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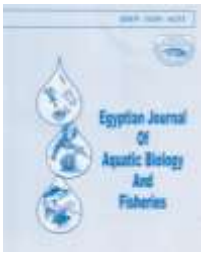

Mangrove Leaves Aqueous Extract Mediated Green Synthesis of TiO_2 and Boron-doped TiO_2 Nanoparticles and their Ecotoxic Effect on Rotifers

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ABSTRACT

Nanotechnology evolution and the production of nano-medicine from various sources had proven to be of high value. Titania nanoparticles (NPs) are photoactive, potentially producing in vivo toxicity in the presence of light. The smaller the size of NPs is gaining importance in increasing research for the treatment of various diseases. In the present study, titania (TiO_2) and boron doped titania (B-TiO_2) NPs were synthesized using mangrove leaf aqueous extract and characterized using FTIR and XRD techniques. The toxic activity was evaluated on rotifers using different concentrations of suspended aqueous solution of pure and B-doped titania at various times (0.5, 1, 2, 4 and 24 h) at room temperature. The data show anatase structure with a diameter of 3 to 30 nm. The toxicity analysis revealed that the TiO_2 of different concentrations used doesn't have any toxicity in presence and absence of light source except a decrease in rotifer movement at high concentration, but B-TiO_2 show slow toxicity, which increases with increasing the concentration to attain a maximum value at high concentration at which 5% of rotifers died after 24 h and its immobilization reaches 23% in darkness. Overall, the best data are obtained in presence of visible light source.

INTRODUCTION

Nanomaterials are frequently exceeding different properties to those shown by bulk modes of the same materials (Kaviraj *et al*., 2014; Ragab *et al*., 2016; Serag et al., 2017; 2018; Helmy *et al*., 2018). A better knowledge of the possible dangers of the nanomaterials is required, and especially, the grit of the properties that may harmfully touch human health. This information will be valuable in organization future negative effects by tolerating the operation of precise regulat or dealings for minimalizing experience to such materials. This may be possible across the insertion of rules, or throughout the utilization of other materials to make available their harmless integration into products. Titanium dioxide (TiO_2) is an interesting material used in different applications such as; paints, photocatalyst, sensors, self-cleaning etc (Chen *et al*., 2007; Barmo *et al*., 2013; Vijayakumar *et al*., 2016; Mousa *et al*., 2018).

As a consequence of the use of nano-TiO_2 in paints, antimicrobial, and antifouling agents and cosmetic field, it has been manufactured in a large amount (Chen *et al*., 2007; Bai *et al*., 2015). In spite of the presence of natural nano-TiO_2 in river water (Wigginton *et al*., 2007), in recent times, direct verification of the liberation of synthetic nano-TiO_2 from urban applications into the aquatic location has been recorded (Kaegi *et al*., 2008). It was pointed that nano-TiO_2 leached out from the nano-TiO_2 covering painted house facades and passed into delivery waters; providing concentrations in the scale of a few μg L^{-1} in a small stream (Elavazhagan *et al*., 2008; Bownik & Stępniewsk, 2015). An attractive alternative to invertebrate stock cultures as a source of test animalsis cryptobiotic or dormant eggs (cysts). Because of their capacity for dormancy, cystscan remain on the shelf until test animals are needed, eliminating the need for stockcultures and their inherent variability. Currently, cysts are commercially available for only two aquatic invertebrates: the brine shrimp rotifers of the genus *Brachionus Artemia* cysts have been used in aquatic toxicity testing for some time (Vanhaecke *et al*., 1980; 1981; Persoone *et al*., 1985; Manju *et al*., 2016), but rotifer cysts are only newly available (Snell, 1986; Clément *et al*., 2013). Due to the numerous application of titania NPs also facile, economic and simplicity of green synthesis method the aim of this work was focused to green synthesis of titania and its non-metal borondoped NPs using mangrove plant leaf aqueous extract. Characterization of the NPs prepared was achieved using Fouriertrans form infrared spectroscopy (FTIR) and X-ray diffraction spectroscopy (XRD). The Photocatalytic toxicity of the green synthesis prepared NPs was studied against aquatic rotifers using different amounts of titania and its doped boron at different times (0.5, 1, 2, 4, and 24 h) at room temperature in absence and presence of visible light source.

MATERIALS AND METHODS

Chemicals

Titanium (IV) isopropoxide was purchased from Sigma-Aldrich and *o*-boric acid from Fluka. All chemicals were used directly without any purification. Double distilled water was used.

Collection and processing of plant samples

Healthful, disease-free leaves of mangrove were gathered from Safaga and Hurghada, Egypt during December 2014. Plants with the entire herbarium were classified and substantiated by the Department of Biodiversity, National Institute of Oceanography and Fisheries, Alexandria, Egypt. The collected leaves were washed three times using tap water and then in detergent water and were finally rinsed with double distilled water until no foreign material remained. The freshly cleaned leaves were left to dry for approximately 45 days in a closed room (25–28°C). The dried plant parts were ground to get a powdered sample and stored in an airtight container and kept away from sunlight.

