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Analysis of the Biological Impacts of Nanomaterials on Silkworms (*Bombyx mori*): A Brief Review

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Abstract: The production of high-quality silkworm silk is crucial to sericulture, along with the production of biomass, silk proteins, and animal feed. The unique characteristics of nanomaterials have the potential to advance the development of several industries, including agriculture, cosmetics, and medicine. The use of nanotechnology in sericulture enhances the quality of silk fibre while also increasing the silkworm's survival rate and encouraging their growth and development. Despite the benefits of nanomaterials, there are some worries about how safe it is to use them in the environment, on people, and in experimental models. Studies have revealed that while certain nanomaterials have therapeutic capabilities, others are poisonous to the silkworm's tissues and organs. This review summarises some studies on the benefits of applying nanoparticles and the biological effects of nanomaterials on silkworms.

Keywords: *Bombyx mori*, Nanomaterial, Silk, Sericulture, Silkworm, Nanotechnology

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Introduction

Nanomaterials are used in a variety of scientific domains including the agricultural, cosmetic, food, medical, and pharmaceutical industries due to their distinctive optical, electrical, and photocatalytic capabilities (Sanvicens and Marco, 2008). Over the years, the safety of nanomaterials on people and the environment has been studied using model species such as *Arabidopsis thaliana*, *Xenopus laevis*, *Drosophila melanogaster*, and rats (Spradling *et al*., 2006). New scientific discoveries have been made possible through the use of these model organisms in research. However, the use of mammalian animal models, such as pigs, mice, and primates, has given rise to bioethical issues because testing on the animals may cause them pain or misery. Therefore, it is essential that experiments that use animal models be conducted in accordance with ethical standards. Significant advancements in the sectors of agriculture, cosmetics, food, and medicine have been made possible by the relatively recent scientific field of nanotechnology (Ganon, 2007). Since nanomaterials are new to sericulture, it is essential to take advantage of their effects on silkworms and on silk in order to increase fertility, survival rates, manage pests, and avoid disease. The effects of nanomaterials on the development of silkworms and the growth of larval bodies are highlighted in this review. Additionally, it covers how using nanomaterials affects the quality of silk as well as how they affect the tissues and organs of silkworms.

Applications of nanomaterials in various sectors:

Nanotechnology, which uses materials with diameters between 1 and 100 nm, is regarded as a recent scientific technological revolution. Nanotechnology in agriculture has offered solutions for problems with plant nutrition, pesticide resistance, and plant protection. For instance, 250 g/ml of magnesium dioxide nanoparticles (MgO NPs) has been shown to improve the weight and height of tobacco plants. It has also been successful in reducing the severity of bacterial wilt caused by *Ralstonia solanacearum***.** The antibacterial action of MgO NPs against *R. solanacearum* in tobacco plants may be due to their capacity to produce more reactive oxygen species (ROS) and to cause oxidative stress (Adeyeye and Ashaaolu, 2021). Besides, MgO NPs, other nanomaterials, including titanium dioxide (TiO_2 NPs), zinc oxide (ZnO NPs), copper oxide (CuO NPs), graphene oxide, silver nanoparticles (Ag NPs), quantum dots, and superparamagnetic particles have been reported to exhibit antibacterial properties against *Streptococcus* mutans (Karlson *et al*., 2015) and *Xanthomonas perforans*; antifungal properties against *Fusarium oxysporum* and *Fusarium graminearum*; and antiviral properties (Santos *et al*., 2019). According to recent studies, multi-walled carbon nanotubes (MWCNTs) also improved tomato seed germination by improving seed water intake. These results of nanoparticles enhancing seed germination demonstrate how applying nanotechnology to agriculture can increase crop yield and enhance crop protection through the use of nanoscale insecticides (Kurwel *et al*., 2015).

