7 polyhedra/mL) were fed to fifth instar silkworms. The results showed survival rates (98.67%), cocooning percentages (61.33%), and cocoon survival rates (35.49%) were significantly higher in silkworms treated with TiO_2 NPs, demonstrating reduced mortality compared to the control group. TiO_2 NPs treatment was equally effective in enhancing silkworm resistance to cytoplasmic polyhedrosis virus (CPV), inhibiting BmCPV proliferation [144].

Additionally, Ruyi et al. [146] explored the effectiveness of silver nanoparticle colloids (AgNPC) in mitigating BmNPV infections in silkworms. Their study revealed that AgNPC improved the survival rate of silkworms from 22% to 67% compared to the untreated control group. The adhesion of the virus to Ag NPs resulted in the generation of free radicals, which penetrated and disintegrated the viral capsids, proteins, and DNA of BmNPVs. This degradation ultimately reduced the virus's pathogenicity and enhanced the survival rates of the affected silkworms.

Likewise, Das et al. [147] reported that silica nanoparticles (Si NPs) induced morphological changes in BmNPV polyhedra, subsequently reducing their infectivity in silkworm larvae. In their study, they injected silkworms (*Nistari*) with a complex of silica nanoparticles and BmNPV (5 µL/larva) and monitored their health over 24, 48, 72, and 96 hours. The findings indicated that silica nanoparticle-treated silkworms not only remained healthy but also demonstrated a prolonged survival rate of 61.03% beyond 150 hours. The application of Si NPs roughened the surface of the polyhedra, and with increasing nanoparticle concentration, the polyhedral surfaces became increasingly tattered, resulting in a loss of regular symmetry [147].

Bacterial diseases

Silver nanoparticles (AgNPs) have demonstrated significant antibacterial and anti-virucidal properties against various bacterial pathogens affecting silkworms, such as *Serratia marcescens*, *Bacillus* spp., *Bacillus thuringiensis*, and *Streptococcus aureus*, as well as viral pathogens like the Nuclear Polyhedrosis Virus and Cytoplasmic Polyhedrosis Virus, at a concentration of 1:100 [148]. In a study involving three bacterial species viz., gram-negative *Bacilli-I*, gram-negative *Bacilli-II*, and gram-positive *Cocci*, isolated from the gut of diseased fifth instar silkworms, both in vivo and in vitro antibacterial activities of AgNPs were assessed. The disk diffusion and well diffusion methods utilized sterile filter paper disks charged with AgNPs (100 µg/mL) placed on agar plates, which were subsequently incubated at 37°C for 24 hours. In the in vivo study, thirty diseased fifth instar larvae were divided into two groups: one group received untreated mulberry leaves, while the other was fed mulberry leaves treated with AgNPs for five days. On the sixth day, the larvae were dissected, and their guts were extracted and macerated for the antibacterial assay.

The results indicated that silver nanoparticles produced a maximum zone of inhibition compared to the negative control and significantly reduced gut bacterial growth in larvae fed AgNPs-treated mulberry leaves [149]. Furthermore, AgNPs exhibited the highest zone of inhibition and lowest growth of gut bacteria (*Bacillus* spp.) in the treated larvae [148, 150]. AgNPs were also effective against pebrine disease at the same concentration. Moreover, AgNPs enhance silkworm immunity by inhibiting reactive oxygen species (ROS), thus activating the Toll pathway, which boosts both humoral and cellular responses against *S. aureus* infections [118].

