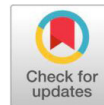


## Research Article

## Open Access

# Synthesis and Characterization of *Psoralea corlyfolia*-mediated titanium dioxide nanoparticles for Antiviral Activity



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## ABSTRACT

Titanium dioxide ( $TiO_2$ ) NPs have chemical properties such as high stability making, highly a perfect choice for photo-catalysts, antimicrobial agents, and in preservatives. Plant extracts may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles.  $TiO_2$  NPs are widely used in silkworms for disease resistance. In this present study, *Psoralea corlyfolia*-mediated  $TiO_2$  nanoparticles significantly influenced the growth of silkworm larvae and reduced the toxicity level of *BmNPV* infestation in treated larvae. The larval mortality was less in the untreated control (3.25 %) which is on par with Ankush (3.65 %) followed by  $TiO_2$  NPs at 75 ppm (4.90 %). The treatment  $TiO_2$  NPs 75 ppm recorded a cocoon weight of 2.23 g. The lowest cocoon weight was recorded in 100 ppm of  $TiO_2$  NPs (2.04 g).

**Keywords:** Titanium dioxide, Silkworms, *BmNPV*, *Psoralea corylifolia*

## Introduction

Nanotechnology has the ability to modify the properties of materials by altering their size, and this has prompted research towards a wide potential use of nanomaterials. Nanomaterials gained attention due to their ability for application in a broad spectrum in wide areas of biology and medicine. Metal nanoparticles are being explored for their use in biosensors, cell and biomolecule labels, and different pharmacological and cancer therapies in the biological area (Jain *et al.*, 2007). It is well known that the physiochemical properties of nanoparticles are strongly dependent on their interaction with binding molecules (Elechiguerra *et al.*, 2005).

The size of the drug nanoparticle and its surface characteristics can be modified to achieve the desired delivery characteristics (Chen *et al.*, 2007). Since the nanoparticle bound drug could not be able to circulate broadly, its negative consequences are limited and a high localized concentration can be achieved where it is needed. In view of the large surface area per unit mass of nanoparticles, the drug loading can be relatively high. Nanoparticle-bound drugs can be readily suspended in liquids and are able to penetrate deep in organs and tissues. Among various semiconductor nanoparticles, titanium dioxide ( $TiO_2$ ) nanomaterial has been broadly recognized as semiconductors as they are ideal components for environment and energy applications because of their identical properties (Mohamed, 2012).

Green synthesis of nanomaterials is an alternative way to physical and chemical methods which leads to eco-friendly

products because of the use of non-toxic materials does not involve high temperature and pressure and estimates lesser cost of production. Green synthesis includes microorganisms, plant extracts, and biomass of the plant (Mohamed *et al.*, 2019).

One of the most significant factors in the success of commercial sericulture is the management of silkworm diseases. To achieve a high quality and steady yield of cocoon, initially, pathogen load and pathogenicity should be reduced and then larval health is to be improved by boosting disease resistance. *Bombyx mori* Nuclear Polyhedrosis Virus (*BmNPV*) is a principal pathogen for the grasserie disease of the silkworm. During all the seasons, *BmNPV* infects all larval instars, but most typically the 4<sup>th</sup> and 5<sup>th</sup> instars, and causes 20-50 per-cent cocoon yield loss. The usage of plant-derived bio-molecules paved the way for minimizing the residual effect and spreading of virulent infections like *BmNPV* (Kumari *et al.*, 2011). The plants possessing antimicrobial and antiviral activities can be used in silkworm rearing which are non-toxic, non-hazardous, and eco-friendly in nature. The bio-molecules present in botanicals such as flavonoids, terpenoids, alkaloids and phenols are found to possess antimicrobial activity and fight against the viral pathogens and could be exploited for eco-friendly disease management in silkworms.

In recent years, the green synthesized nanoparticles opened up new path to treat viral diseases and there have been reports that  $TiO_2$  NPs will help in the recovery of silkworm injury caused by *Bombyx mori* Nuclear Polyhedrosis Virus (*BmNPV*). Titanium dioxide can enter into the body through ingestion, transversal injection or by direct injection into the body. Several research and discussions on the availability of titanium dioxide from the gastrointestinal tract are currently underway. Several research reports indicated that the lowest doses of  $TiO_2$  NPs does not produce apparent toxicity to bacteria, some organisms and mammalian cells (Jeng and Swanson, 2006). Several reports showed that  $TiO_2$  nanoparticles can induce the resistance of silkworm larvae to phoxim toxin (a type of pesticide toxin).

