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ORIGINAL PAPER

Evaluation of *Catharanthus roseus* leaf extract-mediated biosynthesis of titanium dioxide nanoparticles against *Hippobosca maculata* and *Bovicola ovis*

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Abstract The purpose of the present study was based on assessments of the antiparasitic activities of synthesized titanium dioxide nanoparticles (TiO_2 NPs) utilizing leaf aqueous extract of *Catharanthus roseus* against the adults of hematophagous fly, *Hippobosca maculata* Leach (Diptera: Hippoboscidae), and sheep-biting louse, *Bovicola ovis* Schrank (Phthiraptera: Trichodectidae). The synthesized TiO_2 NPs were analyzed by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and atomic force microscopy (AFM). The formation of the TiO_2 NPs synthesized from the XRD spectrum compared with the standard confirmed spectrum of titanium particles formed in the present experiments were in the form of nanocrystals, as evidenced by the peaks at 2θ values of 27.43°, 36.03°, and 54.32°. The FTIR spectra of TiO_2 NPs exhibited prominent peaks at 714 (Ti–O–O bond), 1,076 (C–N stretch aliphatic amines), 1,172 (C–O stretching vibrations in alcoholic groups), 1,642 (N–H bend bond), and 3,426 (O–H stretching due to alcoholic group). SEM analysis of the synthesized TiO_2 NPs clearly showed the clustered and irregular shapes, mostly aggregated and having the size of 25–110 nm. By Bragg's law and Scherrer's constant, it is proved that the mean size of synthesized TiO_2 NPs was 65 nm. The AFM obviously depicts the formation of the rutile and anatase forms in the TiO_2 NPs and also, the surface morphology of the particles is uneven due to the presence of some of the aggregates and individual particles. Adulticidal parasitic activity was observed in varying concentrations of aqueous leaf extract of *C. roseus*, TiO_2 solution, and synthesized TiO_2 NPs for 24 h. The maximum parasitic activity was observed in aqueous crude leaf extracts of *C. roseus* against the adults of *H. maculata* and *B. ovis* with LD_{50} values of 36.17 and 30.35 mg/L, and r^2 values of 0.948 and 0.908, respectively. The highest efficacy was reported in 5 mM TiO_2 solution against *H. maculata* and *B. ovis* (LD_{50}=33.40 and 34.74 mg/L; r^2=0.786 and 0.873), respectively, and the maximum activity was observed in the synthesized TiO_2 NPs against *H. maculata* and *B. ovis* with LD_{50} values of LD_{50}=7.09 and 6.56 mg/L, and r^2 values of 0.880 and 0.913, respectively. This method is considered as an innovative alternative approach to control the hematophagous fly and sheep-biting louse.

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Introduction

The widespread development of tick and fly resistance and high cost of the conventional ectoparasitic drugs have limited the control of veterinary parasites and hence led to evaluation of medicinal plants as an alternative source to control parasites and the tick. The production losses largely result from meat quantity loss caused by tick worry, and the death of cattle from tick fever and the reduction in meat quantity associated with tick infestation were estimated (Sing et al. 1983). *Hippobosca maculata* is a serious pest of equines in India and is also cosmopolitan in distribution

(Parashar et al. 1991). These flies are adapted to a more or less continuous existence on the bodies of their host. Blood sucking flies cause damage to livestock through both direct damage, and as vectors of viral and bacterial infections, the hematophagous flies, Hippoboscidae, are well known to infest sheep and cattle in different parts of the world (Soulsby 1982). Paramphistomosis has been a neglected trematode infectious disease in ruminants but has recently emerged as an important cause of productivity loss. Economic loss caused by paramphistome infections has not been estimated but may be greater than those caused by many other parasites (Hanna et al. 1988). *H. maculata* is responsible for losses in milk, meat, and leather production and for the death of a number of animals, which results in economic losses associated with cattle production, and a recent survey on acaricide resistance conducted through questionnaire reported a large-scale acaricide resistance in India (FAO 2004). *Bovicola ovis* is a scurf feeder which spends its entire life on sheep. It thermoregulates by moving up and down the wool fibers to remain within its narrow thermal tolerance range.