Extract preparation

Mangrove powdered plant leaf (2.0 g) was added to 200 mL of double distilled water in a beaker and stirred for 24 h at 50°C; the double distilled water becomes dark green colour, then filtered and the leaf extract was stored at −24°C for further use.

Biosynthesis of NPs from the aqueous leaf extract of mangrove

Titanium precursor (5.0 g) was added to 50 mL of aqueous mangrove leaf extract with magnetic stirring at 50°C for 10 minutes, then adjusting pH between 7 and 8 using 0.1N of

NaOH and 0.1N HCl solution. Stirring the solution using a magnetic stirrer for 1 h then left aging for 18-24 h at 50°C in an oven (Lenton WF120, UK). Finally, filter, wash, dry, grind and thermally pre-treated in a muffle furnace (Naberthrm GmbH, Germany) at 400°C for 2 h in the air.

Phytochemical analysis

Tannins (**Ferric Chloride test**)

Inserted a few drops of 5% ferric chloride solution into 2 mL of the test solution. Appearing a blue colour confirms the existence of hydrolysable tannins.

Alkaloids

Powdered of the extracted plant sample (100 mg) was dissolved in 5 mL of methanol and filtered. Then 2 mL of filtrate was mixed with 5 mL of 1% aqueous HCl and 1 mL of the mixture was taken separately in test tube followed by addition of few drops of Mayer's reagent, and an appearance of buff-colour precipitate was considered as positive test for the presence of alkaloids (Sofowora, 1983).

Phytosterols (Liebermann-Burchard's test)

Firstly, adding dissolved 2 mg of the powdered plant extract in 2 mL of acetic anhydride in a test tube and heating to boiling point and cooling to room temperature. Then, 1 mL conc. H_2SO_4 is added along the side of the test tube. A formation of a brown ring at the junction and the appearance of dark green color in the upper layer refers to the positive test of phytosterols (Sofowora, 1983).

Triterpenoids (Salkowski test)

Dry extract (2 mg) was shaken with 1 ml of chloroform in a test tube and a few drops of conc. H_2SO_4 were added along the side of the tube. The formation of a red-brown colour at the interface refers to the presence of triterpenoids (Sofowora, 1983).

Flavonoids (Alkaline Reagent test)

Addition of 5 drops of 5% aqueous solution of NaOH to 1 mL of the test solution caused an increase in the intensity of the yellow colour, which turned to colourless on addition of a few drops of 2M HCl which is an indication to the presence of flavonoids (Sofowora, 1983).

Saponins (Foam test)

The formation of stable foam through shaking 5 mL of the test solution in a test tube for five minutes confirmed the test (Sofowora, 1983).

Cardiac glycosides (Keller-Killiani test)

Glacial acetic acid (0.4 mL) and a few drops of 5% ferric chloride solution are added to a small amount of dry extract. Further 0.5 mL of conc. H_2SO_4 was added along the side of the test tube. The formation of blue colour in acetic acid layer confirmed the test (Sofowora, 1983).

Anthraquinone glycosides (**Hydroxyanthraquinone test)**

The appearance of red colour through the addition of a few drops of 10% KOH solution to 1 mL of the extract confirmed the test (Sofowora, 1983).

Test for carbohydrates (Molisch's test)

In a test tube, a few drops of 1% alpha-naphthol was added to 1 mL of test solution and 2-3 mL conc. H_2SO_4 along the side of the tube. The reddish violet or purple ring formed at the junction of two liquids confirmed the test (Sofowora, 1983).

Test for proteins (Biuret test)

Formation of purple or violet colour on mixing well 5 drops of 1% copper sulphate solution with 2 mL of the test solution confirmed the presence of proteins (Sofowora, 1983).

Fats and fixed oils

Formation of a clear blue solution on mixing well 5 drops of the sample with 1 mL of 1% copper sulphate solution and a few drops of 10% NaOH confirmed the test (Sofowora, 1983).

Samples characterization

FT-IR connected with PLATINUM ATR-Bruker Optics 70 was used to analyse FT-IR spectra of the samples at a range of 400-4000 cm^{-1}. The XRD patterns of the samples were measured using Rigaku D/MAX 2500 X-ray diffractometer (CuK = 0.154 nm) radiation under 40 kV and 100 mA. Toxicity of treated and untreated wastewater was observed under the microscope(*Nikon* Eclipse *200* LED Trinocular *Microscope provided with Nikon* DSLR *camera, Japan).*

Toxicity

The effect of suspended solutions of the prepared pure and B-doped TiO_2 on rotifer through bioassays was monitored. All samples were tested without dilution. A small sample of cysts was obtained from genetics laboratory of the national institute of oceanography and fisheries, Alexandria, Egypt. And a rotifer population hatched from these cysts has since been in continuous culture at the same place. This population has been inbred and selected for cyst production for several generations and consequently is highly homozygous. Cyst hatching is started by transferring the cysts to a warmer temperature, lower salinity, and light. Several hundred cysts are placed in 10 mL of seawater medium in a 20 mL dish, which is a convenient size for neonate collection. Procedures for the rotifer acute toxicity bioassay follow the recommendations of Standard Methods for the examination of water and wastewater (1985) and USEPA (1985).

