

Assessment of irradiated TiO₂ nanoparticles on the growth and nutritional components of broccoli

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Abstract

Broccoli is highly tremendously as it is enriched with healthy promoting phytochemicals. This research was undertaken to study the feedback of broccoli to different concentrations of un-irradiated and irradiated (50 kGy of gamma rays) titanium dioxide nanoparticles (TiO₂NPs). Un-irradiated and irradiated titanium dioxide characterization was accomplished by FT-IR, XRD, TGA, SEM and TEM. Foliar spray of titanium dioxide was applied to the broccoli in regards to the results of the characterizations. The growth traits; plant height, leaves No. per plant as well as stem diameter of plants and physical quality of heads were measured. Also, nutritional components of heads were determined. The results detailed that vegetative growth and physical quality of heads positively responded to foliar application of titanium dioxide compared to those obtained from control and 50 ppm from irradiated titanium dioxide (ITiO₂NPs) gave the highest values of all traits. Regarding to nutritional components of heads, the efficiency of photosynthesis increased by using TiO₂ (un-irradiated and irradiated) specially, 50 ppm ITiO₂NPs. Application of Ti significantly increased the osmolytes concentrations such as proline, total free amino acids and soluble sugars as well as the extracts of heads sprayed with TiO₂NPs concentrations displayed a prospective DPPH free radical scavenging action. Likewise, absorption of other nutrient elements and amino acids pool increased by spraying TiO₂NPs. It was found that there are several phytochemical constituents identified by GC-MS that contribute to the biological activity of the methanol extract of broccoli heads that were affected by the use of TiO₂NPs. It is clear that from above results, the using of ITiO₂NPs specially; 50 ppm stimulated growth, resulting in improved quality of broccoli heads which is ultimately reflected in productivity.

Keywords: broccoli; gamma irradiation; nutritional components; physical quality; titanium dioxide (TiO₂NPs)

Introduction

Broccoli (*Brassica oleracea*), Cruciferae, is a widespread international vegetable crop. Its heads are rich with minerals especially K, S, P, Mg (Aboul-Nasr and Ragab, 2000), vitamin A, vitamin C, vitamin B₂, calcium

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and proteins (Aires, 2015). In addition, it has been identified as an anti-cancer source (Talalay and Fahey, 2001). Quality, yield as well as nutritional value of either crop is affected by numerous factors like environmental conditions, fertility of soil, genotype (cultivar, species) as well as soil structure (Cartea *et al.*, 2008). One of the most vital factors that affect plant quality is plant fertilization (Savci, 2012). The agricultural practices similarly fertilization could highly impact levels of bioactive ingredients in brassica (Aires, 2015).

Nanotechnology is procuring attention of its wide range of applications in numerous fields including agricultural industry (Elmer and White, 2018, Dawi *et al.*, 2021), medical field (Mostafavi *et al.*, 2019) and electronics (Kyeremateng *et al.*, 2016). Nanotechnology is flawless principle for imminent revolution of agriculture technology because of total physicochemical properties of the nanoparticles (NPs) being utilized (Lowry *et al.*, 2019). Exposure of plants to diverse NPs constitute both negative and beneficial effects on their development along with growth. Effects of NPs are reliant on the concentration, plant species, type, exposure duration, size as well as source. Nonetheless, it is required to do examination of their applications, ecotoxicology hazards as well as economic feasibility carefully. Nanoparticle's presence in the soil has been expanding drastically because of their release that could take place accidentally, intentionally as well as naturally (Corsi *et al.*, 2018). Among the various traditional ways for preparing inorganic nanoparticles, gamma radiation has a number of advantages over others, including the ability to produce completely reduced and highly pure nanoparticles free of by-products or chemical reducing agents, as well as the ability to control particle size and shape (Abedini *et al.*, 2013; Gharibshahi *et al.*, 2017; Čubová and Čuba, 2020).

Titanium is the 9 mainly copious element in the earth's outer layer and constitutes about 0.57% with weight as well as 0.25% by moles of the outer layer (Buettner and Valentine, 2012). Iron is the mainly copious conversion metal followed by titanium which is about five folds less than iron but 100-folds more than copper. The oxidation positions of Ti^{2+} , Ti^{3+} (titanous), as well as Ti^{4+} (titanic), hence Ti^{4+} is the most stable ion while Ti^{2+} along with Ti^{3+} are unstable ions. Titanium dioxide is the foremost vital substance mostly utilized for paints (Lyu *et al.*, 2017).

Titanium dioxide nanoparticles (TiO_2 NPs) are from the crucial designed NPs assembled in significant amounts with the chemical, cosmetics and food sectors, as well as agriculture (Keller *et al.*, 2013). These particles (TiO_2 NPs) have a further type of titanium in the atmosphere and are assembled around the world approximately at 88,000 Ton each year (Reimann *et al.*, 2001). Titanium concentrations ranges in plants from 1.0 : 578 $mg\ kg^{-1}$, there are various factors that affects plant absorption of titanium like plant species vary in uptake of titanium, the absorption of titanium in plants affected significantly by soil pH, increase applications of titanium enhances concentration of titanium in crops, foliar applications is extra productive for titanium incorporation, titanium concentration in leaves as well as shoots enhanced by titanium spraying however the increase was restricted in soil application as well as plant roots assemble more titanium with low quantity transported to shoots (Wojcik and Wojcik, 2001).

Titanium contributed at small amounts has been proven to have a favourable effect on plant growth, however leads to phytotoxicity at high concentrations. Titanium applied to either leaves or roots at suitable concentrations have appeared to enhance seed germination (Andersen *et al.*, 2016), increase chlorophyll photosynthesis as well as biosynthesis, boost the activity of certain enzymes, improve root absorption of other nutritional elements, improve crop quality as well as yield (Haghighi *et al.*, 2012).

To clarify both the beneficial and detrimental impacts of TiO_2 NPs treatment on plants, 3 main processes have been recommended. The first process shows that TiO_2 NPs control of reactive oxygen species signalling regulation that is influenced by both pro-oxidant along with antioxidant actions. The particular take-up of TiO_2 NPs has been illustrated effectively in photosynthetic organisms, such as plants and algae (Li *et al.*, 2015). The second mechanism deals with $nTiO_2$ -mediated improvement of plant nitrogen metabolism, as $nTiO_2$ can promote the conversion of atmospheric nitrogen to nitrate under UV or sunlight irradiation (Yuan *et al.*, 2013). The third mechanism is related with the shape, size and surface characteristics of $nTiO_2$, which alter its availability to the plants (Zhang *et al.*, 2015).

Since chloroplast one of the most important locates in plants for reactive oxygen species generation in plants (Tripathy and Oelmuller, 2012), the shift in ROS levels caused by TiO₂NPs could be linked to changes in chloroplast task; while, the specific process is unknown. Otherwise, TiO₂NPs has moreover been appeared to promote the manufacture of vitamin E, an imperative lipophilic antioxidant (Szymanska *et al.*, 2016). Seeds treatment with TiO₂NPs suspensions revealed enhanced germination rates, better seedling growth or root lengths enhancement of *Arabidopsis thaliana* (L.) (Szymanska *et al.*, 2016), corn, lettuce, cabbage (Andersen *et al.*, 2016). Additionally, elevated the concentration of chlorophyll in wheat (Kovacik *et al.*, 2014). The contents of nitrogen, phosphorus, calcium and magnesium of tomato plants elevated after titanium utilizing (Kleiber and Markiewicz, 2013). Otherwise, titanium may link with some organic acids like citric acid and vitamin C to permit the chelated titanium to simply be translocated in plants. In addition, titanium could be biomineralized by iron storage protein ferritins (Amos *et al.*, 2013). Meanwhile, Lyu *et al.* (2017) outlined that, when it comes to iron and titanium connections, titanium impacts might be amplified when plants had lack in iron content. More specifically, titanium and iron exhibit antagonistic and synergistic interactions. While plants are deficient in iron content, titanium may increase the expression of genes involved in iron achievement, resulting in increased iron utilization as well as absorption which afterward improves plant development (Schiavo *et al.*, 2016). However, high quantities of TiO₂NPs may lead to cytotoxicity and genotoxicity in plants. Nonetheless, because of its multiple impacts, accurate amounts and approaches of TiO₂NPs adjustments are necessary, previous to their utilization on agricultural plants might be suggested on a large range.

Thus, the foliar application of irradiated TiO₂NPs not tested before, so in the present investigation, the efficiency of un-irradiated and different concentrations of irradiated titanium dioxide foliar application were tested on broccoli to reveal their effect on vegetative traits and nutritional components. For this aim, the study was planned and executed to obtain more information about broccoli's response to both un-irradiated and irradiated TiO₂NPs and also, to the economic importance of broccoli and consumer preference for it.

