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Nanopriming Technology for Boosting Germination of *Capsicum annum* using Mycosynthesized TiO_2 Nanoparticles

Khushbu Gupta[a], Nik Ahmad Nizam Nik Malek[b], Tejpal Singh Chundawat[a,*]

[a] Department of Applied Sciences, The NorthCap University, Sector 23-A Gurugram-122017, Haryana, India.
[a] Centre for Sustainable Nanomaterials (CSNano), Ibnu Sina Institute for Scientific and Industrial Research (ISI-ISIR), Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia.

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*Corresponding author
chundawatchem@yahoo.co.in

Abstract

The aim of this research paper is to investigate the impact of titanium oxide nanoparticles (TiO_2 NPs) on germination of *Capsicum annum* (Pusa Sadabahar). TiO_2 Nps were prepared using *Fusarium oxysporum* extract and then characterized using UV- visible spectroscopy, FTIR, XRD and SEM which confirms that the nanoparticles were elongated spherical with 15 nm diameter. Various concentrations (100-500 µg/ml) of TiO_2 NPs were used to study different aspects of nanoparticles on *Capsicum annum*. The result emphasizes the positive effects of TiO_2 nanoparticles up to the concentration of 400 µg/ml on germination percentage and other morpho-physical parameters such as radical length, plumule length, fresh and dry weight, total seedling length as compared with non-treated plants. Overall, we found better results of using biologically synthesized NPs in chilli plants, while the significant decrease in outcome was also observed at higher concentration. At higher concentration, accumulation of nanoparticles by the plant results in increase in activity of superoxide dismutase and catalase, thus decrease in photosynthesis.

Keywords TiO_2 nanoparticles, *Capsicum annum*, *Fusarium oxysporum*, seed germination, nanopriming, radicle length, plumule length

1.0 INTRODUCTION

Capsicum is a cross-pollinated crop and its cultivated species are of different types such as *Capsicum annum*, *C. frutescens*, *C. chinese*, *C. pubscens*, and *C. baccatum* (Mexicanae, 2012). Chilli is the most widely used commercial crop consumed in the form of green/red ripe fruit powder. It is perennial shrub cultivated throughout the world for its pungency. It is rich in vitamin A & C and uses to cure arthritis, headache, boost up the immune system and lower down the blood cholesterol level.

Nanopriming is an innovative seed priming technology that helps to improve seed germination, seed growth, and yield by providing resistance to various stresses in plants. Nano-priming induces the formation of nanopores in shoot and helps in the uptake of water absorption, activates reactive oxygen species (ROS)/antioxidant mechanisms in seeds, and forms hydroxyl radicals to loosen the walls of the cells and acts as an inducer for rapid hydrolysis of starch. The demand of nanotechnology is continuously increasing in different sectors like textiles, cosmetics, food industry, bioremediation and electronic study (Moll et al., 2016). Nowadays its application has more use in the agricultural sector (Banik and Pérez-de-luque, 2017). Different researchers are studying the different application of nanotechnology for greater yield and to protect the plant from biotic and abiotic stress (Li et al., 2016). Nanoparticles are beneficial to the crops because of their nano-size, easy absorption, less sedimentation and high surface to volume ratio (Biosci, 2014).

The fungus *Fusarium oxysporum* is a source of enzyme, which in turn catalyses reaction for the synthesis of nanoparticles. These enzymes and proteins act as reducing and stabilizing agents during the synthetic reaction (Gupta &

Chundawat, 2019a). The benefits of using fungus over other microbes include their faster growth in the form of mycelia which provides a larger surface area for the conversion of metal salts into their respective metals (Gupta & Chundawat, 2020).

