BIOINSPIRED SYNTHESIS OF TiO_2 NANOPARTICLES AND ITS EFFECT ON *SORGHUM BICOLOR*

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Abstract
Biosynthesis of nanoparticles provides great advantages such as low cost, biocompatibility, non-toxicity and easiest experimental protocol. Titanium dioxide is being one of the fascinating and technologically important materials in almost all the field of nanotechnology. Titanium dioxide nanoparticles are widely used because of its thermodynamic stability, anticorrosion, high photocatalytic activity, wide band gap, high transmittance in visible and infrared spectral range. In the present study, Titanium dioxide nanoparticles were synthesized from titanium isopropoxide as a precursor using Bacillus subtilis and the pure α-amylase enzyme. TiO_2 nanoparticles were characterized by Fourier-transform infrared spectroscopy (FTIR), UV-Visible spectroscopy, X-ray diffraction (XRD) analysis and scanning electron microscopy (SEM). The TiO_2 nanoparticles were found to be spherical, ellipsoidal and irregular in shape. Individual nanoparticles as well --as a few aggregates are found having the size of 5-20 nm. The XRD shows the crystallographic plane of anatase structure of TiO_2 nanoparticles. The synthesized TiO_2 nanoparticles also induced vigour index, antidiabetic and antioxidant property of Sorghum bicolor. TiO_2 treatments have potential to enhance the growth cycle, food chain and economics of Sorghum bicolor.

Keywords: TiO_2 nanoparticles, Bacillus subtilis, α-amylase, Sorghum bicolor, Antidiabetic, Antioxidant

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1. INTRODUCTION

Nanotechnology is a promising field, which covers a wide range of processes, material and applications. The main focus of this new field is the synthesis, characterization, fabrication and modification of material at nanoscale level (Handford and Cambell, 2014). Nanoparticles are non porus solid, atomic or molecular aggregates with dimension between 1 and 100 nm that can eventually modify their physico-chemical properties compared with the complex material. Nanoparticles can be made from a variety of complex materials and they can act depending on chemical composition, size or shape of the particles (Gupta and Tripathi, 2011). Nanoparticles are broadly classified into two groups of organic and inorganic nanoparticles. Organic nanoparticles include carbon nanoparticles the inorganic nanoparticles include magnetic nanoparticles, noble metal nanoparticles (Gold and Silver), semiconductor nanoparticles (TiO_2, ZnO) and metal-based materials (Al_2O_3, $BaTiO_2$ and ZrO_2) (Lide 1991). Smaller particle size enables the development of smaller sensors, which can be utilized more easily into remote locations. Biological synthesis of nanoparticles has grown markedly to create novel materials that are eco-friendly, cost effective and stable with great importance in wider application in the areas of electronics, medicine, food and agriculture (Gupta and Tripathi, 2011).Although nanoparticles can be synthesized through an array of conventional methods, the biological route of synthesis is advantageous as it provides rapid synthesis, controlled toxicity, control of size characteristics, low cost and eco-friendly approach (Waghmode *et al.*, 2019). Nanoparticles are extensively used for the removal of biological and chemical contaminants from the environment (Nia *et al.*, 2015). Nanoparticles are synthesized by different methods like physical, chemical, biological and hybrid methods (Tharanya *et al.*, 2015).

In present work *Bacillus subtilis* and α-amylase enzyme were used for the biosynthesis of TiO_2 nanoparticles. *Bacillus subtilis* is an oxygen tolerant, spore forming bacteria which can survive even in the harsh unfavourable conditions, which makes it a suitable genus for the biosynthesis of metal nanoparticles. This approach is useful for enhancing the downstream processes which has also been made to perceive the mechanism for biosynthesis at extreme level (Kirthi *et al.*, 2011). Enzymes provide a perfect set of structurally diverse biological system which provides unique nano forms (Mishra and Sardar, 2012). Pure α-amylase enzyme acts as a sole reducing agent and catalysed the formation of TiO_2 nanoparticles (Ahmad *et al.*, 2014). Many features of Nanotechnology to agricultural activities are still being explored (Dehkourdi and Mosavi, 2013). TiO_2 nanoparticles act as an effective nano pesticide for cotton leaf worm (Shaker *et. al.*, 2017). TiO_2 promotes seed germination and plant growth in edible cowpea (Owolade and Ogunleti, 2008), where as in the case of spinach, TiO_2 nanoparticles enhanced the protein and chlorophyll content (Yang *et al.*, 2007 and Siddiqui *et al.*, 2015).

Over the years, occurrence of diabetes has increased globally. Diabetes mellitus is intensifying to an alarming epidemic level. It is a group of metabolic diseases characterized by chronic hyperglycemia due to defects in insulin secretion, insulin action, or both. It is classified as one of the foremost causes of high mortality and morbidity rate. Uncontrolled diabetes can lead to stupor, coma and if not treated death, due to ketoacidosis or rare from nonketotic hyperosmolar syndrome. This condition is related to the formation of free radicals including advanced glycation end products (AGES) and to an increase in inflammatory processes. It confers an enormous economic burden owing to its management costs and its complications (Tan *et al*., 2019, Kharroubi and Darwish, 2015). Cost effective therapies are being targeted nowadays mainly the biosynthetic drugs (Pedroso *et al.*, 2019, Sathiavelu *et al.*, 2013, Yadav *et al.*, 2008, Rajurkar and Hande 2011) together with nanoparticles. The nanoparticles used in agriculture purpose for plant growth have received a lot of attention due to their impact on phytochemical synthesis in plants(Ebadollahi *et al.*, 2019, Chung *et al.*, 2019, Jasim *et al.*, 2017, Quiterio-Gutierrez *et al.*, 2019). Literature survey revealed that *Sorghum bicolor* contain phytochemical components which possess antioxidant and antidiabetic activity (Awika and Rooney, 2004, Morais Cardoso *et al.*, 2015). The phytochemicals of *Sorghum bicolor* are mainly phenolic compounds, policosanols and plant sterols. These phytochemicals have different types of antioxidant component which shows different antioxidant activity depend on their harvest time (Jeon *et al.*, 2017). In present study we have tested the effect of TiO_2 nanoparticles on the antidiabetic and antioxidant properties of *Sorghum bicolor*.

2. MATERIALS AND METHODS

2.1 Biosynthesis of TiO_2 nanoparticles and its characterization

2.1.1 Chemicals and reagents

Culture media Luria Bertani agar was purchased from Hi-media (India). Titanium isopropoxide (98%) was procured from Avra Synthesis Pvt. Ltd. (India). Bacterial strains *Bacillus subtilis* was isolated from soil on Luria Bertani agar plates. Unienzyme tablet was used as amylase source prepared by Torrent Pharmaceuticals Ltd., Ahmadabad. Deionised water was used throughout the experiment. The glass wares were washed in chromic acid and thoroughly washed with double distilled water. All other reagents used in reaction were of analytical grade with maximum purity (Lide1991,Tharanya*et al.*, 2015,Siddiqui *et al.*, 2015].