An important area of research in nanotechnology is the synthesis of nanoparticles of different chemical compositions, sizes, and controlled monodispersity. Currently, there is an ever-growing need to develop environmentally benign nanoparticle synthesis processes. Nanoparticles play an indispensable role in drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants, and tissue engineering (Morones et al. 2005). The plant-mediated biosynthesis of nanoparticles is advantageous over chemical and physical methods because it is a cost-effective and environment-friendly method, where it is not necessary to use high pressure, energy, temperature, and toxic chemicals (Goodsell 2004).

Current applications to livestock for flies control primarily use products containing organophosphates or synthetic pyrethroids. However, some flies species have been becoming resistant to these insecticides as a result of repeated exposure (Foil et al. 2004), and these chemicals may have negative effects on non-target organisms including humans. Parasite control, in general, is based on the use of chemical insecticides. Unfortunately, repeated use of chemicals has many negative side effects, including the possibility of causing the development of chemical resistance in some ticks population, and food and environmental contamination if these products are improperly used. Public concerns about the environmental impact and safety of chemical applications are driving research into alternative, sustainable methods for parasites control, including biological control (Chandler et al. 2000). There is a development of experimental processes for the synthesis of nanoparticles of different sizes, shapes, and controlled dispersity. With the development of new chemical or physical methods, the concern for environmental contaminations are also heightened as the chemical procedures involved in the synthesis of nanomaterials generate a large amount of hazardous by-products. Thus, there is a need for "green chemistry" that includes a clean, nontoxic, and environment-friendly method of nanoparticle synthesis (Mukherjee et al. 2001).

In recent years, nanoparticle composites have become important owing to their small size and large surface area and because they exhibit unique properties not seen in bulk materials. As a result, nanoparticles (NPs) have useful applications in photovoltaic cells, optical and biological sensors, conductive materials, and coating formulations (Templeton et al. 2000), and the use of plants for nanoparticle synthesis can be advantageous over other biological processes because it eliminates the elaborate process of maintaining cell cultures and can also be suitably scaled up for large-scale nanoparticle synthesis (Shankar et al. 2004). Nanoparticles have attracted considerable attention because of their various applications. Jha et al. (2009) reported that the synthesized TiO_2 NPs using *Lactobacillus* sp. and *Sachharomyces cerevisae* possessed anti-bacterial and anti-fungal properties. Use of plant extract for the synthesis of nanoparticles could be advantageous over other environmentally benign biological processes because it eliminates the elaborate process of maintaining cell cultures. Recently, green TiO_2 nanoparticles have been synthesized using natural products like *Nyctanthes arbor-tristis* extract (Sundrarajan and Gowri 2011).

Catharanthus roseus L. (*Vinca rosea*) belonging to family Apocynaceae is known with various names in India and all over the world. Hot water decoction of the leaves or the whole plant is used for treatment of diabetes in several countries (Novello and Sprague 1957). *C. roseus* reported as antineoplastic agents to treat leukemia, Hodgkin's disease, malignant lymphomas, neuroblastoma, rhabdomyosarcoma, Wilms' tumor, and other types of cancers (Fischhof et al. 1996). Nayak (2006) reported that the dried or wet leaf extracts of *C. roseus* were applied as a paste on wounds in some rural communities. The fresh juice from the leaves of *C. roseus* made into a tea has been used by Ayurvedic physicians in India and other countries for external use to treat skin problems, dermatitis, eczema, and acne (El-Sayed and Cordell 1981).

