Application of Nanotechnology in Sericulture: A review

Abstract

Nanoparticles (NPs) have emerged as promising tools for enhancing mulberry cultivation and the performance of silkworms, crucial for silk production. Mulberry, a vital plant for silk production, demands proper nutrient management for optimal growth and leaf production. Application of NPs, particularly zinc oxide and iron oxide, has shown significant improvements in mulberry growth parameters. Furthermore, NPs have demonstrated positive effects on silkworm larvae, enhancing feed efficiency and growth parameters, leading to better cocoon traits. Silkworms fed with NPs-treated mulberry leaves exhibited increased body weight, cocoon weight, and silk filament characteristics. Additionally, NPs have shown promise in enhancing silkworm reproduction and fecundity while conferring resistance against diseases like *Bm*NPV. Various NPs, including TiO2, silver and chitosan, have exhibited antibacterial properties against silkworm pathogens, thereby contributing to disease prevention. Overall, NPs offer a sustainable and effective approach to enhance mulberry cultivation, improve silkworm performance and mitigate disease risks, thus potentially revolutionizing silk production practices.

Keywords: Sericulture, Mulberry silkworm (*Bombyx mori*), Nanotechnology, Nanoparticle.

Introduction

The mulberry silkworm economically important insect due to its silk secretion. The production of quality cocoons attributes to nutrition of the silkworm Mulberry leaves serves as ideal nutritional food for silkworm, which expands the quality and quantity of cocoon production. The essential trace elements *viz*., iron, nickel, coper, manganese, potassium, zinc and iodine should be included in nutrition of silkworm growth. Mulberry leaves contains the vitamins which provides minimum requirement of silkworms and these vitamins varies on ecological conditions, fertilizer doses and mulberry varieties. “The mulberry leaf quality is influenced by variety of spacing’s, irrigation levels, nitrogen levels, seasons and the extra foliate that are supplied exogenously through mulberry leaves” (Triped *et al.,* 2009). Enrichment and fortification of mulberry leaves can be enhanced the quality and increase the quantity of cocoon ultimately silk productivity (Krishnaswami *et al.,* 1978).

Nanotechnology is quickly advancing, offering new paths to boost nutrition and revolutionize agriculture. It's gaining massive attention, revolving around tiny particles called nanoparticles, typically 1-100 nanometres in size, made from carbon, metals, metal oxides or organic materials [Hasan 2015]. "Nanoparticles are generally defined as particulate matter with at least one dimension that is less than 100 nm. This definition puts them in a similar size domain as that of ultrafine particles (air borne particulates) and places them as a sub-set of colloidal particles" (SCENIHR 2005). In 2008 the International Organization for Standardization (ISO) defined a nanoparticle as a discrete nano-object where all three Cartesian dimensions are less than 100 nm.

"Nanoparticle components include silica, Fe, ZnO, titanium dioxide, cerium oxide, aluminium oxide, gold nanorods, ZnCdSe/ ZnS core-shell, P/ZnS core-shell and Mn/ZnSe quantum dots. Nanoparticle size, content, concentration and chemistry greatly affect how effective nano-fertilizers are for plant growth. Nutrient release happens when these nano-fertilizers react with water in the soil". [Singh *et al*., 2021].

"Nanoparticle such as Metal oxides, AgO, MgO, ZnO and TiO_2 are inorganic nanomaterials, whereas lipids, polymers and CNTs are organic nanomaterials. Biodegradable, natural and agriculturally safe carriers, such as chitosan, called polymeric NPs" (Ghormade *et al*., 2011). "Owing to the polymeric cationic properties and the ability to interact with negatively charged molecules or polymers, chitosan is a promising agrochemical carrier. Diverse types of nanomaterials like copper, zinc, titanium, magnesium, gold and silver nanoparticles have arisen with effective antimicrobial efficacy against viruses, bacteria and other eukaryotic micro-organisms. Some nanomaterials possess antiviral, antibacterial and antifungal properties and have an excellent capacity to deal with pathogen-related diseases" (Castro *et al*., 2017, Makvandi *et al*., 2020).