2.1.2 Synthesis of TiO_2 nanoparticles by using *Bacillus subtilis.*

Bacillus subtilis was allowed to grow in Luria Bertani broth by incubating for 24 hours at 37^0C in shaking condition at 100 rpm and this was treated as source culture. 100 ml of the culture was taken and diluted with 400 ml of sterile LB broth and allowed to grow for next 24 hours. In order to prepare TiO_2 precursor solution, 4.5 ml of Titanium isopropoxide was drop wise added in previously diluted culture media under vigorous stirring. The stirring was continued for 5 hours to get homogeneous mixing. After that the precursor solution was centrifuged at 4300 rpm for 15 min. Then the pellet was collected and washed for 4 times in deionised water to remove excess broth. Obtained powder was dried at 45^0C in hot air oven and gently grinded using glass mortar and pestle to avoid any structural and morphological changes in the final fine powder (Lide1991,Tharanya*et al.*, 2015).

2.1.3 Synthesis of TiO_2 nanoparticles by using α Amylase Enzyme

Twenty fiveml of 0.025 M Titanium isopropoxide (aqueous) solution was added to 30 ml of α-amylase enzyme. Unienzyme tablet was dissolved in 20 mM sodium acetate buffer of pH 4.5 to get final 2 mg/ml of concentration and incubated at 60^0C for 10 minutes. The solution was cooled and kept at 25^0C with continuous shaking. After 24 hours the mixture was centrifuged at 4000 rpm for 10 minutes. The nanoparticles of TiO_2 obtained in the pellet were washed with deionised water for three times to remove the unbound enzyme. Air dried nanoparticles were used for further characterization (Mishra and Sardar, 2012, Ahmad *et al.*, 2014, Zhao *et al.*, 2014).

2.1.4 Characterization of synthesized TiO_2 nanoparticles

The spectra of TiO_2 nanoparticles synthesized from *Bacillus subtilis* and α-amylase enzyme were measured using FT-IR Perkin Elmer. The UV-Vis spectra of TiO_2 nanoparticles were recorded in Schimadzu UV-2600 spectrophotometer in the 200-800 nm range operated at a resolution of 1 nm (Singh, 2016). The synthesized nanoparticles were used for XRD analysis in Rigaku miniflex 600 X-ray diffractometer. The diffracted intensities were recorded from 0°-70° 2θ angles. Scanning electron microscopy (SEM) analysis was performed on FEI Nova NanoSEM 450 (Tharanya *et al.,* 2015).

2.2 Effect of synthesized TiO_2 nanoparticles on vigour index and in vitro activities of *Sorghum bicolor.*

2.2.1 Chemicals and reagents

0.02 M PO_4 Buffer, 0.06 M NaCl, 1% Starch, Ascorbic Acid was brought from HiMedia Laboratories, Mumbai, India. DNSA reagent (1gm of dinitro salicylic acid and 30 gm of sodium potassium tartrate tetrahydrate to 50ml deionised water, add 20 ml 2N solution and make final volume 100 ml with deionised water), Methanol, DPPH reagent (0.01 gm 2,2-diphenyl-1-picryl-hydrazyl-hydrate to 50 ml ethanol). Unienzyme tablet was used as amylase source prepared by Torrent Pharmaceuticals Ltd., Ahmedabad. All other chemical, reagents, kits and solvents used in this study were of analytical grade.

2.2.2 Pot Assay

Plastic pots having length 11cm and diameter 8cm were filled with 200gm of soil and moisten soil with water. Slurry of *Bacillussubtilis*mediated and enzyme mediated TiO_2 nanoparticles with different concentrations (25 ppm, 50 ppm, 75 ppm and 100 ppm) was prepared. Surface sterilized seeds of *Sorghum bicolor* were soaked for half an hour in TiO_2 slurry. The soaked seeds were sowed in the previously moistened soil. The primitive growth of the seed was observed after four days. During the plant growth the pots were watered twice a day to maintain moisture condition of the soil. Plants were allowed to grow for 10 days. The vigour index was calculated by measuring the length of root and height of the shoot (Dehkourdi and Mosavi, 2013).

2.2.3 Plant extract preparation

Plants were washed with deionised water to remove soil. Plant extracts were prepared in deionised water by adding 10 ml of water filter the extract through filter paper (blotting paper) and then centrifuged at 4000 rpm for 10 minutes. Supernatant was used for the antioxidant assay and antidiabetic assay (Dehkourdi and Mosavi, 2013).

2.2.4 Antidiabetic Assay of *Sorghum bicolor* extracts

For determination of antidiabetic activity, α-Amylase inhibitory test, Glucose diffusion inhibitory test (Ebadollahi *et al.*, 2019), α-glucosidase inhibitory test, sucrose inhibitory test (Dsouza and Lakshmidevi, 2015), has been generally used. α-Amylase inhibitory test was employed for antidiabetic assay of *Sorghum bicolor* extracts.

α-Amylase inhibitory test

Extract of *Sorghum bicolor* grown in the presence of TiO_2 were used as the test sample. Aqueous extract of 500 µl each, was added into 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing 0.5mg/ml of α-amylase enzyme. The mixture was pre-incubated for 10 min at 25°C. After pre-incubation 500 µl of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006M sodium chloride) was added to each tube at 5s intervals and incubated at 25°C for 10 min. 1ml of DNSA reagent was added to the mixture and tubes were kept in boiling water bath for 5min. The reaction mixture was cooled and volume was made to10ml by adding distilled water. Absorbance was measured at 540nm [Ebadollahi *et al.*, 2019, Malapermal *et al.*, 2017].

2.2.5 Antioxidant Assay of *Sorghum bicolor* extracts

Antioxidant activity can be determined by DPPH and ABTS radical scavenging activities has been generally used (Morais Cardoso *et al.*, 2015). DPPH assay was carried out.

DPPH assay

The antioxidant assay was carried out by DPPH (2,2-diphenyl-1-picrylhydrazyl) method. DPPH was solubilized in the methanol to have a solution of 0.3 mM. Aqueous extract was taken in a tube and mixed with methanolic DPPH reagent in 1:1 proportion, the solution was mixed thoroughly. Tubes were incubated for 30 min in dark. Absorbance was taken at 515 nm (MoraisCardoso *et al.*, 2015, Jeon *et al.*,2017, Laware and Rasker 2014).

3. RESULTS AND DISCUSSION

3.1 FT-IR analysis of TiO_2 nanoparticles

The FT-IR of Perkin Elmer make with UATR instrument was used for measuring spectra of synthesized TiO_2 nanoparticles. The FT-IR spectra of synthesized TiO_2 nanoparticles exhibited prominent peaks at 999.56, 1092.78, 1537.89, 1635.96, 1641.34, 1661.90, 3026.21, 3275.73, 3363.78 cm^{-1}. The shift from 3026.21 to 3275.73 cm^{-1} showed O-H stretching due to alcoholic group. The shift from 1537.89 to 1641.34 cm^{-1} showed presence of c=c ring stretching.