Nanomaterials are employed in many fields thus, it is critical to determine how they will affect the environment and public health (Ghormade *et al*., 2011). Animal models are frequently used to clarify the mechanisms behind biological processes, to test the effectiveness of novel chemicals and medications, and to assess the interactions between nanoparticles and living things. The lungs of humans are said to suffer when nanomaterials (NM) are exposed to the environment. Mice exposed to zinc oxide (ZnO) (size of functionalized nanoparticles: 130 nm, size of non-functionalized nanoparticles: 100 nm) have been found to develop pulmonary inflammation. On the other hand, mice exposed to 100 mg/ml of Ag NM (20 nm) did not show any lung-harming effects (Prasad *et al*., 2010). Additionally, it has been noted that anatase TiO_2 NM causes lung inflammation in mouse studies. Male Sprague-Dawley rats exhibited neurotoxic behaviour following an intracerebroventricular injection containing 2 mg/ml of TiO_2 NPs (Fomutu *et al.*, 2022).

Nanoparticles released into the environment are hazardous to living things and can kill them or seriously impair their brain processes. For instance, Liu *et al*. (2009) reported that there was no toxic effect on the growth and development of *Drosophila melanogaster* (fruit fly) from the egg stage to the adult form when carbon-based nanomaterials (fullerene C60, carbon black, single-walled nanotubes, multiwalled nanotubes) were ingested through food. However, these flies' ability to move about was compromised as a result of ingesting carbon black and single-walled nanotubes. According to Demir *et al*. (2011), somatic recombination caused genotoxicity in the wing spot experiment of fruit flies using Ag NPs (0.1, 1, 5, and 10 mM). Fruit fly larvae exposed to TiO_2 NPs showed cytotoxic effects in the midgut and imaginal disc tissues (Alaraby *et al.*, 2016).

Fruit fly wing spot assays were unaffected by TiO_2 NPs, however, when compared to the DNA damage brought on by bulk TiO_2, there was a notable rise in damage.

Fruit flies treated with 20 mg/l of Ag NPs were unable to complete their life cycle (Tortella *et al*., 2020). Later generations of flies showed fecundity levels comparable to those of the fruit flies from the control group after a protracted exposure to Ag NPs, nevertheless. *Caenorhabditis elegans* (nematode) is a perfect model organism to study environmental nanotoxicity at the nanoscale level due to its natural habitat, fast life cycle (4 days), sensitivity, and moderate body size. Fast embryonic development outside of the parent zebrafish is a desirable trait that makes it possible to see cell development from the outside (Tortella *et al*., 2020). Ag NP exposure reduced hatching rates and increased embryo death in zebrafish. When compared to colloidal Au 120 hours after fertilisation, colloidal Ag caused a greater mortality of zebrafish embryos (Bar-Ilan *et al*., 2012).

According to Muller *et al*.(2015), after being exposed to copper oxide (CuO), 1.88 M of dissolved Cu^{2+} inhibited the proteolytic activity of the zebrafish hatching enzyme 1 (ZHE1), delaying the hatching of zebrafish embryos by 50%. The d'Amora group compared the effects of graphene oxide, oxidised carbon nano-horns, and nano-oxidized carbon onions on the growth of zebrafish. When compared to oxidised carbon nano-horns, concentrations of graphene oxide exceeding 50 g/ml resulted in a greater death rate, a slower rate of hatching, reduced mobility, and postponed embryonic development. It is crucial to investigate the advantages of nanotechnology, which is currently in the commercialization exploration phase. The food industry is currently interested in investigating the advantages of utilising nanotechnology in food production and food packaging (Berekaa, 2015). For instance, it was noted that the use of silver nanoparticles as an addition in the creation of food containers (Ag NPs) contributed to the reduction of bacterial development, extending the shelf life of foods kept in those containers.

Silkworm as a model organism:

When opposed to mammalian models, which are more expensive and require lengthy tests, invertebrate model organisms are favoured due of their quick production times and profusion. In contrast to mammalian model organisms, which require complicated laboratory equipment, the rearing conditions of invertebrate model organisms do not necessitate sophisticated infrastructure. This might be the rationale behind scientists' preference for using invertebrates in experimental studies. Additionally, invertebrates are regarded as superior models for researching behavioural and defence systems. Model species like *Caenorhabditis elegans* and *Drosophila melanogaster* have been employed a lot in research on cellular toxicity, how new medications affect people, and environmental pollution (Kholy and Nagggar, 2023).