Thymoquinone-encapsulated chitosan nanoparticles (Tq-Chs NPs) have shown inhibitory effects against pathogenic bacteria infecting *Bombyx mori* larvae, acting as potent antioxidants and anti-inflammatory agents, with these effects potentially enhanced by incorporating Tq into chitosan nanoparticles [151]. Additionally, biosynthesized silver and chitosan nanoparticles at low concentrations (1 ppm, 10 ppm, and 100 ppm) were found to significantly prevent silkworm pathogens [111, 152]. Chitosan, at various concentrations, also exhibited antibacterial activity against bacterial pathogens (*Bacillus* spp., *Micrococcus* spp., and *Serratia* spp.) affecting tropical tasar silkworms under in vitro conditions. Notably, a low concentration (0.2%) of chitosan from silkworms, as well as chitosan nanoparticles, was identified as the minimum inhibitory concentration (MIC) against these bacterial pathogens [153, 154]. Furthermore, AgNPs synthesized using crude flower extracts demonstrated synergistic antibacterial activity against Flacherie and Sappe microbial strains, including *Bacillus subtilis*, *S. aureus*, *Escherichia coli*, *Bacillus cereus*, *Aerobacter cloacae*, and *Salmonella typhi* [103].

Fungal diseases

Poly(N-isopropylacrylamide) (PNIPAM) nanoparticles exhibit notable antifungal properties against the microbial infestation caused by *Aspergillus niger* in Tasar silkworms. PNIPAM nanoparticles can form a microfilm that acts as a protective barrier both externally and internally around the infected silkworms, effectively preventing further microbial invasion. Research indicates that the application of PNIPAM at concentrations of 0.4 and 0.6 mg/mL leads to a significant reduction in microbial infestations in these silkworms [155].

In another study, the fungi *Beauveria bassiana* and *Metarhizium anisopliae*, isolated from the gut of diseased *Bombyx mori* fifth instar larvae, were cultured in petri plates to evaluate the antifungal efficacy of silver nanoparticles against silkworm pathogens causing white and green muscardine. Ramamoorthy et al. [156] demonstrated that silver nanoparticles at a concentration of 100 µL exhibited the maximum zone of inhibition, while the control group showed the least inhibition. Additionally, silver nanoparticles from *P. hornemannii* at the same concentration displayed significant antifungal activity, yielding inhibition zones of 22.6 mm against *B. bassiana* and 21.0 mm against *M. anisopliae*. The antifungal mechanism of silver nanoparticles is attributed to their interaction with fungal mycelia, disrupting cellular membranes and affecting membrane potential. These studies have produced encouraging results through advanced techniques; however, further investigation is required to explore the management of fungal, bacterial, and viral diseases in silkworms. Future research should also consider the synergistic effects of various nanoparticles and their combinations to enhance disease resistance in silkworms.

Green Synthesis of Nanoparticles

Beauveria bassiana, a pathogenic fungus, is transmitted through direct inoculation or by feeding silkworm larvae contaminated leaves. To mitigate these fungal infections, it is crucial to maintain optimal commercial and environmental conditions, along with aseptic practices to promote silkworm health. Prolonged use of fungicides can lead to reduced sensitivity to entomopathogenic fungi, resulting in adverse effects such as decreased cocoon and pupal weights [157]. Consequently, the application of chemical fungicides is not advisable for silkworm caterpillars. To address this issue, an innovative approach known as "green nanotechnology" has emerged, combining principles of green chemistry and engineering to enhance environmental sustainability while addressing challenges posed by entomopathogenic fungi. This technology also reduces the costs associated with nanoparticle (NP) development through biological means, a process referred to as biogenesis.

Current research trends in the scientific community emphasize the use of nanotechnology and nanoparticles, characterized by ultrafine structures measuring less than 100 nm, as effective solutions for treating fungal diseases in silkworms [158]. Traditional methods for synthesizing NPs often involve physical and chemical techniques that demand high energy and temperature, alongside stabilizing agents, which can be detrimental to both human health and the ecosystem [159]. In contrast, green nanotechnology presents a superior alternative, offering a one-step, eco-friendly process that utilizes fewer toxic chemicals and less energy for initiating reactions, while also being cost-effective [160]. Green NPs can be produced through two main categories of organisms: microorganisms, including bacteria and fungi, and plants ''[161]. This approach not only supports the health of silkworms but also promotes a more sustainable method of disease management in sericulture.