Materials and Methods

Irradiation treatment

The titanium dioxide (TiO₂NPs) was purchased from Sigma-Aldrich as nano-powder with ≥99.5% metal basis and were filled inside a glass bottle and then irradiated by a ⁶⁰Co source at a dose rate of 1.0 kGy/h with dose level 50 kGy. The irradiation treatments have done at the Egyptian Atomic Energy Authority (EAEA), National Centre for Radiation Research and Technology (NCRRT), Nasr City, Cairo - Egypt utilizing the research irradiator (⁶⁰Co Gamma cell 220).

Characterization of titanium dioxide nanoparticles

Fourier transforms infrared (FT-IR) spectroscopy: Un-irradiated as well as irradiated TiO₂NPs infrared spectra were achieved utilizing an FT-IR spectrometer (Bruker, Unicomb, Germany with a resolution of 4 cm⁻¹ in the 4000-500 cm⁻¹ wave number range).

X-Ray diffraction (XRD)

The XRD measurements of un-irradiated as well as irradiated TiO₂NPs, were acquired with an XD-DI utilizing (Shimadzu Diffractometer D6000, Japan), utilizing nickel-filtered as well as Cu-K target. The XRD runs were done out over the 2θ range from 4 °C to 90 °C at a scan speed of 8 °C /min, 30 mA as well as voltage 40 kV.

Thermogravimetric analysis (TGA): The TGA determination of both un-irradiated and irradiated titanium dioxide (TiO₂NPs), were executed on a Shimadzu-50 instrument (Kyoto, Japan) with heating rate of 10 °C/min on nitrogen flowed (20 ml/min) from room temperature to 600 °C.

Scanning electron microscopy (SEM): Sample's surface was examined by JSM-5200 SEM, Japan with voltage accelerated at 25 kV following gold deposition in vacuum for three minutes (Mangano *et al.*, 2017).

Transmission electron microscopy (TEM): The TiO₂NPs structure was noticed utilizing TEM (JEOL - JEM 1400CX ELECTRON MICROSCOPE, Japan) at speeding up voltage 100kV (Sezen *et al.*, 2018).

This study was achieved at the NCRRT green house throughout winter season of 2019/2020. Broccoli seeds (*Brassica oleracea* var. *italica*) were obtained from Gaara and Parteners Company, Bab El-Khalk, Cairo - Egypt. Seeds were sown in foam trays (one seed per cell) on mid-September 2019. After 45 days broccoli seedlings were transplanted to the soil. The agricultural needs required for optimal growth were met as well as the chemical and physical properties of farm soil were conducted as displayed in Table 1. Experimental plots were composed in Randomized Block Design (RBD) system with three replications. Four concentrations (25, 50, 100 and 200 ppm) were assembled from irradiated titanium dioxide (ITiO₂NPs) plus the control (H₂O) and un-irradiated titanium dioxide (UITiO₂NPs) (25 ppm) were utilized for spraying the broccoli plants. Foliar application of UITiO₂NPs and ITiO₂NPs was provided three times after week 2, 4 and 8 of transplanting, respectively.

Table 1. Physical and chemical properties of the investigation site

Physical and chemical properties									
A. Physical properties									
Sand %	Silt %	Clay %				Soil texture			
57.3	21.2	21.5				Sandy clay loam			
B. Chemical properties									
E.C. (ds/m)	pH	Cations (meq/l)				Anions (meq/l)			
		Ca ⁺⁺	Mg ⁺⁺	K ⁺	Na ⁺	CO ₃ ⁼	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁼
2.2	7.32	10.5	3.5	0.50	11.2	1.5	4.5	8.0	11.7

Vegetative plant growth

Three plants from random sample were taken from each treatment to evaluate plant height, leaves No./plant and stem diameter.

Physical quality of heads

Broccoli heads of each treatment were gathered at the horticultural ripeness stage to measure certain vegetative growth parameters, those being, head height, head diameter, head weight and florets No./plant.

Nutritional components of heads

Pigment's determination: Broccoli heads chlorophyll a, b, and carotenoids were measured following a spectrophotometric technique Vernon and Seely (1966). Pigment's concentration was evaluated in mg per gram of FW.

Ascorbic acid content

Ascorbic acid (vitamin C) content was determined in fresh head samples (mg/100g FW) by utilizing 2, 6-dichlorophenolindophenol for titration as suggested by AOAC (1990).

Preparation of head extracts

Briefly, 5.0 g of the fresh head samples were grinded with liquid nitrogen after that 25 ml of 80% methanol and shaking for 24 h at room temperature. Whatman filter paper number one was used to filter the extracts, then the extraction was repeated twice (Sobhy *et al.*, 2009). The resulting methanolic extracts volume were adjusted and used for the analysis of total soluble sugars, total free amino acids, total soluble protein, total phenolic, flavonoid, and DPPH analysis.

Total soluble sugars

Head extract of broccoli were utilized to determine the total soluble sugars with phenol-sulphuric protocol as explained by Dubois *et al.* (1956). Measurement the optical density at 490 nm utilizing spectrophotometer (Jasco V530) against blank. The results were indicated as mg of glucose corresponding gram of fresh sample.

Proline content

Concentration of proline was measured following Bates *et al.* (1973) method. The findings were calculated in mg proline per gram fresh weight.

Determination of total free amino acids

Colorimetrically determination of total free amino acids by utilizing ninhydrin method as stated by Jayaraman (1985). Optical density was evaluated at 570 nm as well as free amino acids content was calculated as mg glycine per gram of fresh sample.

Total soluble protein

Determination of total soluble protein content in the extract was done in state of the protocol explained by Bradford (1976).

Determination of total phenolic compounds

Fresh broccoli extracts were spectrophotometrically utilized to determine the total phenolic content with Folin Ciocalteu reagent method using gallic acid for the standard curve in state to Shahidi and Naczki (1995). Total phenolic content of samples was measured at 725 nm as well as indicated as mg gallic acid (GAE)/g fresh weight.

Total flavonoid content

Aluminium colorimetric protocol Marinova *et al.* (2005) was utilized to determine flavonoid content. The absorbance values were determined at 510 nm by spectrophotometer. Standard curve of quercetin was utilized and findings were indicated as mg quercetin corresponding to gram fresh sample.

Antioxidant activity

The electron donation ability or hydrogen atom of the correlating extract was determined by bleaching of a purple coloured methanolic reagent of 2,2-diphenyl-1-picrylhydrazyl in state to Gulluce *et al.* (2004). The optical density has been measured against the blank at 517 nm.

Nutritional elements (nitrogen, phosphorus, calcium, magnesium) as well as titanium and iron determination:

Total nitrogen content was calculated by revised Kjeldahl's protocols (Motsara and Roy, 2008). The percentages of titanium, phosphorus, magnesium, iron as well as calcium in digested broccoli dry samples were determined by Inductively Coupled Plasma Instrument (ICP) in state to the protocol explained in the AOAC (2000).

Amino acids profile of broccoli heads

Amino acids profile of broccoli heads was analysed utilizing HPLC. Dried as well as defatted sample of 100 mg weighted in the screw-capped tubes and 5 ml of HCl 6.0 N was placed. The hydrolysis tubes have been linked to a system that permits the connection of nitrogen as well as vacuum lines without disturbing the sample. The tubes were placed in an oven for 24 h at 110 °C (AOAC, 1990). The tubes were filtered as well as evaporated for dryness in a rotary evaporator.

The HPLC analysis was achieved utilizing an Agilent 1260 series. The partition has been achieved utilizing Eclipse Plus C18 column (4.6 mm x 250 mm i.d., 5 μm). The mobile phase contained buffer (sodium borate as well as dibasic sodium phosphate), pH 8.2 (A) and ACN:MeOH:H₂O, 45:45:10 (B) at a flow rate of 1.5 ml/min. The column temperature was preserved at 40 °C. The outcome acquired was distinguished with those of standard solution of amino acids. Free amino acids determination was established by distinguishing the retention times and peak areas of amino acids standards compared to the components available in the sample.

Identification of bioactive compound by GC-MS

Broccoli heads extract were attached for GC-MS investigation utilizing a HP G1800A appliance. Working by a capillary column HP-5 (length 30 minutes, i.d. 0.25 mm); carrier gas: helium; flow rate: one ml.min⁻¹; 250 °C for inlet temperature; 280 °C for detector temperature; 3 minutes at 100 °C for programmed temperature, subsequently 10-250 °C, as well as 30-280 °C by a mass detector. Recognition peaks were realized with distinguishing the mass spectra of eluting ingredients by those of the Wiley library (Wiley7, NIST 0.1; Wiley, West Sussex, UK) with retention times.

Statistical analysis

Three replicates were used in a randomized complete block design and the data were displayed as mean \pm standard deviation. Statistical analysis has been done using one-way ANOVA, and the differences in averages were evaluated utilizing Duncan's multiple range tests (Duncan, 1955) at $p \leq 0.05$.

Results and Discussion

Characterizations of titanium dioxide nanoparticles

Nanotechnology is the research plus utilization of tiny materials (1-100 nm), a definite quality which constructs these small unique entities. Therefore, nanoparticles application is one of the proposals to improve plant performance and growth (Duhan *et al.*, 2017).

