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Research article

Synthesis of Titanium dioxide (TiO_2) nanoparticles and impact on morphological changes, seeds yield and phytotoxicity of *Phaseolus vulgaris* L.

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Abstract: The use of nanotechnology can fulfill food problems through crop improvement in future time. Nanoparticles can enhance the morphological growth and fruit yield of plants. Titanium is considered beneficial for the growth rate of plants. Titanium dioxide (TiO_2) nanoparticles were synthesized by precipitation method. The nanoparticles were carried out at room temperature using TiO_2, NaOH and HCl as precursors. Its characterizations were completed by PSA (Particle Size Analyzer), UV-Vis Spectroscopy, XRD (X-Ray Diffraction) recorded result was ranging from 60 to 200 nanometers and crystalline nature and SEM (Scanning Electron Microscopy) of TiO_2 nanoparticles. In this research study, we treated *Phaseolus vulgaris* seeds with five different type 15, 30, 60, 120 and 240 mg l^{-1} TiO_2 nanoparticles concentrations for 1 week (7 days) in the culture tubes and examined morphological such as germination rate and time of seeds, root and shoot growth, height, number of leaves, pods and seeds and biochemical changes. Low concentrations 30 and 60 mg l^{-1} boost root and shoot growth rate of seedlings and mature plants parameters. The high concentration 240 mg l^{-1} affected these parameters in a negative manner. At the low concentrations increased height of plants, number of leaves and changed structure and chlorophyll content and weight of seed in treated plants.

Keywords: Bean seeds - Phytotoxicity - TiO_2 nanoparticles - Yield.

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INTRODUCTION

Titanium is a heavy metal and resistant to chlorine and corrosion (Natural process that converts refined metal) in seawater. Titanium can be combined with one or more metallic or non-metallic elements such as aluminum, iron, vanadium and molybdenum. Ti is the 9th abundant element on the earth crust (Kolli & Devaraj 2018). Titanium dioxide is a chemically inert, semiconductor material that exhibits photocatalytic activity with the energy equal to or greater than its band gap energy in the presence of light (Skocaj *et al*. 2011). TiO_2 nanoparticles are the most white color pigment and used in many products such as plastics, papers, paints, etc. many types of minerals used in the production of TiO_2 such as raw rutile phase abundant in earth crust (Gea *et al*. 2019). TiO_2 has a large band gap high dielectric constant, high refraction index, strong chemical stability, wear-resistant properties, high transmission near-infrared regions, hardness, good photocatalytic activity, excellent mechanical strength. TiO_2 has three crystals phase Rutile, Anatase and Brookite. (Akgul 2019) The photocatalytic activity of TiO_2 can induce hydroxyl radical and superoxide by irradiation of sunlight and it is unfavorable for the application of sunscreen (Ibrahim *et al*. 2019).

The recent research in plant science shows many effects of nanoparticles on plants such as genotoxicity, phytotoxicity, plant morphology and physiology in several plants (Shekhawat *et al.* 2014, Mattiello *et al*. 2015, Roohizadeh *et al.* 2015, Hussain *et al.* 2015, Singh *et al.* 2017, Rai & Gupta 2019). Nanoparticles produce positive and negative effects on chlorophyll in plants. The several sizes of nanoparticles used in the research studies in plant science and the small size of nanoparticles is always produced maximum toxicity of plants

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comparison to large nanoparticles (Rastogi *et al*. 2017). TiO_2 nanoparticles regulate and improve growth rate of *Vicia faba* L. plant (Abdel *et al*. 2018). TiO_2 nanoparticles in photosynthesis, nitrogen metabolisms, growth of plants and the chromosomal mutation in eukaryotes and higher plant used in the form of indicator component. It was found in various research studies in chromosomal changes, cytotoxicity, DNA damage and formation of micronucleus (Cunningham *et al*. 2018).

Phaseolus vulgaris L. is an herbaceous annual plant, known as Common bean or French bean or kidney bean. It belongs to family Fabaceae and cultivated in different parts of the world for edible purposes. It was originated in Central and South America. The plant is erect height up to 20 to 60 cm long. The leaves grow alternately on the stem and divided into 3 oval leaflets with smooth edges. The flowers with five petals butterfly like-shape and it has single large petals which upright in directions and two horizontal and two bottom of the flower. The flower has 10 stamens, 9 surrounding the ovary and 1 separate above the ovary. It contains both pistil and stamen in same flower (Hermaphroditic). It is self-fertile and effects of genetic diversity (Razvi *et al*. 2018). The plants are grown in climate of up to 3000 meters above sea level. Plants required 18 to 24° C temperature and optimum pH of soil 6 to 6.8 for pods production (Das *et al.* 2018). In India beans are major crops of rural and urban areas and bean seeds are eaten as pulses. Immature seeds, dry seeds and pods used as vegetables. The *P. vulgaris* is independently domesticated worldwide and important sources of nutrition for millions of people all over the world. It is grown for eaten fresh of its immature pods in temperate reason (Loko *et al*. 2018). In the past one decade bean production increased by formers through the repeated selection process of rich verities generation to generation. There are currently some crop verities commercialized while other crop verities neglected. It is a cash crop for small formers and cultivars in India bean producer as a small scale and it is a major legume food crop in many countries of the world and it is consumed traditional types of dishes (Kotue *et al*. 2018). It has more nutritional value and flavor to carbohydrate-rich meals. It is providing 22 % protein (phaseolin) and their many health benefits e.g. antioxidant and soluble fibers. The Common beans seed contains many important compounds such as fibers, protein, flavonoids, carotenoid, oligosaccharides, saponin, lectins, and condensed tannin. It provides many benefits for human health problems such as reducing cholesterol, colon cancer, regulates blood glucose, heart diseases and maintains insulin level. The 100g of boiled beans seeds contains approximate 140 calories, 5.7 gram protein, and 5.9 g fats and 18 g carbohydrate (Yang *et al*. 2018). It exhibits very important genetic variability which is important for biofortification. The phonotypic and genetically *P. vulgaris* evaluated through the morphological traits, biochemical- nutritional traits, seed protein and allozymes of grain and DNA marker that are preserved to understand the biogeographic distribution, diversification process, utilization and conservation Kingdom (Assefa *et al*. 2019). It is important for providing fodder and contributes soil fertility improvement by fixation of atmospheric nitrogen in crop fields. The leguminous plants can fix nitrogen in symbiosis with many soil bacteria such as *Rhizobium* species and enhance the productivity of plants (Mwenda *et al*. 2018).

