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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_2NPs 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 theuntreated 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_2NPs (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 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 (*Bm*NPV) is a principal pathogen for the grasserie disease of the silkworm. During all the seasons, *Bm*NPV infects all larval instars, but most typically the 4^{th} and 5^{th} 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 *Bm*NPV (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 (*Bm*NPV). 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_2 nanoparticles are toxic to mammals lowest doses of TiO_2 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_2 Nanoparticles using botanical extract

Green synthesis of TiO_2 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 60ºC 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_2 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_2 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. *Bm*NPV was induced at the first day of the third instar. The *Psoralea corylifolia* mediated TiO_2 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_2 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_2NPs was recorded and their functional groups with peak values are presented in Plate 1 and Table 1. In TiO_2NPs, the peak value 3332 cm^{-1} corresponds to the alcohols group (O-H stretching). C-O stretching (vibration) shows the presence of phenol group with a peak value 1388 $cm^{-1.}$ Halogen compound C-Cl stretching lies in the frequency range of 800-600 cm^{-1} with the peak values 671 and 601 cm^{-1}. S-S stretching vibration has aryl disulfide group in the range of 500-430 and peaks are 478 and 455 cm^{-1}. Similarly, Dobrucka (2017)used FTIR spectroscopy to determine different groups on *Echinaceapurpurea* powder and their role in nanoparticle synthesis, The FTIR spectrum of TiO_2 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.

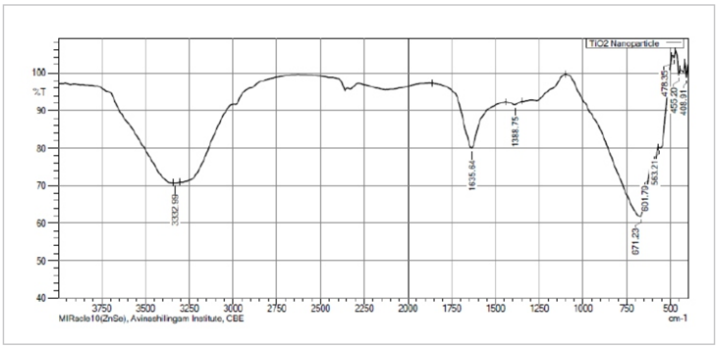

Plate 1. FT-IR spectra of Psoralea corylifolia-mediated TiO2 nanoparticles

Table 1. Characteristic absorbance frequencies of functional groups for TiO_2 nanoparticle under FT-IR analysis

Functional groups	Vibration and intensity	Frequency in (cm^{-1})	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_2 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_2 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_2 nanoparticle was placed on the XRD grid, and the crystallinity was determined (Plate 2). The peaks appeared at 2θ value ranging from 25 to 90º. 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 90.6º. The observed patterns correspond to the anatase and rutile structure of titanium dioxide nanoparticles.

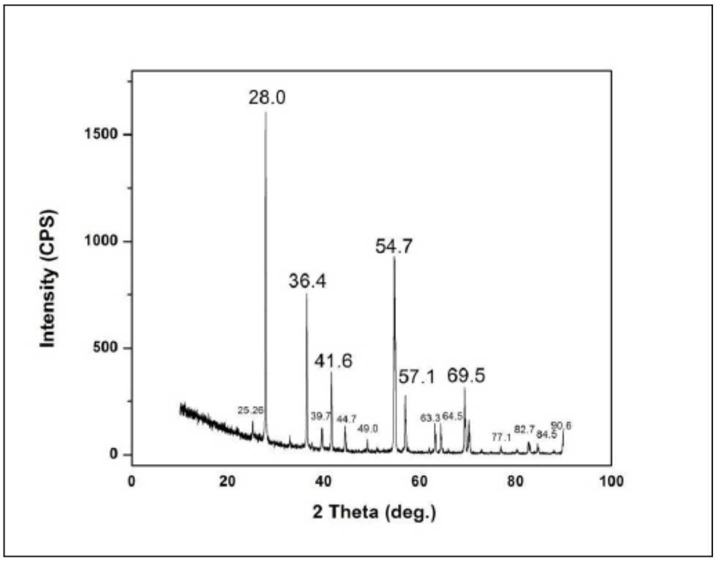

Plate 2. XRD analysis of Psoralea corylifolia mediated titanium dioxide nanoparticles

Scanning Electron Microscopy (SEM) with EDAX analysis

The SEM investigation of biologically synthesized TiO_2 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_2NPs (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_2 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.