Nanoparticles display distinctive physical, chemical and biological characteristics when compared to their counterparts at larger scales. This phenomenon arises from their comparatively larger surface area-to-volume ratio, heightened reactivity or stability in chemical processes, bolstered mechanical strength and other factors. [Assessment R 2007 cited by Elia 2017]. These properties of nanoparticles have led to use various applications including medicine, engineering, catalysis and environmental remediation. The inclusion of nanomaterial in sericulture are novel; therefore, it is imperative to know and exploit their effects on mulberry silkworms and silk productivity. This review endeavours to elucidate the impact of nanoparticles on the growth and development of mulberry plants and silkworms. It comprehensively examines relevant data and delves into how nanoparticles affect larval growth, cocoon productivity, and resistance to disease in silkworms. Moreover, it explores potential opportunities within the captivating realm of nanomaterials in sericulture.

UPTAKE MECHANISM OF NANOPARTICLES

"The process of uptake typically entails the passage of nutrients from the soil toward the root surface, the transport of ions via the membranes of root surface cells, the radial transport of ions into the root xylem vessels, the transport in the xylem and the distribution of ions in the aboveground parts of the plant" [Bowling, 1976]. Recent studies have focused on determining the total nutrient uptake over time, as well as the nutrient uptake of a particular root and its growth rate.

"After entering the soil, plant nanoparticles can take two routes through tissues: apoplast or symplast. Apoplastic transport moves water and substances through extracellular spaces, xylem vessels, and cell walls of neighboring cells, all outside the plasma membrane". (Sattelmacher, 2001). "In symplastic movement transportation takes place through the specialized structure called plasmodesmata" (Roberts and Oparka 2003). "Radial movement of nanoparticles within plant tissues was mainly triggered by apoplastic pathway which assists nanomaterials to reach the root central cylinder and the vascular tissues" (Larue *et al*. 2012; Zhao *et al*. 2012; Sun *et al*. 2014). "After reaching the central cylinder, nanoparticles move upward to the aerial part through xylem" (Cifuentes *et al*., 2010; Wang *et al*., 2013; Sun *et al*., 2014).

"In case of foliar applications, nanoparticles cross cuticular barrier by lipophilic or hydrophilic pathway" (Schonherr 2002). "In lipophilic pathway diffusion takes place through cuticular waxes, whereas in hydrophilic pathway dispersion through polar aqueous pores of cuticle or stomata takes place" (Eichert *et al*., 2008; Eichert and Goldbach, 2008).

APPLICATION OF NANOPARTICLES

Cancer therapy- Nanoparticles specifically target cancer cells, where they undergo either endocytosis or phagocytosis to eliminate the diseased cells.

Drug delivery -Nanoparticles enclosing drugs enhance the stability and solubility of the medication, facilitating targeted delivery to particular tissues or cells.

Antioxidant - Certain oxide nanoparticles exhibit properties similar to antioxidant molecules as a result of their inherent physicochemical characteristics.

Biomolecule detection -Nanoparticles adhere to biomolecules on their surface, enabling the detection of these biomolecules through bio-tagging or labeling techniques.

Plant growth -Nanoparticles can serve as nano-fertilizers by encapsulating fertilizers, leading to improved growth, yield and quality of crops due to positive morphological and biological effects.