The silkworm (*Bombyx mori*) is an insect (invertebrate) that is frequently used as a model organism in life sciences as it contains numerous mutant strains, a complete genome that has been sequenced, and a protein database that is accessible. The use of the silkworm as a model organism has many benefits. These animals are prolific breeders, require less sophisticated infrastructure for rearing, have a brief life cycle, a moderate body size that makes it simple to manipulate genes and organs, a distinct genetic background, and a wealth of mutation resources. Comparatively, it is simpler to extract genes and organs from silkworms than it is from fruit flies, whose tiny body size necessitates the use of a microscope. The fruit fly is a common invertebrate insect with distinguishing characteristics, and researchers utilise its genes to study ageing, cancer, organogenesis, neurological development, and other topics. In contrast to mammalian models, which generate fewer offspring at a time, it is claimed that the fruit fly produces genetically identical progeny despite having a quicker life cycle. Also, similar to the response shown in mammalian models, the fruit fly has been observed to react to medications that affect the central nervous system (CNS). Studies have been done on the genetic resemblance between various human-related genes affected by specific diseases and silkworm genes.

The silkworm was employed as a model organism by Matsumoto and colleagues to research the effects of human insulin on hyperglycemic silkworms (Kikuyama and Tsutsui, 2011). This study's findings suggested that when insulin was given, the sugar levels in the silkworm hemolymph decreased. The scientists also noted that silkworms fed a high-glucose diet for longer than 18 hours displayed a hyperlipidemic phenotype, and that pioglitazone or metformin treatment increased the silkworms' ability to tolerate glucose. By decreasing xanthine oxidase synthesis and increasing body oxidative stress response, the downregulation of the PARK7/DJ-1 gene produced p-translucent (op) silkworms, which makes the p-translucent silkworm a useful model for studying Parkinson's disease (Meng *et al*., 2017). The majority of research on the effectiveness of novel medications for conditions that affect humans, as well as drug screening for antibiotics, uses mammalian model organisms. According to recent studies, the way that silkworms and *Mus musculus* (mice) react to antifungals is similar. The response of the silkworm to amphotericin B and fluconazole was reported to be consistent with the response seen in a murine model after the silkworm was exposed to the median effective dose (ED_{50}) and the median lethal dose (LD_{50}) of *Candida tropica*lis and *Candida albicans*, respectively. While amphotericin B was most effective at preventing fungal growth in mice, fluconazole was effective at preventing the growth of fungi in silkworms. Ishii *et al*. (2010) employed the *Bombyx mori* silkworm as a model to investigate the role of cytokines in the immunological response of insects. In the silkworm *Bombyx mori*, it was demonstrated that the activation of a paralytic peptide caused cellular and humoral immunological responses, which support the host defence (Tanaka and Yamakawa, 2011). This further establishes the viability of using silkworms as a model organism for the investigation of biological processes such as homeostasis, gene control, and cell metabolism (Li *et al*., 2022). Since it has produced pertinent data in numerous domains and its use doesn't raise any bioethical concerns, the silkworm is gaining recognition in research as a model organism. Despite the advances made in genomics, there are still limitations to using silkworms as a model to research mammalian toxicology because these two kinds of animals have different biological systems and organs. According to Paudel *et al*. (2018), silkworms lack neurotoxin receptors, which may explain why they have higher concentrations of neurotoxic chemicals than mice. The silkworm cannot fully express or duplicate the mammalian biological system because it is an invertebrate with a distant link to mammals. This restricts their use as a model organism to assess the effectiveness of medications in several toxicological studies. The silkworm serves as a model organism that offers new insights on how to approach scientific issues and comprehend specific biological processes.