TiO_2 nanoparticles was recorded to boost the growth of plants like spinach by improving nitrogen metabolism and photosynthesis at some concentration (Yang et al., 2006). Based on various research it was established that several oxide nanoparticles such as Fe_2O_3, Al_2O_3, CeO_2, NiO, CuO, ZnO, ZrO_2, MgO, TiO_2, and SnO_2 speed up the process of seed growth and plant germination (Gao et al., 2008; Hong et al., 2005; Yang et al., 2007; Zheng et al., 2005). Lately, Dehkourdi and Mosavi (2013) treated parsley seeds using anatase form of TiO_2 nanoparticles, which showed an increase in radicle, plumule length, vigour index, chlorophyll content, germination percentage & fresh weight of the plant. In the same way, Feizi et al. (2013) confirmed that nano-TiO_2 increases the germination rate in *Salvia officinalis*. Earlier studies, showed positive development at the time of vegetative growth of a plant, like Hong *et al.* (2005) demonstrated an increase in oxygen evolution rate in spinach plants with the exposure of *n*TiO_2. One more research on treated spinach showed an increase in the rate of photosynthetic carbon accumulation in plants (Gao et al., 2008). Tomato plants were treated using *n*TiO_2 and stressed under mild heat to observe the photosynthetic rate improvement in plants as compared with controlled ones (Qi et al., 2013). The absorption of NPs in plant cell wall resulted in morphological and physiological changes which promote the growth of the plant. NPs play important role in plant genomics, gene interaction along with crop improvement without the use of excess fertilizers (Pozveh et al., 2014). Nowadays, the application of nanoparticles in the field of agriculture is still in the research phase and need some more experiments before commercialization.

2.0 MATERIALS AND METHOD

2.1 Materials

Capsicum plant variety PSB (Pusa Sadabahar) was obtained from IARI, New Delhi, India. Sigma Aldrich provided the analytical grade reagents such as nutrient agar, potato dextrose agar (PDA), titanium tetraisopropoxide, peptone, beef extract, sodium chloride, maltose and yeast extract.

2.2 Preparation of Fusarium oxysporum extract and synthesis of TiO_2 nanoparticles

The potato dextrose agar (PDA) medium was used for the growth of fungus *Fusarium oxysporum* at 28°C. The fungus was then inoculated in MGYP medium containing 2% malt extract, 11% glucose, 5% yeast extract and 10% peptone. The culture was incubated for 3 days with agitation at 28°C. The culture containing growth was centrifuged to separate fungal extract from its biomass. The prepared fungal extract was kept in a refrigerator at 4°C to promote further experimental usage (Gupta & Chundawat, 2019b). TiO_2 NPs was prepared by dissolving 20 mL of prepared extract with 50 ml of titanium tetraisopropoxide, under magnetic stirrer for 4h at pH 7 (Ahmad et al., 2020). After 4 h, the nanoparticles were precipitated and procured through centrifugation. The washing of the synthesized nanoparticles was done with double distilled water to remove all contamination. The obtained nanoparticles were oven-dried at 90°C for 8h and were calcined at 500ºC for 4h. The formation of light brown powder suggests that their capping was done with insoluble polysaccharides and carboxylic groups present in extract of fungus *Fusarium oxysporum*.

2.3 TiO_2 nanoparticles characterization

The nanoparticles characterization is the essential part for the assurance of shape, size, morphology and phase purity and surface charge. To confirm the nanoparticle formation, the solution was scanned using UV-Visible spectrophotometer (Model Cary) by taking spectra from 200-800 nm with distilled water as blank. Fourier transform infrared spectra (FTIR) of TiO_2 nanoparticles were recorded using FTIR (Perkin Elmer) with the help of KBr pellet (400-4000 cm^{-1}). The crystal structure, particle size and phase identification of metal nanoparticles were executed on Philips Expert pro-XRD system (DY 1650) at 2θ angle ranging from 20-90°. The size of synthesized nanoparticles along with its surface morphology was analysed by SEM (Model-FEI Quanta 200 SEM).