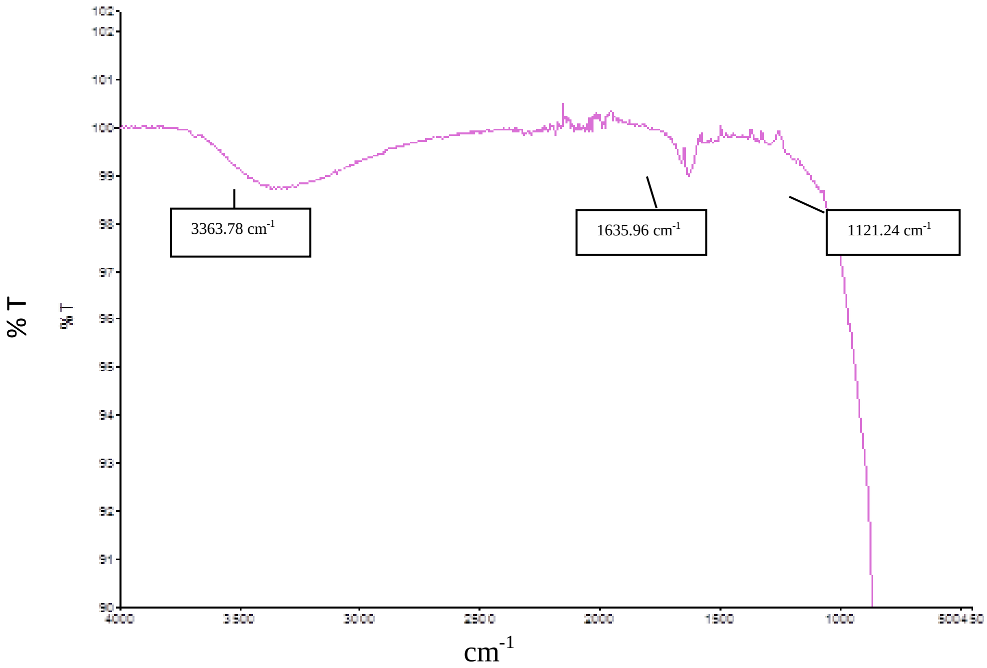
In fig 1. (A), the peak at 1121.24 cm^{-1}showed presence of Standard TiO_2. In fig 1. (2) and (3), The shift from 999.56 to 1092.78 cm^{-1} shows presence of TiO_2. The characteristic wide peaks in the region of 800-1200 cm^{-1} are related to the bending vibration of the Ti-O bonds (Tharanya *et al.*, 2015, Bhujbal *et al.*, 2017, Kong and Yu, 2007).

3.2 UV-VIS Spectroscopic analysis of TiO_2 nanoparticles

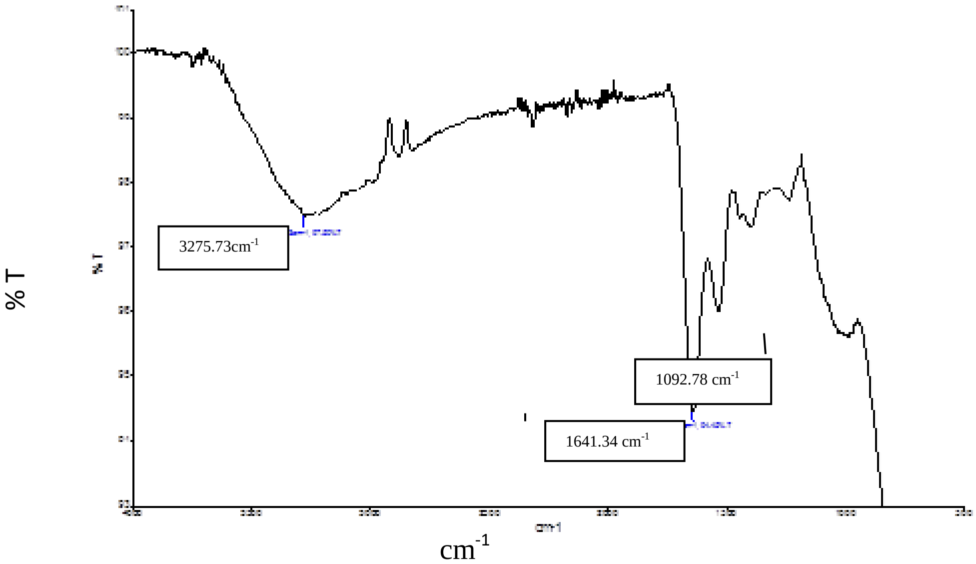
The optical properties of synthesized TiO_2 nanoparticles were studied using Shimadzu UV-2600 spectroscopy. Fig. 2, shows the absorbance of the samples in nano range at room temperature. The spectra exhibited between 200-800 nm ranges were studied. In fig 2. (A) spectra observed at 366 nm showed presence of standard TiO_2 nanoparticles. In fig 2. (B), Bacillus mediated TiO_2 nanoparticles showed spectra at 363 nm and in fig 2. (C), Alpha amylase mediated nanoparticles showed spectra at 367 nm. The wavelength of synthesized TiO_2 nanoparticles appeared in between 360-370 nm in UV-Vis spectroscopy, which indicates the presence of TiO_2 nanoparticles in the sample (Tharanya *et al.*, 2015).

3.3 XRD analysis of TiO_2 nanoparticles

The synthesized TiO_2 nanoparticles were used for the X-Ray Diffractometer analysis. XRD pattern for *Bacillus subtilis* mediated TiO_2 nanoparticles showed distinct diffraction peaks at 28.32^0, 45.22^0, 52.48^0 and 60.02^0. Alpha amylase mediated nanoparticles showed diffraction peaks at 28.58^0, 28.89^0, 41.32^0, 43.92^0 and 62.36^0 which is regarded as an attribute indicator of the biologically synthesized nanoparticles TiO_2 crystallites. The sample showed weak and broad peaks indicating the amorphous nature of synthesized TiO_2 and a slight increase in the intensity of peaks correspond to anatase form of TiO_2 (Lide 1991, Tharanya *et al.*, 2015, Bhujbal *et al.*, 2017).