Impact of Green NPs on Silkworm Fungal Diseases

Green nanoparticles (NPs) are recognized for their exceptional efficiency and ultrafine structures. Metal or metal oxide NPs, including those made from zinc, silver, aluminum, and titanium oxide, exhibit toxic effects on a variety of organisms such as crustaceans, bacteria, fungi, plants, and pathogens [162]. For instance, a study investigating silver nanoparticles (AgNPs) derived from *Portieria hornemannii* demonstrated significant antifungal activity against *Beauveria bassiana* and *Metarhizium anisopliae*. These AgNPs were analyzed using a UV-visible spectrophotometer, and their structure and size distribution were examined through transmission electron microscopy (TEM), revealing an average size range of 9.0 to 80 nm. The AgNPs from *P. hornemannii* exhibited the highest zone of inhibition against *B. bassiana* and *M. anisopliae* at concentrations between 100 and 200 ppm [156]. The large surface area-to-volume ratio of these NPs enhances their binding activity, allowing for increased interaction with microbial cell membranes and subsequent disruption of cell wall integrity [163]. The antifungal properties of AgNPs also extend to the mycelia of fungi such as *Trichophyton mentagrophytes* and *Candida spp.*, where they specifically target cell membranes, altering membrane potential [164].

Furthermore, studies have indicated that green synthesized zinc oxide (ZnO) NPs exhibit antifungal effects on *Rhizopus stolonifer* and *A. flavus*, causing greater protein leakage compared to other fungal species [165]. Natural extracts have also been explored for their antifungal properties; for instance, garlic extract at a 1:5 concentration was found to be the most effective against *B. bassiana*, while ginger (6.00 and 10.66 mm) and onion (7.16 mm and 12.00 mm) at a 1:1 concentration exhibited only slight inhibition of fungal growth [166]. Additionally, the application of 50% garlic extract on silkworms demonstrated a 50.17% protection rate against muscardine infections, leading to increased cocoon weight, shell ratio, and shell weight [167]. This underscores the potential of both green NPs and natural extracts in managing fungal infections in silkworms, highlighting their role in promoting sericulture health.

Impact of Green NPs on Silkworm

Nanomaterials are attracting considerable interest due to their unique physical, chemical, and biological properties [168]. Numerous studies have explored the effects of various green nanoparticles (NPs) on *Bombyx mori*. For example, gold NPs synthesized from *Allium cepa* (onion) have shown a significant impact on silkworms, notably enhancing silk protein yield and cocoon weight at low concentrations [112]. Similarly, bio-silver NPs derived from *Morus alba* (mulberry) leaf extract have been reported to promote growth in silkworm larvae at lower concentrations (1 ppm, 10 ppm, and 100 ppm) [111]. Green AgNPs synthesized from the extract of *Acalypha indica* at concentrations of 10% and 20% exhibited growth-promoting effects at lower silver nanoparticle (AgNP) concentrations [106].

While most studies indicate the beneficial effects of green NPs on silkworms at lower concentrations, some nanoparticles, such as CdTe QDs, exhibited detrimental impacts on hemolymph, leading to reduced larval growth and silk production. The enhanced surface area-to-volume ratio of these NPs increases their binding capacity, thereby promoting interactions with microbial cell membranes and potentially disrupting cell wall structures [163]. Moreover, the application of nanoparticles has demonstrated therapeutic properties for diseased silkworms, significantly influencing various biochemical and antioxidant enzyme attributes. Consequently, these nanoparticles could serve as effective alternatives to traditional fertilizers and may aid in enriching plants with nutritional value [169].