Preparation suspended TiO_2 solution

B-TiO_2 were prepared by adding 1.0 g/100 mL H_2O then sonication for 1 h.

Exposure conditions

The toxicity of TiO_2 and B-TiO_2 were tested by exposing 5 mL of cultured rotiferto different concentrations of freshly prepared suspended titania samples (0, 1, 2, 3, 4, and 5 mL) in 20 mL glass beakers with keeping constant shaking 100 rpm using shaker (*JSOS-500* orbital shaker) for (0.5, 1, 2, 4 and 24 h) in ordinary laboratory conditions and observingthe immobilization and deceleration of rotifer.The test was done in the presence and absence of light. Halide lamp (400 W) was used as a visible light source. The distance between the lamp and the aqueous suspension was kept at 15 cm. Each exposure was performed in triplicate and average for each one was taken.

RESULTS AND DISCUSSION

Phytochemical screening

The aqueous extract of mangrove leafwas qualitatively analysed for the existence of numerous phytoconstituents. The results obtained are listed in Table 1 and containchiefly saponins, phytosterols, phenolic compounds, quinones, carbohydrates, and protein. The high saponin content present in the extract suggests that saponins are the most favourable starting material for preparation of the TiO_2 NPs in the current work.

Table 1: Qualitative phytochemical screening of aqueous extracts of Mangrove leaves along with blank (water) and positive control.

Tested constituents	Test name	Blank	Control	Mangrove
Tannin	Ferric chloride	-	+	+
Alkaloids	Mayer's	-	+	+
Phytosterols	Liebermann Burchard	-	+	+
Triterpenoids	Salkowski	-	+	+
Flavonoids	Alkaline Reagent	-	+	+
Saponins	Foam	-	+	+
Cardiac Glycoside	Keller Killani	-	+	+
Anthraquinone glycosides	Hydroxyanthraquinone	-	+	-
Carbohydrates	Molisch's	-	+	+
Proteins	Biuret	-	+	-
Fats and fixed oils	Copper sulphate	-	+	-
Amino acid	Millon's test	-	+	-

+ Sign indicates presence, - sign indicates absence.

The qualitative analysis of the major bioactive constituents Mangrove leaves aqueous extracts listed in the Table 1 also revealed the existence of hydrolysable tannins, alkaloids, phytosterols, triterpenes, flavonoids, saponins,cardiac glycosides, carbohydrates, while proteins, amino acid, anthraquinone glycosides fats and fixed oils were found to be absent.

FTIR spectroscopy

The FT-IR spectra of anatase phases TiO_2 and B-TiO_2 NPs samples are shown in Fig. 1. The peaks at 3200-3500 cm^{-1} were assigned to the stretching vibration and bending vibration of surface –OH group and the band at 420-490 cm^{-1} observed for all samples was assigned to the Ti-O stretching vibration. The peaks at 912 and 850 cm^{-1} in chemically synthesized TiO_2 were shifted to 732 cm^{-1} in mangrove green synthesis TiO_2 NPs and to 726 and 674 cm^{-1} in mangrove green synthesis Boron-TiO_2 NPs.

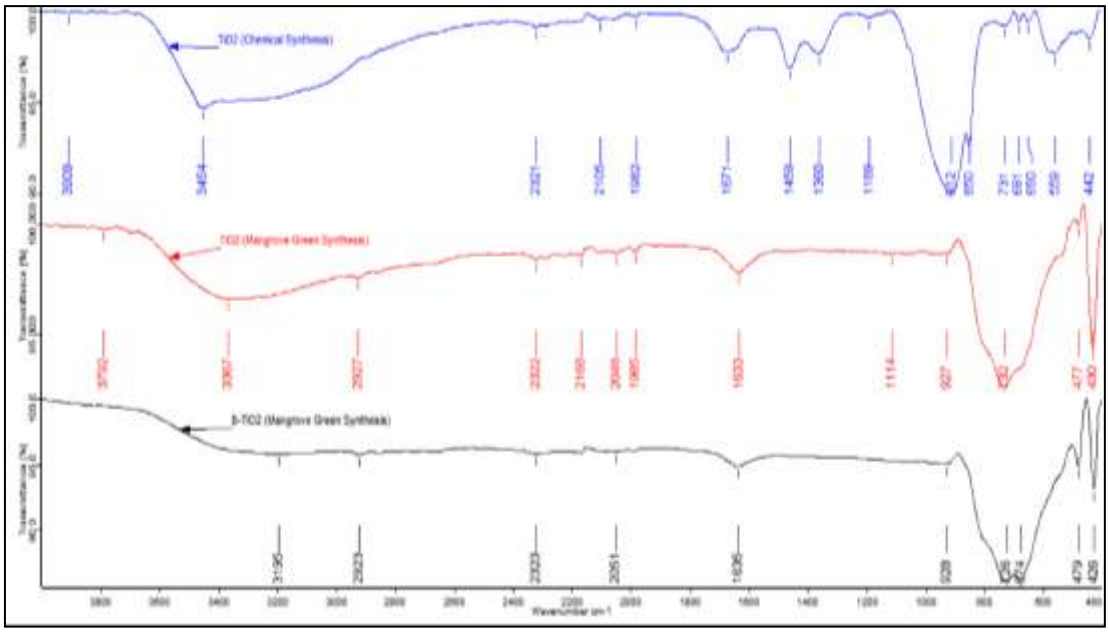

Fig. 1: FTIR of TiO_2 chemically synthesized and greenly synthesized TiO_2 NPs and B-TiO_2 NPs using aqueous mangrove extract.