Effect of nanomaterials on silkworm development and growth:

The silkworm larvae's feeding efficiency is crucial since it determines how quickly they grow and develop. Low concentrations of nanoparticles (NPs) improve the body growth and feeding effectiveness of larvae (Li *et al*., 2022). The ingestion and digestibility of mulberry leaves were improved in silkworm larvae fed with 5 or 10 mg/l of TiO_2 NPs (with sizes in the range of 5-6 nm), which significantly accelerated their body weight gain when compared to the control group and to larvae treated with various concentrations of TiO_2 NPs (40, 80, and 160 mg/l) after 168 h. Silkworms feed on mulberry leaves during their larval stage because these leaves provide all the nutrients they need for growth and development (Zhang *et al*., 2014). The silkworm's fat body is capable of storing, using, and transferring the nutrients needed for the silkworm larvae's growth and development. Das *et al*. (2013) examined the effects of 5 mg/l of TiO_2 NPs on the silkworm fat body's nutrition metabolism. In contrast to the control group, the application of TiO_2 NPs stimulated the silkworm's insulin signalling system by improving the metabolism of carbs, proteins, and lipids. In contrast to the control group, Ramos *et al*. (2020) found that feeding silkworm larvae 5 g/ml of TiO_2 NPs resulted in the development of bigger testes and ovaries. According to Pandiarajan *et al*. (2016) when compared to silkworms exposed to 10 and 100 ppm of Ag NPs, silkworm larvae treated to 1 ppm of Ag NPs showed enhanced larval development rate and cocoon weight. Additionally, it was found that, when compared to silkworms exposed to various Ag NP concentrations, those fed with 100 ppm of Ag NPs had the highest mortality rates. When compared to the control group and groups that received Ag NPs at lower concentrations, Meng *et al*. (2017) found that silkworm fed with 800 mg/l of Ag NPs had a greater mortality.

Reactive oxygen species (ROS) are considered as a typical by-product of oxygen metabolism and are involved in cell signalling and homeostasis. Oxidative stress, which damages DNA, proteins, and lipids, is brought on by high quantities of ROS in living things. The imbalance of free radicals within the organism is the cause of this. Since it activates cells' defence systems and antioxidant enzymes, ROS generation in living things is crucial. According to Xu *et al*. (2017), silkworm larvae subcutaneously injected with 10-70 g/ml of ZnO NPs stimulated the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-PX). The activation of these enzymes is linked to the production of apoptosis-related genes in the silkworm midgut, including Dronc, Caspase, and Trt. These enzymes are responsible for removing excess ROS. The study team also found that the expression levels of these enzymes in the midguts of silkworms were significantly higher in the group that had been exposed to ZnO NPs than in the control group, and that the toxicity of ZnO NPs was time-dependent because the antioxidant enzymes remained active.

The organs and tissues of experimental animal models suffer when exposed to high quantities of nanoparticles. For instance, it has been noted that Drosophila and CD-1 mice cannot reproduce when exposed to high quantities of TiO_2 NPs (Philbrook *et al*., 2011). Additionally, three months of therapy with silica nanoparticles in mice led to reports of liver injury. When compared to control groups, larger concentrations and sublethal dosages of certain nanomaterials cause higher mortality, poor cocoon quality, and reduced body weight. 5th-instar silkworm larvae were given sublethal dosages (0.08 and 0.32 nM) of cadmium telluride quantum dots (CdTe QDs) of 530 and 720 nm diameters by Liu *et al.* (2009) for a 48 h period. When compared to the control group, the larvae exposed to quantum dots had reduced larval body mass, and the QD 530 nm group's mortality rates were much greater. They showed that because of their lower particle size and inhibitory effects on hematopoiesis, CdTe QDs with a particle size of 530 nm caused a higher level of hemocyte death when compared to the CdTe QDs 720 nm group.

ROS generation increased in silkworms fed at 100 ppm of Ag NPs, leading to cell death, necrosis, and DNA damage. Comparing this mortality rate to silkworm groups fed at 1 and 10 ppm of Ag NPs revealed that it was greater. These findings are in line with research by Meng *et al*. (2017) who found that although silkworm growth rates were accelerated by Ag NP concentrations more than 800 mg/l, they also perished. Similar studies showed that, despite increased concentrations of Ag NPs having a favourable effect on silkworm body weight, exposure to Ag NPs had negative effects on tissues and may have had deleterious effects on major organs. Chen *et al*. (2019) investigated the toxicity of Ag NPs at three different concentrations (100, 200, and 400 mg/l) on silkworm midgut tissues. Exposure of 400 mg/l Ag NPs produced tissue damage, including damage to the silkworm midgut, and increased Ag NP concentrations caused poisoning symptoms to manifest.