Impact of Nanoparticles on Reproduction and Fecundity

B. mori larvae were fed mulberry leaves that had been soaked in a 5 mg/L solution of titanium dioxide (TiO_2) nanoparticles, and subsequently dried. After feeding, the larvae were dissected, and the mature testes and ovaries of the silkworms were collected and stored at -80 °C. The administration of TiO_2 NPs resulted in enhanced metabolism of proteins and carbohydrates, which is essential for meeting the energy requirements for gonad growth and development. Notably, the silkworms exhibited a higher density of oocyte differentiation and formation within their ovaries, leading to an increased egg yield. This finding suggests that TiO_2 nanoparticles not only facilitate nutrient accumulation and transformation during reproductive development but also enhance oviposition capabilities in *B. mori*. The study recorded a significant increase in egg production, with the highest number reaching 620 grains and an egg weight of 6.49×10^{-4} grams, while the incidence of unfertilized eggs was minimized to just five grains [170].

Advancing Economic Traits of Silkworms with Nanotechnology

Impact of Nanoparticles on Silkworm Cocoon Quality

Enriching mulberry leaves represents a viable strategy to enhance both cocoon and silk productivity while maintaining quality through nutrient fortification. Nutritionally fortified leaves, supplemented with ascorbic acid, folic acid, selenium, and metal nanoparticles, have demonstrated significant improvements in the growth and development of silkworms, which in turn enhances the economic characteristics of the resulting cocoons [171]. In one study, silkworms fed mulberry leaves treated with riboflavin nanoparticles at a concentration of 60 μg/ml exhibited notable increases in cocoon weight (1.19 g), cocoon shell weight (0.27 g), pupal weight (0.92 g), cocoon shell ratio (22.689%), silk filament weight (0.53 mg), silk filament length (807 m), and denier (3.903). This improvement is attributed to the influence of vitamin nanoparticles on the growth and metabolism of silkworm larvae, consequently enhancing the cocoon and reeling parameters [104].

Additionally, Patil et al. [112] reported that silkworms fed mulberry leaves treated with gold nanoparticles at 300 ppm showed significantly enhanced cocoon and reeling traits. Specifically, the cocoons weighed 23.75 g for ten cocoons, with a shell weight of 4.12 g, a cocoon shell ratio of 16.54%, and a total cocoon yield of 819.3 g per dfl. The cocoon length measured 3.93 cm, breadth 2.36 cm, and filament length reached 867.46 m, with a denier of 4.05 and fibroin content of 78.07%. This enhancement is likely due to the specific dosage of gold nanoparticles, which are thought to directly stimulate silk gland activity, thereby yielding superior-quality cocoons [112]. Similarly, the impact of silver nanoparticles and spirulina on the economic traits of silkworms was evaluated, revealing that those fed with mulberry leaves treated with Ag NPs and spirulina at 300 ppm exhibited significantly higher cocoon weight (1.84 g), cocoon shell ratio (41.3%), silk filament weight (0.32 g), and fibroin content (79.20%). This positive effect is likely due to the nutritional benefits of silver nanoparticles and their influence on food consumption, which directly affects larval traits and improves cocoon quality [105]. Furthermore, silkworms (LNB4 x D2) fed with Ag NPs treated MR2 mulberry leaves at a concentration of 25% displayed the highest cocoon length (3.66 cm), width (2.40 cm), and weight (2.21 g), underscoring the stimulatory effect of Ag NPs on feeding activity. Consequently, Ag NPs enhance food digestibility, which leads to increased cocoon dimensions and weight [107].

Moreover, silkworms that consumed mulberry leaves treated with 50 ppm spirulina-mediated TiO_2 NPs exhibited increased cocoon weight, shell ratio, silk filament length, filament weight, and denier, while also showing a reduction in rendita [172]. Notably, silkworms fed with TiO_2 NPs treated mulberry leaves at a concentration of 5 mg/L achieved significantly superior cocoon weight (2.12 g), cocoon shell weight (0.56 g), cocoon shell ratio (26.32%), average cocoon filament length (1207.06 m), reelability(67.57%), and neatness (98.33 points). This improvement is attributed to the increased feed efficiency of *B. mori* due to the TiO_2 NP-treated leaves, which subsequently enhances cocoon quality and reeling parameters [102]. The effects of TiO_2 NP-treated mulberry leaves on cocoon parameters (Suju x Minghu) indicated that significantly superior cocoon and reeling traits were observed in silkworms fed with TiO_2 NPs at 10 mg/L. This enhancement likely results from TiO_2 NPs promoting the growth and development of *B. mori*, potentially by enhancing food digestion and nutrient absorption, thereby improving silk protein synthesis [101].