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Although, high doses of TiO<sub>2</sub> nanoparticles are toxic to mammals lowest doses of TiO<sub>2</sub> NPs benefits the growth and immune functions of silkworms (Li *et al.*, 2020).

## Materials and Methods

### Materials

Leaf samples of *Psoralea corylifolia* were collected and thoroughly washed with running tap water followed by rinsing with distilled water twice and shade dried at room temperature for two weeks. The dried plant leaves were ground into fine powder using a mixer and packed into air-tight containers and stored in normal conditions for future use.

### Preparation of TiO<sub>2</sub> Nanoparticles using botanical extract

Green synthesis of TiO<sub>2</sub> nanoparticles was performed as described earlier by Krishnasamy *et al.* (2015) with modifications. Soxhlet apparatus was used for extraction purpose. 25 g of the powdered leaves of *P. corylifolia* were weighed into 250 ml of chloroform and percolated overnight. The sample tube of the unit was fitted with a filter disc at the bottom and filled with ground samples, sealed with another filter disc and compressed. This was fitted to an electric heating mantle with soxhlet unit, filled with 250 ml chloroform and a temperature of 60°C was maintained for 6 hours.

0.1 mM titanium dioxide aqueous solution was prepared in a beaker and stirred using a magnetic stirrer for two hours to dissolve the powder TiO<sub>2</sub> into water completely. 10 ml of the *P. corylifolia* leaf extract was added to 90 ml of the prepared titanium dioxide solution and stirred at room temperature for 24 hours. After 24 hours, the resultant mixture formed the TiO<sub>2</sub> nanoparticles were used for the study.

### Characterization of nanoparticles

Nanoparticles are generally characterized by their size, shape, surface area, and dispersity (Jiang *et al.*, 2009). The Homogeneity of these properties is important in many applications. The common techniques of characterizing nanoparticles are as follows; Fourier transform infrared spectroscopy (FT-IR), powder X-ray diffraction (XRD), Scanning electron microscopy (SEM), and Transmission electron microscopy (TEM). These studies were carried out at the Department of Nanoscience and Technology, TNAU, Coimbatore, Tamil Nadu.

### FT-IR analysis

FT-IR spectroscopy is useful for characterizing the surface chemistry (Chithrani *et al.*, 2006). Organic functional groups (e.g., carbonyls, hydroxyls) attached to the surface of nanocomposites and the other surface chemical residues are detected using FT-IR.

### Powder X-ray diffraction (XRD) analysis

XRD was used for the phase identification and characterization of the crystal structure of the nanocomposites (Sun *et al.*, 2000). X-rays penetrate into the nanomaterial and the resulting diffraction pattern was compared with standards to obtain structural information.

### Scanning Electron Microscopy (SEM) with EDAX analysis

Electron microscopy is another commonly used method of characterization. Scanning electron microscopy and Transmission electron microscopy were used for morphological characterization at the nanometer to micrometer scale (Schaffer *et al.*, 2009).

### Transmission Electron Microscopy (TEM) analysis

Transmission electron microscopy has a 10,000-fold highest resolution compared with scanning electron microscopy and is used to characterize the individual particles of the sample.

### Nanoparticles evaluation under silkworm rearing against silkworm pathogens

First and second instar silkworm larvae were reared with fresh mulberry foliage. From the third instar, silkworms were reared in experimental conditions with 50 larvae per replication. *BmNPV* was induced at the first day of the third instar. The *Psoralea corylifolia* mediated TiO<sub>2</sub> nanoparticles were used for treatments with different concentrations, viz., 25, 50, 75, and 100 ppm at the second day of third and first day of the fourth instar. Ankush (a general bed disinfectant) for grasserie pathogen were used as check. During this period, all the economic parameters were recorded.

## Results and Discussion

In the present study, *Psoralea corylifolia* biosynthesized titanium dioxide nanoparticles were characterized and confirmed through FTIR, XRD, SEM and TEM analysis. The investigation revealed the formation of TiO<sub>2</sub> nanoparticles. The FTIR analysis of *P. corylifolia* mediated nanoparticles alkanes; halogen compound amyl disulfides S-S stretching is obtained. This confirms the nanoparticles are loaded with plant phytoconstituents.