Fourier transforms infrared (FT-IR) spectroscopy. The FT-IR transmission spectrum analysis of UITiO₂NPs and ITiO₂NPs as illustrated in Figure 1A. The FT-IR scale of TiO₂NPs plainly confirms three major bands, the first band is the widest, as it was noticed at 3318 cm⁻¹ as well as 3371 cm⁻¹ for both un-irradiated and irradiated TiO₂NPs, sequentially linking to stretch pulsation of the O-H group of the TiO₂NPs. The second band was noticed throughout 1623 cm⁻¹, linking to indirect type of H₂O Ti-OH; the final band is a notable peak at 1027 cm⁻¹ connected to Ti-O types (Mugundan *et al.*, 2015). Exact outcomes were acquired by (Colthup *et al.*, 1990) who discovered the FT-IR analysis concludes TiO₂NPs absorption spectra which is roughly around 3426 cm⁻¹, that specifies the existence of hydroxyl group with stretched bonds. The 1630 cm⁻¹ absorption peak might be connected to hydroxyl (bending) indicating the water as moisture in the sample. Also, the existent outcome is in line with Pulisova *et al.* (2012) who discovered the absorption from 3200 to 3600 cm⁻¹ with greatest at 3400 cm⁻¹ could be promoted to the stretching pulsation of the H-bonded OH groups of titanium hydroxide along with the absorbed water. The absorption about 1630 cm⁻¹ is because of the bending vibration of absorbed H₂O molecules. The absorption peak varies between the wave range numbers 650-700 cm⁻¹ is the typical TiO₂NPs anatase phase climax as recognized previously (Irshad *et al.*, 2020).

X-Ray diffraction (XRD): The XRD crystallographic appearance particle/pattern size division plot of titanium nanoparticles as observed in Figure 1B. The titanium dioxide nanoparticles XRD particle had 2 theta peaks at 25, 38, 48, and 55 validating pure TiO₂NPs. In addition, reveal optical as well as structural outcomes manifested that γ -rays did not alter the irradiated TiO₂NPs (B) crystallinity. The peak enlargement indicated the presence of nanoparticles of TiO₂NPs. Enormous sharp peak stipulates the crystalline phase of TiO₂NPs, the strength of the diffraction signal elevated by utilizing irradiation at dose level (50 kGy). This is in a harmony

with Irshad *et al.* (2020) who discovered utilizing the synthesis protocol would not affect this property of TiO₂NPs.

Thermogravimetric analysis (TGA): The TGA approach in which the mass of an example is observed against temperature or time. Figure 2 observes TGA curves for both UITiO₂NPs (A) and ITiO₂NPs (B) by wt%. The TGA curves indicated that there was no significant variation between UITiO₂NPs (A) and ITiO₂NPs (B) while ITiO₂NPs have more resistance to thermal degradation.

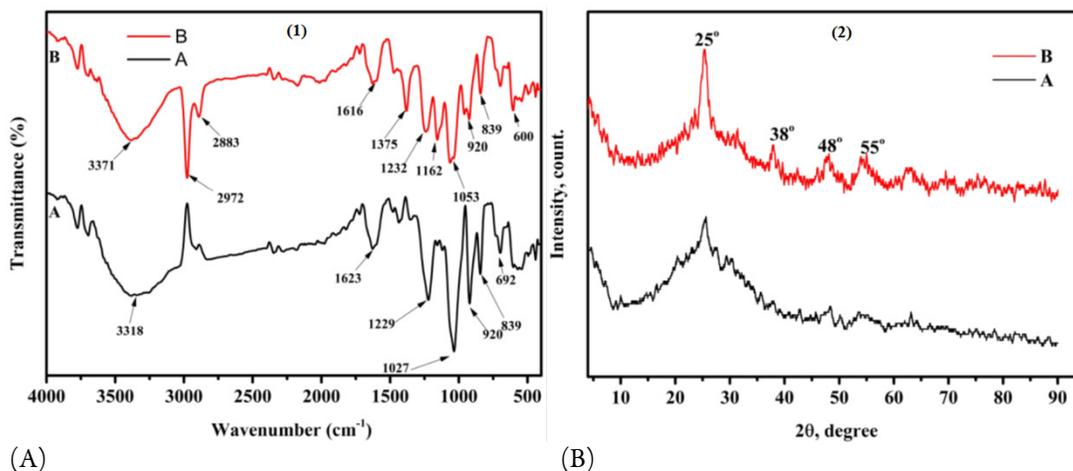


Figure 1. Fourier transforms infrared (FT-IR) spectra (1), X-Ray diffraction (XRD) spectra (2) of UITiO₂NPs (A) and ITiO₂NPs (B). UITiO₂NPs: un-irradiated titanium dioxide nanoparticles, ITiO₂NPs: irradiated titanium dioxide nanoparticles

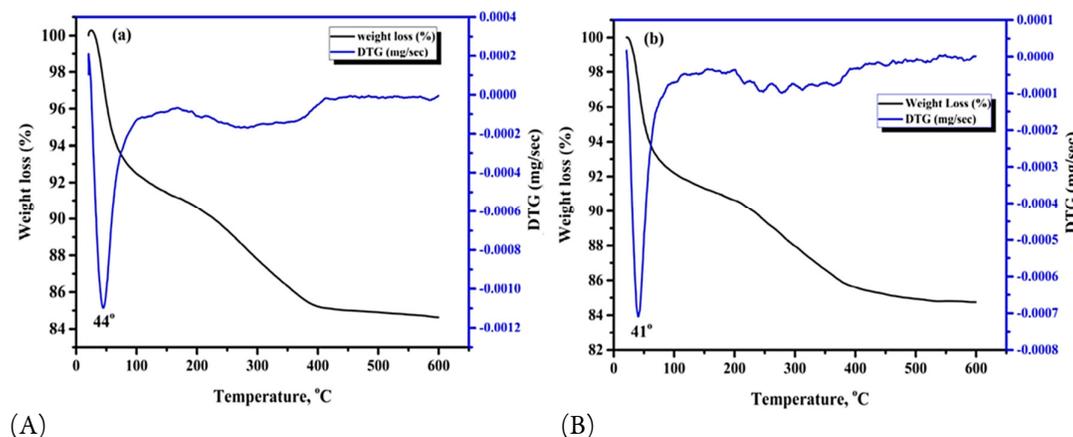


Figure 2. Thermogravimetric analysis (TGA) spectra of UITiO₂NPs (A) and ITiO₂NPs (B) UITiO₂NPs: un-irradiated titanium dioxide nanoparticles, ITiO₂NPs: irradiated titanium dioxide nanoparticles.

Scanning electron microscopy (SEM) and transmission electron microscopy (TEM): Appearance of UITiO₂NPs along with ITiO₂NPs were assessed by analysis of SEM. Utilizing high resolution scanning electron microscopy, the surface structure of the assembled sample was probed. Figure 3 reveals SEM images for distinct magnification. The image (A) appears micrographs of UITiO₂NPs conveyed the establishment of clusters in the shape of disordered, irregular as well as heterogeneous plates involving of fine particles, moreover, could notice a broad distribution of cluster sizes. Nonetheless, it was noticed that clusters acquired by ITiO₂NPs more arranged as well as smaller in size (B).

In connection to TEM (Figure 4) UITiO₂NPs (A) and ITiO₂NPs (B) manifest a pile in addition to the range of particle size between 2.15-11.6 nm for UITiO₂NPs and 1.29-7.30 nm for ITiO₂NPs. It would be assumed that γ -rays are the favoured practice for metallic nanoparticles synthesis due to it being reproducible which might manage the form of the particles yielding monodisperse tinny nanoparticles, simple, inexpensive as well as usage of less toxins precursors as recognized by Cele (2018). Scanning electron microscope indicated that TiO₂NPs have been in a circular form among tiny aperture and commonly available in the group that constructs the cluster exterior form (Sethy *et al.*, 2020). Therefore, Gharibshahia *et al.* (2017) concluded that intra-band quantized excitation of conduction electrons can transpire in metal nanoparticles from the lowest energy states to the highest energy states when the particles received energy from electromagnetic radiation.

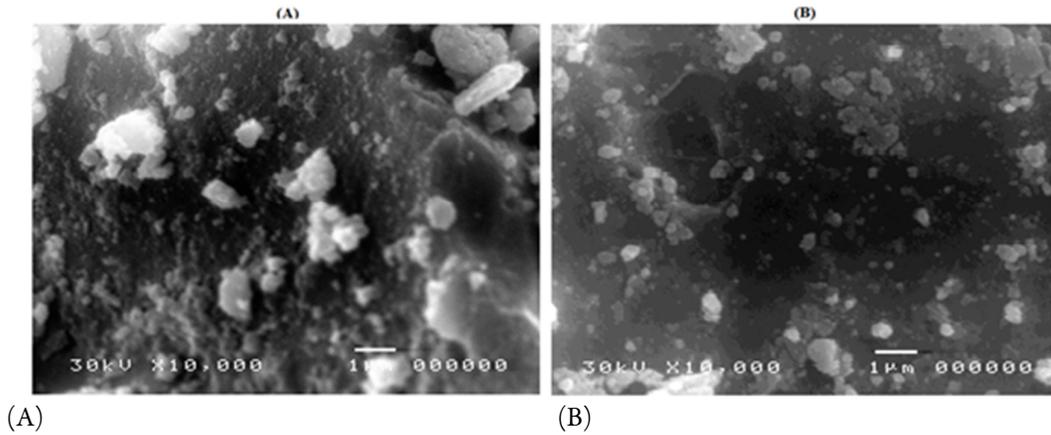


Figure 3. Scanning electron microscopy (SEM) of UITiO₂NPs (A) and ITiO₂NPs (B)
UITiO₂NPs: un-irradiated titanium dioxide nanoparticles, ITiO₂NPs: irradiated titanium dioxide nanoparticles.