Investigating the impact of nano zinc and nano copper on cocoon parameters revealed that silkworms fed with mulberry leaves treated with Nano Zn and Nano Cu at 500 ppm showed significantly better cocoon weight (19.64 g for ten cocoons), cocoon shell weight (4.65 g for ten shells), cocoon shell ratio (23.67%), and silk productivity (0.62 cg/day). This improvement may be attributed to the stimulation of metabolic activities in silkworms, resulting in enhanced growth and development, as well as increased silk production [95]. Similarly, silkworms fed with nano zinc oxide at 20 ppm-treated mulberry leaves exhibited superior cocoon weight (14.63 g for ten cocoons), shell weight (2.49 g for ten shells), cocoon shell ratio (17.98%), filament length (823.83 m), and filament denier (2.45). This enhancement is likely due to the adequate nutrient supply provided by the nano zinc oxide-treated leaves, which accelerates enzyme activity and auxin metabolism in plants, leading to improved larval traits and subsequently increased cocoon and reeling parameters [98].

In summary, various nanoparticles, including Ag NPs, TiO_2, and nano Zn coupled with Cu, have been shown to enhance the economic traits of cocoons [95, 101, 102, 107]. Furthermore, carbon nanotubes (CNT) have been utilized to produce high-strength silk fibers, demonstrating enhanced stress, strain, conductivity, and thermal stability, with mechanical properties comparable to super silk and spider silk fibers [82]. The mechanical properties of silk are further improved by feeding silkworms with MoO_2 nanoparticles [173]. Ultimately, the yield of cocoons in commercial silkworm rearing can be significantly enhanced through the application of various nanofertilizers, necessitating standardization based on the type and combination of nanoparticles used.

Impact of Nanomaterials on the Silk Fibers

The allure of silkworm silk in the textile industry can be attributed to its lustrous sheen and remarkable mechanical properties [174-176]. Consequently, enhancing the quality and luster of this silk has become a focal point of research. Studies indicate that the incorporation of nanomaterials, including carbon nanotubes (CNTs), titanium dioxide, copper, and graphene, can significantly improve both the mechanical properties and secondary structures of silkworm silk [177, 178]. When assessing silk quality, scientists typically consider the mechanical properties, crystalline structure, and thermal stability of silk fibers. Research has demonstrated that hindering the conformational transition of silk fibroin from a random coil/α-helix to a β-sheet leads to increased breaking elongation and toughness, thereby enhancing the mechanical properties of the silk [179].

The convergence of nanoscience and nanotechnology has revitalized materials science, driving advancements in the sericulture sector and resulting in an array of improved materials, such as polymers and textiles through nanostructuring and nanoengineering. Structured nanomaterials, exemplified by nanocoatings, nanocomposites, nanofibers, and nanofinishing, hold significant promise for transforming the textile industry. These innovations enable functionalities such as self-cleaning surfaces, conductive textiles, antimicrobial properties, controlled hydrophilicity or hydrophobicity, and protection against fire and UV radiation—all without compromising the inherent properties of fibers and fabrics. The textile sector is increasingly leveraging these nanotechnological advancements, leading to the development of nanoproducts ranging from nanocomposite fibers to intelligent, high-performance polymeric nanocoatings, thereby enhancing conventional textiles with improved functionalities.