X-Ray Diffraction measurements

The as-prepared pure TiO_2 sample showed (101), (004), (200), (100) and (204) peaks at 2θ values of 25.24, 37.85, 48.00, 54.91 and 62.64° indicating that the as-prepared pure TiO_2 has an anatase crystal structure (Fig. 2). XRD of all the doped samples also showed that the peak positions of the obtained anatase structure are shifted a slightly towards the left as compared with that of pure anatase TiO_2. This shift refers to the incorporation of doped ions into the anatase. The crystallite sizes of the specimens were estimated from full-width at half-maximum of the (101) anatase peak by the Debye-Sherrer equation (2) (Li *et al*., 2005).

$$C_s = \frac{0.9\lambda}{\beta cos\theta} \qquad (2)$$

where C_s is the crystallite size, β is the full width at half-maximum($FWHM_{hkl}$) of the more intense peak.

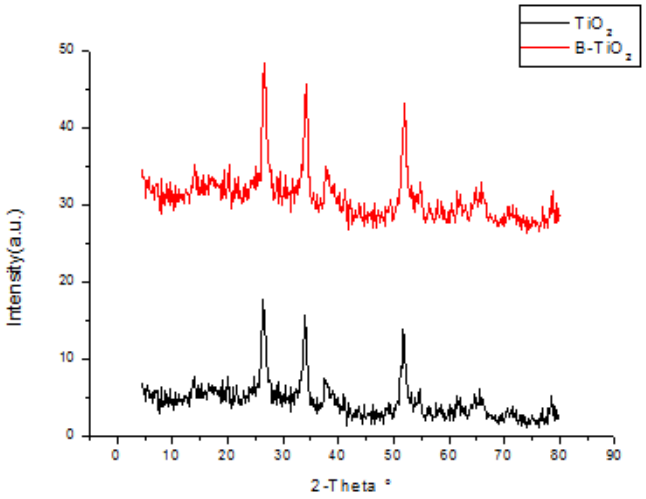

Fig. 2: XRD of greenly sensitized TiO_2 and B-TiO_2 using mangrove aqueous extract.

Determination of Eco-toxicity of TiO_2 and B-TiO_2NPs

The toxicity effect of TiO_2 and B-TiO_2 NPs were evaluated on rotifer at room temperature (25 ± 2 °C) using different volumes of doped and un-doped TiO_2 NPs. The preparedsuspensions of pure and doped titania samples (1%) were employed in differentconcentrations mainly, 0, 1, 2, 3, 4 and 5 mL in 5 ml of collected rotifers.

The mixtures were shaken at 130 rpm using shaker (orbital shaker *JSOS-500* 500 × 500 mm). And the immobilization was observed under the microscope (*E200*-LED *Nikon* Microscope) after (0.5, 1, 2, 4 and 24 h) at room temperature (Tables 2 and 3) show rotifers percentage that immobilized during different times (0.5, 1, 2, 4, and 24 h) after exposed to TiO_2 and B-doped NPs. These results demonstrate that the different added suspended volumes of TiO_2 NPs show very low immobilization degree with a maximum value of 4% after exposing to high volume of the suspended TiO_2 (5 mL) and a high time of exposure (24 h). B-TiO_2 have the same toxic effect, where immobilization increases by increasing the concentration of doped titania to reach the maximum value (4-5%) after exposure for 24 h in darkness, while in presence of light source immobilization reaches to 2% in case of B-TiO_2. The data show that TiO_2 has no effect on rotifer immobilization using different concentrations

at different times except for suspended volumes 4 and 5 mL of titania which have the slight effect and that may related to testing conditions.

Table 2: Rotifers immobilization (%) after exposing to different volumes of TiO_2 NPs at different times (0.5, 1, 2, 4 and 24 h).

Added volume of TiO_2 NPs suspension	Rotifer immobilization (%) by TiO_2 NPs after (h)				
	0.5	1	2	4	24
0	–(–)	–(–)	–(–)	–(–)	–(–)
1	–(–)	–(–)	–(–)	–(–)	–(–)
2	–(–)	–(–)	–(–)	–(–)	–(–)
3	–(–)	–(–)	–(–)	–(–)	–(–)
4	–(–)	–(–)	–(–)	1±0.01(–)	3±0.01(–)
5	–(–)	–(–)	–(–)	2 ±0.01(–)	4±0.01(–)

Values under light exposure found in ( ).