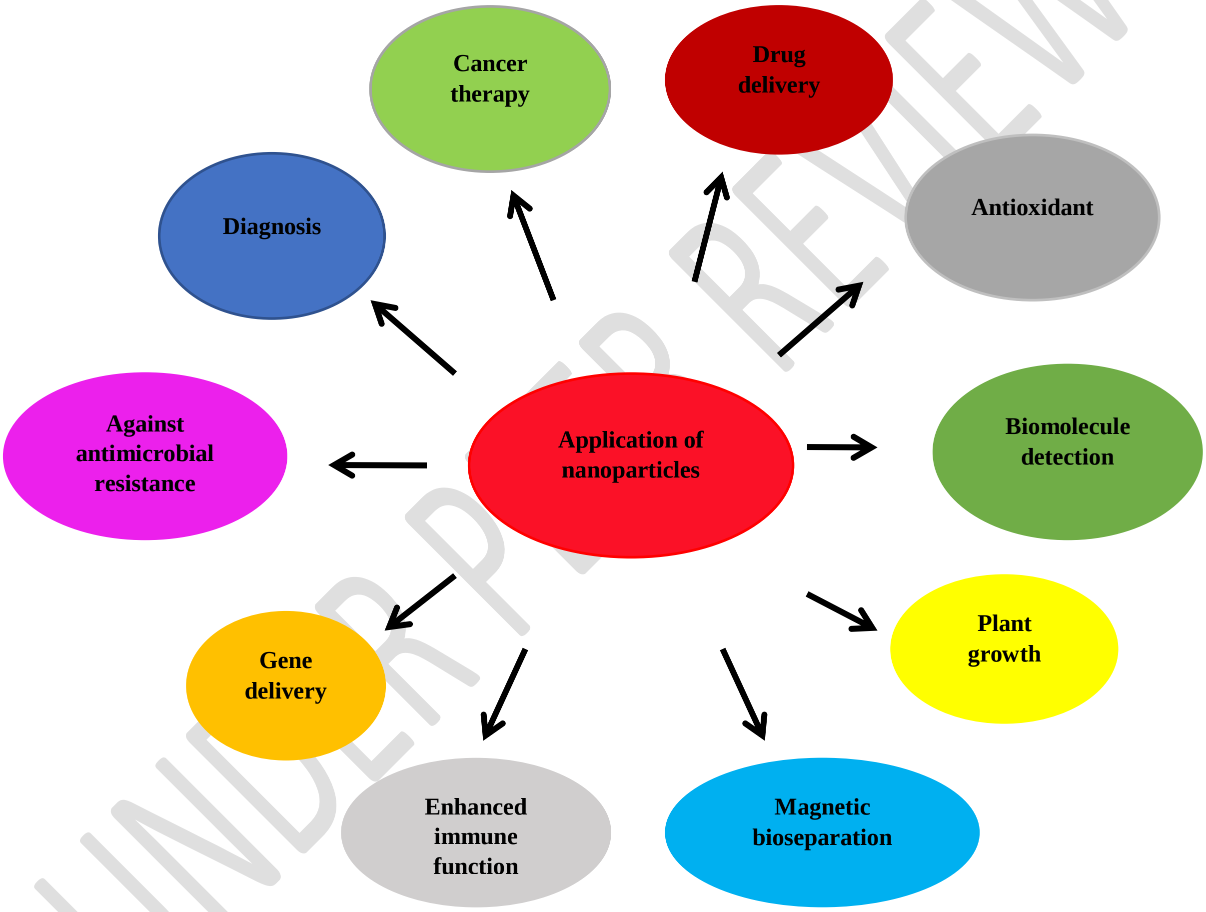

Fig 1. Application of nanoparticles in different fields

Magnetic bio-seperation - Magnetic nanoparticles selectively absorb the desired product onto their surface, facilitating its separation from the solution.

Enhanced immune function -Nanoparticles have the capability to trigger an immune response by functioning as adjuvants.

Gene delivery-Nanoparticles offer an alternative to vectors in the transfer of genes within the field of genetic engineering.

Against antimicrobial resistance- In combating antimicrobial resistance, nanoparticles surpass barriers due to their unique physio-chemical properties. These properties empower nanoparticles to employ diverse bactericidal pathways, thus achieving antimicrobial activity through novel means.

Diagnosis -Combining diagnostic and therapeutic agents within a single nanoparticle formulation.