Effects of nanomaterial exposure on silkworm tissues and organs:

Nanotechnology is only recently being used in various field of study, hence it is crucial to assess how it may affect model organisms' tissues and organs. The silk gland is mostly found in the silkworm larval stage and is in charge of both storing and synthesising the silk proteins -- sericin and fibroin. When consumed by the silkworm, chemical residues from the use of fungicides and pesticides on crops, may contaminate mulberry leaves and cause harm or death to vital tissues and organs. Li *et al*. (2018) investigated the effects of feeding TiO_2 NPs to phoxim exposed silkworms as a therapy. When silkworm larvae were fed mulberry leaves containing phoxim residue, the silk gland was injured, exhibiting significant vacuolization in the gland lumen and sparse epithelial cell growth. It has been suggested that feeding silkworms with TiO_2 NPs can repair the harm done by phoxim exposure. Additionally, it has been demonstrated that phoxim exposure may lead to a decrease in the levels of the sericin genes ser2 and ser3, which accounts for the vacuolation of the silk gland and slowed cocooning pace.

Rajasekharreddy *et al*. (2017) examined the effects of flavonoids (FLV), biosynthesized flavonoid Ag NPs (FLV-Ag NPs), and Ag NPs on *Staphylococcus aureus*-infected silkworms. They indicated that these silkworms' hemocyte density was raised by the administration of FLV and FLV-Ag NPs, which explained their bactericidal activity against *S. aureus*. Furthermore, bacterial growth in control groups was noticeably higher than in those given biosynthesized FLV-Ag NPs, which prevented bacterial growth.

Research findings have shown that Ag NPs are harmful to bacteria and other pathogens, and they are well known for their appealing physicochemical properties and their antibacterial uses . In addition to the antimicrobial uses of Ag NPs, Ismail *et al*. (2021) reported that an antiviral assay using Ag NPs made with *Spirulina platensis* was successful in reducing *Bombyx mori* nuclear polyhedrosis virus (BmNPV) infection in silkworms by increasing hemocyte density. Invertebrates' increased hemocyte population suggests that a defence mechanism is in place to deal with invading cells. Quantum dots are used in biological imaging because they have distinct optical properties. They have a size-dependent cytotoxicity as well. To examine the variations in the immunological responses and programmed cell death generated in hemocytes, dosages of 32 mM of CdTe QDs, 1 g/l of citric acid-nitrogen-doped carbon dots (C-NCDs), and 0.39 g/l of Si NPs were administered to silkworms. It was demonstrated that Si NPs' induction of autophagy and apoptosis was reversed, and that experimental groups exposed to C-NCDs and CdTe QDs triggered haemocyte autophagy, apoptosis, and necrosis.

Rehman *et al*. (2021) investigated the effects of adding Si NPs to the silkworm's haemolymph. 3.9 g of Si NPs was more harmful to haemocytes than the groups exposed to 0.39 and 0.039 g of Si NPs. Since a large dose of Si NPs (3.9 g) did not result in a rise in ROS, it was hypothesised that the haematopoiesis would self-repair and that the damage to the hematopoietic tissues would be minimal. The effectiveness of employing silver nanoparticle colloids (AgNPC) to lessen BmNPV infection in silkworms was investigated by Sayed *et al*. (2020). When compared to the untreated control group, it was demonstrated that AgNPC were able to increase the silkworm survival rate from 22 to 67%. Free radicals were created as a result of the virus adhering to AgNps, and these radicals entered and destroyed the virus capsids, proteins, and DNA of BmNPVs. As a result, the pathogenicity of the virus was lessened and the infected silkworms' survival rate was increased.