MATERIALS AND METHODS

Synthesis of TiO_2 nanoparticles by precipitation method

The 80 g TiO_2 dispersed in 10 M of Sodium Hydroxide (NaOH) and the solution heated under refluxing at 100°C for the 3 hrs with continuous magnetic stirring, on the magnetic stirrer. The 1 M Hydrochloric acid (HCl) was added the in suspension of maintaining the pH value 5 of the suspension continues magnetic stirring for 5 hrs at 400 rpm. The obtained solution centrifuged at 5000 rpm for 25 minutes supernatant discarded and obtained particles washed with double distilled water and dried in hot air oven for 6 hrs at 90° C and again heated at 500° C to formed Anatase phase of TiO_2 nanoparticles (Tiwari *et al*. 2017).

Characterizations of TiO_2 nanoparticles

Several techniques and instruments were used for characterization process of TiO_2 nanoparticles such as XRD (X-Ray Diffraction), UV Vis- Spectroscopy, SEM (Scanning Electron Microscopy) (Mahajan *et al.* 2011) and PSA (Particle Size Analyzer) in Central Instrumentation Facility (CIF) Jiwaji University, Gwalior M.P. India.

Preparation of TiO_2 nanoparticles concentrations

The TiO_2 nanoparticles were mixed in distilled water in the concentration bottles to prepared of different concentrations such as 15, 30, 60, 120 and 240 mg l^{-1} or (1.5, 3.0, 6.0, 12.0, and 24.0 mg/100 ml distilled water) respectively. These concentrations shaked very well and sonicated with ultra-wave sonicator for 60 minutes respectively. All concentrations were stored at 4°C in the refrigerator for further uses (Fig. 1).

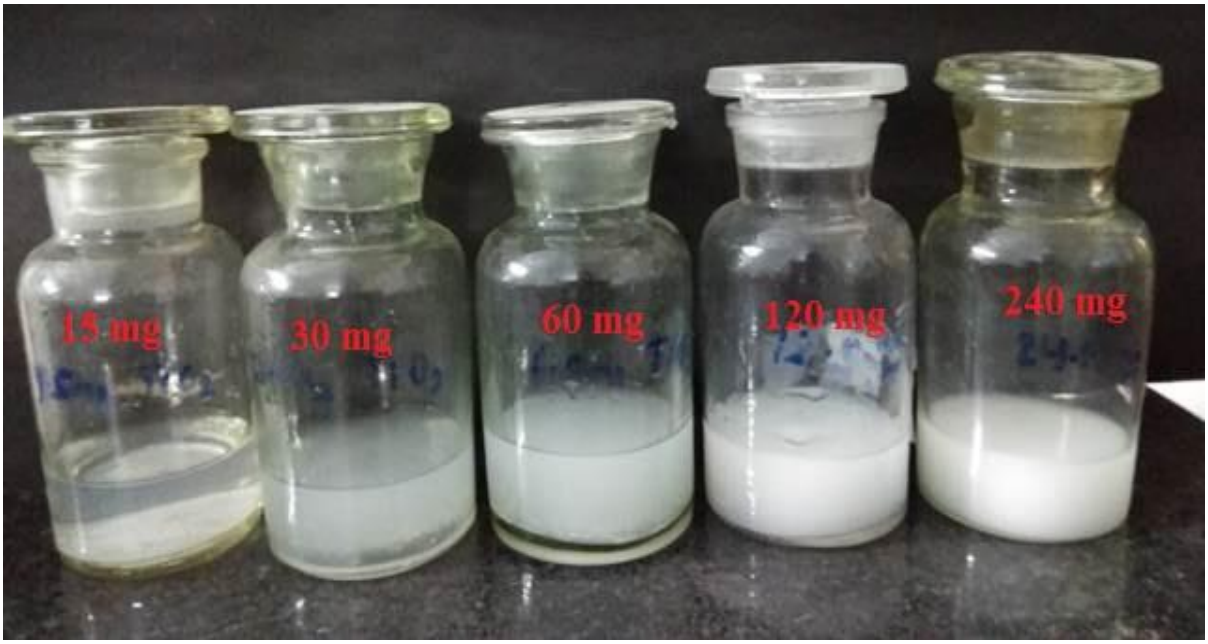

Figure 1. Different concentrations of TiO_2 nanoparticles for the treatment of *Phaseolus vulgaris* L. seeds.

Biological materials

The seeds of *Phaseolus vulgaris* L. procured from local market of Gwalior, and maintained in the laboratory and plants were cultivated in the Botanical garden (Natural condition) of School of Studies in Botany, Jiwaji University Gwalior, India

Treatment of seeds with TiO_2 nanoparticles

Seeds of *Phaseolus vulgaris* L. were surface sterilized with 4% Sodium hypochlorite solution for 5 minutes and washed with distilled water (4 times). Seeds dose concentrations and control (10 seeds/dose) were performed to three replicates (10×3 = 30) of each concentration. Surface sterilized seeds sown in test tubes were treated with the different concentrations (such as 15, 30, 60, 120 and 240 mg l^{-1}) of TiO_2 nanoparticles and left for 24 hr at room temperature. After 24 hrs seeds transferred to new test tubes containing 1 ml of TiO_2 nanoparticles concentration and this process were repeated continuously for 7 days. The distilled water used for control (Kushwah *et al*. 2018).

Estimation of Chlorophyll

The 0.5 g fresh leaves were collected from matured plants and washed with tap water then double distilled water. The 0.5 g leaves sample cut into small pieces and transferred into 10 ml acetone (80% acetone), and crushed with the help of mortar and pestle. The homogenate centrifuged at 5000 rpm for 25 minutes at 4 °C and supernatant were collected in the test tubes and 0.5 ml of it is mixed with 4.5 ml solvent. The solution was analyzed for Chlorophyll-a, Chlorophyll-b, and Carotenoids content by spectrophotometer. The equation used for the analysis of Chlorophyll-a, Chlorophyll-b, and Carotenoids (given below) (Sumanta *et al*. 2014),

Equations

$$\text{Chlorophyll-a} = 12.25A_{663.2} - 279A_{646.8}$$

$$\text{Chlorophyll-b} = 21.5A_{646.8} - 5.1A_{663.2}$$

$$\text{C} \times + \text{c (carotenoid)} = (1000A470 - 1.82Ca - 85.02Cb)/198$$

Statistical analysis

The statistical analysis was done with three replicates of each treatment and experimental results were presented as Mean ± SE (Standard Error) ($P \leq 0.05$) each experimental value was compared to its corresponding control.