EFFECT OF NANOPARTICLES ON MULBERRY

Nanoparticles as fertilizer for mulberry propagation

Mulberry is a high biomass producing, fast-growing, perennial, woody plant belonging to the genus Morus under the family Moraceae. Lu *et al*. (2003) suggested that, “a proper nutrient management is required for appropriate root establishment, growth and leaf production”. Hansch and Mendel (2009) stated that “micronutrients like iron, copper, zinc, manganese are required in very low amount, but presence of correct balance of these elements is essential for growth and quality leaf production”. Geetha *et al*. (2016) suggested that “in case of multi micronutrient deficiency in Mulberry, yield can be reduced even up to fifty percent. Chemical and chelated fertilisers must be applied in order to remedy the deficiency”. Chelated fertilisers are expensive and frequently used on crops with high value. Nanoparticles can be synthesized from plant extract, which aid in seed germination, pesticide residue degradation and improved soil quality. (Ghrair *et al*., 2010; El-Temsah *et al*., 2014).

Nithya *et al*. (2018) recorded “significantly highest shoot height (96.63 cm), number of branches per plant (8.47), number of leaves per shoot (18.60), number of leaves per plant (157.15), leaf area (96.90 cm^2) and leaf yield (0.46 kg /plant) in the foliar application of nano Zinc Oxide at 20 ppm in V-1 mulberry variety. They also reported that nano zinc fertilizer treatment to be cost effective with higher B:C ratio (2.93) and highest net returns per hectare as compared to $ZnSO_4$ fertilizer”. Das and Mandal (2020) reported that “nano-silver solution act as effective preservatives and enhance the activity of enzymatic and non-enzymatic antioxidants thereby reducing the harmful effect of accumulated free radicals and reactive oxygen species (ROS). Prevention of ROS generation helps in preventing plastid membrane peroxidation and thus maintaining chlorophyll content, extending the shelf life. They also reported that, the total chlorophyll, total protein, total sugar, reducing sugar, proline, total phenol, ascorbic acid, carotenoid and flavonoid contents were maximum after 7 days of leaf preservation in biosynthesized silver nanoparticles".

"Soil and foliar application of iron oxide nanoparticles and EDTA functionalized iron oxide nanoparticles on mulberry improves the overall growth parameters. The application of iron oxide nanoparticles @ 10 mg/kg in soil significantly improved sprouting percentage (82%), number of leaves (52.73% improved over control), plant biomass (37.20% and 90.24% increase of shoot and root biomass over control, respectively), root attributes (34% increment for root length) and also shortened the first leaf appearance period in mulberry" (Haydar *et al*., 2021). "Similarly, growth parameters, including shoot height, number of branches per plant, number of leaves per plant and total leaf area recorded in mulberry plant raised with foliar application of nano nitrogen fertilizer" (Pooja *et al*., 2022). Also, noted that increased maximum leaf yield and improved quality parameters, such as total carbohydrates, crude protein, crude fibre, chlorophyll content and leaf elemental compositions, with foliar application of nano nitrogen fertilizer.

EFFECT OF NANOPARTICLES ON LARVAE

Effect on larval Feed efficacy

"The feeding efficiency of silkworm larvae is important as it accounts for their growth rate and development. Low concentrations of nanoparticles (NPs) enhance larval body growth and feeding efficiency. Silkworm larvae fed with TiO_2 NPs (5 or 10 mg/L) improved the ingestion and digestibility of mulberry leaves, which significantly accelerated their body weight gain" [Li *et al*., 2016].

Prabhu *et al*., (2012) recorded "the superior feed efficacy *viz*, food consumption (gm) food utilization (gm) approximate digestibility (%) food consumption index (%) and Co-efficient of food utilization (%) in silkworm fed with MR2 mulberry leaves treated with 25 % silver nanoparticles solution". "Similarly feeding low concentration of TiO_2 NPs showed significantly improved the amount of ingested food (g/larvae), the amount of digestion (g/larvae) and percentage of ingested food (%) in silkworms" [Zhang *et al*. 2014; Li *et al*. 2016]. Riboflavin NPs fortified mulberry leaves enhanced the feed efficacy specially with higher production rate (mg/day) and metabolism rate (mg/day) in silkworm (Kamala and Karthikeyan 2019). Similarly, *Bombyx mori* L. larvae, pupae and cocoons showed improved body weight and shell weight as well as increased feeding efficiency when exposed to biosynthesized AgNPs (Surendra *et al*. 2023).