Materials and methods

Materials

The leaves of *C. roseus* were collected from Melvisharam (12°56′23″N, 79°14′23″E), Tamil Nadu, India. Taxonomic identification was done by Dr. C. Hema from the

Department of Botany, Arignar Anna Government Arts College for Women, Walajapet, Vellore, India. The voucher specimen was numbered and kept in our research laboratory for further reference. Titanium dioxide (TiO_2 purity 99.0%) was purchased from Himedia, Mumbai, India.

Parasite collection

The newly attached adults *H. maculata* Leach (Diptera: Hippoboscidae) were collected from the softer skin inside the thigh, flanks, abdomen, brisket, and forelegs of naturally infested cattle and sheep. *H. maculata* adults have a short, straight capitulum, and brown to cream colored body. The adult of *B. ovis* Schrank (Phthiraptera: Trichodectidae) were collected from the body of goats. The sheep body louse is a pale yellow insect, brown transverse stripe on the abdomen, and a broad, red-brown head. The parasites were identified by Department of Veterinary Parasitology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India.

Synthesis of titanium dioxide nanoparticles

The fresh leaf of *C. roseus* broth solution was prepared by taking 10 g of thoroughly washed and finely cut leaves in a 250 mL Erlenmeyer flask along with 100 mL of sterilized double distilled water and then boiling the mixture for 10 min before finally decanting it. The extract was filtered with Whatman filter paper no. 1 and stored at −15°C and could be used within 1 week. The filtrate was treated with 20 ml of aqueous leaf extract added in 80 ml of 5 mM (39.94 mg of TiO_2 powder in 100 mL Milli-Q water) solution in an Erlenmeyer flask under stirring at 50°C. After 4 h of continuous stirring, the formed light green color change indicated the formation of TiO_2 NPs.

Filter paper impregnated bioassay test

The adults of *H. maculata* and *B. ovis* were collected from various parts of both sheep and goats. The aqueous leaf extract of *C. roseus*, TiO_2 solution, and synthesized titanium dioxide nanoparticles (TiO_2 NPs) were used in filter paper impregnated tests in sealed glass jars. Different concentrations of aqueous extract and TiO_2 solution ranging from aliquot of 6.12, 12.5, 25, 50, and 100 mg/L and synthesized TiO_2 NPs of 5, 10, 15, 20, and 25 mg/L were prepared and a series of filter paper envelopes (Whatman filter paper no. 1, ~216 cm^2) with micropores treated with each concentration of samples. Each envelope was treated with 3 mL solution uniformly distributed with a pipette on internal surfaces. Five envelopes were impregnated with each tested solution. The control papers were impregnated with distilled water only. *H. maculata* and *B. ovis* (20–50 individuals) were collected directly from infested animals. Each test was performed by placing 20 adults of *H. maculata* and *B. ovis* into the series of filter paper envelopes (Whatman filter paper no.1, ~216 cm^2) with micropores treated with each concentration. The numbers of dead adults were counted for 24 h. The death of the parasites was confirmed when there was cessation of motility or waggling of the appendages upon touching with a needle. The dose–response data were subjected to probit analysis to determine the LD_{50} values for the 24 h exposure under constant climatic conditions (25°C, 12/12 h L/D; Nyamador et al. 2010).

Dose–response bioassay

The crude leaf extract of *C. roseus*, TiO_2 solution (5 mM), and synthesized TiO_2 NPs were subjected to dose–response bioassay for parasitic activity against the adults of *H. maculata* and *B. ovis*. Different concentrations ranging from 6.12 to 100 mg/L (for aqueous plant extracts, TiO_2) and 5.0 to 25 mg/L (for synthesized TiO_2 NPs) were prepared for parasitic activity. The numbers of dead parasites were counted after 24 h of exposure, and the percentage of mortality was reported from the average of five replicates. However, at the end of 24 h, the selected test samples turned out to be equal in their toxic potential.

Data analysis

The average parasite mortality data were subjected to probit analysis for calculating LC_{50}, and other statistics at 95% fiducial limits of upper confidence limit and lower confidence limit were calculated by using the software developed by Reddy et al. (1992). Results with $p<0.05$ were considered to be statistically significant.