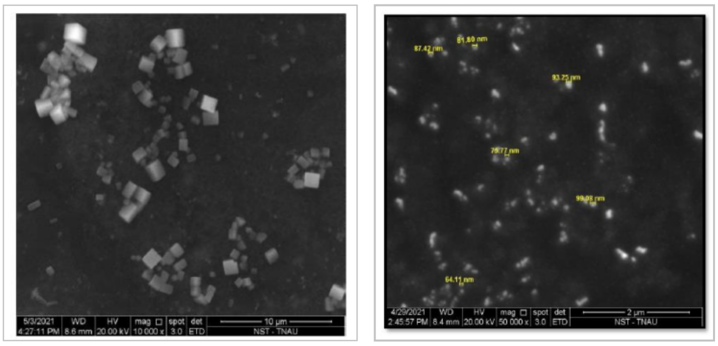

Plate 3a. SEM images of Psoralea corylifolia mediated titanium dioxide nanoparticles

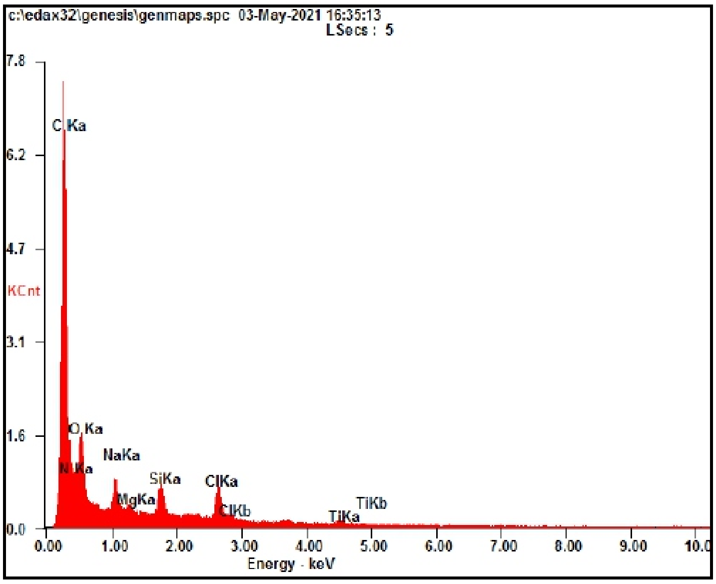

Plate 3b. SEM with EDAX element of TiO2 nanoparticles

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/Fe3O4 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_2 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_2 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).