2.4 Effect of nanoparticles on morpho-physical parameters of *Capsicum annum*

Seeds of *C. annum* were first washed with teepol and then immersed in 5% sodium hypochlorite solution to make their surface sterile. After that, the seeds were cleaned with double distilled water under laminar airflow and then soaked in double-distilled water for 2h having a different concentration of TiO_2 nanoparticles from100-500 µg/ml. 10 seeds were transferred over each dish having 3-4 stacks of filter paper. The seeds placed over Petri plates were kept at a distance of 1 cm from each other. Distilled water containing nanoparticles (100-500 µg/ml) was added to each petri dish. Petri dishes were covered and was maintained at 26±2°C and illuminated with 8 hours light and 16 hours dark in the culture room. For morphological studies, the time of germination was permitted up to 15 days and a petri dish without TiO_2 nanoparticles was taken as control.

2.4.1 Germination Percentage

Germination was calculated after every 2 days up to the day when radical length reached up to 1 mm. The germination percentage was formulated by counting the number of germinated seeds out of total seeds kept for the experiment using the formula given below.

$$\text{Germination Percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

2.4.2 Radicle length, plumule length and total seedling length (cm)

Shoot length of three selected seedlings was measured in cm using meter scale after 15 days of germination and observation was recorded from both controls as well as nanoparticles treated plants. Root length was also measured from both treated and non-treated plants. In the same way, total seedling length was also calculated (**Figure 1**).

Figure 1 Picture of the plant *C. annum*

2.4.3 Plant fresh and dry weight (g)

On 15^{th} day of germination, seedlings were randomly selected and weighed for fresh and dry weight with the help of electronic weighing balance. Seeding was kept under oven at 80°C for 5 days, for the measurement of dry weight.

3.0 RESULTS AND DISCUSSION

3.1 UV-visible spectroscopy

Figure 2 shows the band at 358 nm which arises due to transfer of charges from the valence band which is formed by 2p orbitals of O^{2-}anions to the conduction band which is formed by 3d t_2 orbital of the Ti^{4+} cations. The anatase phase of TiO_2 nanoparticles was also confirmed through this band of UV spectra (Saravanan et al., 2018; Sethy et al., 2020; Wang et al., 2019).

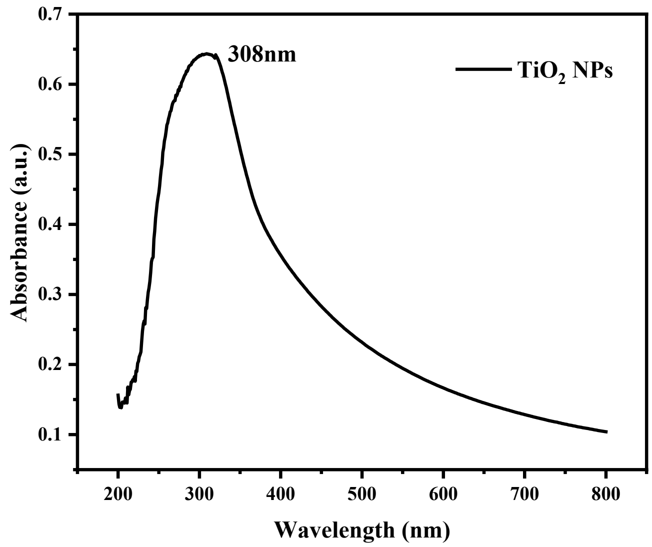


Figure 2 UV-visible spectroscopy of TiO_2 nanoparticles.

3.1 Fourier Transform Infra Red Spectroscopy

Different functional groups responsible for nanoparticle formation were determined by FTIR spectra of green synthesized TiO_2 NPs. The FTIR spectra showed prominent peaks at 3284, 1647, 1379, 1096 $cm^{-1}$and 592 cm^{-1} (Fig. 3). A broadened peak at 3284 cm^{-1} was due to OH stretching of alcoholic groups. C-C ring stretching was indicated by the peak at 1647 cm^{-1}. The observed band at 1379 cm^{-1} was due to bending vibration of the CH_2 in the proteins and lipids. The peak at 1096 cm^{-1} represented amide linkages formed between the fungal proteins and formed TiO_2 which is created during the reaction period. TiO_2 nanoparticles presence was confirmed by the peak at 592 cm^{-1}. No peak present at 2900 cm^{-1} for C–H stretching which affirms the organic compound removal post calcination for 4h at 500°C (Martra et al., 2000; Vishnu Kirthi et al., 2011).