(a)



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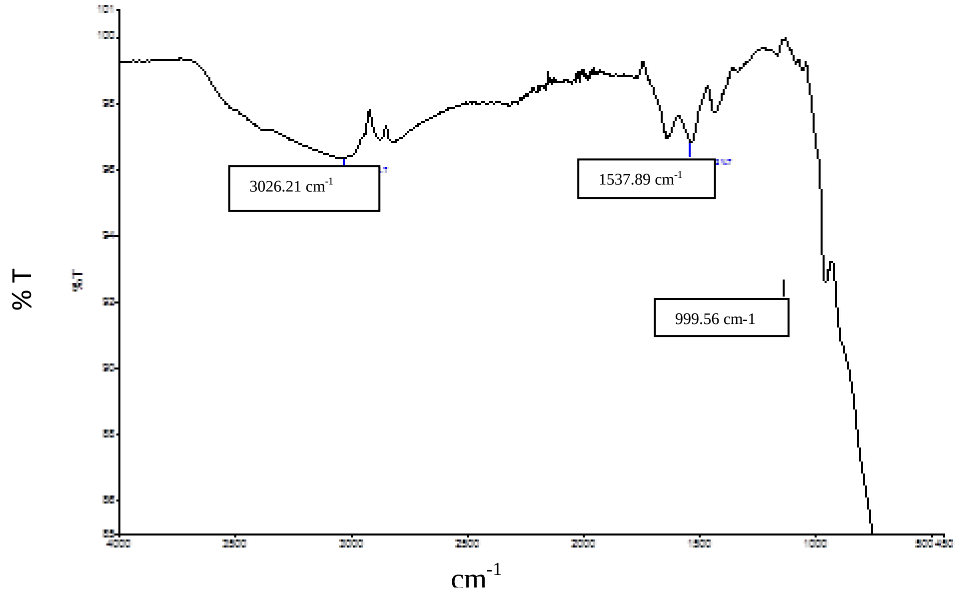
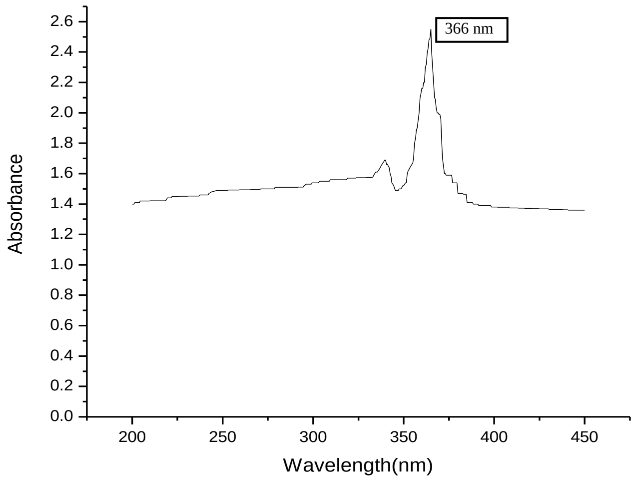


(c)

Figure 1. FT-IR spectra of (a) Standard TiO_2 nanoparticles (b) TiO_2 nanoparticles synthesized from *Bacillus subtilis*. (c) TiO_2 nanoparticles synthesized from α-amylase enzyme.



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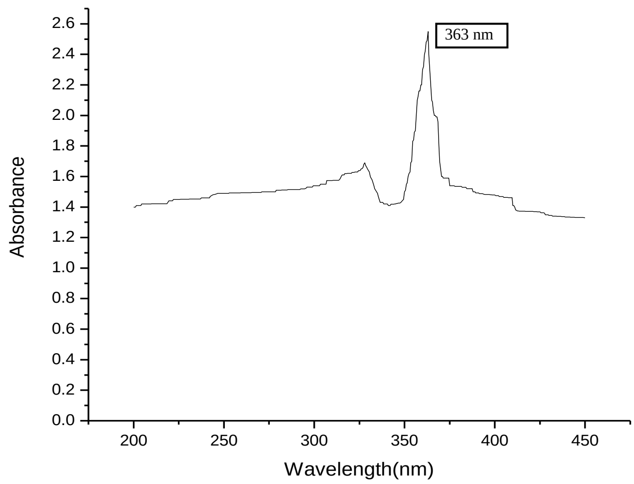

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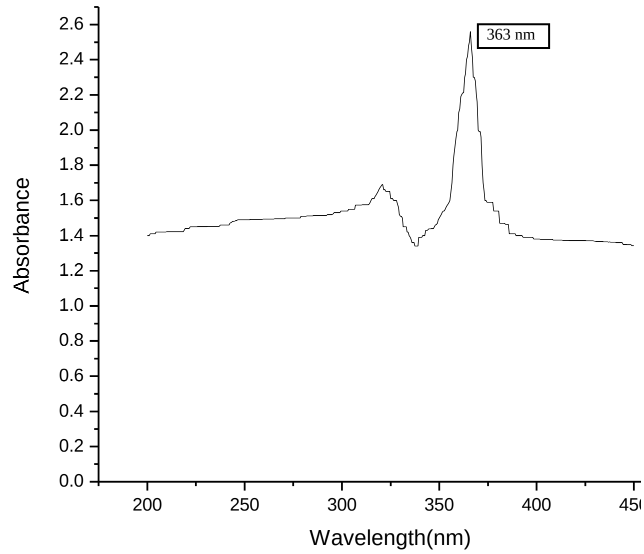
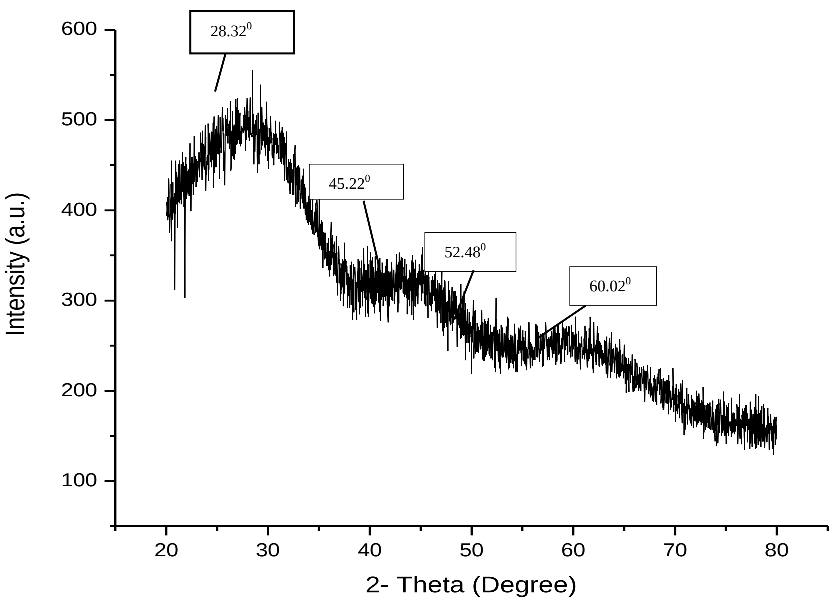
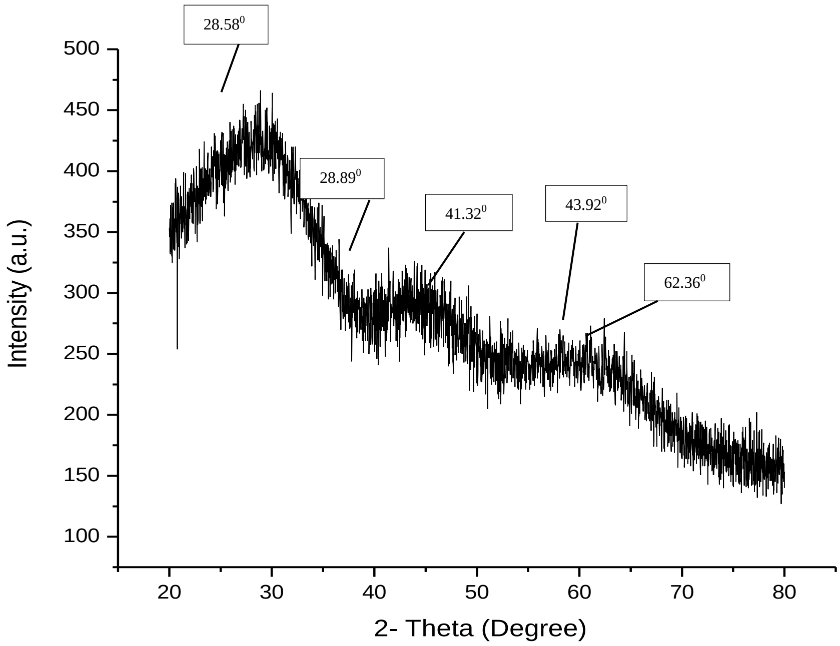