Characterizations of TiO_2 nanoparticles

PSA (Particle Size Analyzer): Particle Size Analyzer is based on dynamic light dispersion and it is measured the average size of any nanomaterials. The synthesized TiO_2 nanoparticles mixed in double-distilled water and Sonicated (Ultra wave sonicator) for 60 minutes. Then analyzed by Particle Size Analyzer and obtained results were 60 to 200 nanometers in size under dynamic light dispersion which showed in PSA (Particle Size Analyzer) analyzed graph (Fig. 2) (Simadzu SALD- 2300 Version 3.1.1).

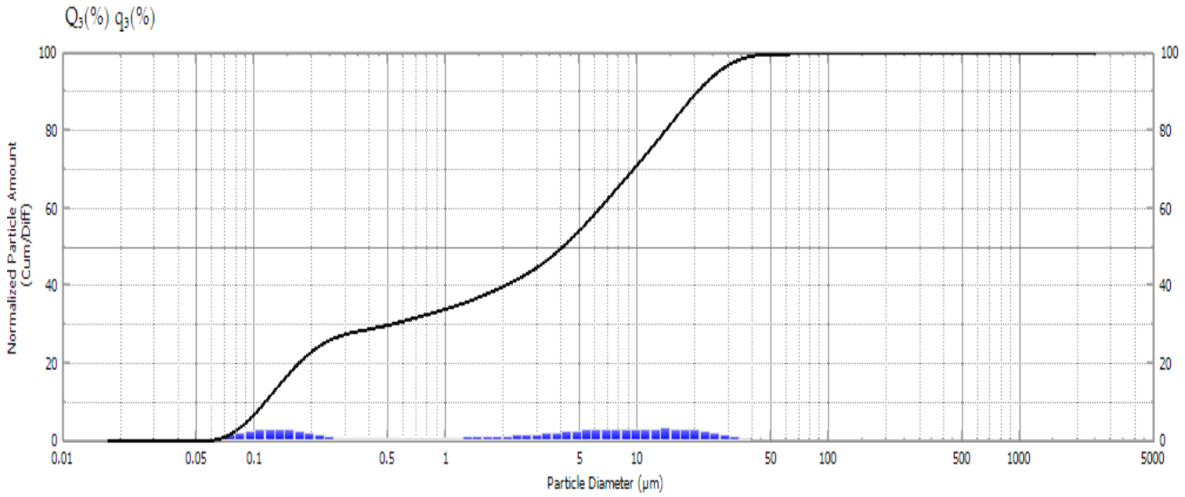

Figure 2. PSA (Particle Size Analyzer) analyzed graph of Titanium dioxide nanoparticles.

XRD (X-ray Diffraction): The analysis of synthesized TiO_2 nanoparticles by X-ray Diffraction was 2θ range of 24° to 64°. The peaks generated by XRD analysis confirmed TiO_2 nanoparticles. The peaks at 25.28, 37.8, 48.04, 53.89, 55.06 and 62.68 were corresponding (101), (004), (200), (211) and (204) planes of Titanium dioxide nanoparticles and confirmed anatase phase structure. The obtained data matched from the Joint Committee on Powder Diffraction Standards (JCPDS card no 21-1272). This is to confirm that synthesized TiO_2 nanoparticle was in crystalline nature and nano form (Fig. 3). (XRD model no mini Flex 600).

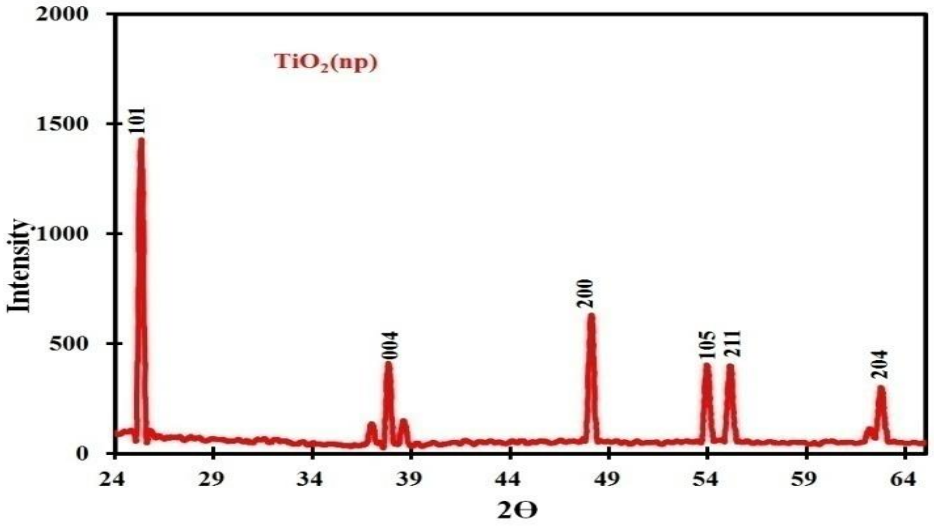

Figure 3. XRD (X-ray Diffraction) analysis pattern of Titanium dioxide nanoparticles.

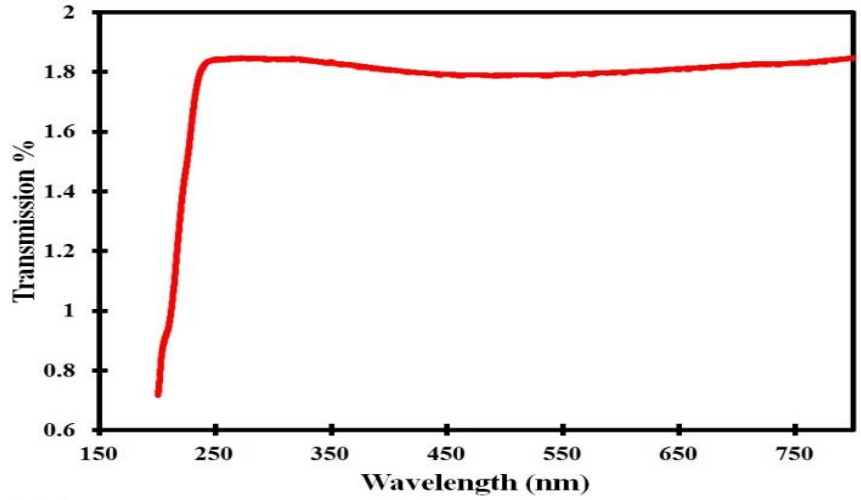

Figure 4. UV-Vis Spectroscopy analysis of TiO_2 nanoparticles recorded in 250 nm wavelength.

