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ASSESSING THE IMPACTS OF TITANIUM DIOXIDE NANOPARTICLES ON SEED GERMINATION AND SEEDLING GROWTH IN WHEAT

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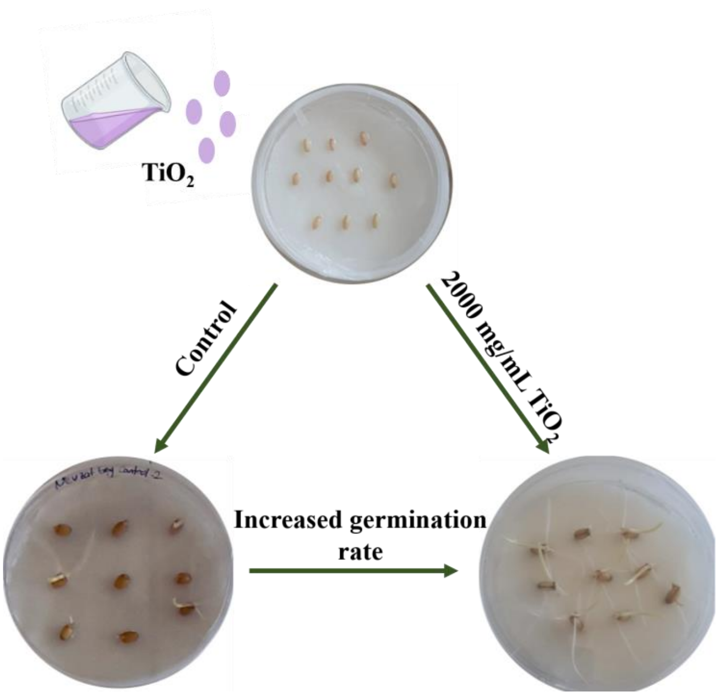
Highlights

- We evaluate the impact of TiO_2 nanoparticles not only on the germination of seeds but also on the growth of wheat (*Triticum aestivum* L.) through soil application.
- TiO_2 treatment at concentrations up to 2000 mg/L in seeds has a favorable effect on root and shoot length of wheat.
- TiO_2 treatment up to a dosage of 60 mg/kg in the soil treatment has a favorable effect on plant shoot length.
- The most significant stage in demonstrating the favorable impacts of TiO_2 NPs on plant germination rate and root and shoot growth in a dose-dependent way is to determine the optimal concentration.
- The beneficial effects of TiO_2 nanoparticles on seed germination and plant growth may have a big financial impact on horticulture, agriculture, and the energy industry—particularly on the manufacturing of biofuels.

Graphical Absract



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ABSTRACT: Wheat is the main food source for key nutrients in humans, hence any new research into boosting wheat quality and yield is vital. Recent advances in nanotechnology have made nanoparticles appropriate for use in agriculture. Titanium dioxide (TiO_2) nanoparticles have a considerable impact on plants, but further research is required to make them commercially feasible. Herein, we evaluate the impact of TiO_2 nanoparticles not only on the germination of seeds but also on the growth of wheat (*Triticum aestivum* L.) through soil application. The experimental findings reveal that TiO_2 treatment at concentrations up to 2000 mg/L in seeds planted in Petri dishes has a favorable effect on wheat root and shoot length, whereas it has a suppressive effect at higher concentrations. As expected, a similar trend is observed for plant shoot length in the soil treatment with beneficial effects recorded up to a dosage of 60 mg/kg. In conclusion, the beneficial effects of TiO_2 nanoparticles on seed germination and plant growth may have a big financial impact on horticulture, agriculture, and the energy industry—particularly on the manufacturing of biofuels.

Keywords: *Germination, Root Length, Shoot Length, TiO_2 Nanoparticles, Wheat, Triticum Aestivum L.*

1. INTRODUCTION

The growing world population has raised the need for food, which has become a serious worldwide concern; boosting agricultural production and minimizing plant yield losses are required to address this issue. Wheat recognized as an essential ingredient in human nutrition, is critical in satisfying dietary requirements; hence, extensive breeding experiments are conducted to improve wheat quality and yield. Besides breeding research, nanotechnology has recently been employed to accelerate the germination and growth of wheat, minimize development time, and produce more efficient seeds [1-3]. Inorganic and organic nanoparticles are employed in a variety of agricultural applications, including the biosensors, nanopesticides and nanofertilizers, the genetic modification of plants and animals to incorporate desired traits, and the development of plant growth regulators through rapid advances in nanotechnology [4, 5]. TiO_2 has been shown in the literature to have greater beneficial impacts on plant growth and seed germination than other inorganic nanoparticles [6-11].