Characterization of TiO_2 nanoparticles

The resulting pellet was dissolved in de-ionized water and filtered through Millipore filter (0.45 μm). The synthesized nanoparticles were identified by X-ray diffraction (XRD) spectroscopy (PerkinElmer Spectrum One instrument, PW1830 instrument operating at a voltage of 40 kV and a current of 30 mA with CuKα radiation). Fourier transform infrared (FTIR) spectra of the samples were measured using PerkinElmer Spectrum One instrument in the diffuse reflectance mode at a resolution of 4 cm^{-1} in KBr pellets. Powder samples for the FTIR were prepared similar to powder diffraction measurements. The FTIR spectra of synthesized TiO_2 NPs taken were analyzed, which discussed for the possible functional groups for the formation of nanoparticles. For the scanning electron microscopic studies, 25 μL of sample was sputter-coated on copper stub, and the images of nanoparticles were studied using scanning electron microscopy (SEM; JEOL, ModelJFC-1600). Topography was studied using atomic force microscopy (AFM; PARKS scanning probe microscope) working in the non-contact mode. AFM images have been processed using XEI software given by PARKS system (Horcas et al. 2007).

Results

In the filter paper impregnated bioassay method, the aqueous leaf extracts, TiO_2 solution (5 mM), and synthesized TiO_2 NPs of *C. roseus* were noted; however, the synthesized TiO_2 NPs showed 100% mortality against the adults of *H. maculata* and *B. ovis* at the concentration of 5,10,15,20, and 25 mg/L. The mortality of aqueous leaf extracts of *C. roseus* against the adults of *H. maculata* and *B. ovis* showed the values of LD_{50}=36.17 and 30.35 mg/L, and r^2=0.948 and 0.908, respectively. Values of the efficacy of 5 mM TiO_2 solution against *H. maculata* and *B. ovis* showed the LD_{50} values of 33.40 and 34.74 mg/L, and r^2 values of 0.786 and 0.873, respectively. The synthesized TiO_2 NPs by *C. roseus* extract against the adults of *H. maculata* and *B. ovis* reported the LD_{50} values of 7.09 and 6.56 mg/L, and r^2 values of 0.880 and 0.913, respectively (Table 1). In the present study, the parasitic activity results showed the highest mortality in synthesized

Table 1 Parasitic activity of synthesized TiO_2 NPs of *C. roseus* against *H. maculata* and *B. ovis*

Species	Extract/materials	Concentrations (mg/L)	Percent mortality[a] (mg/L)±SD	LD_{50} (LCL–UCL) (mg/L)	Slope	r^2
H. maculata	Aqueous extract	100	84	36.17 (30.8–42.45)	32	0.948
		50	62			
		25	32			
		12.5	21			
		6.12	11			
	TiO_2 (5 mM solution)	100	70	33.40 (25.9–42.97)	22	0.786
		50	59			
		25	47			
		12.5	22			
		6.12	9			
	Synthesized TiO_2 NPs	25	100	7.09 (6.19–8.12)	38	0.880
		20	82			
		15	38			
		10	22			
		5	13			
B. ovis	Aqueous extract	100	82	30.35 (30.1–41.42)	18	0.908
		50	64			
		25	36			
		12.5	18			
		6.12	7			
	TiO_2 (5 mM solution)	100	80	34.74 (29.4–41.02)	19	0.873
		50	67			
		25	35			
		12.5	19			
		6.12	5			
	Synthesized TiO_2 NPs	25	99	6.56 (5.47–7.86)	44	0.913
		20	63			
		15	44			
		10	29			
		5	11			

Control—nil mortality

UCL upper confidence limit, *LCL* lower confidence limit, r^2 regression coefficient

[a] Mean value of five replicates LD_{50} lethal dose that kills 50%

TiO_2 NPs than the aqueous leaf extract of *C. roseus*. Each test included a control group (distilled water) with five replicates for each individual concentration. All the tested components that showed lethal effect and mortality were positively dose dependent. The results showed that the optimal hours for measuring the percent mortality adults of *H. maculata* and *B. ovis* synthesized TiO_2 NPs were 13%, 22%, 38%, 82%, and 100%, and 11%, 29%, 44%, 63%, and 99% at 1, 6, 12, 18, and 24 h, respectively. The comparative study of larvicidal and adulticidal activity of different nanoparticles are shown in Table 2.