UV-vis Spectroscopy: The TiO_2 nanoparticles were analyzed by UV-vis Spectroscopy (UV visible spectroscopy model UV-1280 Multipurpose) and the graph shows the transmission spectra on TiO_2 nanoparticles. The wavelength of spectrum was 250 nm and spectrums were transmitted ground state to higher energy state to determine the optical properties (transmittance and reflectance) and energy band gap was found 1.75 eV to 2.5 eV. The result was matched by plotting graph which was confirmed that TiO_2 is pure form (Fig. 4) (Kushwah & Patel 2019).

Scanning electron microscopy (SEM): Scanning electron microscopy is one of the common methods for imaging the microstructure and morphology of materials. In SEM, an electron beam material with low energy is irradiated and scans the surface of the sample. The morphological size of TiO_2 nanoparticles of the Anatase phase was spherical from 60 to 200 nanometers (Model no. Zeiss EVO MA10) (Fig. 5).

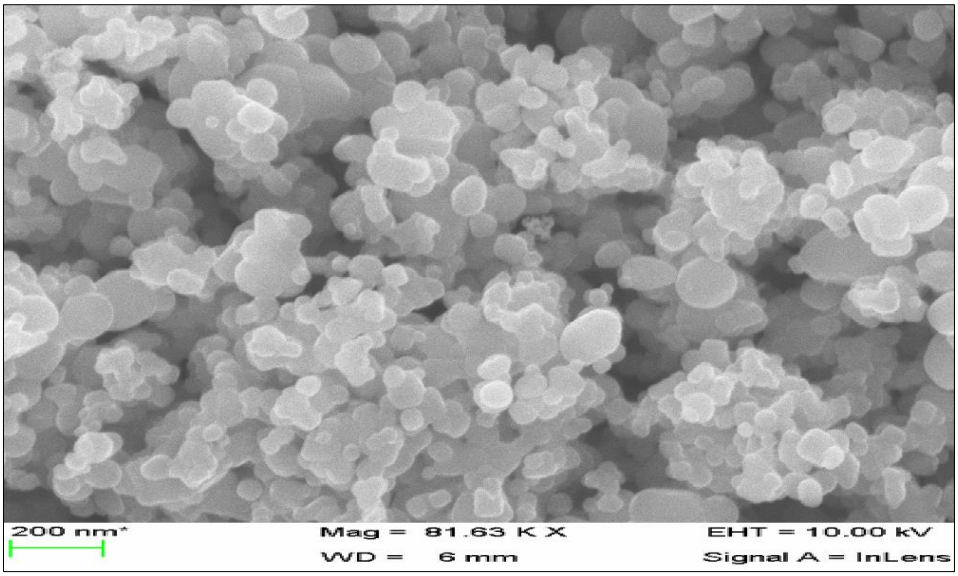

Figure 5. SEM Micrograph image of Anatase phase of TiO_2 nanoparticles.

RESULTS

Germination of seeds

This experiment was done (January to April 2019) average temperature 14°C to 31°C *Phaseolus vulgaris* seeds were in test tubes for germination in the laboratory. The seeds germination started within 2 days at 30, 60 and 120 mg l^{-1} while 3rd days at 15 and 240 mg l^{-1} concentrations of TiO_2 nanoparticles and also control. The germinated seedlings of treated seeds were cultured for 7 days in test tubes. Many treated seeds were not germinated at different concentrations and many seedlings were in unhealthy conditions and died after 7 days (Fig. 6; Table 1). As the concentration of nanoparticles increases, their density also increases. The nanoparticles aggregate at the hilum (Seed pore) of the seeds to form a membrane. Due to this membrane, the movement of essential elements was stopped which reduces the germination rate and plant survival.

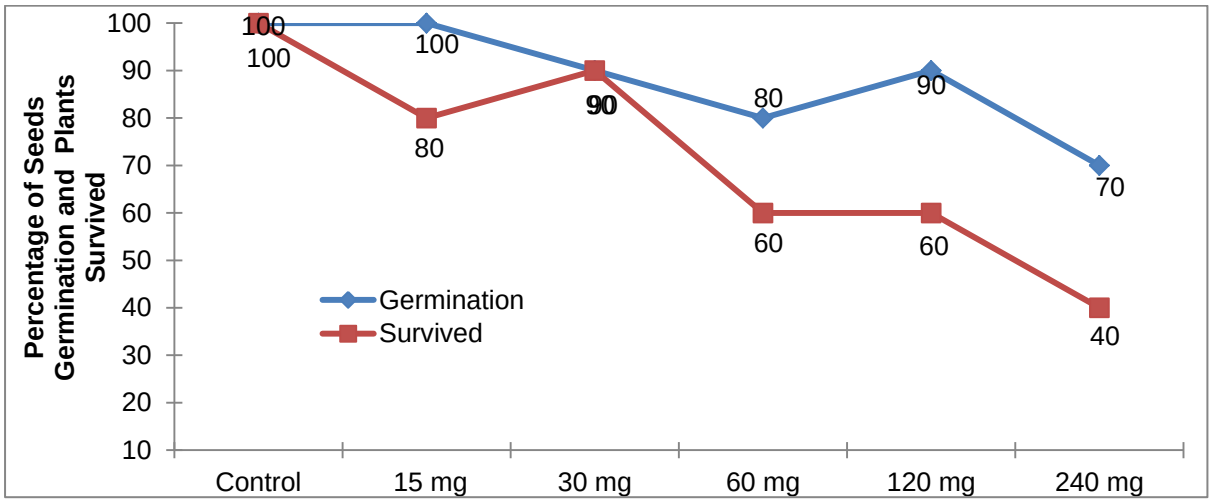

Figure 6. Seeds germination and survival percentage of *Phaseolus vulgaris* L. plants from different concentrations of TiO_2 nanoparticles.