TiO_2 nanoparticles are not only one of the most attractive materials in agriculture and food technology but are also used commercially in a wide range of other industries, such as cosmetics, medicines, catalysts, solar cells and microelectronic devices [12-18]. TiO_2 usage in various sectors can lead to its accumulation in rivers and water treatment systems, meaning that the probability of reaching the plants during irrigation is high. Understanding the impact of TiO_2 on plants is therefore crucial for both the environment and human health.

A research in the literature reported that nano-anatase TiO_2 dramatically boosted spinach leaf biomass, total nitrogen, and oxygen, chlorophyll, and protein levels [19]. In another work, they discovered that TiO_2 NPs can mitigate the negative impacts of drought stress on plant physiological

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processes by lowering MDA and H_2O_2 levels and stabilizing photosynthetic pigments[6]. On the other hand, Amini et al. [20] revealed that TiO_2 NPs have an active role in signal transduction, defense, metabolism, and regulation under stress conditions. Badshah et al. [21] investigated the effects of green synthesized TiO_2 NPs (25, 50, 75, and 100 μg/mL) on wheat cultivars under salt stress. The results show that TiO2 NPs improved germination rates as well as morphological and metabolic characteristics in both salt-stressed and control environments. Specifically, 25 μg/mL and 50 μg/mL concentrations improved plant length, fresh and dry weight, leaf area, and chlorophyll content under stress conditions. However, 75 μg/mL and 100 μg/mL concentrations showed adverse effects on germination, agronomic, physiological, and biochemical attributes. Moreover, Faraji and Sepehri [22] explored the effects of TiO_2 NPs (0, 500, 1000, and 2000 mg/L) and sodium nitroprusside (SNP) (0 and 100 μM) on seed germination and seedling growth of wheat under drought stress caused by polyethylene glycol (PEG) (0, -0.4 and -0.8 MPa). They reported that drought stress induced by PEG decreased germination percentage (GP), germination rate (GR), germination energy (GE), fresh root weight (RFW), root length (RL), vigor index (VI), fresh shoot weight (SFW), shoot length (SL) and the germination time (MGT) increased in wheat seeds. It has been reported that MGT decreases significantly under severe drought stress. However, using TiO_2 NPs and SNP alone or in combination has significantly increased GP, GE, GR, RL, SL, RFW, SFW, and VI. After all these observations, they concluded that applying TiO_2 NPs and SNP alone or in combination could substantially reduce the adverse effects of PEG-induced drought stress on seed germination and early seedling growth of wheat. In another study, Feizi et al. [23] evaluated the effects of TiO_2 concentrations (in the range of 1-500 ppm) on seed germination and wheat seedling growth. Their results showed that only the TiO_2 NPs treatments affected the mean germination time among the wheat germination indices. The lowest and highest mean germination time was found at control and 10 ppm concentration, respectively. The authors reported that shoot length, seedling length, and root dry matter were positively affected by TiO_2 NPs, and plant seedling lengths were higher than control (without TiO_2) at 2 and 10 ppm concentrations of TiO_2. According to the EU's Scientific Committee on Emerging and Newly Identified Health Risks, NPs are not dangerous, but their safety is still debated.

The purpose of this study was to investigate the effects of TiO_2 NPs on morphological characteristics of wheat (*Triticum aestivum* L.) such as germination, root, and shoot length. To assess shoot and root development in two bread wheat varieties (Adana 99 and Nevzatbey), we evaluated the impact of four different concentrations (500, 1000, 2000, and 3000 mg/L) in a Petri dish and five variable concentrations (20, 40, 60, 80 and 100 mg/kg--TiO_2/soil) in the soil.

2. MATERIAL and METHOD

2.1. Plant Material and Sterilization

Two varieties of wheat, Adana 99 and Nevzatbey, were used in the study. Adana 99 and Nevzatbey wheat *seeds* were supplied by Prof. Dr. Nevzat AYDIN. Wheat seeds were sterilized with 70% Ethyl Alcohol (C_2H_5OH), distilled water, and 0.5 % sodium hypochlorite (NaClO), respectively. Then, the seeds were rinsed in pure water four times for 5 minutes.