Furthermore, research into the differential role of gold nanoparticles synthesized from *Allium cepa* has revealed notable effects on the sericin and fibroin content of silkworms. Specifically, gold nanoparticle treatment significantly altered the percentages of these proteins in the fifth instar, demonstrating that a 300 ppm dose of green gold nanoparticles resulted in a fibroin percentage of 78.07%, while sericin decreased from 39.46% (control) to 21.92%. This indicates that gold nanomaterials not only modify fibroin protein levels but also enhance various silk and cocoon traits [112]. Wu et al. [180] reported that copper and titanium NPs did not significantly affect silkworm weight but improved the quality of silk fibers.

Recent studies have delved into the impacts of nanomaterials on silkworms and their silk fibers. Cai et al. [179] investigated the production of intrinsically modified silk fibers by feeding silkworms a titanium dioxide (TiO_2) artificial diet at varying concentrations (1, 2, 3, and 4%). They found that a 1% TiO_2 diet markedly improved the mechanical properties and ultraviolet resistance of silk fibers compared to control mulberry feed. However, increasing concentrations of TiO_2 diminished breaking strength and UV resistance. Notably, 1% TiO_2 nanoparticles effectively restricted the conformational transition of silk fibroin, resulting in a more pronounced crystallization effect.

In another study, Cheng et al. [181] showed that copper (Cu) or silver (Ag) nanoparticles incorporated into the silkworm larval diet were absorbed into the silk fibers, augmenting their mechanical properties and promoting silk protein crystallization. This finding aligns with Wu et al. [180], who reported that silkworm silk enriched with Cu exhibited impressive tensile strength (360 MPa) and strain (38%), surpassing the values of natural silk fibers (control) by 89% and 36%, respectively. Zhang et al. [182] explored the effects of glucose-coated Ag nanoparticles on the silkworm diet, revealing that silk fibers exposed to these nanoparticles exhibited a smooth surface and significant changes in diameter. Concentrations of 0.20% and 0.02% of Ag nanoparticles were shown to promote silk protein synthesis, enhance mechanical properties, and improve the antibacterial characteristics of the silk.

Additionally, single-walled nanotubes (SWNTs) and graphene were observed to similarly limit the conformational transition of silk fibroin, thereby positively affecting the silk fiber's secondary structure and enhancing its electrical conductivity [175]. Graphene's outstanding electrical conductivity, optical properties, and mechanical strength were imparted to silk fibers [183-186]. Interestingly, silkworms fed lower concentrations (0.2 wt %) of SWNTs demonstrated improved mechanical properties compared to those fed 2 wt % of graphene, which yielded lesser enhancements.

Furthermore, Ma et al. [187] assessed the impact of vascular injection of graphene quantum dots (GQDs) on silk mechanical properties. GQDs, known for their exceptional mechanical properties and reinforcement capabilities [185], were injected into silkworms, resulting in silk fibers with improved crystallization and mechanical properties. Xu et al. [188] demonstrated that feeding silkworms a composite of unpurified CNTs and lignosulfonate resulted in excess lignosulfonate coating that hindered CNT integration into silk fibers. However, purifying the CNT/lignosulfonate composites led to a higher CNT content and an ordered graphitic structure in the resulting silk fibers, achieving mechanical strength of 1.07 GPa and strain of 16.8%.

Consequently, the effects of bovine serum albumin (BSA)-stabilized gold nanoclusters (BSA-Au NCs) were investigated by Ma et al. [187] through intravascular injection at varying concentrations (9.38, 1.88, 0.938, and 0.188 μg). The results indicated that silkworms injected with 9.38 μg of BSA-Au NCs exhibited enhanced silk mechanical properties, with elongation at break, breaking strength, and toughness modulus of 38.02%, 368.88 MPa, and 104.12 MPa, respectively, compared to the control group. Notably, the crystalline structures of BSA-Au NCs silk remained unchanged, yet the crystallization of silk proteins was significantly enhanced.