(c)

Figure 2. UV-Vis spectra of (a) Standard TiO_2 nanoparticles (b) TiO_2 nanoparticles synthesized from *Bacillus subtilis* (c) TiO_2 nanoparticles synthesized from α-amylase enzyme.



(a)



(b)

Figure 3. XRD pattern of (a) Synthesized TiO_2 nanoparticles from Bacillus subtilis, (b) Synthesized TiO_2 nanoparticles from α-amylase enzyme.

3.4 SEM analysis of TiO_2 nanoparticles

The Scanning electron microscopic images of TiO_2 nanoparticles synthesized from *Bacillus subtilis and* α-amylase enzyme are showed in fig. 4, The formation of TiO_2 nanoparticles and their morphology in the SEM study demonstrate d that synthesized nanoparticles were spherical, ellipsoidal and irregular in shape, individuals as well as a few aggregates were seen (Lide 1991, Tharanya*et al.*, 2015).

3.5 Pot Assay

Vigour index determines the emergence of seeds under favourable as well as unfavourable conditions. Seed vigour is a concept describing various aspects of seed performance in field as purity, health and viability of seed play an important role in germination (Venter, 2000). From Table 1, the data results were analysed statistically as the mean $\pm$SD with one-way analysis of Variance (ANOVA) (Anupama *et al.*, 2014). Taking into account p value and f value, it can be concluded that due to the treatment of TiO_2 nanoparticles, vigour index significantly improved in both between and within groups (Rastegar *et al.*, 2011).

3.6 Antidiabetic assay using α-amylase inhibitory test

The results of this experiment are summarized in fig. 6, antidiabetic activity was carried out by α-amylase inhibitory test using aqueous extracts of *Sorghum bicolor* grown in presence and in absence of TiO_2 nanoparticles. The plant extract of *Sorghum bicolor* grown in absence of TiO_2 nanoparticles (control) showed 68% of antidiabetic activity. The plant showed maximum inhibition of the α-amylase enzyme with the highest value of 92.64% seen at 25mg/ml concentration of *Bacillus subtilis* mediated TiO_2 nanoparticles. *Sorghum bicolour* showed maximum inhibition of the α-amylase enzyme with highest value of 94.11% at 100mg/l concentration of α-amylase mediated TiO_2 nanoparticles. [Ebadollahi *et al.*, 2019, Malapermal *et al.*, 2017].

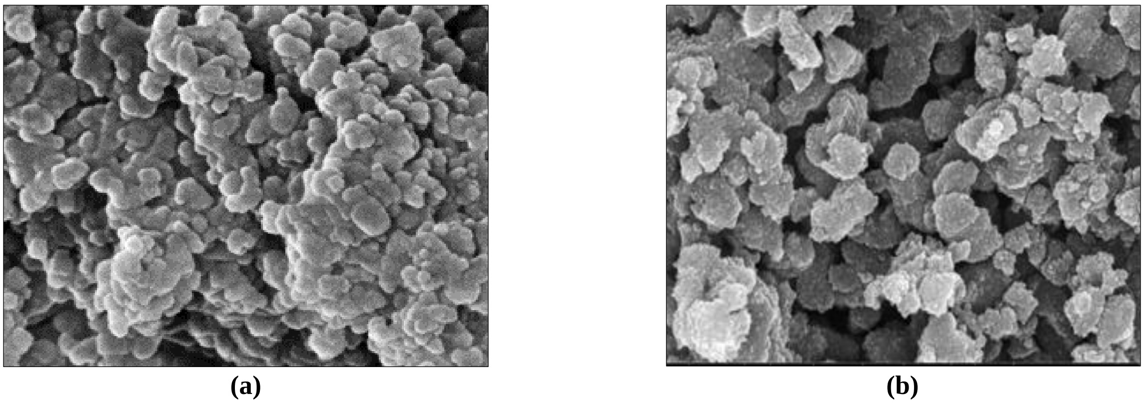


Figure 4. Scanning electron microscopic images of (a) Synthesized TiO_2 nanoparticles from Bacillus subtilis. (b) Synthesized TiO_2 nanoparticles from α-amylase enzyme