2.2. Preparation of TiO_2 Solutions

The Nanosized TiO_2 powder was AEROXIDE® TiO_2 P25, supplied by Sigma Aldrich Company. The primary particle size of nanosized TiO_2 was 21 nm, and purity was > 99.5%. 500, 1000, 2000, and 3000 mg/L TiO_2 solutions were prepared from the main stock. 80 mL of ultrapure water was used to disperse 0.5 g of TiO_2 using sonication at 300 W and 40 kHz for 2 hours. Once the sonication process was complete, the solution was diluted with distilled water to a final volume of 100 mL.

2.3. Seed Germination

Experiments were carried out in two replications. After the seed sterilization, autoclaved filter papers were placed on the Petri dishes, and nine wheat seeds were placed at an equal distance. Then, 5 ml of the prepared TiO_2 solutions were added to each Petri dish at the determined concentrations with a micropipette. After sowing the seeds, the Petri dishes were closed by parafilm. The Petri dishes were kept in the dark for two days, then transferred to a plant growth cabinet and left to grow for 21 days at 24°C, under a 16-hour light and 8-hour darkness cycle [24]. During this period, the root and shoot lengths of the germinated wheat in the sterile cabinet on the 3rd, 7th, 15th, 18th, and 21st days were measured and noted. Seeds with a root of 2 mm were considered to have started to germinate, and the following formula was used to calculate the germination rate.

$$Germination\ Rate\ (\%) = \frac{\text{The Number of Germinated Seeds}}{\text{The Total Seeds Placed in Petri Dish}} \times 100$$

Under greenhouse conditions, an equal amount of soil was placed in the pots, and two seeds from Adana 99 and Nevzatbey varieties were planted and grown in each pot under equal conditions; for soil treatment five different concentrations of TiO_2 NPs (0, 20, 40, 60, 80 and 100 mg/kg – $w(TiO_2)/w(Soil)$) were applied. The pots were watered regularly in equal amounts at certain intervals. Plants were grown in the greenhouse and shoot length measurements were made at certain intervals during the 15th and 30th days.

2.4. Statistical Analysis

The obtained data were subjected to variance analysis with the "SPSS 16 for Windows" program, and the Duncan (DMRT) test was used to compare the means. $P < 0.05$ was accepted as significant in all statistical evaluations.

3. RESULTS and DISCUSSIONS

TiO_2 solutions were prepared at concentrations of control (0), 500, 1000, 2000, and 3000 mg/L to evaluate the effects of TiO_2 NPs on germination, root, and shoot length of wheat (*Triticum aestivum* L). Nine seeds were sown in each Petri dish in two replications and the sown seeds were treated with TiO_2 solutions prepared at different concentrations.

Germination rates of the wheat seeds planted in Petri dishes according to different days given in Table 1. According to Table 1, the germination rate of Adana 99 variety seeds planted in Petri dishes on the third day in the control group was 61.11%. Germination rates on the 7th, 10th, and 15th days were calculated as 66.67%. At 500 mg/L TiO_2 concentration, germination rates were calculated as 66.67% on the 3rd day and 77.78% on the other days. At 1000 mg/L TiO_2 concentration, the germination rate did not change from the 3rd day and gave 77.78%. The germination rate at 2000 mg/L gave the highest rate with 83.33%. The germination rate was 66.67% on the 3rd day at 3000 mg/L TiO_2 concentration and 72.22% on the other days. While the lowest germination rate result was observed in the control group, the highest germination rate was determined at 2000 mg/L concentration. According to the results obtained, it can be stated that TiO_2 NPs have a positive effect on the germination of the Adana 99 variety. The germination rate, which was calculated as 55.56% on the 3rd day in the control group, was calculated as 61.11% on the other days for Nevzatbey (Table 1). While the germination rates were calculated as 66.67% on the 3rd day at 500 mg/L TiO_2 concentration, it was 72.22% on the 7th, 10th, and 15th days. While the germination rate was 72.22% on the 3rd day at 1000 mg/L, it was calculated as 77.78% in subsequent days. The germination rate results of 2000 mg/L and 3000 mg/L TiO_2 concentrations were calculated as 72.22% and 66.67 respectively. While the highest germination rate was observed at 1000 mg/L, the lowest germination rate was observed in the control group.