Survived plants

We observed similar germination percentage of treated seeds of *P. vulgaris* plants at 15 mg l^{-1} concentrations of TiO_2 nanoparticles, but at 240 mg l^{-1} germination percentage decreased due to the high toxicity of nanoparticles. The highest seeds germination was 100% at 15 mg, 90% at 30 mg, 80% at 60 mg, 90% at 120 mg and 70% at 240 mg l^{-1}. The survival percentage of plant was 80% at 15 mg, 90% at 30 mg, 60% at 60 and 120 mg, and 40% at 240 mg l^{-1} concentrations of TiO_2 nanoparticles. Percentage of survival was not equal to the germination rate, much-sprouted seed died with negative effects of TiO_2 nanoparticles. The growth rate of survival plants was slow at 240 mg l^{-1} concentrations of TiO_2 nanoparticles (Fig. 6).

Root and shoot growth

The root and shoot growth of seedlings were increased, TiO_2 nanoparticles affect seed germination and growth rate. The average value of seedling growth was highly increased as compared to control plants. After 7 days maximum root length was 5.93 cm at 30 mg l^{-1} and 4.66 cm at 240 mg l^{-1} highest concentration. While 4.13 cm root length was measured in the control plant (Table 1). TiO_2 nanoparticles enter the root system through water molecules and interact with cellular organelles, and changed the growth rate of seedlings. After 7 days shoot length was 13.66 cm at 30 mg l^{-1} and shoot length recorded 5.63 cm in 240 mg l^{-1} highest concentration. The shoot length was recorded 10.43 cm in the control plant. We find TiO_2 nanoparticles affects seedling growth rate of *P. vulgaris* plants. In several seedling growths were inhibited by the negative effects of TiO_2 nanoparticles. At 240 mg l^{-1} concentration of nanoparticles reduces morphological characters of *P. vulgaris* plants. After 7 days germinated healthy seedlings were transferred in pots (Soil pH 6.7) for further growth of plants (Fig. 7; Table 1).

Table 1. Values are Mean ± SE of root and shoot growth of seedlings of *Phaseolus vulgaris* L. after 7 days.

Concentrations in mg l^{-1}	First seed germination from sowing (in days)	First shoot from sowing (in days)	Root growth of seedlings after 7 days in cm	Shoot growth of seedlings after 7 days in cm
Control	3	4	4.13 ± 0.18	10.43 ± 0.53
15	3	4	4.56 ± 0.78	9.26 ± 0.86
30	2	3	5.93 ± 0.96	13.16 ± 3.48
60	2	3	4.63 ± 0.93	11 83 ± 2.51
120	2	3	4.56 ± 0.92	8.43 ± 1.94
240	3	4	4.66 ± 1.28	5.63 ± 1.18

Figure 7. Germination of seeds and highest root and shoot growth of germinated *Phaseolus vulgaris* L.

Spectrophotometric analysis of chlorophyll

The chlorophyll content of matured *P. vulgaris* plants leaves sample was analyzed by spectrophotometer absorbance reading at different wavelengths for chlorophyll-a, b and carotenoids (µg/ml). The chlorophyll a and

b content was increased 1.56 and 1.91 at 120 mg l^{-1} treated plants compared to control plants. While carotenoids content decreases lower to higher concentrations treated plants compared to control plants (Table 2). The chlorophyll-a 1.51 and b 1.82 at 60 mg l^{-1} treated plants followed by 30 mg and 15 mg l^{-1} treated plants. While the highly decreased chlorophyll- a, b and carotenoids at 240 mg l^{-1} treated plants compared to control plants. The control plants chlorophyll- a, b and carotenoid was 1.53, 1.87, and 0.92 respectively (Table 2).

Table 2. Values are Mean ± SE (Standard Error) of Chlorophyll- a, b and Carotenoid (µg ml^{-1}) of leaves of *Phaseolus vulgaris* L. plants.

Concentrations in mg l^{-1}	**Chlorophyll-a (µg ml^{-1})**	**Chlorophyll-b (µg ml^{-1})**	**Carotenoids (µg ml^{-1})**
Control	1.53 ± 0.08	1.87 ± 0.10	0.92 ± 0.06
15	1.33 ± 0.15	1.74 ± 0.12	0.61 ± 0.08
30	1.33 ± 0.11	1.77 ± 0.16	0.75 ± 0.11
60	1.51 ± 0.19	1.82 ± 0.08	0.69 ± 0.15
120	1.56 ± 0.12	1.91 ± 0.10	0.72 ± 0.10
240	1.11 ± 0.10	1.21 ± 0.15	0.14 ± 0.05

Height of plants

The height of fully matured (60 days) treated plant was highest increased up to 71.66 cm at 30 mg l^{-1} as well as increased 66.33 cm at 60 mg l^{-1} concentration of TiO_2 nanoparticles compared to control plants. The highly decreased height of 40.33 cm at 240 mg l^{-1} followed by 52.66 cm at 120 mg l^{-1} treated plants compared to control plants. In the natural system the height of plants depends on growing age of plants. The highest length of control plants was recorded as 61.66 cm. while 15 mg l^{-1} treated plants height more compared to 120 mg l^{-1}treated plants. The TiO_2 nanoparticles had a positive effect on the growth of plants at low concentrations (Table 3).

Number of leaves and structure

The numbers of compound leaves of fully matured treated plants were increased and 18.66 leaves were recorded at 60 mg l^{-1} and followed by 18.33 leaves at 30 mg l^{-1} concentration plants. The leaves at 15 mg l^{-1} and 120 mg l^{-1} concentration treated plants were reduced 14.66 and 13.66 respectively compared to control (16.66 leaves) plants. At the 240 mg l^{-1} concentration leaves number highly decreased 8.33 compared to control plants as well as the color of leaves was yellow due to the lack of chlorophyll content. In the concentration of 60 mg l^{-1} and 120 mg l^{-1} treated plants leaves structure had changed and veins became thick compared to the control plants they were very much developed. The TiO_2 nanoparticles were used to increase the number of leaves with low concentrations. But the 240 mg l^{-1} concentration of TiO_2 nanoparticles reduced the size of the leaves in *P. vulgaris* plants and also reduces the chlorophyll content (Fig. 8; Table 3).