### Fourier Transform Infra-Red Spectroscopy analysis

FT-IR spectrum of biosynthesized TiO<sub>2</sub>NPs was recorded and their functional groups with peak values are presented in Plate 1 and Table 1. In TiO<sub>2</sub>NPs, the peak value 3332 cm<sup>-1</sup> corresponds to the alcohols group (O-H stretching). C-O stretching (vibration) shows the presence of phenol group with a peak value 1388 cm<sup>-1</sup>. Halogen compound C-Cl stretching lies in the frequency range of 800-600 cm<sup>-1</sup> with the peak values 671 and 601 cm<sup>-1</sup>. S-S stretching vibration has aryl disulfide group in the range of 500-430 and peaks are 478 and 455 cm<sup>-1</sup>. Similarly, Dobrucka (2017) used FTIR spectroscopy to determine different groups on *Echinaceapurpurea* powder and their role in nanoparticle synthesis, The FTIR spectrum of TiO<sub>2</sub> NPs revealed the characteristic bands which indicates the presence of C-O stretching alcohols, carboxylic acids, esters and ethers. Also, C-H rock alkenes, saturated hydrocarbons C=C, O-H bonded alcohols and phenols.

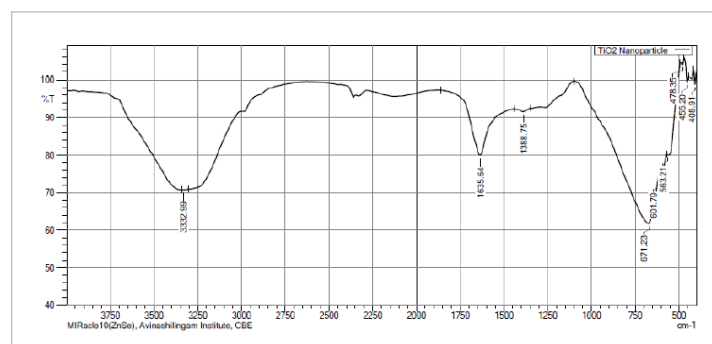


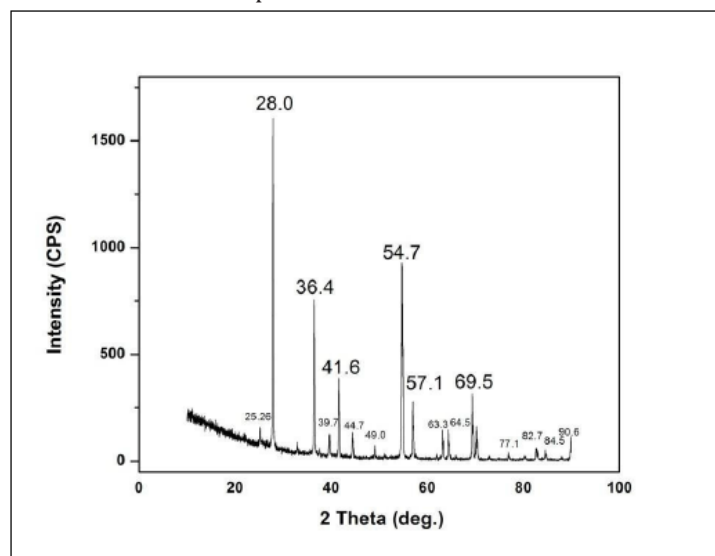
Plate 1. FT-IR spectra of *Psoralea corylifolia*-mediated TiO<sub>2</sub> nanoparticles

**Table 1. Characteristic absorbance frequencies of functional groups for TiO<sub>2</sub> nanoparticle under FT-IR analysis**

Functional groups	Vibration and intensity	Frequency in (cm <sup>-1</sup> )	Peak value
Alcohols	O-H str, sh	3570-3450	3332.99
Phenols	C-O str,s	1400-1310	1388.75
Halogen compound	C-Cl str, s	800-600	671.23
		601.79	
Aryl disulfides	S-S str	500-430	478.35
		455.20	