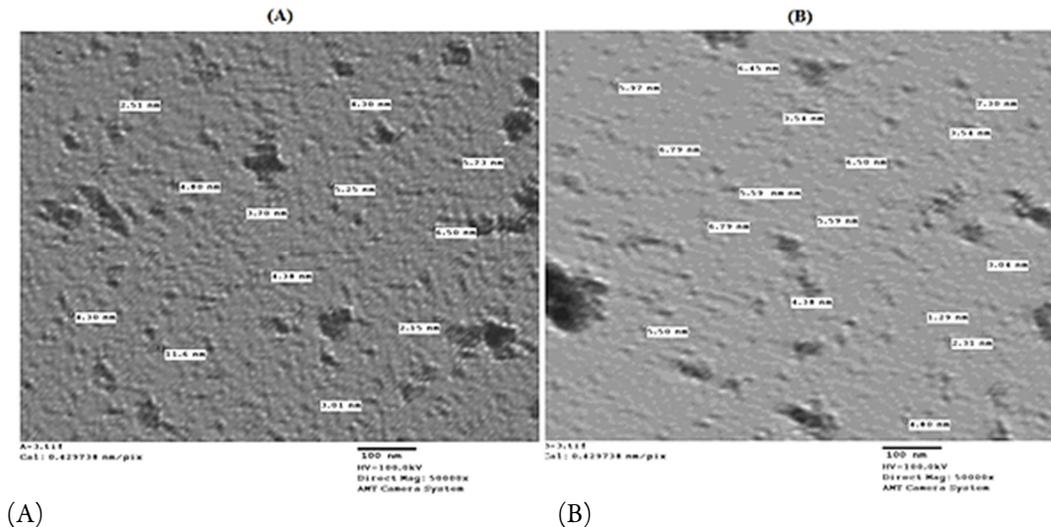


Figure 4. Transmission electron microscopy (TEM) of UITiO₂NPs (A) and ITiO₂NPs (B)
UITiO₂NPs: un-irradiated titanium dioxide nanoparticles, ITiO₂NPs: irradiated titanium dioxide nanoparticles.

Effect of TiO₂NPs on vegetative growth

Findings in Table 2 revealed that the proofed vegetative growth factors firmly responded to foliar spray. The maximum vegetative growth values were exhibited as, plant height, leaves No./plant, and stem diameter were confirmed when plants were sprayed with 50 ppm (55 cm, 22.2 and 4.02 cm respectively) subsequently by 100 ppm (53 cm, 21 and 3.78 cm respectively) from ITiO₂NPs. Nonetheless, the least values were obtained by control treatment which permits 47 cm, 17.4 and 2.98 cm, respectively. It can be noticed that all considered vegetative development factors significantly elevated with increasing the ITiO₂NPs level from 25 to 100 ppm as well as drastically diminished with elevating adding of ITiO₂NPs to 200 ppm, however, it was still higher compared to the control treatment. The remarkable highest values of the studied growth parameters were acquired from the treatment 50 ppm subsequently 100 ppm of ITiO₂NPs afterwards 25 ppm of ITiO₂NPs. The least values in this consideration were acquired from control treatment.

It could be summarized that spraying 50 and 100 ppm of ITiO₂NPs induced favourite effect and significantly elevated all studied vegetative broccoli plant growth parameter. These outcomes advance together with outcomes obtained by Abdel Latef *et al.* (2017) who explored the effects of three diverse concentrations of TiO₂NPs (0.01, 0.02 and 0.03%) on plant development and response to stress in broad bean. They indicated that 0.01% TiO₂NPs elevated plant growth that was enlarged shoot length, root dry weight of plants as well as leaf area under normal as well as saline soil conditions. Furthermore, El-Ghamry *et al.* (2018) noticed that the researched vegetative growth factors as; stem diameter, fresh, dry weight of shoot and root, number of leaves beside lettuce plant height developed on sandy soil drastically elevated as rising the TiO₂NPs level from 0.0 to 25 ppm as well as drastically reduced by elevating in TiO₂NPs concentration. Correspondingly, Gohari *et al.* (2020) noticed that the greatest agronomics character (leaf weight, leaf number and plant height) was noticed in 100 mg/l TiO₂-treated under control conditions. Likewise, Mustafa *et al.* (2021) who observed 40 mg/l of TiO₂NPs is effective to enhance the morphophysiological attributes, that is; fresh and dehydrated weight, chlorophyll contents, leaves No./plant, length of root, shoot beside whole plant. Nonetheless, higher concentration which is 60 as well as 80 mg/l, wheat cultivars suffered a detrimental impact.

Table 2. Impact of foliar spraying different levels of UITiO₂NPs and ITiO₂NPs on vegetative growth of broccoli plants

Treatments		Plant height (cm)	Leaves No./plant	Stem diameter (cm)
Control		47.00±1.58 e	17.40±0.52 d	2.98±0.19 e
UITiO ₂ NPs	25 ppm	50.00±0.71 cd	19.20± 0.92 c	3.36±0.21 cd
ITiO ₂ NPs	25 ppm	51.20±1.10 c	20.00±1.00 bc	3.50±0.14 c
	50 ppm	55.00±1.22 a	22.20±1.30 a	4.02±0.28 a
	100 ppm	53.00±1.41 b	21.00±1.00 ab	3.78±0.13 b
	200 ppm	49.00±0.71 d	18.80±1.31 c	3.20±0.14 d
LSD at (p ≤ 0.05)		1.514	1.321	0.1943

Data are given as mean ±SD (n=3) and various letters inside the same column are significantly differences at (p ≤ 0.05).

UITiO₂NPs: un-irradiated titanium dioxide nanoparticles, ITiO₂NPs: irradiated titanium dioxide nanoparticles

Physical quality of heads

Acquired outcomes stated in Table 3 stipulate that plants sprayed with 50 ppm ITiO₂NPs showed the highest head height, head diameter, head weights in addition to number of florets per plant (19.04 cm, 21.04 cm, 665.18 g and 17.2 respectively) followed by 100 ppm treatment ITiO₂NPs (18.16 cm, 18.74 cm, 509.22 g and 16.8 respectively). Nonetheless, the lowest values were obtained by control in the same traits (15.42 cm, 13.82 cm, 326.74 g and 13.00 respectively). Enhance in quality of head by the using of 50 and 100 ppm ITiO₂NPs might be pointed to the effect of TiO₂ on plant metabolism. Overall, the results propose that Ti have optimistic effects on crop quality as well as plant development. The TiO₂NPs exposure to diverse crops with different doses as well as particle size has been stated by Irshad (2021), for example, TiO₂NPs were noticed to elevate red bean (*Vigna angularis* L.) growth. The absorbed along with transportation of nutrients was

strengthened through one to three weeks of TiO₂NPs treatment. It was delineated that red bean treated by TiO₂ single exposure was noticed to be more dominant in elevating chlorophyll, triumph over oxidative stress, translocation as well as root development as distinguished by zinc oxide indicated little advantages towards enhancing chlorophyll as well as root development flush when utilized with TiO₂ (Jahan *et al.*, 2018). Moreover, TiO₂NPs utilizing to tomato plants enhanced the biomass, triggered enzyme activities, fruits and chlorophyll as well as elevated of tomato plant stress tolerance (Lyu *et al.*, 2017). In addition, soluble solids of fruit, firmness as well as size of 3 raspberry (*Rubus idaeus* L.) varieties elevated later than spraying the fruits by titanium previous to gather (Grajkowski and Ochmian, 2007).

Table 3. Impact of foliar spraying different levels of UITiO₂NPs and ITiO₂NPs on physical quality of broccoli heads

Treatments		Head height (cm)	Head diameter (cm)	Head weight (g)	Florets No./plant
Control		15.42±1.39 e	13.82±1.15 e	326.74±26.94 d	13.00±1.18 e
UITiO ₂ NPs	25 ppm	16.24±0.54 cd	15.42±1.04 cd	424.22±24.25 c	15.40±1.14 c
ITiO ₂ NPs	25 ppm	16.90±1.16 c	16.98±0.66 c	428.20±23.43 c	16.00±1.03 bc
	50 ppm	19.04±0.79 a	21.04±1.43 a	665.18±47.48 a	17.20±0.84 a
	100 ppm	18.16±0.65 b	18.74±1.08 b	509.22±45.70 b	16.80±1.30 ab
	200 ppm	15.90±0.89 de	14.90±1.56 de	380.08±27.63 c	14.20±0.84 d
LSD at (p ≤ 0.05)		0.7521	1,585	48.81	1.145

Data are given as mean ±SD (n=3) and various letters inside the same column are significantly differences at (p ≤ 0.05).