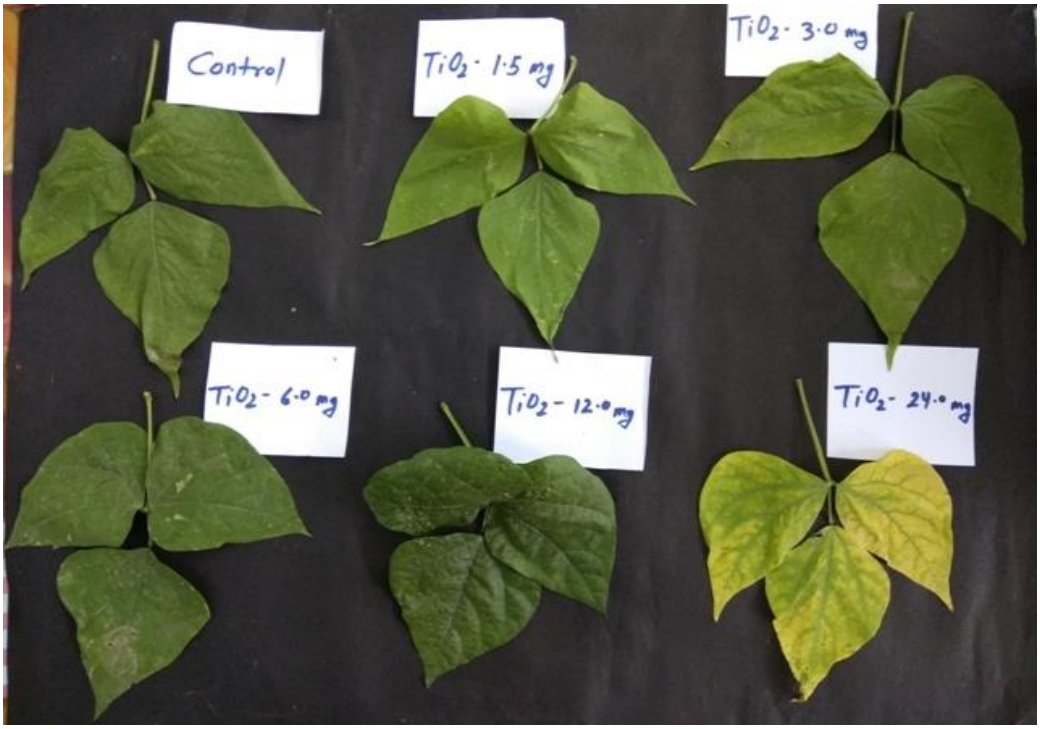

Figure 8. Matured leaf of control and treated with TiO_2 nanoparticles concentrations of *Phaseolus vulgaris* L. plants.

Table 3. Values are Mean ± SE (Standard Error) of height of plants, number of leaves and root length of harvested *Phaseolus vulgaris* L. plants.

Concentrations in mg l^{-1}	Height of matured plants in cm	Number of leaves in plants	Root length of harvested plants in cm
Control	61.66 ± 2.02	16.66 ± 1.44	17.66 ± 1.44
15	56.66 ± 2.84	14.66 ± 2.02	17.33 ± 2.02
30	71.66 ± 6.69	18.33 ± 1.85	20.66 ± 4.25
60	66.33 ± 6.0	18.66 ± 2.02	18.33 ± 2.33
120	52.66 ± 4.25	13.66 ± 1.85	17 ± 2.07
240	40.33 ± 4.33	8.33 ± 1.44	13.66 ± 1.44

Number of flowers in plants

In all treated concentrations and control plants the number of flowers was counted, the maximum number of flowers was 12.66 in the control plant. In the treated plants the number of flowers decreased from lower to higher concentrations compared to control plants. The number of flowers 12.0 at 30 mg followed by 15 mg, 120 mg, and 240 mg l^{-1} treated plants. The high concentrations of 240 mg l^{-1} treated plants highly reduced in the number of flowers compared to treat and control plants (Table 4).

Number of pods in plants

We observed the number of pods had decreased from lower to higher concentrations compared to control plants. The number of pods 10.0 at the 15 mg l^{-1} and 11.33 pods at 30 mg l^{-1} concentrations treated plants. While 9.33 pods at 120 mg l^{-1} followed by 8.33 pods at 60 mg l^{-1} treated plants but highly decreased pods 5.0 at 240 mg l^{-1} treated plants. The highest 11.66 pods recorded in control plants. The control plants pod contain 4 to 5 seeds per pod; and its similar 15 & 30 mg l^{-1} treated plants pod contain 4 to 5 seeds while at 120 and 240 mg l^{-1} concentrations treated plants pod contain 3 to 4 seeds per pod and reduce the number of pods (Table 4).

Table 4. Values are Mean ± SE (Standard Error) of number of flowers, pods, obtained seeds and weight of seeds (in grams) of *Phaseolus vulgaris* L. plants.

Concentrations in mg l^{-1}	Number of flowers in per plant	Number of pods in per plant	Number of obtained seeds per plant	Weight of obtained seed/ 10 seeds in g.
Control	12.66 ± 0.87	11.66 ± 1.20	54.33 ± 2.90	3.30 ± 0.12
15	11.66 ± 1.20	10.0 ± 1.52	47.66 ± 7.88	3.06 ± 0.11
30	12.0 ± 1.15	11.33 ± 1.44	49.33 ± 6.0	3.20 ± 0.19
60	9.33 ± 0.87	8.33 ± 1.44	37.33 ± 5.04	3.41 ± 0.37
120	10.0 ± 0.57	9.33 ± 0.87	36.33 ± 4.80	2.86 ± 0.15
240	7.33 ± 0.87	5.0 ± 1.52	18.33 ± 4.48	1.88 ± 0.05

Number of obtained seeds in plants

The total number of seeds collected in fully mature pods from various treated plants were counted and compared with total seeds collected from control plants. All the treated plants obtained seeds decreased in number from lower to higher concentrations. There was the highest number of total obtained seeds/plant 50.66 in control plants. We observed 49.33 seed at 30 mg l^{-1} followed by 47.66 seed at 15 mg l^{-1}, 37.33 seed at 60 mg l^{-1}, 36.33 seed at 120 mg l^{-1} and 18.33 seed at 240 mg l^{-1} treated plants. The higher concentrations of nanoparticles were reduced the number of seeds yield in *P. vulgaris* plants due to their toxic or negative effects (Table 4).