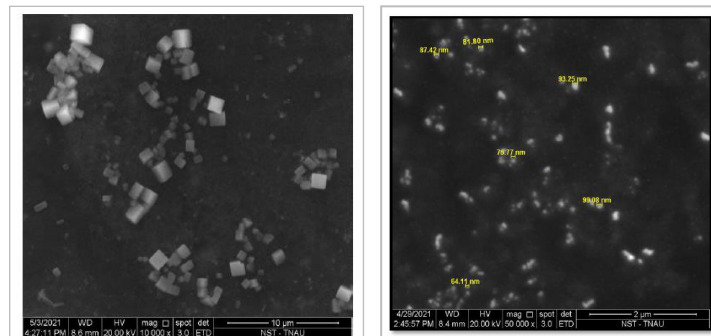
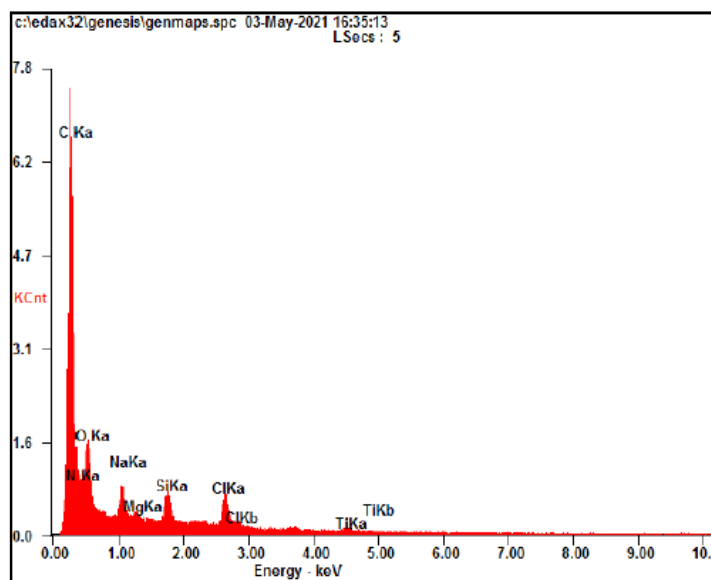
### Powder X-ray diffraction (XRD) analysis

The XRD pattern of *Psoralea corylifolia* mediated TiO<sub>2</sub> NPs revealed the crystallinity patterns which corresponds to the anatase and rutile phases of titanium dioxide nanoparticles. Rajeshkumar (2019) analyzed the *Cassia fistula*-mediated TiO<sub>2</sub> NPs using XRD and confirmed the crystalline nature and particle size of the NPs where he observed the Face- centered cubic (FCC) structure of the NPs showing weak broadened wurtzite structure as earlier reported by Vijayakumar *et al.* (2017). In our study, the fine sample of TiO<sub>2</sub> nanoparticle was placed on the XRD grid, and the crystallinity was determined (Plate 2). The peaks appeared at 2θ value ranging from 25 to 90°. The XRD patterns of the biosynthesized titanium nanoparticles were observed at 25.26, 28.0, 36.4, 39.7, 41.6, 44.7, 49.0, 54.7, 57.1, 63.3, 64.5, 69.5, 77.1, 82.7, 84.7 and 90.6°. The observed patterns correspond to the anatase and rutile structure of titanium dioxide nanoparticles.