UITiO₂NPs: un-irradiated titanium dioxide nanoparticles, ITiO₂NPs: irradiated titanium dioxide nanoparticles

Photosynthetic pigments

Figure 5 showed that chlorophyll a, b content and carotenoids of broccoli heads (0.3737, 0.317 and 0.126 mg/g FW), respectively were significantly (p ≤ 0.05) increased for ITiO₂NPs 50 ppm level compared with the other treatments followed by the level of 100 ppm. On the other side, chlorophylls significantly (p ≤ 0.05) reduced by elevating ITiO₂NPs level to 200 ppm, however, they were still higher compared to the control treatment that was the least values in this regard (0.242, 0.166 and 0.063 mg/g FW). The treatment 50 ppm ITiO₂NPs stated the highest value of total chlorophyll (0.691 mg/g FW). In the opposite, the control indicated the least values (0.403 mg/g FW) of total chlorophyll. There was an encouraging relation between the results of growth and biochemical parameters. These outcomes are similar with Moaveni *et al.* (2011) who described that TiO₂NPs could obtain amount of pigments as well as assist transport of photosynthetic matter through recovery in structure of chlorophyll along with light absorption. Nanoparticles lengthen the photosynthesis mechanism by converting light energy into active electrons as well as chemical activity in chloroplasts. This protocol elevates the photosynthesis efficiency, stimulates the Rubisco (Ribulose-1,5-bisphosphate carboxylase-oxygenase) activase as well as gains carbon photosynthesis in barley. In addition, these outcomes are supported by the results of Lyu *et al.* (2017) who recommended that plants treated with titanium are characterized by a higher chlorophyll content as well as more rigorous photosynthesis. Correspondingly, El-Ghamry *et al.* (2018) stated that chlorophyll a, b and total chlorophyll of lettuce plant significantly elevated with rising of titanium addition from 0.0 to 25 ppm as well as significantly lowered with increasing titanium dioxide concentration. The TiO₂NPs application had remarkable effects on photosynthetic pigments as well as the greatest content of chlorophyll a, b and carotenoids were noticed in 100 mg/l TiO₂ (Gohari *et al.*, 2020). In titanium dioxide high levels, the decrease in chlorophylls may be linked to impairment in photosynthesis, alters in metabolic schemes as well as promote in osmotic pressure which may reduce uptake of water as well as nutrients.

Ascorbic acid (vitamin C) content

The represent results of vitamin C (mg /100g FW) established in broccoli heads as evaluated by different levels of titanium dioxide are seen in Figure 6. Obtained data showed that the average values of vitamin C were significantly ($p \leq 0.05$) elevated with increasing TiO₂ treatments as well as the highest value (77.56 mg/100g FW) for vitamin C was recorded for the ITiO₂NPs treatment (50 ppm) while the smallest value (56.93 mg/100g FW) for vitamin C was proofed for the control treatment. Using titanium could also enhance crop quality; tomato plants grown on Rockwool supplemented with nutrient solution carrying titanium contain high levels of total sugar and vitamin C in the fruits (Kleiber and Markiewicz, 2013). Using titanium could elevate vitamin C and anthocyanin contents of six strawberry cultivars (Skupien and Oszmianski, 2007). These outcomes are sustained with the results of El-Ghamry *et al.* (2018) on lettuce and discovered that vitamin C significantly increased with elevating titanium treatment from 0.0 to 25 ppm and significantly ($p \leq 0.05$) reduced as elevating the titanium element level.

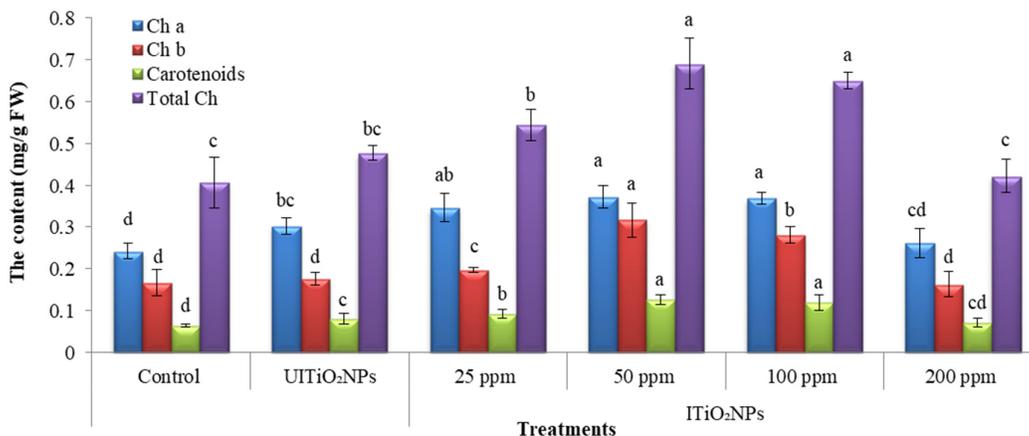


Figure 5. Effect of different concentrations (ppm) of UITiO₂NPs and ITiO₂NPs on pigments content (mg/g FW) of broccoli heads

Vertical bars \pm SD (n=3) and various letters on the bars indicate significantly differences at ($p \leq 0.05$). UITiO₂NPs: un-irradiated titanium dioxide nanoparticles, ITiO₂NPs: irradiated titanium dioxide nanoparticles

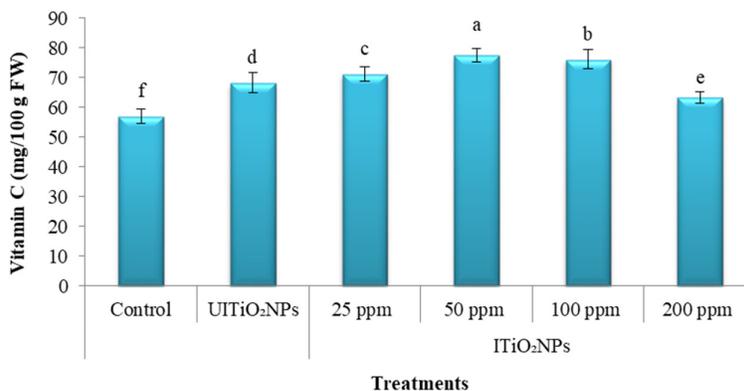


Figure 6. Effect of different concentrations (ppm) of UITiO₂NPs and ITiO₂NPs on ascorbic acid (mg/100 g FW) of broccoli heads

Vertical bars \pm SD (n=3) and various letters on the bars indicate significantly differences at ($p \leq 0.05$). UITiO₂NPs: un-irradiated titanium dioxide nanoparticles, ITiO₂NPs: irradiated titanium dioxide nanoparticles

Effect of TiO₂NPs on content of osmolytes

The results indicated that 50 ppm ITiO₂NPs application increased the contents of total soluble sugars and proline in broccoli heads over that of respective untreated control (Figure 7). Both 50 ppm and 100 ppm ITiO₂NPs were more effective in enhancing this constitutes comparative the control. The treatment 50 and 100 ppm ITiO₂NPs gave the highest values of soluble sugars (5.22 and 5.20 mg/g FW, respectively), and proline (1.21 and 1.16 mg/g FW, respectively). Likewise, the free amino acids and total soluble proteins affected by the use of different levels of un-irradiated and irradiated titanium dioxide. The data showed an increasing in the mean values of the concentrations for the above-mentioned nutrients, as the values increased with increasing TiO₂NPs treatment from 25 to 100 ppm and then decreased with increasing ITiO₂NPs to 200 ppm, but they were still higher than the control treatment. The treatment 50 ppm ITiO₂NPs showed the highest values of total free amino acids and total soluble proteins content (1.23 as well as 0.860 mg per gram FW, respectively). It is clear from these results, the usage of ITiO₂NPs at 50 ppm affirmatively adjust plant development as well as this increment might be linked with enhanced photosynthesis pigments.