Table 3: Rotifers immobilization (%) after exposing to different volumes of B-doped TiO_2 NPs at various times(0.5, 1, 2, 4 and 24 h)

Added volume of B-TiO_2 NPs suspension	Rotifer immobilization (%) by B-doped TiO_2 NPs after (h)				
	0.5	1	2	4	24
0	–(–)	–(–)	–(–)	–(–)	1±0.01(–)
1	–(–)	–(–)	1±0.01(–)	1±0.01(–)	1±0.01(–)
2	–(–)	–(–)	1±0.01(–)	1±0.01(–)	1±0.01(–)
3	–(–)	–(–)	1±0.01(–)	1±0.01(–)	1±0.01(–)
4	–(–)	1±0.01(–)	1±0.01(–)	1±0.01(–)	2±0.01(–)
5	–(–)	1±0.01(–)	2±0.01(–)	3±0.01(1±0.01)	5±0.01(2±0.01)

Values under light exposure found in ( )

Determination of deceleration of TiO_2 and B-doped TiO_2 NPs

The deceleration of our samples on rotifer was observed under microscope triplicate, and the average was taken as (%) (Figs. 3 and 4). Tables (4 and 5) show the effect of used NPs on rotifers after exposing to different volumes of NPs at various times. For TiO_2 the maximum deceleration that observed is (9 and 2%) in darkness and in presence of light source respectively, also the deceleration increases by increasing time and increasing concentration (Table 5). This may be due to increasing concentration of TiO_2 particles that agglomerate more to form highly aggregated NPs whichmay cause a decrease in the speed of rotifers. Adding boron to titania increases the deceleration than that of using of pure TiO_2 to attain a maximum value (23% and 9%) at high concentration after 24 h in darkness and in presence of light source, respectively (Table 5).

Table 4: Rotifers deceleration (%) after exposing to different volumes of TiO_2 NPs at different times (0.5, 1, 2, 4 and 24 h).

Added volume of TiO_2 NPs suspension	Rotifers deceleration (%) by TiO_2 NPs after (h)				
	0.5	1	2	4	24
0	–(–)	–(–)	–(–)	–(–)	1±0.01(–)
1	–(–)	–(–)	–(–)	1±0.01(–)	2±0.01(–)
2	–(–)	–(–)	–(–)	3±0.01(–)	4±0.01(–)
3	–(–)	–(–)	1±0.01(–)	5±0.01(1±0.01)	6±0.01(1±0.01)
4	–(–)	–(–)	1±0.01(–)	5±0.01(1±0.01)	7±0.01(2±0.01)
5	–(–)	–(–)	1±0.01(–)	6±0.01(1±0.01)	9±0.01(2±0.01)

Values under light exposure found in ( ).

Table 5: Rotifers deceleration (%) after exposing to different volumes of B-doped TiO_2 NPs at different times (0.5, 1, 2, 4 and 24 h).

Added volume of B-TiO_2 NPs suspension	Rotifers deceleration (%) by B-doped TiO_2 NPs after (h)				
	0.5	1	2	4	24
0	–(–)	–(–)	1±0.01(–)	2±0.01(–)	3±0.01(–)
1	–(–)	–(–)	3±0.01(–)	5±0.01(–)	9±0.01(3±0.01)
2	–(–)	–(–)	5±0.01(–)	7±0.01(2±0.01)	13±0.01(4±0.01)
3	–(–)	6±0.01(2±0.01)	11±0.01(4±0.01)	13±0.01(4±0.01)	15±0.01(5±0.01)
4	–(–)	8±0.01(3±0.01)	12±0.01(4±0.01)	16±0.01(5±0.01)	19±0.01(6±0.01)
5	–(–)	8±0.01(3±0.01)	15±0.01(5±0.01)	20±0.01(7±0.01)	23±0.01(9±0.01)

Values under light exposure found in ( ).

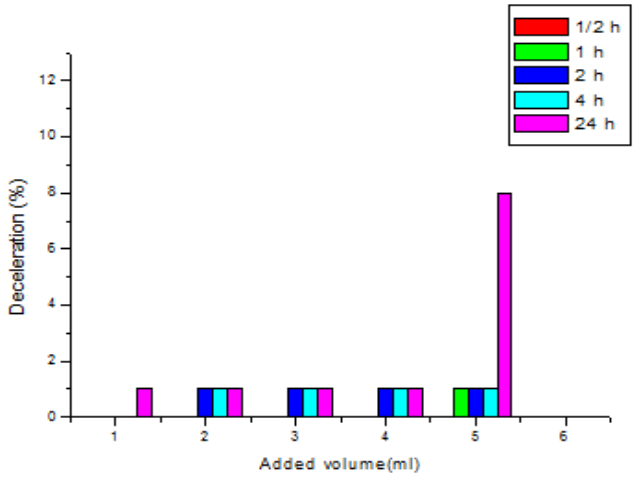

Fig. 3: Rotifers deceleration (%) after exposing to different volumes of TiO_2 NPs at different times (0.5, 1, 2, 4 and 24 h) in darkness.