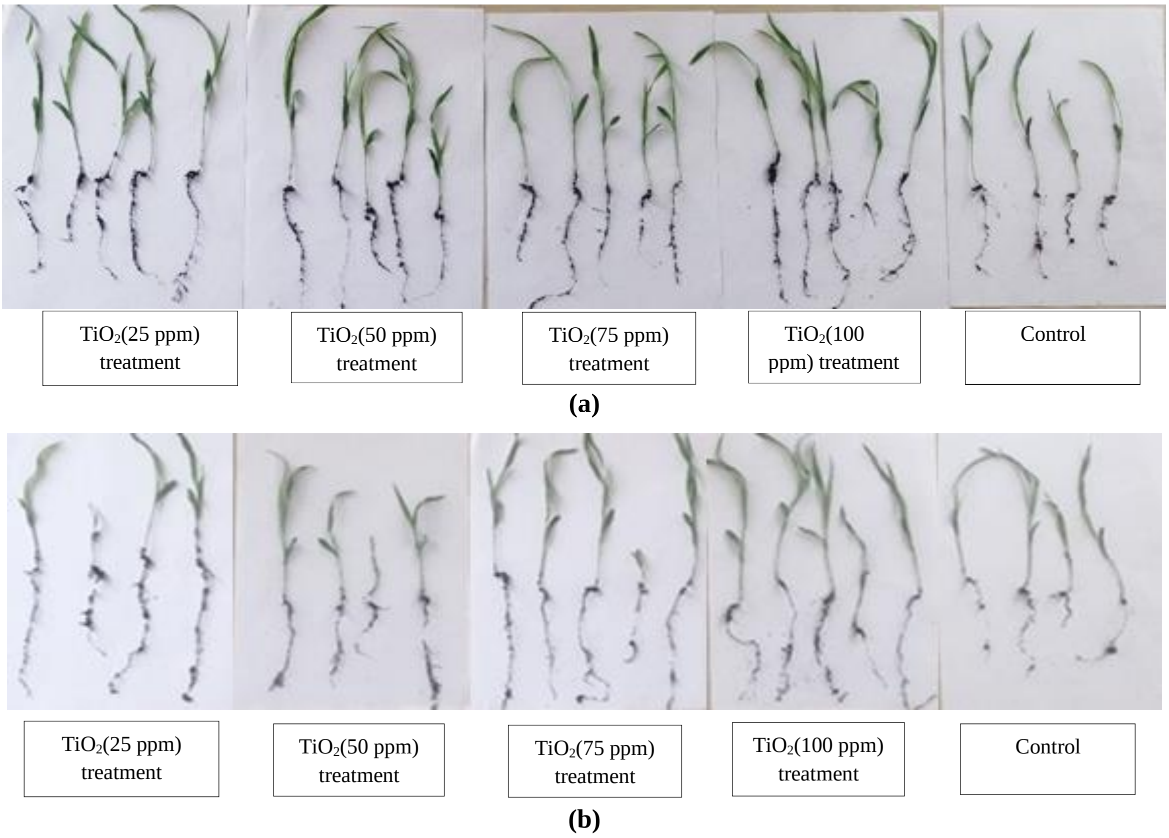


Figure 5. Growth of Sorghum bicolor grown in presence of (a) *Bacillus sublitis* mediated TiO_2 nanoparticles after 10 days, (b) α-amylase mediated TiO_2 nanoparticle after 10 days

Table 1. Effect of TiO_2 nanoparticles on Sorghum bicolor

Sr. No	Treatment	Concentration (ppm)	Shoot length Mean(cm)	Root length Mean(cm)	Seed Germination (%)	Vigour Index
1	*Sorghum bicolor* seed treated with *Bacillus subtilis* mediated TiO_2 NPs	25	2.65	13.8	100	1645
		50	2.74	13.16	100	1590
		75	2.62	10.5	100	1312
		100	3.5	13.44	100	1694
2	*Sorghum bicolor* seed treated with α-amylase mediated TiO_2 NPs	25	3.4	11.84	100	1524
		50	3.26	12.64	100	1590
		75	3.4	10.34	100	1374
		100	3.3	12.9	100	1620
3	Control	-	3	9.55	100	1253

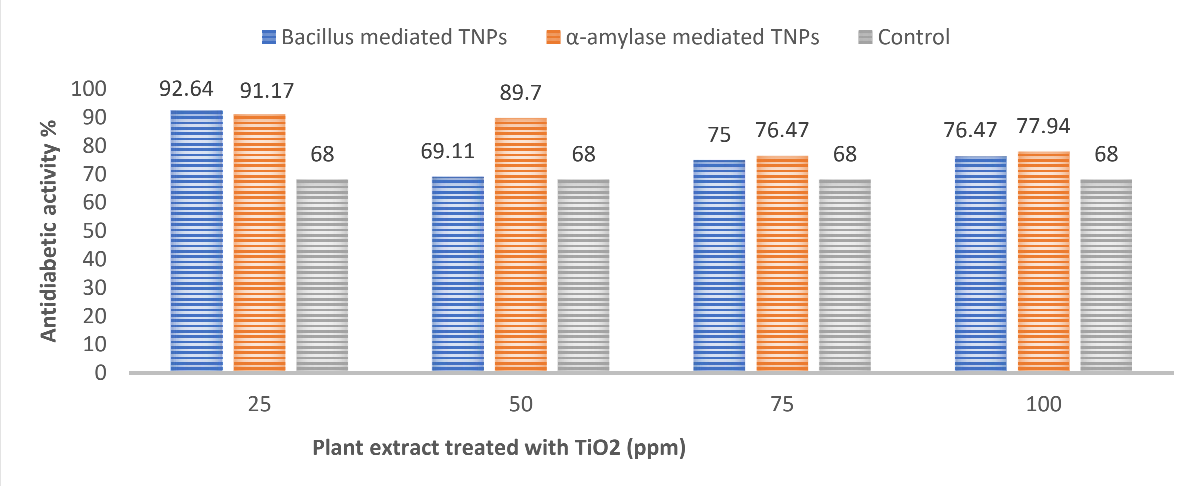


Figure 6. Antidiabetic activity of Bacillus mediated and α-amylase mediated TiO_2 nanoparticles.

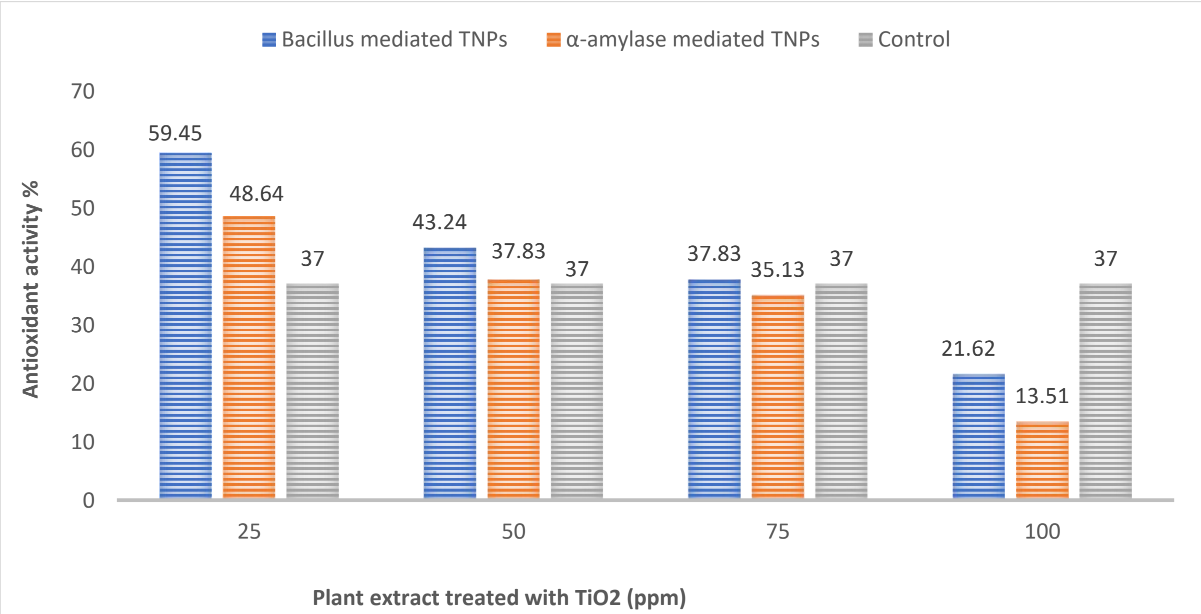


Figure 7. Antioxidant activity of Bacillus mediated and α-amylase mediated TiO_2 nanoparticles.