The effects of nanomaterials on the silk fibers of silkworms:

Due to its gleaming look and superior mechanical qualities, silkworm silk is becoming more and more popular in the textile industry. Studies are being conducted in this field in order to improve the lustre and quality of silkworm silk. It has been suggested that feeding silkworms nanomaterials such as carbon nanotubes (CNTs), titanium dioxide, copper, and graphene will improve the mechanical characteristics and secondary structures of their silk. The mechanical characteristics, crystalline structure, and thermal stability of the silk fibre are typically taken into account while researching silk quality. Silk fibroin's inability to transition from a random coil/helix to a sheet conformation results in higher breaking elongation and toughness modules, which translate to good mechanical properties .

Nanoparticles affect silkworms and the silk fibres. In order to produce inherently changed silk fibres, Arunkumar *et al*. (2023) fed silkworms with artificial diets containing TiO_2 at various concentrations (1, 2, 3, and 4%). When compared to the control (mulberry feed), it was found that feeding silkworms an artificial diet containing 1% TiO_2 considerably improved the mechanical characteristics and ultraviolet resistance of the silk fibre. Breaking strength and UV resistance decreased as the concentration of TiO_2 in the artificial diet increased. The conformation change of silk fibroin from a random coil/helix to a sheet, which results in a more pronounced restricted crystallisation effect, was demonstrated to be constrained by 1% of TiO_2 NPs.

The secondary structure of the silk fibre was positively impacted by the modified diet of the silkworm, and the graphitic structure improved its electrical conductivity because single-walled nanotubes (SWNTs) and graphene prevented the conformation transition of silk fibroin from a random coil/helix to a sheet. The silk fibres were given the high mechanical strength, optical characteristics, and electrical conductivity of graphene. Additionally, it was shown that silkworms fed with a lesser concentration (0.2 wt%) of SWNTs had enhanced mechanical properties compared to those fed with graphene (2 wt%), which had the least improved mechanical qualities. Cheng *et al*. (2017) showed that Cu or Ag NPs added to the feed of silkworm larvae were absorbed into the silk fibres, improving their mechanical characteristics and encouraging the crystallisation of silk protein. According to Wu *et al*. (2017), silkworm silk containing copper had a good tensile strength of 360 MPa and a strain of 38%, which are 89% and 36% higher than the corresponding values for natural silk fibre (control) (Ma *et al*., 2019).

An improved crystallisation of the silk proteins had no effect on the crystalline structures of the BSA-Au NCs silk. Zhang *et al*. (2019) studied the effect of glucose-coated Ag NPs on the diet and protein synthesis of silkworm larvae. Glucose-coated Ag NPs applied to silk fibres resulted in smooth surfaces and considerable changes to the silk diameter. Ag NPs concentrations of 0.20 and 0.02% have been shown to enhance the mechanical, antibacterial, and protein synthesis of silk, as well as the mechanical properties of the silk fibres.

Conclusion

In this review, reports on the use of nanomaterials in sericulture were addressed, with a particular emphasis on the impacts on silkworm growth and development, silk fibre quality, and survival rates. The quality, tissue healing, and overall survival rate of the silkworm have all been reported to improve with the addition of nanomaterials via food or injections. Despite the favourable results of using nanomaterials on silkworms, it has been demonstrated that large doses of several nanomaterials, including Ag NPs, CdTe QDs, and Si NPs, cause an excessive amount of ROS to be produced. This produces oxidative stress, which results in cell death and autophagy. The impact of nanoparticles on the silk fibres was also covered in this review. According to reports, the presence of these nanoparticles improved the elongation and durability of the silk fibre and did not harm the silk's crystalline structure. Additionally, several nanoparticles showed therapeutic effects when exposed to sick silkworms. For instance, it has been suggested that feeding TiO2 NPs to silkworms will repair the harm that exposure to phoxim has done to the silk gland. Additionally, Ag

NPs increased the overall survival rate of BmNPV-infected silkworms.

It is critical to assess the effects of nanomaterials because of the ecosystem's potential toxicity. Experimental trials utilising nanomaterials with various sizes and concentrations must be conducted utilising silkworms as the animal model. In order to comprehend the interaction between NPs and silkworms, more nanomaterials must be utilised to investigate the recovery effects following an exposure of ill silkworms to nanoparticles.

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