These outcomes are agreeing with Abdel Latif *et al.* (2017) who stipulated that the 0.01% TiO₂NPs supplementation increased significantly the levels of osmolytes (total amino acids, soluble sugars as well as proline) in plants developed on salt-affected plants as well as normal soil against plants subjected to salinity alone of broad bean. In addition, Khater (2015) stated that TiO₂NPs foliar spraying led to an outstanding increase in total sugars and total free amino acids of Coriander plants. The definite mechanism by using nanoparticles (NPs) adjust plant secondary metabolites is not yet completely clarified. Recently, harmonized phytochemical as well as genomic researches established that nanoparticles may perform as elicitors for plant secondary metabolite induction via prompting diverse cellular signalling transduction passageways (like, ROS metabolism, calcium flux and mitogen-activated protein kinases). Accordingly, the noticed changes in the declared pathways may due to alterations in gene expression stages as well as metabolic enzyme elevation which might modify induction of secondary metabolite (Ebadollahi *et al.*, 2019). In addition, Ze *et al.* (2011) stated that TiO₂NPs could stimulate light uptake by chloroplasts through the genes regulation of linked to light harvesting complex II that is in accordance by the obtained inspection of soluble sugars rise upon TiO₂NPs supplementation. These detections together reinforce the positive regulatory role of UITiO₂NPs and ITiO₂NPs on photosynthetic system. The effect on development enhancement has been assisted by the escalation rate of total amino acids that would be linked with the TiO₂NPs property to enhance plant nitrogen status (Yuan *et al.*, 2013).

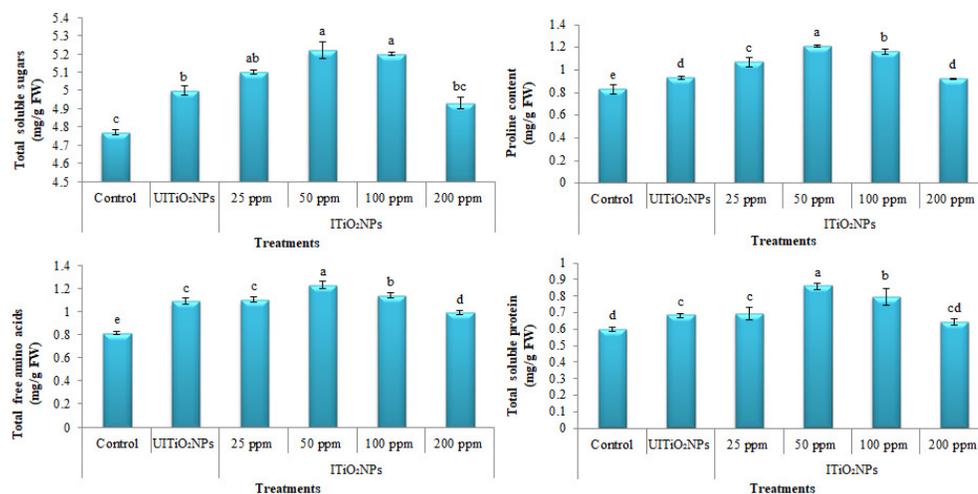


Figure 7. Effect of different concentrations (ppm) of UITiO₂NPs and ITiO₂NPs on osmolytes (mg/g FW) of broccoli heads

Vertical bars \pm SD (n=3) and various letters on the bars indicate significantly differences at ($p \leq 0.05$). UITiO₂NPs: un-irradiated titanium dioxide nanoparticles, ITiO₂NPs: irradiated titanium dioxide nanoparticles

Phenolic and flavonoid contents as well as DPPH scavenging activity assay

Un-irradiated and irradiated TiO₂NPs different concentrations significantly ($p \leq 0.05$) elevated phenolic compounds as well as flavonoids content in broccoli heads from 0.496 and 0.047 mg/g FW (control) respectively to 1.18 and 0.088 mg/g FW (50 ppm ITiO₂NPS) respectively (Figure 8). These outcomes showed that concentrations of both (un-irradiated and irradiated) TiO₂NPs had stimulating effect on accumulation in broccoli heads from phenolic content. These outcomes are in a harmony with Khater (2015) who stated that foliar spraying of TiO₂NPs leads to rise of the total phenols of Coriander plants. It is common that the greater levels of phenolics in heads could be elucidated with the function of TiO₂NPs in the biosynthesis of acetate shikimate pathway, which results in a greater induction of flavonoids along with phenols (Sousa *et al.*, 2008).

The DPPH radicals have been utilized broadly to estimate of antioxidant action of diverse plants extracts (Bagetti *et al.*, 2011). The major results of radical DPPH scavenging activity of broccoli heads are illustrated in Figure 8. Broccoli heads extract sprayed with TiO₂NPs various concentrations (un-irradiated and irradiated) displayed a probable activity of scavenging DPPH free radical compared to control.

The heads extracts were able to directly react and quenching DPPH radical. The treatment 50 ppm ITiO₂NPs displayed the greatest activity (85.09%) when distinguished with control (70.37%). Outcomes indicated that phenolic compounds and flavonoids substances are correlated with DPPH scavenging activity assay which means that phenolic substances along with flavonoids content are participate in the antioxidant activity of broccoli methanolic extracts. Phenolic substances are essential for plant development and growth. The phenols have expanded remarkable awareness due to their prospective protecting role against oxidative damage done by different stresses. In present study, UITiO₂NPs and ITiO₂NPs plants at certain levels produced greater phenols and flavonoids substances than control samples. These outcomes are confirmed by Ghorbanpour (2015) results who stated that TiO₂NPs had affirmative effects on secondary metabolite substances that comprises phenolic as well as flavonoids substances of *Salvia officinalis* plant. It is common that phenolic compounds due to their antioxidant action under stress environment could keep cells from the free radicals by reducing their poisonous on organelle structures (Mittler, 2002). Likely to current findings, an affirmative relation between antioxidant activity and phenols substances of *Salvia officinalis* plant has been stated by Ghorbanpour (2015).

Nutritional elements (nitrogen, phosphorus, calcium, magnesium)

Nutritional constituents; nitrogen, phosphorus, calcium, as well as magnesium level (ppm) in broccoli heads are determined by diverse concentrations of TiO₂NPs are illustrated in Table 4. The results manifested a notable effect for TiO₂NPs, on the mean results of the nutrients levels as indicated before, where the values significantly ($p \leq 0.05$) elevated with rising titanium treatment. The ITiO₂NPs levels 50 and 100 ppm illustrated the highest values of the broccoli heads nutritional elements. In the other side, the control treatment illustrated the smallest values. It could be expressed that foliar spraying of UITiO₂NPs and ITiO₂NPs on broccoli revealed the highest content of nitrogen, phosphorous, calcium, magnesium along with the treatment 50 ppm ITiO₂NPs provided the greatest values (145.32, 26.50, 7.50 and 4.00 ppm), respectively as illustrated in Table 4. The control treatment emerged in the lowest N, P, Ca as well as Mg contents 40.60, 16.60, 4.10 and 2.90 ppm, respectively as distinguished to other treatments. Current findings corroborate with Khater (2015) views on Coriander plants as found by El-Ghamry *et al.* (2018) on lettuce plants. Using titanium also augmented plants capability to assume further minerals. The substances of magnesium, calcium, phosphorus along with nitrogen of tomato plants elevated after titanium appliance (Kleiber and Markiewicz, 2013). Moreover, Ogunkunle *et al.* (2020) mentioned that NPs encouraged the accessibility of micronutrients in plants and stress related enzymes actions have been supported moreover with TiO₂NPs appliance.

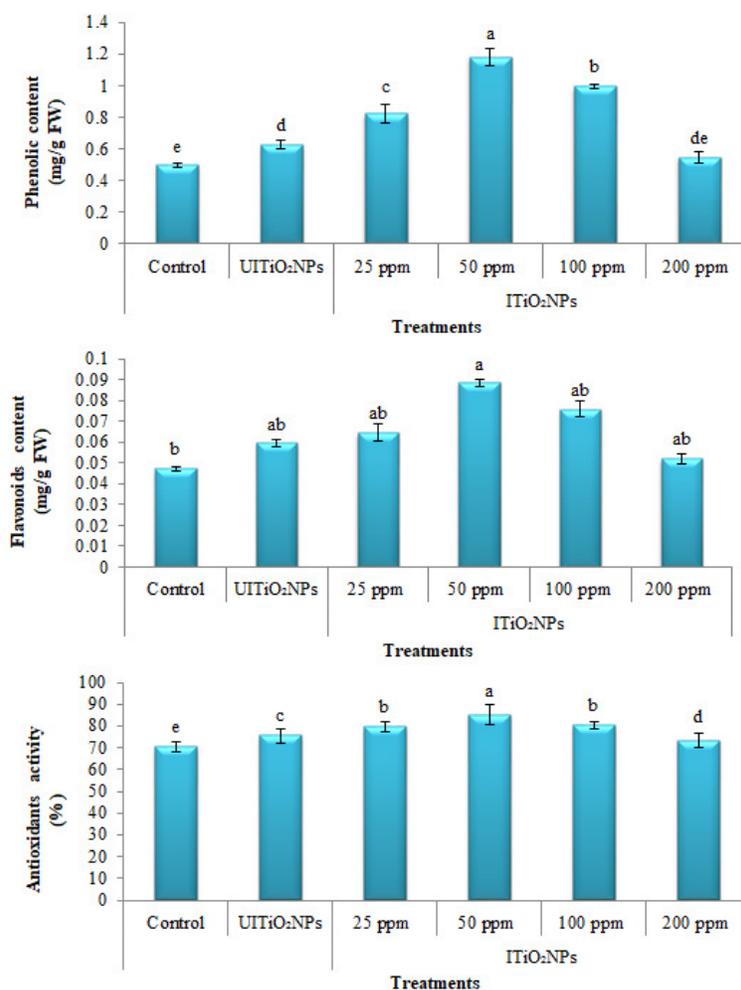


Figure 8. Effect of different concentrations (ppm) of UITiO₂NPs and ITiO₂NPs on phenolic compounds, flavonoids content (mg/g FW) and (%) antioxidant activity of broccoli heads
Vertical bars ±SD (n=3) and various letters on the bars indicate significantly differences at (p ≤ 0.05). UITiO₂NPs: un-irradiated titanium dioxide nanoparticles, ITiO₂NPs: irradiated titanium dioxide nanoparticles