Characterization of TiO_2 NPs

The formation of TiO_2 NPs synthesized using *C. roseus* leaf extract was supported by X-ray diffraction measurements. XRD analysis showed three distinct diffraction peaks at 27.43°, 36.03°, and 54.32° which indexed the planes 110, 101, and 211, respectively, of the cubic face centered TiO_2 (JCPDS no. 21–1272). The average grain size formed in the biosynthesis was determined using Scherrer's formula, $d=0.89\lambda/\beta\cos\theta$ was estimated as 100 nm for the higher intense peak (Fig. 1). The sharp peaks and absence of unidentified peaks confirmed the crystallinity and higher purity of prepared nanoparticles.

FTIR spectroscopy analyses were carried out to identify the biomolecules responsible for capping of the bioreduced TiO_2 NPs synthesized using plant extract. Figure 2 shows the synthesized TiO_2 NPs using *C. roseus* leaf aqueous extract where the absorption peaks were located at 714 (Ti–O–O bond), 1,076 (C–N stretch aliphatic amines), 1,172 (C–O stretching vibrations in alcoholic groups), 1,642 (N–H bend bond), and 3,426 (O–H stretching due to alcoholic group). SEM analyses of the synthesized TiO_2 NPs clearly showed clustered and irregular shapes, mostly aggregated and having an average size of 25 to 110 nm with interparticle distance. It is clear in Fig. 3a, b the clustered structures with sizes of 65 nm. The line profile image was drawn by the XEI software and the horizontal line at 6 μm on a 2D AFM image. The height of the particles existing across the line can be evaluated by placing two cursors. The AFM was performed in order to know the topological map of the surface of the synthesized nanoparticles. The surface area of the nanoparticles has increased dramatically showing with the increase in the peaks Fig. 4a, b. The AFM obviously depicts the formation of the rutile and anatase forms in the TiO_2 NPs, and also, the surface morphology of the particles is uneven due to the presence of some of the aggregates and individual particles.

Discussion

The adulticidal activity of aqueous leaf extracts and synthesized TiO_2 NPs of *C. roseus* showed maximum activity observed in aqueous extract of *C. roseus* and the synthesized TiO_2 NPs against *H. maculata* and *B. ovis*. The nanoparticle synthesis is important as the instability or aggregation of nanoparticles would lead to a decrease in their biological activities (Kvitek et al. 2008). The larvicidal aqueous crude leaf extracts and synthesized

Table 2 The comparative study of larvicidal and adulticidal activity of different species and different nanoparticles

Source	Common name	Parts used	Treated samples	Particle size (nm)	Biological activity	Target organisms	LC_{50}	Reference
Manilkara zapota	Zapota	Leaf	Ag NPs	70–140	Larvicidal	*Rhipicephalus (Boophilus) microplus*	16.72 mg/l	Rajakumar and Rahuman (2011)
Mimosa pudica	Sensitive plant	Leaf	Ag NPs	25–60	Larvicidal	*A. subpictus* *C. quinquefasciatus*	13.90 mg/l 11.73 mg/l	Marimuthu et al. (2011)
						R. microplus	8.98 mg/l	
Tinospora cordifolia	Guduchi	Leaf	Ag NPs	55–80	Larvicidal	*A. subpictus* *C. quinquefasciatus*	6.43 mg/l 6.96 mg/l	Jayaseelan et al. (2011a)
					Adulticidal	*P. humanus capitis*	12.46 mg/l	
Zinc nitrate	Zinc nitrate	–	ZnO NPs	60–120	Larvicidal	*A. subpictus* *C. quinquefasciatus*	11.14 mg/l 12.39 mg/l	Kirthi et al. (2011)
					Adulticidal	*R. microplus*	29.14 mg/l	
						P. humanus capitis	11.80 mg/l	
Cu (II) acetate hydrate	Cu (II) acetate hydrate	–	Cu NPs	35–80	Larvicidal	*A. subpictus* *C. quinquefasciatus*	23.47 mg/l 15.24 mg/l	Ramyadevi et al. (2011)
Citronella oil (*Cymbopogon nardus*)	–	–	Nano emulsion	120–200	Repellent	*A. aegypti*	_	Sakulku et al. (2009)
Permethrin	Permethrin	–	Permethrin	151±27	Larvicidal	*C. quinquefasciatus*	0.117 mg/l	Anjali et al. (2010)