Weight of obtained seeds

The size and weight of mature dry seeds increase 3.41 g (per 10 seeds) at 60 mg l^{-1} treated plants compared to control plants. The control plant seeds weight was 3.30 g (per 10 seeds). While other seed weight was 3.20 g at 30 mg l^{-1} followed by 3.06 g at 15 mg l^{-1}, 2.86 g at 120 mg l^{-1} and highly decreased 1.88 g at 240 mg l^{-1} concentration treated plants. The high concentrations of TiO_2 nanoparticles on the yield of P. vulgaris seeds caused toxicity or negative effects and affect the size of the seed (Table 4).

Root length of harvested plants

The root length of harvested treated plants increases morphologically with many adventitious fibrous roots measured 20.66 cm plants at 30 mg l^{-1} followed by 18.33 cm at 60 mg l^{-1} concentrations compared to control plants. The harvested root length was 17.66 cm of control plants. While other treated plants harvested roots were 17.33 cm at 15 mg l^{-1} followed by 17.0 cm at 120 mg l^{-1} and highly decreased 13.66 cm at 240 mg l^{-1} concentrations. TiO_2 nanoparticles promote adventitious and fibrous roots in *P. vulgaris* plants at lower concentrations (Table 3).

Sterile plant

We found one sterile plant at 120 mg l^{-1} concentration treated *Phaseolus vulgaris* plants. This plant was pure sterile, with no flowers in this plant and no fruit (pods) and compound leaves with healthy conditions counted until the harvesting stage (Fig. 9). It may be the effect of TiO_2 nanoparticles suppressed the florigen hormone. Shalit *et al*. (2009) reported that the florigen hormone produced in the leaves, which acts in the shoot apical meristem of the buds. Florigen hormone is responsible for flowering in plants.

Figure 9. One sterile plant without flowering and fruiting at 120 mg l^{-1} treated plants.

Fourier Transform Infrared Spectroscopy (FTIR)

Functional activity occurring in plants was studied under the influence of nanoparticles with the help of FTIR (Fig. 10). A sample of 5.0 g dried plant was grinded and the extract was extracted with distilled water with the help of Soxhlet. Liquid extracts were used to identify the functional group of the active compounds of *P. vulgaris* plants. The absorption spectrum of FTIR on the control plants sample exhibited peak at 3339.65 cm^{-1} (Transmission 51.16%) confirmed the presence of Alcohol (H bonded, OH stretch), Primary amines (Asym NH_2 stretch), Primary amides (Asym NH_2 stretch) and Secondary amides (NH stretch). The next peak at 1634.47 cm^{-1} (Transmission 71.36%) represented the presence of Tertiary amides, Hydrazones (C=N stretch), Primary amides (NH_2 scissors) and Alkyl derivatives (C=C stretch). In the 30 mg l^{-1} (variation plants) plants Sample exhibited peak at 3339.64 cm^{-1} (Transmission 51.06%) and peak at 1634.73 cm^{-1} (Transmission 71.2%) there functional group of active compounds was similar to the control sample. There was no change in them, FTIR data interpretation with (https://www.protea.ltd.uk/ir-search.html).

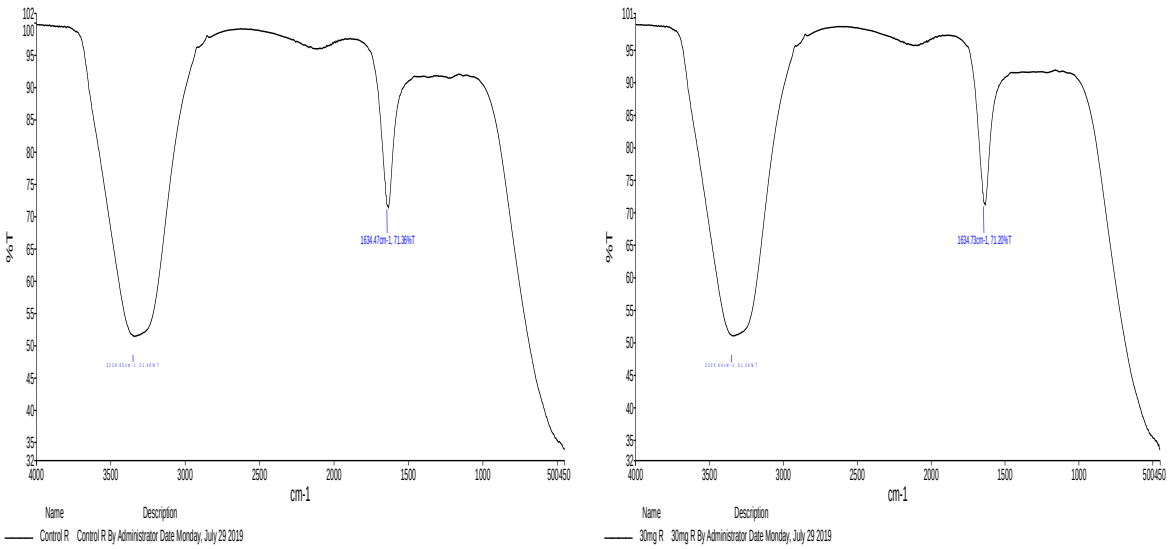

Figure 10. FTIR analyses of Extract of Control and 30 mg l^{-1} concentration treated *Phaseolus vulgaris* L. plants.