Table 1. Germination rates of the wheat seeds with time-varying

Day	Adana 99		Nevzatbey	
	Concentration (mg/L)	**Germination rate (%)**	**Concentration (mg/L)**	**Germination rate (%)**
3	Control	61,11 a	Control	55,56 a
7	Control	66,67 a	Control	61,11 a
10	Control	66,67 a	Control	61,11 a
15	Control	66,67 a	Control	61,11 a
3	500	66,67 ab	500	66,67 ab
7	500	77,78 ab	500	72,22 ab
10	500	77,78 ab	500	72,22 ab
15	500	77,78 ab	500	72,22 ab
3	1000	77,78 bc	1000	72,22 b
7	1000	77,78 ab	1000	77,78 b
10	1000	77,78 ab	1000	77,78 b
15	1000	77,78 ab	1000	77,78 b
3	2000	83,33 c	2000	72,22 b
7	2000	83,33 b	2000	72,22 ab
10	2000	83,33 b	2000	72,22 ab
15	2000	83,33 b	2000	72,22 ab
3	3000	66,67 ab	3000	66,67 ab
7	3000	72,22 ab	3000	66,67 ab
10	3000	72,22 ab	3000	66,67 ab
15	3000	72,22 ab	3000	66,67 ab

**The difference between the means shown with different letters in the same column is significant at the $p \leq 0.05$ level.

The lowest germination rate of the two investigated wheat cultivars was observed in the control group. Since the calculated germination rate at different concentrations of TiO_2 is higher than the control group, it is determined that the TiO_2 NPs positively affect the germination rate. NPs can induce active oxygen owing to their photocatalytic activity. Thus, they can support the germination of the plant by increasing the stress resistance and water and oxygen uptake of the seed [25]. Feizi et al. [23] reported that an appropriate concentration of nano-TiO_2 can promote wheat seed germination but has an inhibitory effect at high concentrations. Similarly, in another study in which the effect of NPs on wheat germination under stress was observed, the positive role of TiO_2 NPs and SNP on wheat germ germination indices was stated. As a result of the study, it was reported that applying TiO_2 NPs and SNP could be a promising approach for reducing the effects of stress on wheat seed germination and early growth [24]. Shoot and root lengths of Adana 99 and Nevzatbey wheat varieties were measured at 7, 10, 15, and 21 days to evaluate the effects of TiO_2 NPs. The results of the effect of TiO_2 applied at different concentrations on plant shoot and root length of the wheat varieties are shown in Table 2. Accordingly, the Nevzatbey cultivar had the highest shoot and root length on the 21st day, with a concentration of 2000 mg/ml and values of 15.71 and 15.99, respectively. By increasing the concentration to 3000 mg/ml, shoot and root lengths generally decreased compared to other concentration ratios. Taking into consideration the assessment of shoot and root lengths in Table 2, both shoot and root lengths at 3000 mg/ml were much lower for the Adana 99 variety than the control. Low TiO_2 concentrations may not show any significant favorable benefits. It is clear that further increases in concentrations may result in toxicity, even if other research indicate the presence of beneficial benefits at high concentrations [26].

Table 2. The effect of TiO_2 application at different concentrations and days on shoot and root length of the wheat varieties

TiO_2 (mg/L)	Day	Adana 99		Nevzatbey	
		Shoot Length (cm)**	Root Length (cm)**	Shoot Length (cm)**	Root Length (cm)**
Control	7	7,00 a	6,82 a	6,23 a	5,68 a
	10	7,41 ab	6,67 a	10,68 a	10,71 a
	15	11,32 a	10,99 a	12,84 ab	12,08 a
	21	12,53 a	12,21 a	13,35 a	12,38 a
500	7	7,22 ab	6,95 a	6,55 a	5,95 ab
	10	7,71 bc	7,93 b	10,88 ab	11,08 ab
	15	12,04 ab	12,59 b	13,30 abc	13,88 b
	21	13,27 b	13,84 b	13,73 a	14,25 b
1000	7	7,52 ab	6,99 a	7,01 ab	6,36 ab
	10	7,96 cd	8,40 c	11,66 bc	11,83 bc
	15	12,36 b	12,86 b	13,75 bc	14,36 b
	21	14,18 c	14,87 c	15,22 b	15,70 c
2000	7	7,77 b	7,22 a	7,48 b	6,71 b
	10	8,26 d	8,75 c	12,14 c	12,49 c
	15	12,50 b	13,28 b	14,12 c	14,60 b
	21	14,59 c	15,08 c	15,71 b	15,99 c
3000	7	6,94 a	6,62 a	6,39 a	5,85 ab
	10	7,15 a	6,40 a	10,47 a	10,33 a
	15	11,20 a	10,64 a	12,40 a	11,96 a
	21	12,42 a	11,97 a	12,93 a	12,33 a

**The difference between the means shown with different letters in the same column is significant at the $p \le 0.05$ level.