Table 4. Effect of foliar spraying different concentrations of un-irradiated and irradiated TiO₂NPs on nutritional elements (ppm) of broccoli heads

Treatments		N	P	Ca	Mg
		Conc. (ppm)			
Control		40.60±4.00 d	16.60±1.44 c	4.10±0.20 e	2.90±0.16 c
UITiO ₂ NPs	25 ppm	122.26±10.69 bc	20.20±2.59 b	4.60±0.29 d	3.10±0.11 de
ITiO ₂ NPs	25 ppm	134.40±13.00 ab	20.90±1.00 b	5.30±0.35 c	3.30±0.2 cd
	50 ppm	145.32±13.93 a	26.50±2.19 a	7.50±0.66 a	4.00±0.24 a
	100 ppm	143.36±13.08 a	26.30±1.88 a	6.60±0.39 b	3.60±0.17 b
	200 ppm	110.88±14.21 c	19.90±2.15 b	6.50±0.27 b	3.40±0.21 bc
LSD at (p ≤ 0.05)		20.23	3.194	0.4813	0.2759

Data are given as mean ±SD (n=3) and various letters inside the same column are significantly differences at (p ≤ 0.05). UITiO₂NPs: un-irradiated titanium dioxide nanoparticles, ITiO₂NPs: irradiated titanium dioxide nanoparticles

Concentrations of Ti and Fe in broccoli heads as affected by different rates of titanium dioxide application

The results outlined in Table 5 showed the levels of Ti and Fe in ppm as influenced by evaluated concentrations of TiO₂NPs in broccoli heads. The data illustrated that titanium levels of broccoli heads (ppm) elevated with rising Ti application levels. The titanium values of broccoli were 0.12, 0.19, 0.21, 0.25, 0.28 and 0.41 (ppm) at zero, 25 ppm UITiO₂NPs, 25, 50, 100 and 200 ppm (ITiO₂NPs) used concentrations. Instead of this trend; iron concentration in broccoli heads was elevated with enhancing titanium concentrations up to 50 ppm and then the values were declined by increasing TiO₂NPs however it was still greater than the control treatment, as the greatest value for iron concentration was at 50 ppm of ITiO₂NPs treatment (2.77 ppm) while the smallest value was acquired from the control treatment (0.68 ppm).

Iron values in broccoli heads remarkably elevated by increasing titanium concentration up to 50 ppm because of Ti and Fe have antagonistic and synergistic connection. Plants which suffer from Fe shortage, Ti aids prompt genes expression transmitted to iron achievement, thus improving Fe absorption as well as consumption, thereby improving plant development. The relation of plants with titanium and with iron may lead to incidence the titanium bind proteins in plants which also selectively binds by titanium or non-specifically distribute among iron or further nutrients. While titanium concentrations are elevated in plants, might lead to phytotoxicity (Lyu *et al.*, 2017).

These outcomes agree with results of Radkowski (2013) who reported that Ti element inspires iron uptake from soil as well as elevates nitrogen fixation. Additionally, El-Ghamry *et al.* (2018) established that nitrogen, phosphorus, potassium and iron of lettuce plants notably enhanced by increasing of used titanium from 0.0 to 25 ppm as well as drastically decreased with escalation in titanium dioxide. Various clarifications have been suggested considering the effect of titanium as a favourable element to plants that includes the participation in nitrogen fixation in the nodules of legumes, effect on plant metabolism via elevating uptake of further nutrients like magnesium, iron as well as contributing in the redox reaction system (Ti⁴⁺/Ti³⁺ with Fe³⁺/Fe²⁺) therefore enhancing the iron activity in plants tissue or decreasing the efficiency of the photosystem II at the high level from titanium as interface by iron in the electron transport sequence (Lyu *et al.*, 2017).

Table 5. Effect of foliar spraying different concentrations of un-irradiated and irradiated TiO₂NPs on Ti and Fe concentrations (ppm) of broccoli heads

Treatments		Ti	Fe
		Conc. (ppm)	
Control		0.12±0.010 e	0.68±0.108 e
UITiO ₂ NPs	25 ppm	0.19±0.020 d	1.02±0.098 cd
ITiO ₂ NPs	25 ppm	0.21±0.017 cd	1.22±0.082 c
	50 ppm	0.25±0.030 bc	2.77±0.204 a
	100 ppm	0.28±0.026 b	1.93±0.227 b
	200 ppm	0.41±0.036 a	0.83±0.098 de
LSD at (p ≤ 0.05)		0.05753	0.2933

Data are given as mean ±SD (n=3) and various letters inside the same column are significantly differences at (p ≤ 0.05).

UITiO₂NPs: un-irradiated titanium dioxide nanoparticles, ITiO₂NPs: irradiated titanium dioxide nanoparticles

Amino acids profile of broccoli heads

Findings in Table 6 demonstrate broccoli heads amino acid profile affect by different concentration of TiO₂NPs. Seventeen amino acids were detected in the samples: aspartic acid (Asp), glutamic acid (Glu), serine (Ser), histidine (His), glycine (Gly), threonine (Thr), arginine (Arg), alanine (Ala), tyrosine (Tyr), valine (Val), cystine, methionine (Met), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), lysine (Lys) and proline (Pro).

Essential amino acids that have been found are Thr, Arg, Val, Met, Phe, Ile, Leu and Lys and classified histidine as a nonessential amino acid (Kmieciak *et al.*, 2010). Most of these essential amino acids increased using TiO₂NPs except Arg which decreased using both un-irradiated and irradiated TiO₂NPs. In the amino acids

profile content of broccoli heads Glu and Asp acids were predominant and the left behind amino acids are present in lower proportions and the result was fully consistent with the results of Kmiecik *et al.* (2010).

When comparing the control and treatment groups, it is obvious that TiO₂NPs enhanced the amino acid pool. In comparison to the control sample (265.95 µg/g DW), 50 ppm ITiO₂NPs had the greatest amount of total amino acid (273.03 µg/g DW), taken after by 100 ppm ITiO₂NPs (271.70 µg/g DW). Because methionine is the only sulfur-including amino acid required by mammals, it must be obtained completely through food. Methionine amino acid provided 2.77 µg/g DW when ITiO₂NPs at 50 ppm was applied taken after by 100 ppm ITiO₂NPs (2.73 µg/g DW), while control had the smallest level (2.63 µg/g DW). Aromatic amino acids such as phenylalanine and tyrosine were also found to enhance with elevating levels of TiO₂NPs in both UITiO₂NPs and ITiO₂NPs samples, with the maximum amount at 200 ppm of ITiO₂NPs (13.45 µg/g DW). Necessary amino acids are important for human body needs in their food to perform metabolic processes (Wu, 2009), and the findings for the current study showed that it improved with the using of ITiO₂NPs, particularly at 50 ppm taken after by 100 ppm concentrations.

Table 6. Effect of foliar spraying different concentrations of un-irradiated and irradiated TiO₂NPs on amino acid content of broccoli heads (µg/g DW)

Amino acid	Conc. µg/g DW					
	Control	UITiO ₂ NPs	ITiO ₂ NPs			
			25 ppm	50 ppm	100 ppm	200 ppm
Aspartic	38.60	38.87	39.20	39.77	39.45	38.91
Glutamic acid	47.77	48.04	48.22	48.11	48.16	48.13
Serine	16.38	16.49	16.54	16.69	16.57	16.45
Histidine	5.92	5.73	5.68	5.60	5.58	5.44
Glycine	13.87	13.75	13.57	13.11	13.07	12.90
Threonine	10.24	10.59	10.86	11.53	11.33	10.37
Arginine	19.55	19.27	19.17	19.09	18.89	18.72
Alanine	14.10	14.74	15.04	15.16	14.92	14.34
Tyrosine	3.53	3.63	3.74	3.97	4.11	4.23
Valine	17.11	17.45	17.66	17.98	18.21	18.43
Cysteine	1.33	1.29	1.19	0.95	0.81	0.71
Methionine	2.63	2.66	2.69	2.77	2.73	2.74
Phenylalanine	8.18	8.44	8.65	8.89	9.19	9.22
IsoLeucine	7.51	7.70	7.75	7.79	7.64	7.55
Leucine	20.04	20.47	20.49	20.96	20.62	20.17
Lysine	21.14	21.32	21.57	21.85	21.67	21.23
Proline	18.05	18.36	18.63	18.81	18.75	18.47
Total sulfur AA	3.96	3.95	3.88	3.72	3.54	3.45
Total aromatic AA	11.71	12.07	12.39	12.86	13.30	13.45
Total essential AA	106.40	107.90	108.84	110.86	110.28	108.43
Total non-essential AA	159.55	160.90	161.81	162.17	161.42	159.58
Total	265.95	268.80	270.65	273.03	271.70	268.01

UITiO₂NPs: un- irradiated titanium dioxide nanoparticles, ITiO₂NPs: irradiated titanium dioxide nanoparticles

Identification of bioactive compound by GC-MS

The optimum method for determining bioactive components is to use GC-MS (Muthulakshmi *et al.*, 2012). Methanol extract examination of broccoli heads using GC-MS was performed in this study. The constituents were recognized through, GC-MS based on peak area and retention duration. There was a total of 25 substances discovered (Table 7).