Effect on larval growth parameters

During the larval stages, silkworms feed on mulberry leaves which have all the required nutrients needed for their growth and development. Mulberry leaves treated with silver nanoparticles or along with spirulina led to significant increase in length and weight of fifth-instar silkworm larvae due to enhanced nutrition efficiency (Thangapandiyan and Dharanipriya, 2019; Meng *et al*., 2017). Similarly, Prabhu *et al.* (2011) reported the increased length, width and weight of 3rd, 4th and 5th instar larvae when fed with silver nanoparticles. Pandiarajan *et al*. (2016) reported that "the exposure of silkworm larvae to Ag NPs (1 ppm) improved the larval growth rate and the cocoon weight". Tian *et al*. (2016) focused on studying "the impact of TiO_2 NPs (5 mg/L) on the nutrient metabolism of the silkworm fat body. The treating mulberry leaves with TiO_2 NPs activated the insulin signalling pathway of the silkworm by enhancing the metabolism of carbohydrates, proteins and fat when compared to the control group".

Nithya (2018) found that larval weight and effective rate of rearing (ERR) increased after feeding with nano zinc oxide treated mulberry leaves. Also found that reduced Moulting duration (h) and 5th instar larval duration (days) with the same. Further, Pramila *et al*., (2019) reported that full grown larval weight (g/10), 5th instar larval duration (h), total larval duration (h) and ERR (%) significantly increased in silkworm fed with nano zinc along with nano copper. Pooja *et al*. (2022) reported that significant improvement in the larval traits of silkworm fed with mulberry leaves with foliar application of nitrogen nano-fertilizer.

Influence of nanoparticles on cocoon traits

Patil *et al*. (2016) stated that the silkworms fed with mulberry leaves treated with gold nanoparticles (300 ppm) were significantly superior in cocoon and reeling traits. Similarly, silkworms fed with nanoparticles of riboflavin treated mulberry leaves showed significantly highest cocoon weight, shell weight, pupa weight and shell ratio (Kamala and Karthikeyan, (2019). They also found that nanoparticles of riboflavin enhanced the denier, silk filament weight (g) and filament length(m). Further, silver nanoparticles or along with spirulina improved the cocoon weight (g), silk filament weight (g), sericin content (%) and fibroin content (%) (Thangapandiyan and Dharanipriya, 2019). The silkworms that were fed mulberry leaves treated with 50 ppm of spirulina-mediated TiO2NPs showed increased cocoon weight (g), shell ratio (%), silk filament length (m), filament weight (g), denier, and decreased rendita (kg). (Vasant *et al*. 2023). Similarly, Ag NPs, TiO_2 and Nano Zn along with CuNPs enhanced the economic traits of cocoons [Prabhu *et al*., 2011; Zhang *et al*., 2014; Li *et al*., 2016; Charya *et al*. 2019; Pramila *et al*., 2019].

According to Nithya (2018) "adequate supply of zinc nanoparticles which accelerates the activity of enzymes and auxin metabolism in the plants that increased the larval parameters, thereby cocoon parameters of silkworms". "Nano micronutrients might have stimulated the metabolic activities in silkworm resulting in better growth and development, resulting in production of good quality cocoons" (Pramila *et al*., 2019).

"By feeding silkworm with the carbon nanotube (CNT) obtained high strength silk fibre (SF) from silkworm. It proved that the stress, strain, conductivity and thermal stability of SF have been visibly enhanced, with the mechanical properties being comparable with those of super SF and even the spider silk fibre" (Wang *et al*. 2014). "The mechanical properties of the resulted silk were enhanced after feeding silkworms with MoO_2 nanoparticles" (Liang *et al*., 2020).

Influence of nanoparticles on reproduction and fecundity

"Feeding the silkworm with TiO_2 NPs is found to increase the metabolism of proteins and carbohydrates to meet the energy demand for growth and development of gonads. Silkworms exhibited the denser oocytes differentiation and formation in ovaries resulting high density of eggs, indicates that TiO_2 NPs not only increase the nutrient accumulation and transformation during the reproductive development but also improve the oviposition ability in *B. mori"* (Ni *et al*., 2015).