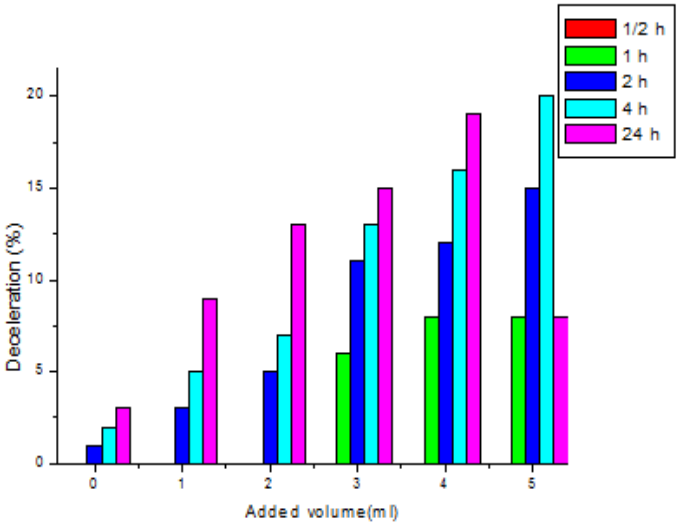

Fig. 4: Rotifers deceleration (%) after exposing to different volumes of B-TiO_2 NPs at various times (0.5, 1, 2, 4 and 24 h) in darkness.

Due to the leakage of toxicity data of TiO_2 NPs on rotifers and numerous studies on other aquatic organisms (Adams *et al*., 2005; Hund-Rinke, Simon, 2006; Federici *et al*., 2007; Heinlaan *et al*., 2008; Baun *et al*., 2008; Drobne *et al*., 2009; Wang *et al*., 2009; Clément *et al*., 2013; Minetto *et al*., 2014) we did this study.

Aggolomeration have noticeable effect on toxicity of NPs (Franch *et al*., 2004; Guzman *et al*., 2006). From Figs. (5-8), we can conclude that by increasing time the nanoparticles agglomeration increases, which also increases by increasing the concentration of nanoparticles and this may have negative effect on rotifers immobilization as soon as deceleration. Many future efforts should be made in order to understanding the some roles of different properties such as shape, size, degree of agglomeration, catalytic and chemical of particles contribute to toxic effects on algae, bacteria, vertebrates and invertebrates species.