**Plate 2. XRD analysis of *Psoralea corylifolia* mediated titanium dioxide nanoparticles**

### Scanning Electron Microscopy (SEM) with EDAX analysis

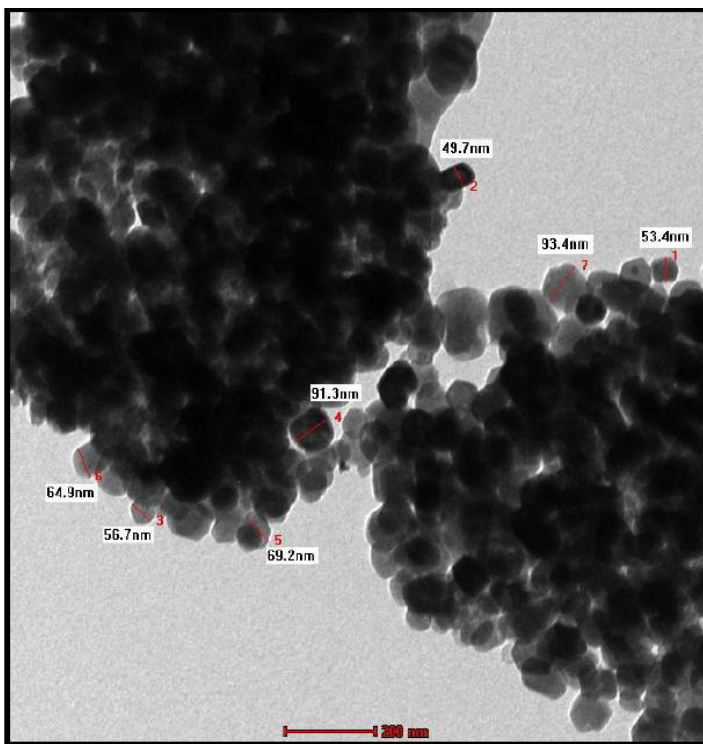
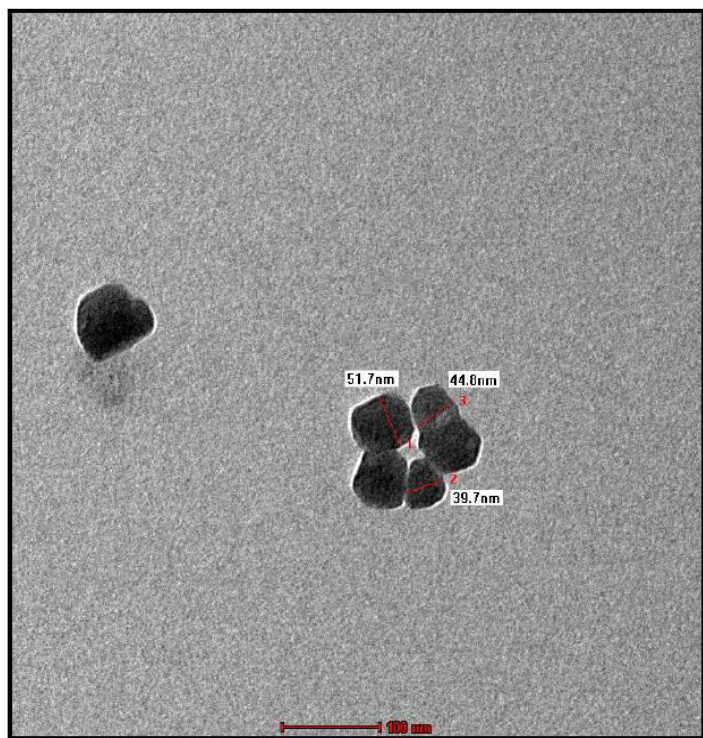
The SEM investigation of biologically synthesized TiO<sub>2</sub> NPs was in cubic structure distributed evenly with sizes ranging from 64 to 99 nm (Plate 3a). The energy dispersive X-Ray Spectroscopy (EDAX) analysis shows the elemental composition, which is present in the TiO<sub>2</sub> NPs (Plate 3b). The titanium peaks appeared at 4 to 5 keV, carbon peaks at 0.5 keV, oxygen peaks at 0.5 to 1.0 keV, silica peaks at 1.8 to 2.0 keV, and various metal oxides are found and the presence of Carbon (C) carbon, oxygen, magnesium and chlorine indicates the presence of biomolecules adsorbed on the surface of titanium nanoparticles. The energy dispersive analysis proves that the particles are crystalline in nature and indeed metallic TiO<sub>2</sub> NPs. Similarly, Krishnasamy *et al.* (2015) biosynthesized *Azadirachta indica* leaf extract-mediated nanoparticles and analyzed for FESEM images. The results indicated the biosynthesized titanium nanoparticles were spherical in shape and their size ranged from 25 to 87 nm.

**Plate 3a. SEM images of *Psoralea corylifolia* mediated titanium dioxide nanoparticles****Plate 3b. SEM with EDAX element of TiO<sub>2</sub> nanoparticles**

### Transmission Electron Microscopy (TEM) analysis

Transmission electron microscopy (TEM) has a 10000-fold highest resolution compared with scanning electron microscopy. The TEM images and size distribution of talc/Fe<sub>3</sub>O<sub>4</sub> nanocomposites showed that the mean diameter of the nanoparticles existed from 1.2 to 3.2 nm (Kalantari *et al.*, 2013).

Hariharan *et al.* (2017) revealed the morphology, crystallinity, and size of the green synthesized TiO<sub>2</sub> NPs by TEM images where the shape of the nanoparticles was hexagonal to irregular in shape with moderate variation in size ranging from 13-34 nm. In our study, the morphology, crystallinity and size of the biologically synthesized TiO<sub>2</sub> NPs were determined and the shape of the nanoparticles was hexagonal and irregular in shape with moderate variation in size ranging from 30 to 90 nm (Plate 4).



**Plate 4. TEM images of *Psoralea corylifolia* mediated TiO<sub>2</sub> nanoparticles**

### Evaluating the effects of *Psoralea corylifolia* mediated titanium dioxide nanoparticles

#### (TiO<sub>2</sub> NPs) on *BmNPV* treated silkworm, *Bombyx mori*

In recent years, nanoparticles emerged as new route to treat viral diseases and there were reports that titanium dioxide nanoparticles have ability to treat the injury of silkworms caused by *BmNPV* (Das *et al.*, 2013). Periodical bed cleaning and providing good quality leaves will help in reducing the spread of diseases (Nirupama, 2014). The results of the experiment to evaluate the effects of TiO<sub>2</sub> NPs of *BmNPV* treated silkworm larvae with different treatment concentrations, *viz.*, TiO<sub>2</sub> NPs at 25, 50, 75 and 100 ppm with Ankush as the standard check is described below.