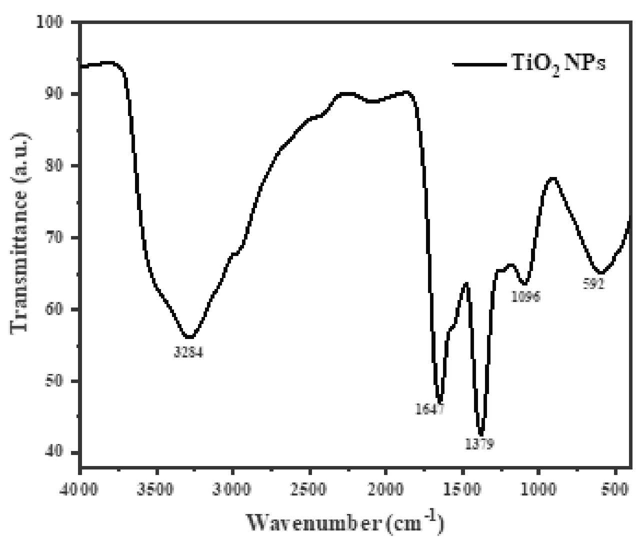


Figure 3 FTIR of TiO_2 nanoparticles.

3.2 X-Ray Diffraction

The peaks in 2θ range of 10°- 80° were seen at 25.23°, 37.86°, 47.95°, 53.97°, 54.96°, 62.63°, 68.87°, 70.12° and 75.11° and fittingly matches with Miller indices (hkl) values: (101), (004), (200), (105), (211), (204), (116), (220) and (215), respectively which establishes the anatase form of TiO_2 nanoparticles (**Figure 4**). Results were verified with JCPDS card no. 78-2486. Debye Scherrer's equation was put to use for calculating the average particle size which was calculated to 14.02 nm (**Table 1**).

$$D = \frac{0.9\lambda}{\beta\cos\theta}$$

λ = 0.15406 nm, β= FWHM (Radians), θ = Peak position (Radians)

X-ray diffraction analysis was used to evaluate the strain and dislocation density of synthesized nanoparticles. Various properties of materials are strongly impacted by the dislocation of particles. The broadening of the peak may likewise happen because of strains present in crystal structure emerging from defects such as twinning and dislocation (Bagheri et al., 2013; Chenari et al., 2016).

δ(nm^{-2}) is the dislocation density= $1/D^2$

ε(Strain)= β(radians)/4tanθ (radians)

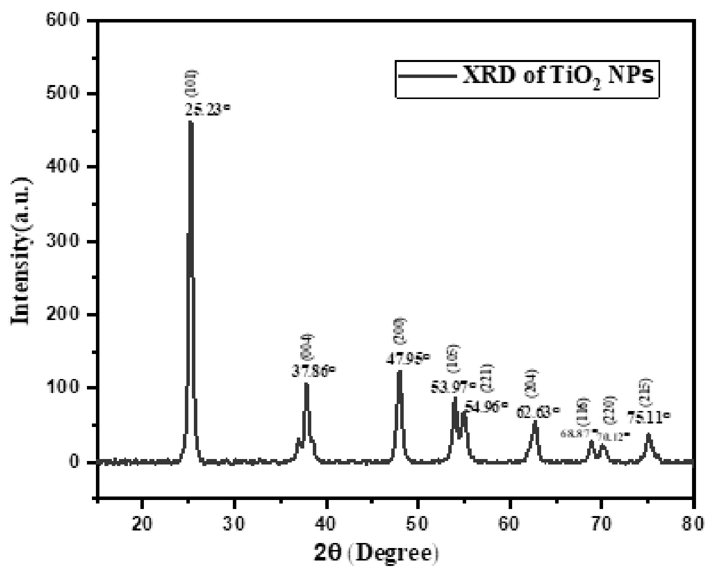


Figure 4 XRD pattern of TiO_2 nanoparticles

Table 1 XRD details.