Future Perspectives

The future prospects of nanotechnology in sericulture appear exceptionally promising, with the potential to transform the industry in multiple ways. The incorporation of nanoparticles into silkworm nutrition can significantly enhance growth rates, resulting in accelerated development and increased silk production. Furthermore, these nanoparticles are crucial for improving disease resistance in silkworms, facilitated by advanced formulations that protect against viral and bacterial infections. Integrating nanoscale materials into silkworm diets not only enhances silk quality but also improves its mechanical properties, luster, and durability. Additionally, nanotechnology paves the way for innovative solutions in smart packaging and storage of silkworms and silk products, effectively reducing spoilage and damage. Moreover, the application of nanotechnology promotes environmental sustainability by reducing the reliance on chemical pest control and optimizing the efficiency of water and nutrient usage. Advances in this field could also lead to precise gene editing in silkworms, resulting in the development of novel silk varieties with specialized properties tailored for medical and industrial applications. In addition, nanoparticle-based drug delivery systems can improve silkworm health without adverse effects, while innovations in waste utilization through nanotechnology can convert sericulture by-products into valuable resources. Consequently, these advancements have the potential to transform sericulture into a more efficient, cost-effective, and eco-friendly industry, with extensive applications in biotechnology and materials science.

Challenges and Constraints

Despite its significant potential, the application of nanotechnology in sericulture faces several critical limitations. One primary concern is the toxicity of nanoparticles, which may adversely impact silkworm health, leading to unpredictable biological consequences and a decline in silk quality. Additionally, the environmental ramifications are noteworthy; deploying nanomaterials in open fields could result in soil and water contamination, thereby affecting local ecosystems. Cost and scalability represent considerable obstacles; the production and application of nanomaterials on a large scale in sericulture can be prohibitively expensive, potentially rendering it economically unfeasible for small-scale farmers. Furthermore, the absence of standardized protocols for the use of nanoparticles complicates the establishment of safe and effective guidelines, leading to variability in results. Moreover, the limited research on the long-term effects and potential risks of nanotechnology restricts our understanding of its interactions with biological systems in silkworms. Regulatory uncertainty further complicates matters, as existing agricultural regulations may not sufficiently address the use of nanotechnology in sericulture, creating additional barriers to widespread adoption. Finally, ethical concerns arise regarding genetic manipulation facilitated by nanotechnology, prompting questions about its potential ecological and societal impacts. Addressing these limitations is essential for the sustainable integration of nanotechnology into the sericulture industry.

Conclusion

In conclusion, nanotechnology plays a crucial role in enhancing sericulture by improving both productivity and sustainability in silk production. The application of nanofertilizers has been shown to significantly increase mulberry yields through enhanced nutrient delivery, improved root development, and increased leaf biomass, all of which are vital for sustaining healthy silkworm populations. The judicious use of nanomaterials such as silver nanoparticles (Ag NPs), titanium dioxide nanoparticles (TiO_2 NPs), and silica nanoparticles (Si NPs) has yielded positive outcomes in tissue repair, survival rates, and overall silk production. However, it is essential to manage dosage levels carefully, as excessive exposure to these nanoparticles may lead to the generation of reactive oxygen species (ROS), which can cause cellular damage. Notably, research indicates that these nanomaterials can enhance the mechanical properties of silk fibers without compromising their crystalline structure. Moreover, they exhibit therapeutic potential in addressing silkworm infections, thereby improving survival rates in those affected by pathogens such as *Bombyx mori* nucleopolyhedrovirus (BmNPV). By incorporating green nanotechnology, the sericulture sector can advance disease management and productivity while minimizing environmental impact. Future research should prioritize understanding the physiological effects of nanomaterials on silkworms and their implications for silk production, as well as assessing their environmental safety. Optimizing the application of nanotechnology will enable the sericulture industry to become more resilient and efficient, ultimately benefiting sericulturists and enhancing the global quality of silk.

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