In this present study, the TiO<sub>2</sub> NPs greatly influenced the growth of the larvae. The larval mortality was less in the untreated control (3.25 %) which is on par with Ankush (3.65 %) followed by TiO<sub>2</sub> NPs at 75 ppm (4.90 %). (Table 2). The larval weight was significantly influenced by the mulberry leaves treated with TiO<sub>2</sub> NPs during rearing (Table 2). The treatment Ankush and untreated control recorded the larval weight of 3.85 g and 3.89 g followed by TiO<sub>2</sub> NPs at 75 ppm (3.81 g), 50 ppm (3.75 g), 25 ppm (3.69 g) and 100 ppm (3.61 g) respectively. Larval duration was less in the untreated control (207.00 h) which is on par with Ankush (211.00 h). This was followed by TiO<sub>2</sub> NPs 75 ppm (215.00 h), 50 ppm (218.00 h) (Table 2). The cocoon weight was greatly influenced by the mulberry leaves treated with TiO<sub>2</sub> NPs during rearing. The treatment TiO<sub>2</sub> NPs 75 ppm recorded cocoon weight of 2.23 g. The lowest cocoon weight was recorded in 100 ppm of TiO<sub>2</sub> NPs (2.04 g) (Table 3). Gobena and Bhaskar (2015) conducted an experiment and revealed that *Psoralea corylifolia* treated mulberry leaves fed silkworms showed maximum effective rate of rearing (ERR) (98.33 and 100.00 %), cocoon weight (18.06 and 18.79 g/10 worms), pupal weight (14.30 and 15.23 g/10 worms), shell weight (3.68 and 3.72 g/10 worms) and cocoon shell ratio (20.38 and 19.80 %) for the first and second rearing respectively. In this study, the shell weight of *BmNPV* treated larvae was greatly influenced by the mulberry leaves treated with TiO<sub>2</sub> NPs during rearing. The untreated control recorded highest shell weight of 0.44 g followed by Ankush (0.40 g) which is on par with TiO<sub>2</sub> NPs 75 ppm (0.38 g) and 50 ppm (0.36 g). The lowest shell weight was observed in TiO<sub>2</sub> NPs 100 ppm (0.28 g) respectively (Table 3). Similarly, the untreated control recorded a maximum shell ratio of 18.48 % which is on par with Ankush (17.39 %) and TiO<sub>2</sub> NPs 75 ppm (17.04 %). This was followed by TiO<sub>2</sub> NPs 50 ppm (16.58 %) and 25 ppm (16.11 %) (Table 3). Xu *et al.* (2015) demonstrated that feeding TiO<sub>2</sub> NPs to silkworms increases the disease resistance in silkworms against *BmNPV* by strengthening immunity and suppressing viral proliferation in silkworms. The study carried out by Li; Ni; *et al.* (2016) using TiO<sub>2</sub> NPs proved that the TiO<sub>2</sub> NPs treated silkworms (5 and 10 mg/L) had shown improved feeding efficiency and increased cocoon weight, shell weight, and shell ratio.

**Table 2. Effect of TiO<sub>2</sub> nanoparticles application on larval characters of BmNPV infected B. mori**

No.	Treatment	Larval mortality (%)	Larval weight (g)	Larval duration (hrs)
T <sub>1</sub>	TiO <sub>2</sub> Nanoparticles @ 25 ppm	6.65 <sup>ab</sup>	3.69 <sup>ab</sup>	221.00 <sup>bc</sup>
T <sub>2</sub>	TiO <sub>2</sub> Nanoparticles @ 50 ppm	5.25 <sup>ab</sup>	3.75 <sup>ab</sup>	218.00 <sup>abc</sup>
T <sub>3</sub>	TiO <sub>2</sub> Nanoparticles @ 75 ppm	4.90 <sup>ab</sup>	3.81 <sup>ab</sup>	215.00 <sup>abc</sup>
T <sub>4</sub>	TiO <sub>2</sub> Nanoparticles @ 100 ppm	9.45 <sup>b</sup>	3.61 <sup>b</sup>	225.00 <sup>c</sup>
T <sub>5</sub>	Ankush	3.65 <sup>a</sup>	3.85 <sup>ab</sup>	211.00 <sup>ab</sup>
T <sub>6</sub>	Treated control (BmNPV)	90.00 <sup>c</sup>	1.74 <sup>c</sup>	248.00 <sup>d</sup>
T <sub>7</sub>	Untreated control	3.25 <sup>a</sup>	3.89 <sup>a</sup>	207.00 <sup>a</sup>
	<b>SEd</b>	<b>2.40</b>	<b>0.13</b>	<b>5.72</b>
	<b>CD (0.05)</b>	<b>5.15</b>	<b>0.27</b>	<b>12.27</b>