3.7 Antioxidant assay using DPPH method

Antioxidants activity was carried out using DPPH Assay of aqueous extracts of *Sorghum bicolour* grown in presence and in absence of TiO_2 nanoparticles. Antioxidant activity depends on harvest time and concentration of phenolic content present in extract due to the maximum correlations between phenolic compounds and DPPH radical scavenging mechanism. In fig.7, antioxidant components and their activities maintain higher values at low concentration of TiO_2 nanoparticles. The plant extract of *Sorghum bicolour* grown in absence of TiO_2 nanoparticles (control) showed 37% of antioxidant activity. The plant extract showed high antioxidant activity value of 59.45% in presence of 25mg/l concentration of *Bacillu ssubtilis* mediated TiO_2 nanoparticles than α-amylase mediated TiO_2 nanoparticles of value 48.64% seen at 25mg/l. (MoraisCardoso *et al.*, 2015, Laware and Rasker, 2014).

4. CONCLUSION

The work explains the use of inexpensive, nontoxic and eco-friendly easily available microorganisms and enzymes for the rapid synthesis of TiO_2 nanoparticles. The synthesized TiO_2 nanoparticles were characterized by using UV-Vis spectra ranges between 360-370 nm. FT-IR spectra clearly indicates O-H stretching due to alcoholic group, presence of c=c ring stretching and presence of TiO_2at region 800-1200 cm^{-1} shows bending vibrations of Ti-O bonds. The crystallinity of the TiO_2 nanoparticles was confirmed by XRD analysis and it revealed the presence of amorphous nature and anatase phase of TiO_2. SEM images of *Bacillus subtilis* mediated and α-amylase mediated TiO_2 nanoparticle showed individual nanoparticles as well as a few aggregates having the size of 5-20 nm are found. The bacterial biosynthesis of the TiO_2 nanoparticles provides a fast, and purest form of producing nanoparticles. The use of a specific enzyme in the *in vitro* synthesis of nanoparticles is important as it reduces the steps involved in the processing required for the use of these nanoparticles in many aspects. TiO_2 nanoparticles have potential to enhance the growth rate of *Sorghum bicolor*. The plant showed highest vigour index at highest concentration (100ppm) of *Bacillus subtilis* mediated TiO_2 nanoparticles than α-amylase mediated TiO_2 nanoparticles. The *Bacillus subtilis* mediated TiO_2 nanoparticles also responsible for significant changes in antidiabetic and antioxidant property of *Sorghum bicolor*. The plant extract showed high antidiabetic activity at low concentration (25ppm) of Bacillus mediated TiO_2 and at high concentration (100ppm) of α-amylase mediated TiO_2. The plant extract showed highest antioxidant activity in presence of less concentration (25ppm) of both *Bacillus subtilis* mediated and α-amylase mediated TiO_2 nanoparticles, but *Bacillus subtilis* mediated TiO_2 nanoparticles showed high antioxidant activity than α-amylase mediated TiO_2 nanoparticles. This study provides scientific evidence on effect of TiO_2 nanoparticles to enhance growth, antidiabetic and antioxidant potential of *Sorghum bicolor*.

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CONFLICT OF INTEREST

Authors declared that they have no conflict of interest.

5. REFERENCES

[1]. Ahmad, R., Mohsin, M., Ahmad, T. and Sardar, M. (2014). Alpha amylase assisted synthesis of TiO_2 nanoparticle: Structural characterization and application as antibacterial agents. Journal of Hazardous Materials, 283:171-177.

[2]. Anupama, N., Murali, M., Jogaish, S. and Amrutesh, K. N. (2014). Crude oligosaccharides from *Alternaria solani* with *Bacillus subtilis* enhance defence activity and induce resistant against early blight disease and tomato. Asian Journal of Science and Technology, 5(7):412-416.

[3]. Awika, J. M. and Rooney, L. W. (2004). Sorghum phytochemicals and their potential impact on human health. A review, Phytochemistry, 65:1199-1221.

[4]. Bhujbal, N. N., Takle, S. P., Khore, S. K., Naik, S. D., Landge, S. L., Kale, B. B. and Sonawane, R. S. (2017). Cr doped TiO_2 catalysin photocatalytic degradation of jakofix red dye [HE7B]. IJSRST, 3(1):2395-6011.

[5]. Chung, I. M., Rajakumar, G., Subramanian, U., Venkidasamy, B. and Thiruvengadam, M. (2019). Impact of Copper Oxid Nanoparticles on Enhancement of Bioactive Compounds Using Cell Suspension Cultures of Gymnemasylvestre (Retz.) R. Br. Appl. Sci, 9: 2165.

[6]. Dehkourdi, E. H. and Mosavi, M. (2013). Effect of Anatase Nanoparticles (TiO_2) on Parsley Seed Germination (Petroselinum crispum) In Vitro. Biological Trace Element Research, 155:283–286.

[7]. Dsouza, D. and Lakshmidevi, N. (2015). Models to study invitro antidiabetic activity of plants: A review. International Journal of Pharma and Bio Sciences, 6(3):732-741.

[8]. Ebadollahi, R., Jafarirad, S. and Kosari-Nasab, M. (2019). Effect of explant source, perlite nanoparticles and TiO2/perlite nanocomposites on phytochemical composition of metabolites in callus cultures of Hypericum perforatum. Scientific Reports, Nature research, 9: 12998.

[9]. Gupta, S. M. and Tripathi, M. (2011). A review of TiO_2 nanoparticles, Chinese Science Bulletin, 56:1639-1657.

[10]. Handford, C. E. and Cambell, K. (2014). Implications of nanotechnology for agri-food industry: Opportunity benefits and risks. Trends in Food Science and Technology, 40(2): 226-241.

[11]. Jasim, B., Thomas, R., Mathew, J. and Radhakrishnan, E. K. (2017). Plant growth and diosgenin enhancement effect of silver nanoparticles in Fenugreek (*Trigonellafoenumgraecum L.*). Saudi Pharmaceutical Journal, 25:443–447.

[12]. Jeon, S. H., Kim, I. S., Park, S. K., Jung, K. Y., Kim, S. W. and Cho, Y. S. (2017). Dependence of Sorghum bicolor antioxidant activity on harvest time. ScienceAsia, 43:155-162.