Peak	Planes	FWHM	Height	d-spacing	Size	δ= $1/D^2$*10^{-3}	δ= β/4tanθ *10^{-3}
25.238	101	0.4671	469.632	3.525	17.426	3.292	9.104
37.86	004	0.6811	93.894	2.374	12.330	6.577	8.664
47.950	200	0.6191	119.107	1.895	14.043	5.070	6.074
53.978	105	0.557	82.284	1.697	15.985	3.913	4.778
54.969	211	0.743	65.922	1.669	12.049	6.887	6.233
62.635	204	0.796	49.986	1.482	11.680	7.330	5.709
68.87	116	0.555	26.132	1.362	17.339	3.326	3.535
70.122	220	0.771	21.322	1.340	12.575	6.323	4.798
75.113	215	0.783	33.117	1.263	12.786	6.116	4.447

3.3 Scanning Electron Microscopy

SEM image shows the surface topography and size of TiO_2 NPs. The SEM micrograph of TiO_2 nanoparticles synthesized using fungal extract showed that nanoparticles were spherical with some agglomeration (**Figure 5**). The average size of the nanoparticles comes out to be 15 nm in diameter length.

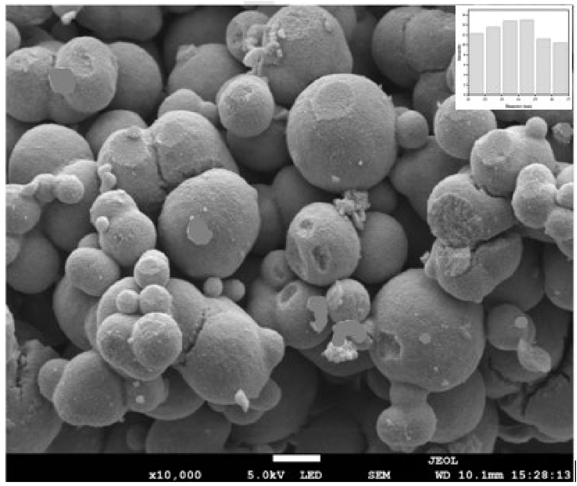


Figure 5 SEM of TiO_2 NPs.

3.4 Effect of nanoparticles on morpho-physical parameters of *Capsicum annum* (Pusa Sadabhar)

3.4.1 Germination rate (%)

The mean germination percentage ranged from 80.1% to 84.5% with the increase in the concentration of TiO_2 nanoparticles in plant *C. annum*. It might accelerate the amylase and protease activities and sugar which are required for early seed germination. Beyond 400 µg/ml, nanoparticles showed adverse effects as germination % was decreased and was observed to be lower than that of control (**Figure 6**). At higher concentration nanoparticles disrupt stages of cell division, causing disturbed metaphase, chromatin bridge, multiple chromosomal breaks, and cell disintegration. Use of titanium oxide nanoparticles on different plants has been also manifested (Haghighi and Teixeira Da Silva, 2014; Jiang et al., 2017). In the same way, Laware & Raskar (2014) found improved germination in tomato plants with the use of TiO_2 nanoparticles. Tumburu et al. (2015) also found the increase in seed germination of *Arabidopsis thialiana* on exposure with TiO_2 NPs at a lower concentration.