LC_{50} lethal concentration that kills 50% of the exposed larvae

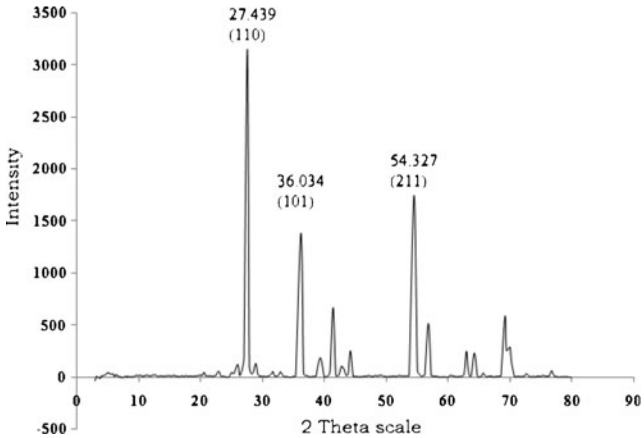

Fig 1 XRD patterns of TiO_2 nanoparticles synthesized from aqueous leaf extract of *C. roseus*

silver nanoparticles (Ag NPs) of *Mimosa pudica* showed the highest mortality in synthesized Ag NPs against the larvae of *Rhipicephalus* (*Boophilus*) *microplus* (8.98 mg/L, r^2=0.479; Marimuthu et al. 2011). In *Nelumbo nucifera* leaf, synthesized Ag NPs maximum efficacy was observed in crude methanol, aqueous, and synthesized Ag NPs against the larvae of *Anopheles subpictus* (LC_{50}=8.89, 11.82, and 0.69 ppm; LC_{90}=28.65, 36.06, and 2.15 ppm) and against the larvae of *Culex quinquefasciatus* (LC_{50}=9.51, 13.65, and 1.10 ppm; LC_{90}=28.13, 35.83, and 3.59 ppm), respectively (Santhoshkumar et al. 2011). The synthesized zinc oxide nanoparticles against *R. microplus* and *Pediculus humanus capitis*, and the larvae of *A. subpictus* and *C. quinquefasciatus* showed LC_{50} values of 29.14, 11.80, 11.14, and 12.39 mg/L, respectively (Kirthi et al. 2011). Jayaseelan et al. (2011b) reported the aqueous extract and synthesized Ag NPs of