[13]. Kharroubi, A. T. and Darwish, H. M. (2015). Diabetes mellitus: The epidemic of the century. World Journalof Diabetes, 6(6): 850–867

[14]. Kirthi, A. V., Rahuman, A. A., Rajkumar, G., Marimuthu, S., Santoshkumar, T., Jayaseelan, C., Elango, G., Zahir, A. A., Kamaraj, C. and Bagvan, A. (2011). Biosynthesis of titanium dioxide nanoparticles using bacterium *Bacillus subtilis*. Material Letters, 65:2745-2747.

[15]. Kong, J. and Yu, S. (2007). Fourier transform infrared spectroscopic analysis of protein secondary structures. Acta Biochimia et BiophysicaSinica, 39(8):549-559.

[16]. Laware, S. L. and Rasker, S. (2014). Effect of titanium dioxide nanoparticles on hydrolytic and antioxidant enzymes during seed germination in onion. International Journal of Current Microbiology and Applied Sciences, 3(7):749-760.

[17]. Lide, D. R. (1991). Biosynthesis of TiO_2 and its application. Handbook of chemistry and physics, 71st Edition F1 CRC, Boca Raton.

[18]. Malapermal, V., Botha, I., Krishna S. B. N. And Mbatha., J. N. (2017). Enhancing antidiabetic and antimicrobial performance of *Ocimumbasilicum*, and *Ocimum sanctum (L.)* using silver nanoparticles. Saudi Journal of Biological sciences, 24:1294-1305.

[19]. Mishra, A. and Sardar, M. (2012). Alpha-amylase mediated synthesis of silver nanoparticles. Science of Advanced Materials, 4:143-146.

[20]. MoraisCardoso, L. D., Pinheiro, S. S., Martino, H. S. D. and Pinheiro-Sant'Ana, H. M. (2015). Sorghum (*Sorghum bicolor I.*): Nutrients, Bioactive Compounds, and potential impact on human health. Critical Reviews in Food science and Nutrition, 1-77.

[21]. Nia, Y., Millour, S., Noel, L., Krystek, P., Jong, W. and Guerin, T. (2015). Determination of Ti from TiO_2 nanoparticles in biological materials by different ICP-MS instrument: Method validation and applications. J NanomedNanotechnol, 6:269.

[22]. Owolade, O. F. and Ogunleti, D. O. (2008). Effects of titanium dioxide on the diseases, development and yield of edible cowpea. Journal of Plant Protection Research, 48(3):329-335.

[23]. Pedroso T, F. D., Bonamigo, T. R., Vasconcelos J, S. P., Félix, J. M., Cardoso C, A. L., Souza R, I. C., Santos A, C. D., Volobuff C, R. F., Formagio A, S. N. and Trichez V, D. K. (2019). Chemical constituents of *Cochlospermum regium* (Schrank) Pilg. root and its antioxidant, antidiabetic, antiglycation, and anticholinesterase effects in Wistar rats. Biomedicine and Pharmacotherapy, 111: 1383-1392.

[24]. Quiterio-Gutiérrez, T., Ortega-Ortiz, H., Cadenas-Pliego, G., Hernández-Fuentes, A. D., Sandoval-Rangel, A., Benavides-Mendoza, A., la Fuente M. and Juárez-Maldonado, A. (2019). The Application of Selenium and Copper Nanoparticles Modifies the Biochemical Responses of Tomato Plants under Stress by Alternaria solani. International Journal of Molecular Sciences, 20:1950.

[25]. Rajurkar, N. S. and Hande, S, M. (2011). Estimation of phytochemical content and antioxidation activity of some selected traditional Indian medicinal plants. Indian Journal of Pharmaceutical Sciences, 73(2):146-151.

[26]. Rastegar, Z., Sedghi, M. and Khomari, S. (2011). Effects of accelerated aging on soybean seed germination indexes at laboratory conditions. Notulae Scientia Biologicae, 3(3):126-129.

[27]. Sathiavelu, A., Sangeetha, S., Archit, R. and Mythili, S. (2013). In Vitro anti-diabetic activity of aueous extract of the medicinal plants *Nigella sativa*, *Eugenia jambolana*, *Andrographis paniculata* and *Gymnemasylvestre*. International Journal of Drug Development and Research, 5(2):0975-9344.

[28]. Shaker, A. M., Zaki, A. H., Abdel-Rahim, E. F. M. and Khedr, M. H. (2017). TiO_2 nanoparticles as an effective nanopesticide for cotton leaf worm. Agricultural Engineering International: CIGR Journal, 61-68.

[29]. Siddiqui, M. H., Al-Whaibi, M. H., Firoz, M. and Al-Khaishany, M. Y. (2015). Role of nanoparticles in plants. Nanotechnology and Plant Science, 19-35.

[30]. Singh, P. (2016). Biosynthesis of titanium dioxide nanoparticles and their antibacterial property. International Journal of Chemical and Molecular Engineering, 10(2):275-278.

[31]. Tan, S.Y., Mei Wong, J. L., Sim, Y. J., Wong, S. S., Mohamed Elhassan, S. A., Tan, S. H., Ling Lim, G. P., Rong Tay, N. W., Annan, N. C., Bhattamisra, S. K. and Candasamy, M. (2019). Type 1 and 2 diabetes mellitus: a review on

current treatment approach and gene therapy as potential intervention. Diabetes and Metabolic Syndrome: Clinical Research and Reviews, 13 (1): 364-372.

[32]. Tharanya, P., Vadakkan, K., Hemapriya, J., Rajeshkannan, V. and Vijayanand, S. (2015). Biogenic approaches for the synthesis of Titanium dioxide nanoparticles using a halophilic bactrial isolate- *Chromohalobactersalexigens* strain PMT-1. Int. J. Curr. aca. Rev, 3 (10):334-342.

[33]. Venter, A. V. D. (2000). What is seed vigour?. Journal of New Seeds, 2(3):62-72.

[34]. Waghmode, M., Gunjal, A., Mulla, J., Patil, N. and Nawani, N. (2019). Studies on the titanium dioxide nanoparticles: biosynthesis, application and remediation. A review, SN Applied Sciences, 1:310.

[35]. Yadav, J. P., Saini, S., Kalila, A. N. and Dangi, A. S. (2008). Hypoglycemic and Hypolipidemic activity of ethanolic extract of *Salvadoraoleoides* in normal and alloxan-induced diabetic rats. Indian J Pharmacol, 40(1):23-27.

[36]. Yang, F., Liu, C., Gao, F., Su, M., Wu, X., Zheng, L., Hong, F. andYang, P. (2007). The improvement of spinach growth by nano-anatase TiO_2 treatment is related to nitrogen photoreduction. Biol Trace Elem Res, 119:77-88.

[37]. Zhao, Y., Lin, K. And Zhang, W. (2014). Nano-titanium dioxide (TiO_2)-induced changes affecting Cu^{2+}-mediated alternations in bacterium *Bacillus subtilis* and α-amylase. Toxicological and Environmental Chemistry, 92(10)1851-1856.