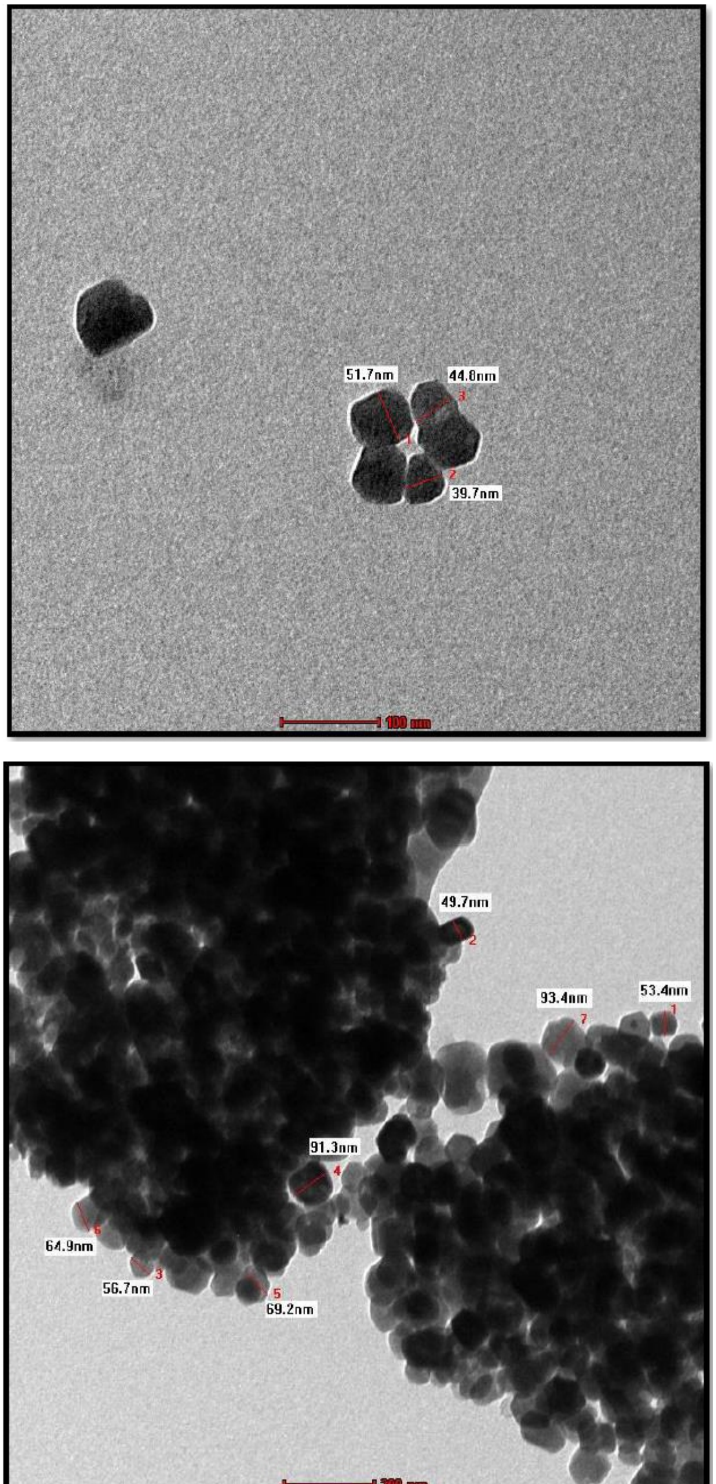

Plate 4. TEM images of Psoralea corylifolia mediated TiO2 nanoparticles

Evaluating the effects of *Psoralea corylifolia* mediated titanium dioxide nanoparticles (TiO_2 NPs) on *Bm*NPV 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 *Bm*NPV (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_2 NPs of *Bm*NPV treated silkworm larvae with different treatment concentrations, *viz.,*TiO_2 NPs at 25, 50, 75 and 100 ppm with Ankush as the standard check is described below.

In this present study, the TiO_2 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_2 NPs at 75 ppm (4.90 %). (Table 2). The larval weight was significantly influenced by the mulberry leaves treated with TiO_2 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_2 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_2 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_2 NPs during rearing. The treatment TiO_2 NPs 75 ppm recorded cocoon weight of 2.23 g. The lowest cocoon weight was recorded in 100 ppm of TiO_2 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 *Bm*NPV treated larvae was greatly influenced by the mulberry leaves treated with TiO_2 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_2 NPs 75 ppm (0.38 g) and 50 ppm (0.36 g). The lowest shell weight was observed in TiO_2 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_2 NPs 75 ppm (17.04 %). This was followed by TiO_2 NPs 50 ppm (16.58 %) and 25 ppm (16.11 %) (Table 3). Xu *et al.* (2015)demonstrated that feeding TiO_2 NPs to silkworms increases the disease resistance in silkworms against *Bm*NPV by strengthening immunity and suppressing viral proliferation in silkworms. The study carried out by Li; Ni*; et al.* (2016) using TiO_2 NPs proved that the TiO_2 NPs treated silkworms (5 and10 mg/L) had shown improved feeding efficiency and increased cocoon weight, shell weight, and shell ratio.

Table 2. Effect of TiO_2 nanoparticles application on larval characters of BmNPV infected B. mori

No.	Treatment	Larval mortality (%)	Larval weight (g)	Larval duration (hrs)
T_1	TiO_2 Nanoparticles @ 25 ppm	6.65^{ab}	3.69^{ab}	221.00^{bc}
T_2	TiO_2 Nanoparticles @ 50 ppm	5.25^{ab}	3.75^{ab}	218.00^{abc}
T_3	TiO_2 Nanoparticles @ 75 ppm	4.90^{ab}	3.81^{ab}	215.00^{abc}
T_4	TiO_2 Nanoparticles @ 100 ppm	9.45^{b}	3.61^{b}	225.00^{c}
T_5	Ankush	3.65^{a}	3.85^{ab}	211.00^{ab}
T_6	Treated control (*Bm*NPV)	90.00^{c}	1.74^{c}	248.00^{d}
T_7	Untreated control	3.25^{a}	3.89^{a}	207.00^{a}
	SEd	**2.40**	**0.13**	**5.72**
	CD (0.05)	**5.15**	**0.27**	**12.27**

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

Table 3. Effect of TiO_2 nanoparticles application on cocoon characters of BmNPV infectedB. mori

No.	Treatment	Cocoon weight (g)	Shell weight (g)	Shell ratio (%)
T_1	TiO_2 Nanoparticles @ 25 ppm	2.11^{cd}	0.34^{d}	16.11^{b}
T_2	TiO_2 Nanoparticles @ 50 ppm	2.17^{bcd}	0.36^{cd}	16.58^{b}
T_3	TiO_2 Nanoparticles @ 75 ppm	2.23^{abc}	0.38^{bc}	17.04^{ab}
T_4	TiO_2 Nanoparticles @ 100 ppm	2.04^{d}	0.28^{e}	13.72^{c}
T_5	Ankush	2.30^{ab}	0.40^{b}	17.39^{ab}
T_6	Treated control (*Bm*NPV)	1.20^{e}	0.14^{f}	11.66^{d}
T_7	Untreated control	2.38^{a}	0.44^{a}	18.48^{a}
	SEd	**0.08**	**0.02**	**0.77**
	CD (0.05)	**0.17**	**0.03**	**1.65**

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

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