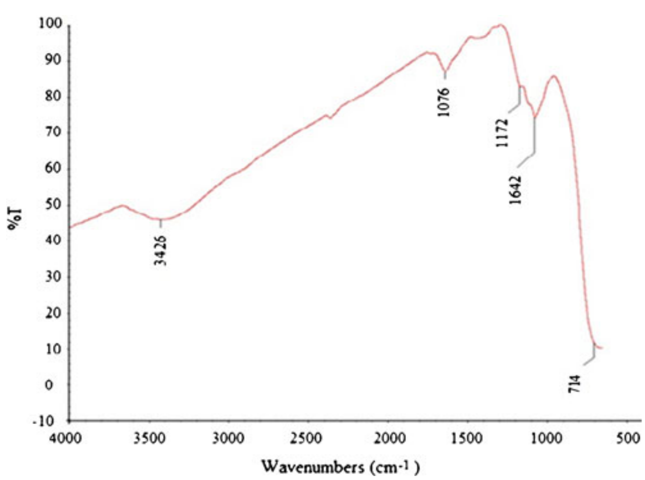

Fig 2 FTIR spectrum of TiO_2 nanoparticles synthesized by *C. roseus*

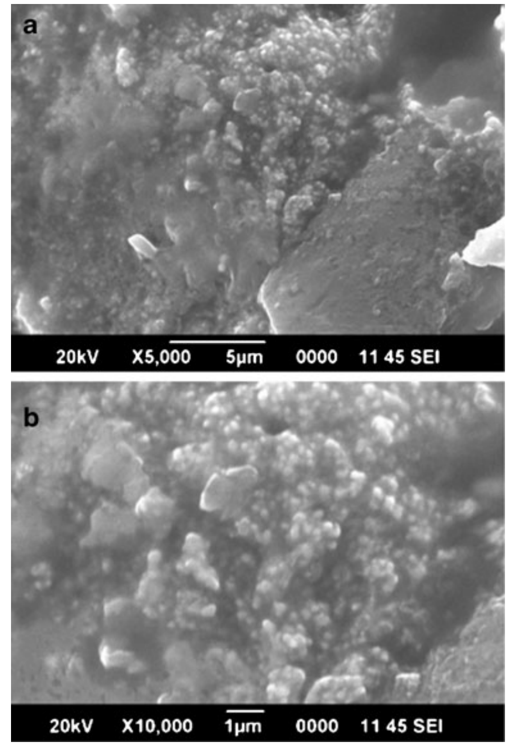

Fig 3 Scanning electron microscopic observation of synthesized TiO_2 nanoparticles **a** Magnification, ×5,000 **b** Magnification, ×10,000

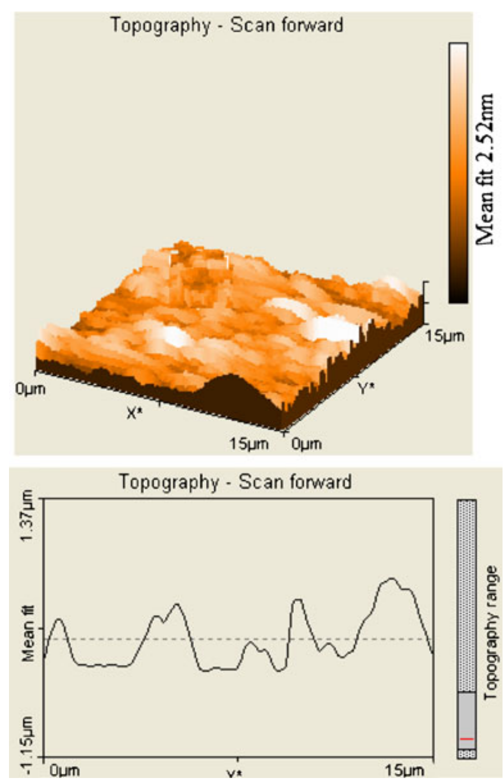

Fig 4 AFM image of the synthesized TiO_2 NPs by aqueous leaf extract of *C. roseus* showing **a** increased surface area and **b** topographical range in 15 μm^2

Musa paradisiaca in *Haemaphysalis bispinosa* with LC_{50} values of 28.96 and 1.87 mg/L and in *H. maculata* 31.02 and 2.02 mg/L, respectively. A cardiac glycosidal (cardenolide) extract isolated from *Calotropis procera* was tested for their effects against the larvae and adult stages of *Hyalomma dromedarii* (Al-Rajhy et al. 2003). *Melaleuca alternifolia* oil and neem seed oil exhibited acaricidal effects against nymphal *Ixodes ricinus* and adults of *Hyalomma anatolicum excavatum* ticks, respectively (Abdel-Shafy and Zayed 2002; Iori et al. 2005).Organophosphates are still very effective against *B. ovis*, but control is hampered by inadequate application via plunge or shower dipping (Levot 1995). Heath et al. (1995) reported that the approach would be through the effect exhibited by azadirachtin, the most active ingredient from *Azadirachta indica*, which decreases the number of the sheep-biting louse *B. ovis* by 85% to 100% on treated sheep.