In a column means followed by a common letter(s) are not significantly different by LSD (0.05).

**Table 3. Effect of TiO<sub>2</sub> nanoparticles application on cocoon characters of BmNPV infected B. mori**

No.	Treatment	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
T <sub>1</sub>	TiO <sub>2</sub> Nanoparticles @ 25 ppm	2.11 <sup>cd</sup>	0.34 <sup>d</sup>	16.11 <sup>b</sup>
T <sub>2</sub>	TiO <sub>2</sub> Nanoparticles @ 50 ppm	2.17 <sup>bcd</sup>	0.36 <sup>cd</sup>	16.58 <sup>b</sup>
T <sub>3</sub>	TiO <sub>2</sub> Nanoparticles @ 75 ppm	2.23 <sup>abc</sup>	0.38 <sup>bc</sup>	17.04 <sup>ab</sup>
T <sub>4</sub>	TiO <sub>2</sub> Nanoparticles @ 100 ppm	2.04 <sup>d</sup>	0.28 <sup>e</sup>	13.72 <sup>c</sup>
T <sub>5</sub>	Ankush	2.30 <sup>ab</sup>	0.40 <sup>b</sup>	17.39 <sup>ab</sup>
T <sub>6</sub>	Treated control (BmNPV)	1.20 <sup>e</sup>	0.14 <sup>f</sup>	11.66 <sup>d</sup>
T <sub>7</sub>	Untreated control	2.38 <sup>a</sup>	0.44 <sup>a</sup>	18.48 <sup>a</sup>
	<b>SEd</b>	<b>0.08</b>	<b>0.02</b>	<b>0.77</b>
	<b>CD (0.05)</b>	<b>0.17</b>	<b>0.03</b>	<b>1.65</b>

In a column means followed by a common letter(s) are not significantly different by LSD (0.05).

## References

- Chen, Yan, J Vellore Mohanraj, Fang Wang, and AE Heather Benson. 2007. "Designing chitosan-dextran sulfate nanoparticles using charge ratios." *Aaps Pharmscitech*. 8 (4):131-139.
- Chithrani, B Devika, A Arezou Ghazani, and CW Warren Chan. 2006. "Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells." *Nano letters*. 6(4):662-668.
- Das, Sumistha, Ankita Bhattacharya, Nitai Debnath, Alokmay Datta, and Arunava Goswami. 2013. "Nanoparticle-induced morphological transition of *Bombyx mori* nucleopolyhedrovirus: A novel method to treat silkworm grasserie disease." *Applied microbiology and biotechnology*. 97 (13):6019-6030.
- Dobrucka, Renata. 2017. "Synthesis of Titanium dioxide nanoparticles using *Echinacea purpurea herba*." *Iranian journal of pharmaceutical research*. 16 (2):756.
- Elechiguerra, Jose Luis, L Justin Burt, R Jose Morones, Alejandra Camacho-Bragado, Xiaoxia Gao, Humberto H Lara, and Miguel Jose Yacaman. 2005. "Interaction of silver nanoparticles with HIV-1." *Journal of nanobiotechnology*. 3 (1):1-10.
- Gobena, Waktole Sori, and RN Bhaskar. 2015. "Fortification of mulberry leaves with medicinal botanical plant extracts effect on silkworm, *Bombyx mori* L. (PM × CSR2) (Lepidoptera: Bombycidae) larval growth and cocoon traits." *Journal of Biological Sciences*. 15 (4):199-206.
- Hariharan, D, K Srinivasan, and LC Nehru. 2017. "Synthesis and characterization of TiO<sub>2</sub> nanoparticles using *Cynodon dactylon* leaf extract for antibacterial and anticancer (A549 Cell Lines) Activity." *Journal of Nanomedicine Research*. 5 (6):1-5.
- Jain, K Prashant, Wenyu Huang, and A Mostafa El-Sayed. 2007. "On the universal scaling behavior of the distance decay of plasmon coupling in metal nanoparticle pairs: A plasmon ruler equation." *Nano letters*. 7 (7):2080-2088.
- Jeng, Hueiwang Anna, and James Swanson. 2006. "Toxicity of metal oxide nanoparticles in mammalian cells." *Journal of Environmental Science and Health Part A*. 41 (12):2699-2711.
- Jiang, Jingkun, Günter Oberdörster, and Pratim Biswas. 2009. "Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies." *Journal of Nanoparticle Research*. 11 (1):77-89.
- Kalantari, Katayoon, Mansor Bin Ahmad, Kamyar Shameli, and Roshanak Khandanlou. 2013. "Synthesis of talc/Fe<sub>3</sub>O<sub>4</sub> magnetic nanocomposites using chemical co-precipitation method." *International journal of nanomedicine*. 8:1817.
- Krishnasamy, Anbalagan, Sundaresan Mohanraj, and Velan Pugalenti. 2015. "Rapid phytosynthesis of nano-sized titanium using leaf extract of *Azadirachta indica*." *International Journal of ChemTech Research*. 8 (4):2047-2052.