The prevailing compounds were Carvacrol, Eugenol, Caryophyllene, Nizatidine, Myristaldehyde, Acetic acid, 10,11-dihydroxy-3,7,11-trimethyl-dodeca-2,6-dienyl ester, Hexadecanoic acid, methyl ester, Palmitic acid, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, 9-Octadecenoic acid (Z)-, methyl ester, Methyl stearate, 9,12-Octadecadienoic acid (Z,Z)-, Oleic acid, 9,12-Octadecadienoyl chloride, (Z,Z)-, 9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester.

Table 7. Identification of bioactive compound by GC-MS affected by different concentrations of un-irradiated and irradiated TiO₂NPs of broccoli heads

RT	Name of the compound	Area %					
		Cont.	UITiO ₂ NPs	ITiO ₂ NPs			
				25 ppm	50 ppm	100 ppm	200 ppm
3.94	5,8,11,14-Eicosatetraenoic acid, phenylmethyl ester, (all-Z)-	0.11	0.12	0.17	0.20	0.27	0.26
8.19	Carvacrol	3.23	3.45	3.54	3.78	3.66	3.47
9.2	Eugenol	2.84	2.59	2.41	2.03	1.66	1.23
10.24	Caryophyllene	1.54	1.12	0.83	0.74	0.51	0.50
11.43	1-Dodecanol	0.45	0.52	0.64	0.69	0.71	0.68
12	Nizatidine	20.22	21.21	21.53	21.81	21.69	21.47
14.06	Myristaldehyde	1.60	0.54	0.55	0.47	0.39	0.30
14.89	Acetic acid, 10,11-dihydroxy-3,7,11-trimethyl-dodeca-2,6-dienyl ester	1.86	0.67	0.32	0.17	0.14	0.11
15.42	Hexadecane, 1,1-bis(dodecyloxy)-	0.86	0.51	0.45	0.37	0.33	0.24
16.47	Z,Z,Z-4,6,9-Nonadecatriene	0.17	0.23	0.24	0.30	0.31	0.43
16.58	2H-Pyran-3-ol, tetrahydro-2,2,6-trimethyl-6-(4-methyl-3-cyclohexen-1-yl)-, [3S-[3 α ,6 α (R*)]]-	1.76	1.45	1.31	1.15	0.96	0.61
17.94	10,13-Octadecadienoic acid, methyl ester	0.71	0.35	0.30	0.26	0.19	0.11
18.05	13-Heptadecyn-1-ol	1.32	0.93	0.76	0.69	0.64	0.40
19.33	Limonen-6-ol, pivalate	0.34	0.51	0.53	0.59	0.64	0.67
19.53	Hexadecanoic acid, methyl ester	3.87	4.00	4.22	4.43	4.58	4.61
20.30	Palmitic acid	1.12	1.37	1.54	1.75	1.61	1.44
22.20	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	10.67	11.26	11.55	11.47	11.24	11.00
22.39	9-Octadecenoic acid (Z)-, methyl ester	13.74	14.05	14.35	14.48	14.32	14.13
22.50	Cholestan-3-ol, 2-methylene-, (3 α ,5 α)-	0.11	0.12	0.13	0.13	0.12	0.11
22.8	Methyl stearate	3.53	3.62	3.76	3.84	3.57	3.27
22.99	9,12-Octadecadienoic acid (Z,Z)-	10.21	11.46	11.63	11.79	11.62	11.51
23.05	Oleic acid	3.53	4.08	4.57	4.76	4.62	4.24
23.93	9,12-Octadecadienoyl chloride (Z,Z)-	2.05	3.24	3.91	4.06	3.65	3.28
25.96	1-Isopropenyl-4,5-dimethylbicyclo[4.3.0]nonan-5-ylmethyl phenyl sulfoxide	1.09	1.12	1.16	1.24	1.43	1.64
27.56	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	2.86	2.91	2.96	3.07	3.25	3.14

UITiO₂NPs: un- irradiated titanium dioxide nanoparticles, ITiO₂NPs: irradiated titanium dioxide nanoparticles

There were several phytochemicals constituents that contribute to the biological action (Source: Dr. Duke's Phytochemical and Ethnobotanical Databases (online database)) of methanol extract of broccoli heads that were affected by different concentration of TiO₂NPs. Among the phytochemicals identified that increased by treatments, 5,8,11,14-Eicosatetraenoic acid, phenylmethyl ester, (all-Z)- an unsaturated fatty acid ester that has cardio protective activity. Carvacrol is responsible for the biological activities like antimicrobial, antitumor, antimutagenic, antigenotoxic, anti-inflammatory, analgesic, antispasmodic, angiogenic, antihepatotoxic antiparasitic, antielastase, antiplatelet, AChE inhibitory, insecticidal and hepatoprotective activities. Limonen-

6-ol, pivalate, an antioxidant and anti-inflammatory. Hexadecanoic acid or Hexadecanoic acid methyl ester can be used as antioxidant, flavor, pesticide, antibacterial and antifungal. 9-Octadecenoic acid (Z)-, methyl ester have anti-inflammatory, antiandrogenic cancer preventive, dermatitigenic hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge properties. Also, Oleic acid may be employed as monoacylglycerol, antioxidant and anti-atherosclerotic. Finally, 9,12-Octadecadienoic acid (Z,Z)-, unsaturated fatty acid it is an anti-inflammatory, hypocholesterolemic, cancer preventive, insectifuge, antiarthritic, hepatoprotective, antiandrogenic, nematocide, antihistaminic, antieczemic. 9,12-Octadecadienoyl chloride which has the property of cancer preventive, anti-inflammatory, hepato-protective, insectifuge, hypocholesterolemic. According to Mohammad and Abdul Kareem (2019), TiO₂NPs boosted all plant features, counting morphological, biochemical, active constituents, and efficiency, which were linked to improved photosynthesis. Moreover, TiO₂NPs also have a variety of significant achievements on the morphological, physiological, and biochemical aspects of specific plant species (Lei *et al.*, 2008). The addition of 100 mg/l TiO₂NPs boosted the key components of the essential oil profile, this could be due to an increase in the expression of certain biosynthetic enzymes involved in the creation of constituents and its accessibility as suggested by the findings of Gohari *et al.* (2020).

Conclusions

Titanium nanoparticles, which were newly discovered and have dramatic impact on plant morphological, physiological, and biochemical features, have the potential to increase overall plant performance. Nevertheless, appropriate to size effect, small-diameter TiO₂NPs might be able to infiltrate the cells directly. As a result, it can be seen that irradiated titanium dioxide produced superior findings than non-irradiated titanium dioxide, implying that irradiation reduced the size of irradiated particles more than non-irradiated particles. As a result, it had a high absorption rate. The ITiO₂NPs has beneficial effects on plant development and crop quality of broccoli at (50 and 100 ppm) treatment, however the good effects at 50 ppm treatment were more visible than the favourable effects at 100 ppm treatment, under the same conditions of this experiment. Finally, the findings of this inquiry, has been shown that ITiO₂NPs is a positive advantageous as it is enhancing plant development. In addition, the current study's findings demonstrated that these irradiation nanoparticles are extremely effective at encouraging plant growth. Traditional techniques of encouraging plant development can be replaced with these strong and cheap INPs. Additional nanotechnology advancements in this industry might have far-reaching financial consequences and many advantages for customers, manufacturers, and plant growers. Future research on ITiO₂NPs will be needed to gain a better understanding of their production, use and mechanistic properties.

Authors' Contributions

A.A.A. - Conceptualization, designed the study, supervision, project administration, curation, editing and reviewed the initial and final draft; R.W.M. Contributed in the collection of data, prepared the initial draft, the methodology, analyzed the data, mathematical processing, visualization, review; Y.A.L. - Literature review and contributed in methodology data processing, design, ; G.S. - Contributed to the conceptualization of idea, contributed in study design and reviewed the initial draft the manuscripts' review writing and curation, and editing. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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