The physicochemical parameters including morphology, crystallinity, agglomeration state, and surface active groups play significant role in toxicity of the metal oxide NPs (Handy et al. 2008). The XRD peaks at 2θ=25.25° (101) and 48.0° confirm the characteristic facets for anatase form of TiO_2 (Liu et al. 2005). In a related pervious study, it has been observed that the crystal structure of nano-TiO_2 contributed to cytotoxicity, with anatase TiO_2 showing more toxicity than rutile TiO_2 (Sayes et al. 2006). A couple of distinct Bragg reflections corresponding to (110), (101), and (211) sets of lattice planes were manifested in the X-ray diffraction patterns.

FTIR spectra of TiO_2 NPs exhibited prominent peaks at 3,426, 1,642, 1,172, 1,076, and 714 cm^{-1}. The 3,427 cm^{-1} absorbance bands are known to be associated with the stretching O–H due alcoholic group. In particular, the 1,642 cm^{-1} indicates the presence of H bend bond for 1° for proteins. The band observed at 1,172 cm^{-1} is due to C–O stretching vibrations in alcoholic groups in carbohydrates. The bands in alcoholic groups are due to C–O stretching vibrations in carbohydrate observed at 1,076 cm^{-1} got oxidized to unsaturated and 714 cm^{-1} peak at due to Ti–O–O bond (Sadiq et al. 2011; http://orgchem.colorado.edu/hndbksupport/specttutor/irchart.html).

SEM analysis showed the particle size between 25 and 110 nm as well as the cubic structure of the nanoparticles (Khandelwal et al. 2010). The size of the TiO_2 nanoparticles was measured by AFM in contact mode with silicon cantilevers with force constant of 0.02–0.77 Nm^{-1} and tip height of 10–15 mm. For imaging by AFM, the sample was suspended in acetone and spins coated on a silicon wafer. The acetone vaporized leaving the particles behind (Mahapatraa et al. 2008).

Mouchet et al. (2008) reported that a high mortality rate (85%) was noted at the highest double-walled carbon nanotube concentration (500 mg L^{-1}) against the larvae of *Xenopus laevis*. Baun et al. (2008) indicated the toxicity of C60, carbon nanotubes, and zinc oxide to an aquatic invertebrate, *Daphnia magna*. Although an attempt to develop essential oil for pesticides and insecticides has been made in a variety of water-soluble formulations such as nanoemulsion incorporated with β-cypermethrin (Wang et al. 2007) and essential oil-loaded microcapsules for pest control (Moretti et al. 2002). Recent studies demonstrated that TiO_2 NPs were synthesized by two microalgae species *Scenedesmus* sp. and *Chlorella* sp. (Sadiq et al. 2011). The photocatalytic inactivation of *Escherichia coli* and *Pichia pastoris* was studied with combustion synthesized TiO_2 (Sontakke et al. 2010). Prasad et al. (2009) have reported photocatalytic inactivation of *Bacillus anthracis*, which was studied by using titania nanomaterials.

The present green synthesis shows that the environmentally benign and renewable source of *C. roseus* is used as an effective reducing agent for the synthesis of TiO_2 NPs. This biological reduction of metal would be boon for the development of clean, nontoxic, and environmentally acceptable green approach to produce metal nanoparticles, involving organisms even ranging to higher plants. The formed TiO_2 NPs are highly stable and have significant parasitic activity.

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