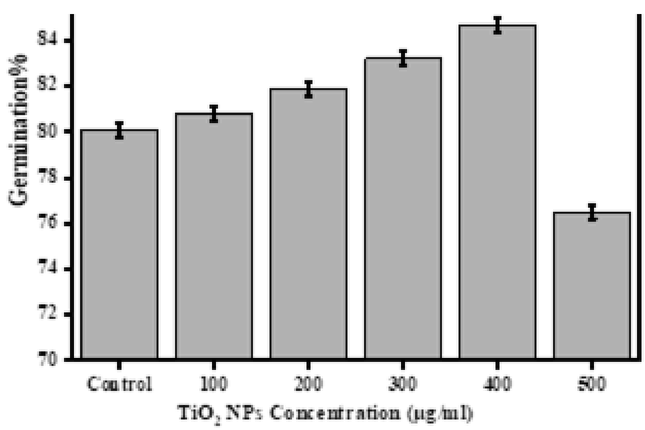
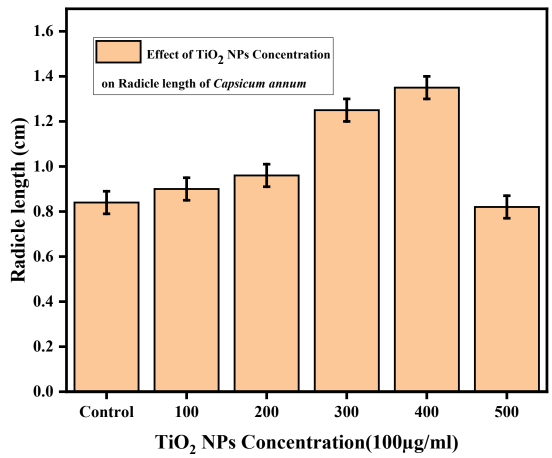


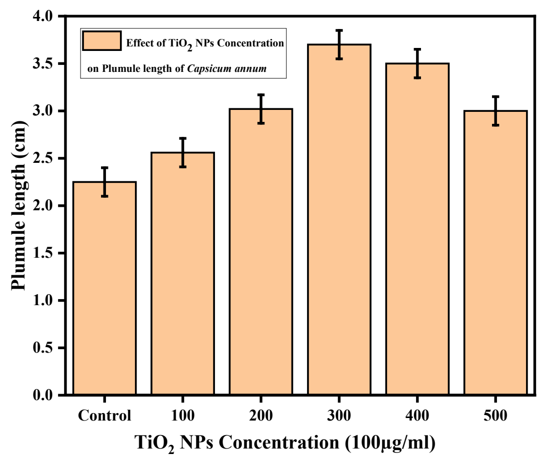
Figure 6 Effect of TiO_2 nanoparticles on germination% of *Capsicum annum.*

3.4.2 Radical length, plumule length and total seedling length (cm)

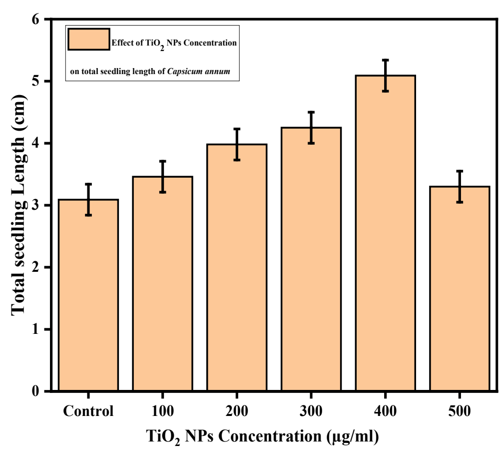
After 15 days of germination, the radical length was measured and increase in radical length was observed with the application of titanium oxide nanoparticles. The mean root length was found minimum (0.84 cm) under controlled condition while maximum (1.34 cm) was observed at 400 µg/ml concentration of TiO_2 nanoparticles (**Figure 7(a)**). Szymanska et al. (2016) found an increase in root length with the application of nanoparticles. Under suitable concentrations, these nanoparticles had a positive effect in root length but under high concentration, they show negative impact too. In the same way zinc oxide nanoparticles help to increase the root length in *Vigna radiata* (Raliya et al., 2015).