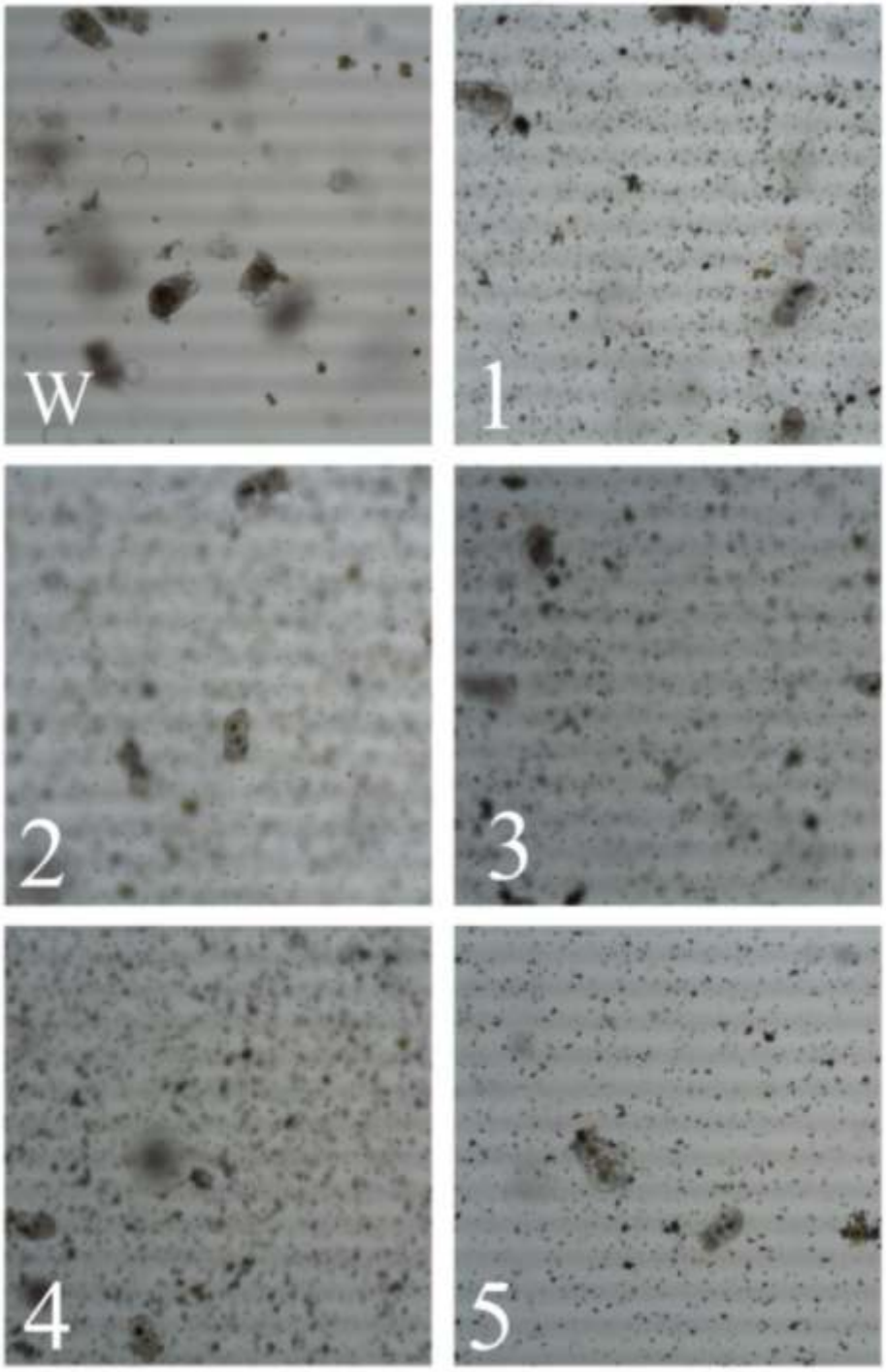

Fig. 5: Rotifers before (W) and after exposing to different volumes (1-5 mL) of TiO_2 suspension 1-5 mL, respectively after 1/2 h.

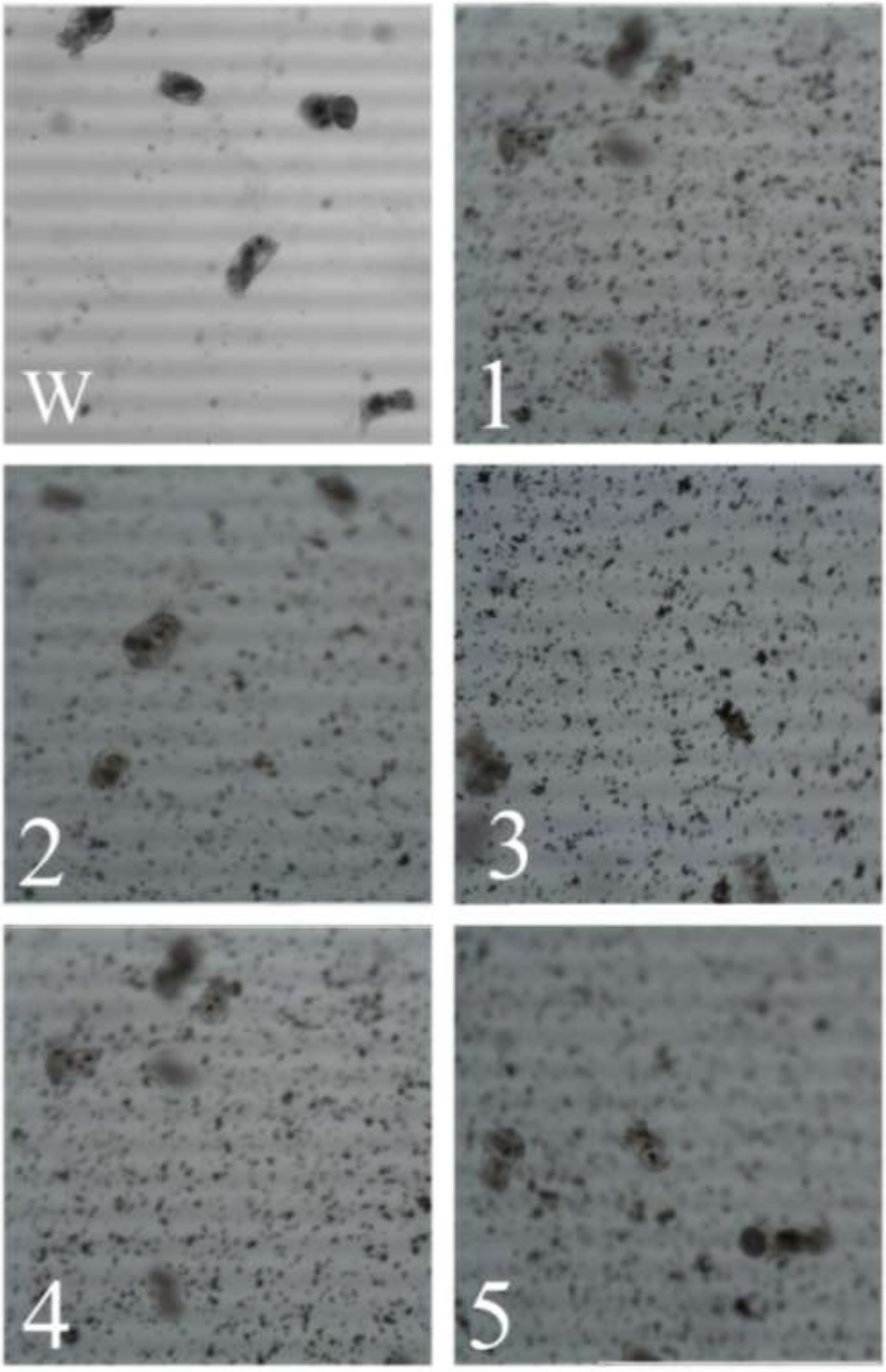

Fig. 6: Rotifers before (W) and after exposing to different volumes (1-5 mL) of TiO_2 suspension 1-5 mL, respectively after 4 h.