13. Kumari, S Saritha, RN Bhaskar, and S Latha. 2011. "In-vivo effect of medicinal botanicals on rearing parameters of the silkworm, PMx CSR2." *Current Biotica*.5 (1):40-45.
14. Li, Mengxue, Fanchi Li, Zhengting Lu, Yilong Fang, Jianwei Qu, Tingting Mao, Hui Wang, Jian Chen, and Bing Li. 2020. "Effects of TiO<sub>2</sub> nanoparticles on intestinal microbial composition of silkworm, *Bombyx mori*." *Science of the Total Environment*.704:135-273.
15. Li, YangYang, Min Ni, FanChi Li, Hua Zhang, KaiZun Xu, XiaoMing Zhao, JiangHai Tian, JingSheng Hu, BinBin Wang, and WeiDe Shen. 2016. "Effects of TiO<sub>2</sub> NPs on silkworm growth and feed efficiency." *Biological trace element research*. 169 (2):382-386.
16. Mohamed, RM. 2012. "UV-assisted photocatalytic synthesis of TiO<sub>2</sub>-reduced graphene oxide with enhanced photocatalytic activity in decomposition of sarin in gas phase." *Desalination and Water Treatment*.50 (1-3):147-156.
17. Mohamed, Hamza Elsayed Ahmed, Shakeeb Afridi, Ali Talha Khalil, Dania Zia, Javed Iqbal, Ikram Ullah, Zabta Khan Shinwari, and Malik Maaza. 2019. "Biosynthesis of silver nanoparticles from *Hyphaene thebaica* fruits and their in vitro pharmacognostic potential." *Materials Research Express*.6 (10):1050.
18. Nirupama, R. 2014. "Screening and identification of silkworm breeds of *Bombyx mori* L. for their resistant and susceptible against white muscardine." *Munis Entomol. Zool*.9:510-517.
19. Rajeshkumar, S. 2019. "Antifungal impact of nanoparticles against different plant pathogenic fungi." In *Nanomaterials in Plants, Algae and Microorganisms*, 197-217. Elsevier.
20. Schaffer, Bernhard, Ulrich Hohenester, Andreas Trügler, and Ferdinand Hofer. 2009. "High-resolution surface plasmon imaging of gold nanoparticles by energy-filtered transmission electron microscopy." *Physical Review B*.79 (4):401-410.
21. Sun, Shouheng, Christopher B Murray, Dieter Weller, Liesl Folks, and Andreas Moser. 2000. "Monodisperse FePt nanoparticles and ferromagnetic FePt nanocrystal superlattices." *science*287 (5460):1989-1992.
22. Vijayakumar, Sekar, Balasubramanian Malaikozhundan, Sathappan Shanthi, Baskaralingam Vaseeharan, and Nooruddin Thajuddin. 2017. "Control of biofilm forming clinically important bacteria by green synthesized ZnO nanoparticles and its ecotoxicity on *Ceriodaphnia cornuta*." *Microbial pathogenesis*107:88-97.
23. Xu, Kaizun, Fanchi Li, Lie Ma, Binbin Wang, Hua Zhang, Min Ni, Fashui Hong, Weide Shen, and Bing Li. 2015. "Mechanism of enhanced *Bombyx mori* nucleopolyhedrovirus-resistance by titanium dioxide nanoparticles in silkworm." *PLoS One*10 (2):e0118222.