DISCUSSION

The development of nanotechnology for the past 20 years has various positive applications in plant science. The seed germination rate of three replicates of *Phaseolus vulgaris* L. differs between each treatment every treatment did not show 100% germination. The greater height was recorded in plants treated with 30 mg l^{-1} concentration as compared to control plants. All treated plants showed differences compared to each other. The high concentration 240 mg l^{-1} of TiO_2 nanoparticles causes toxicity and metabolic disturbances and decreased morphological parameters in plants. Mirzajani *et al*. (2013) reported that high concentrations of TiO_2 nanoparticles affect the plant growth of *Oryza sativa* L. TiO_2 penetrates plant cells which damage the cell wall and plasma membrane. Golami *et al*. (2018) reported TiO_2 nanoparticles increase rate of cell division, cell size, callus induction and hormones (Cytokinin and gibberellins) of *Rosmarinus officinalis* L. plants and showed significant growth in treated plants compared to control plants. Hajra & Mandal (2017) studied TiO_2 nanoparticles affect the growth and development of *Cicer arietinum* L. plants, but high concentrations of TiO_2 nanoparticles decrease the growth rate of plants. The chlorophyll content was increased at 120 mg l^{-1} and other treatments decreased differentially compared to control plants. Kurepa *et al*. (2010) reported that various types of nanoparticles were induced pore size of the cell wall for maximum entry of nanoparticles in cells of *Arabidopsis thaliana* L. and increased plant growth chlorophyll and morphological characteristics. Daghan (2018) studied the increase in 5, 10 and 20 mg l^{-1} TiO_2 nanoparticles applications have significantly reduced the chlorophyll content of old and young leaves of *Zea mays* L. plants. Hong *et al*. (2005) reported that after being treated with TiO_2 nanoparticles increase absorption of light, regulate transmission conversion of light energy, prevention of chloroplast and induce photosynthetic period of chloroplast in plant cells at below the 1000 ppm of the concentrations. Duhan *et al*. (2017) studied nano TiO_2 stimulate photosynthetic rate in plants it showed the degradation of pesticide and great efficiency of pathogen disinfection. Yang *et al*. (2006) reported nano TiO_2 play role in the light absorption, increases photosynthesis rate in Spinach plants. The present study showed that the number of leaves of mature plants in natural conditions more likely nanoparticles determines the leaves growth and structure had changed at 120 mg l^{-1} treated *P. vulgaris* plant. Missaoui *et al*. (2017) reported, at 16 days of nano-size TiO_2 strain, 100 mg l^{-1} higher concentrations were decreased by 20% leaf area of *Trigonella foenum-graecum* L. compared to controls. Yang *et al*. (2007) reported that All leaves treated with nano-anatase TiO_2 (1200 µl) were green. After treatment, one part of the old leaf surface turned green, and the other became yellowish-white. In this study the number of pods was decreased in all concentrations compared to control. The concentration of 240 mg l^{-1} nano TiO_2 pods yield was affected due to toxic effects. Nair (2016) reported in agronomic fields such as plant growth, leaves, yield, seed number, and weight and plant biomass improved with 0.02% TiO_2 nanoparticles of wheat plants. Lopez-Vargas *et al*. (2018) reported that the tomato fruit quality showed differences in all the evaluated variables the fruits increased with 50 mg l^{-1} treatments with Cu nanoparticles. Hong *et al*. (2016) reported that the treatment of CuO nanoparticles in the *Cucumis sativus* L. plants increased fruit yields at 250 mg l^{-1} concentration compared to control plants. In this study the weight of the seeds of *P. vulgaris* increased very minute level at 60 mg l^{-1} treated plants compared to control plants. The lower TiO_2 concentrations increased seed weight due to positive effects of nanoparticles through the interaction of cell organelles. Laware & Raskar (2014) reported that the ZnO nanoparticles increased seed weight of onion at different 10 µg ml^{-1}, 20 µg ml^{-1}, 30 µg ml^{-1} and 40 µg ml^{-1}concentrations. Mahakham *et al*. (2017) reported that Ag nanoparticles increased the seed weight of rice at 10 mg and 20 mg l^{-1} concentration treated plants. Razzaq *et al*. (2016) reported that the 100 grains weight was significantly higher for 25 and 125 ppm compared to other treatments and control of wheat plants. Sheykhbaglou *et al*. (2010) reported that the treatment of iron oxide nanoparticles in the soybean plants increased grain yields at 0.75 g l^{-1} concentration compared to control plants. In this study the root length of harvested plants treated with 30 and 60 mg l^{-1} concentrations were increased, and the root length was much shorter in 240 mg l^{-1} treated plants compared to control plants. Zheng *et al*. (2005) reported the root growth increased with at 100 mg l^{-1} concentration of TiO_2 nanoparticles in the spinach plants. Ghosh *et al*. (2010) reported nano TiO_2 is non-toxic less than 100 mg l^{-1} and showed 100% seed germination and root growth most leguminous plants. Darlington *et al*. (2009) reported nano TiO_2 is not well absorbed by soil in plants compared to water molecules absorption in plants and it showed increases of root growth. Giordani *et al*. (2012) reported that the increased root morphology at 50–500 mg l^{-1} of TiO^2 nanoparticles showed root hair formation on tomato plants. In this study one sterile plant found at 120 mg l^{-1} concentration treated plants. Shalit *et al*. (2009) the florigen hormone produced in the leaves, which acts in the shoot apical meristem of the buds. Florigen hormone is responsible for flowering in plants. In this study the extract of *P. vulgaris*, FTIR peak values at 3339.65 cm^{-1} and 1634.47 cm^{-1} confirmed the presence of Alcohol -

OH stretch, Asym NH_2 stretch, NH stretch Hydrazones C=N stretch, NH_2 scissors and Alkyl derivatives (C=C stretch). Hemmalakshmi *et al*. (2017) reported functional group of active compounds in aqueous extract of *Erythrina variegate* L. the strong instant peaks are identified at 3383.14, 1583.56, 1404.18 and 1072.42 cm^{-1} which represented hydroxyl compound, carboxylic acids, amide and phenol or tertiary alcohol, phosphate ion vibration respectively. Kumar & Ramaswamy (2014) reported that the methanolic extract of *Senna auriculata* L. showed peaks at 3390 cm^{-1} and 1055 cm^{-1} (C-O) for hydroxyl (-OH) group, group 2929 cm^{-1} for (C-H stretching) and 1627 cm^{-1} for (C=C) group.

CONCLUSION

In the present study, the absorption of TiO_2 nanoparticles with the water molecules was confirmed by the roots of *Phaseolus vulgaris* L. plants. TiO_2 promotes seed germination and elongation of roots of seedlings. TiO_2 has unique properties to boost the growth of root and shoot of treated plants. TiO_2 affects the morphology of the mature plant such as root length, number and structure of leaves, plant height, chlorophyll content, and fruit yield. TiO_2 nanoparticles produce both positive and negative effects in *P. vulgaris* plants. Low concentrations of TiO_2 nanoparticles produce positive effects on plants, while higher concentrations produce negative or toxic effects on plant morphology such as the reduction in plant height, leaves, chlorophyll content and yield. In the future time, more research is needed at cellular and genetic levels on plants from different nanoparticles for positive prospects in plant species.

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