Influence of nanoparticles on resistance to silkworm diseases

Feeding TiO_2 NPs inhibits the proliferation of *Bm*NPV in silkworm larvae and improves larval survival rate and cocoon traits after *Bm*NPV infection (Xu *et al*., 2015; Zhao *et al*., 2020; Fometu *et al*. 2022). Das *et al*. (2013) found that silica nanoparticles (NP)-induced morphological transformation of *Bm*NPV polyhedra could reduce the infectivity of *Bm*NPV in silkworm larvae. Silver nanoparticle showed maximum zone of inhibition and lowest gut bacterial (*Bacilli sp.*) growth of larvae. (Li *et al*., 2013; Prabhu *et al*., 2012). Similarly, Silver nanoparticle of *P. Hornemannii* (100 µl) showed maximum zone of inhibition against *B. bassiana* (22.6 mm) and *M. anisopliae* (21.0 mm) (Ramamoorthy *et al*., 2019). By inhibiting reactive oxygen species (ROS), the Ag NPs activates the Toll-pathway in silkworm to boost humoral and cellular immunity against *S. aureus* infection (Rajasekhar reddy *et al*., 2017).

"Thymoquinone-Encapsulated Chitosan Nanoparticles (Tq-Chs NPs) showed an inhibitory impact against pathogenic bacteria infecting *Bombyx mori* larvae, proved their effects as antioxidant and anti-inflammatory agents which could be improved by loading Tq on Chs nanoparticles" (Hasan and Fahamy 2020). "The biosynthesized silver and chitosan nanoparticles at low concentration showed significance in the prevention of silkworm pathogens (1ppm, 10ppm, and 100 ppm)" (Pandiarajan *et al*., 2016; El-Adly 2022). "Chitosan at different concentrations showed antibacterial activity against the bacterial pathogens (*Bacillus spp*., *Micrococcus spp*. and *Serratia spp*.) of tropical tasar silkworm under in vitro conditions. The low concentration (0.2%) of chitosan from silkworm and chitosan NPs was identified as a minimum inhibitory concentration (MIC) against bacterial pathogens" (Priyadarshini *et al*., 2018; Madhusudhan *et al*., 2023). AgNPs synthesised using crude flower extract showed synergistic antibacterial activity against Flacherie and Sappe microbial strains, including *B. subtilis, S. aureus, E. coli, B. cereus, Aerobacter cloacae* and *S. typhi*, according to Surendra *et al*. (2023).

"These NPs have a large surface area to volume ratio which enhances the binding activity or saturation capacity of NPs which is predicted to increase binding to the microbe cell membrane and also destroy the cell wall structure" [Ahmad *et al*., 2013].

Most studies showed positive upshot NPs on silkworms at their lower concentration. In addition, the exposure of diseased silkworms to some nanomaterials also exhibited some therapeutic properties. Applied nanoparticles also exhibited greater impact on several biochemical and antioxidant enzymes attributes. These nanoparticles might be an ideal substitution for the traditional fertilizer and will be helpful in fortification of plants with nutritional value.

CONCLUSION: -

Sericulture implies rearing of silkworm for production of silk and ultimately its usage for textile and garment. Time has come to diversify it to make sericulture more sustainable, lucrative and remunerative one. Modern nano-technological advancement has assumed greater importance in the development of sericulture and its diversification. The science of nanotechnology and its application particularly in the area of enhancing mulberry leaf production, improvement in feed efficacy, development of diseases resistant breed and feed and synthesis of high-quality silk may take sericulture into a new height. The introduction of nanomaterials through diet has been reported to improve quality, tissue repair and the overall survival rate of the silkworm. The present paper, therefore, is a comprehensive document where an attempt to accumulate different reports and research findings on thrust areas of sericulture like spraying effect of NPs on phenotypic characteristics of mulberry, showing enhancement in biomass and different growth attributes and its influence on silkworm have been discussed which will prove to be useful for further development of the silk industry in coming years.

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