(a)



(b)



(c)

Figure 7 Effect of TiO_2 nanoparticles on (a) radical length, (b) plumule length and (c) total seedling length.

At 15 days of seedlings, plumule length was also measured with different concentration of TiO_2 nanoparticles (**Figure 7 (b)**) The mean shoot length was ranged from 2.23 cm to 3.70 cm. Increase in water absorption by TiO_2 particles enriches the nutrient uptake which leads to increase in plant height (Raliya et al., 2016).

After 15 days plant saplings total length (Shoot length + Root length) was measured with meter scale and found maximum 5.09 cm at 400 µg/ml concentration of TiO_2 nanoparticle (**Figure 7 (c)**). Application of titanium oxide nanoparticles caused an increase in plant height in case of eggplant and tomato plant along with the increase of the number of leaves per plant. Andersen et al. (2016) found increased in seedling length with the application of titanium oxide nanoparticles. Application of NPs on the seed particle increases the physiological process thereby induces plant growth and development, resulting in the improved metabolic system for seedling growth (Rizwan et al., 2019).

3.4.3 Fresh/Dry weight of seedlings (g)

Seedlings were uprooted from the agar plate and weighed in the digital weighing balance and fresh weight was found maximum (0.92 g) at 400 µg/ml concentration of nanoparticle while it was found minimum (0.38 g) under controlled conditions. It showed a sharp increase from 100µg/ml to 400 µg/ml concentration of nanoparticle (**Figure 8**).

Plant samples were dried and weighed for a total dry weight of *C. annum*. The dry weight of chilli plant was increasing with increasing concentration of nanoparticle up to 400 µg/ml. In Solanaceae family use of TiO_2 nanoparticles in the seedlings cause increase in fresh and dry weight of seedlings which may be due to the production of new pores on seed coat during penetration. Yaqoob *et al.* (2018) also found similar results after the application of nanoparticles in seeds.

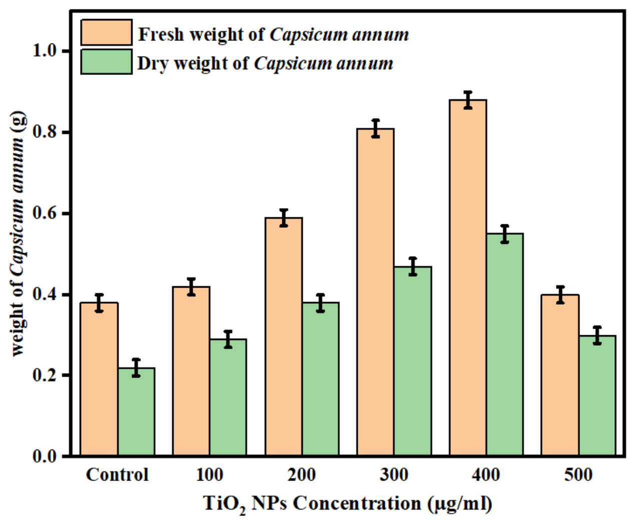


Figure 8 Effect of TiO_2 nanoparticles on fresh and dry weight of seedlings.

4.0 CONCLUSION

For improving agricultural production, synthesis of TiO_2 nanoparticles is an important step as it improves the morpho-physical parameters. The fungus *F. oxysporum* extract used for the synthesis of TiO_2 nanoparticles showed great potential. In the presence of a different concentration of titanium oxide nanoparticles morpho-physical parameter like total seedling length, plumule length, radical length, fresh and dry weight got positively affected. The present finding reveals that the nanoparticles mainly help in early germination of seeds by break down the dormancy period as compare to non-treated plants. The efficient application of NPs as fertilizers can be morally applied at larger scale globally. However, the TiO_2 nanoparticles had shown a positive impact on plant growth, major studies are still in need to figure out the exact translocation of nanoparticles in plants.

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