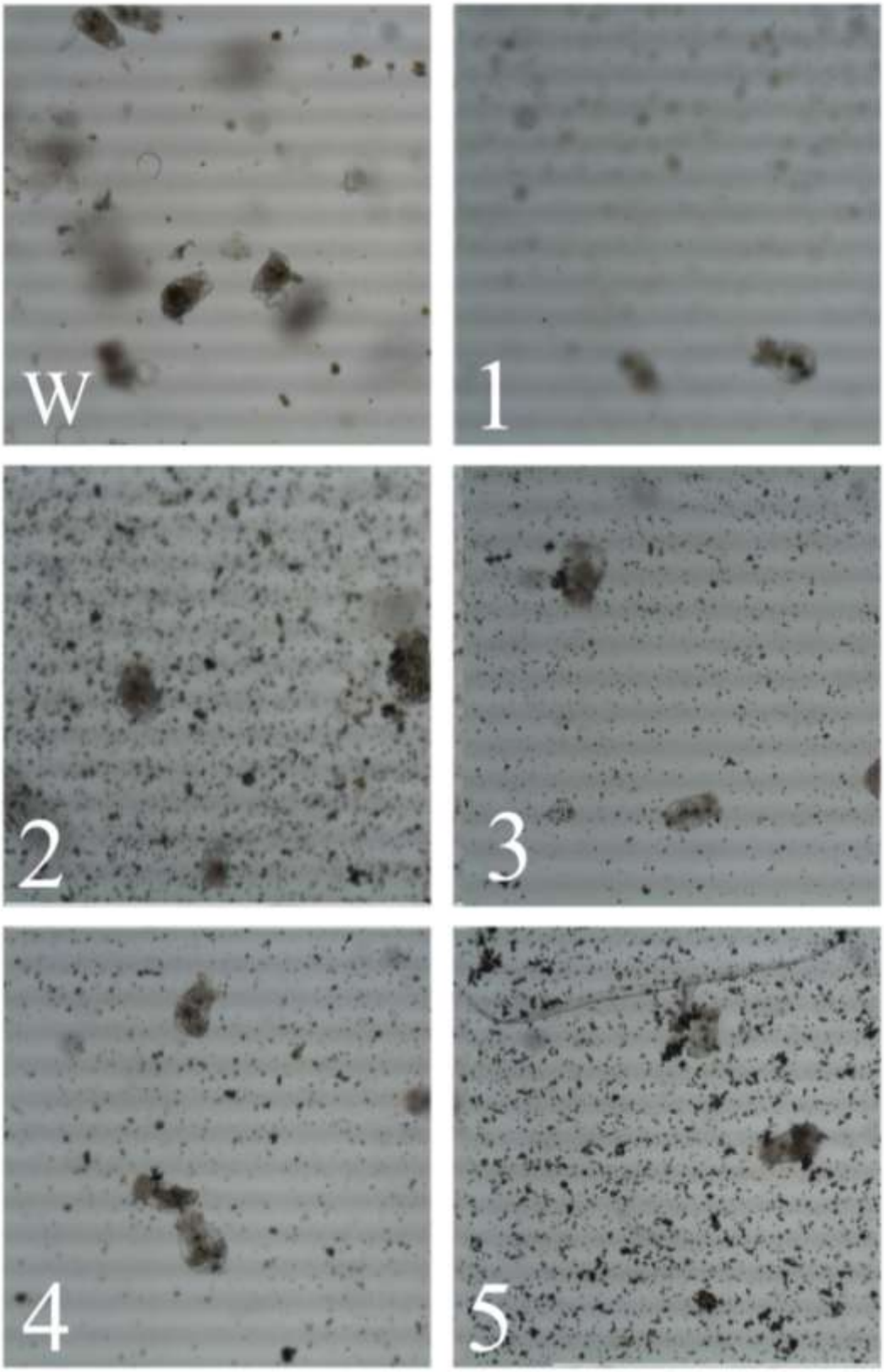

Fig. 7: Rotifers before (W) after exposing to different volumes (1-5 mL) of B-TiO_2 suspension 1-5 mL, respectively after 1/2 h.

CONCULOSION

In the present investigation, the un-doped and non-metal boron doped TiO_2 were synthesized by using the aqueous extract of mangrove plant leaves. Qualitative phytochemical screening of aqueous extract was performed to show major active constituents. Characterization of greenly prepared NPswas done using different techniques. The immobilization and deceleration of greenly prepared NPs were studied to determine its toxicity in the presence and absence of visible light source.

The data show that greenly prepared NPs. have slight toxicity with a maximum toxicityof 5 and 9% in the case of TiO_2 and B-TiO_2, respectively, in darkness after its treatment for 24 h. The maximum immobilization values attained were 9 and 23% for TiO_2 and B-TiO_2,respectively, which may be related to the aggregation of NPs and the data in presence of visible light source are better than in darkness.

DEDICATION

This work is presented to the spirit of Elsayed T. Helmy uncles Mr. Fathy Mohamed Helmy Abou-Elezz and Mr. Hesham Elmenshawy Asking God to bless them.

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