According to the Adana 99 variety shoot lengths at different TiO_2 concentrations (Figure 1a), a linear increase was observed in shoot lengths up to 2000 mg/L concentration on the 7th and 10th days, while a higher increase was observed on the 15th day compared to the other days. The lowest shoot length was determined at 3000 mg/L TiO_2 concentration. When the root lengths observed on the 7th, 10th, 15th, and 21st days of the Adana 99 cultivar were examined (Figure 1b), the same result was reached with the shoot length and the highest root length was observed at 2000 mg/L, while the lowest root length was found at 3000 mg/L. Song et al. [27] also found that low concentrations of titanium dioxide promoted plant growth, but high concentrations inhibited it. According to SPSS data results of the shoot and root lengths of the Nevzatbey variety, root and shoot measurements are shown in Figures 1c and 1d. A linear increase up to 2000 mg/L was observed in both graphs, while a decrease was observed at 3000 mg/L. The experimental findings suggest that TiO_2 NPs can increase plant water and nutrient uptake by expanding root pores due to heightened surface reactivity [28]. However, elevated concentrations of TiO_2 nanoparticles may cause NP aggregation, potentially leading to decreased water availability due to pore clogging in roots [29]. Higher organic matter concentration reduces the bioavailability and mobility of NPs. For this reason, high levels of TiO_2 NPs may also affect shoots and roots [30].

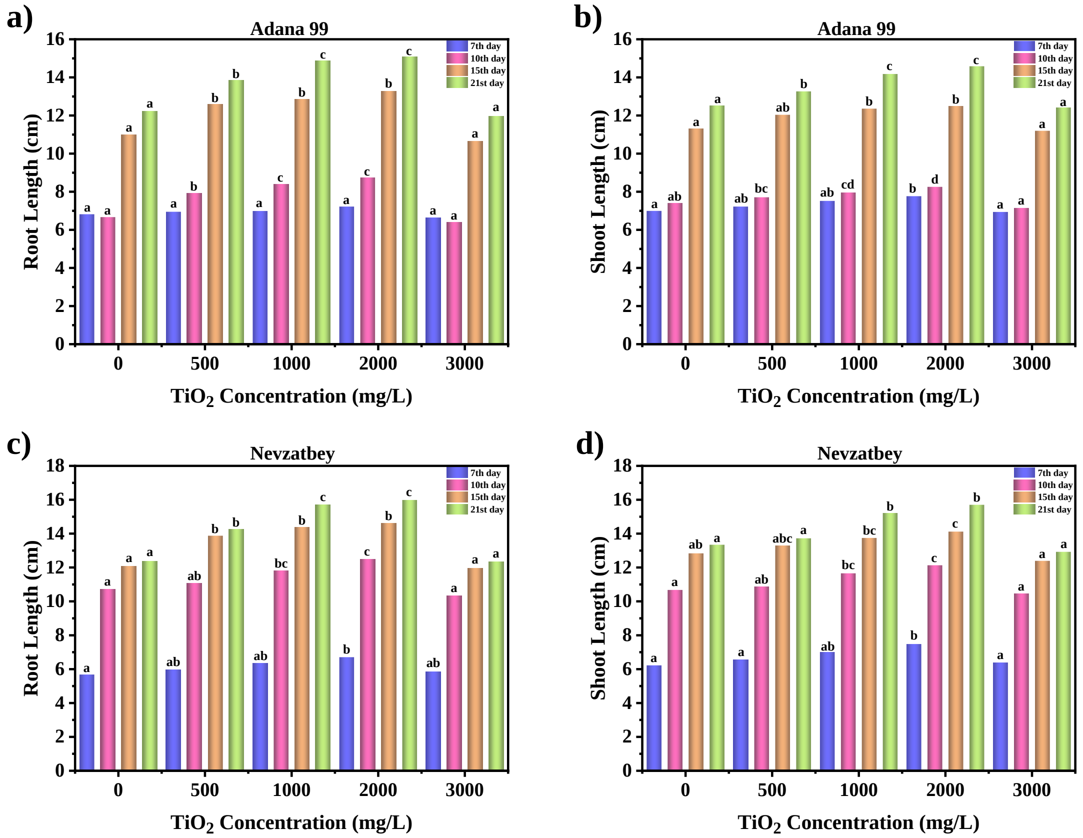

Figure 1. The effect of TiO_2 at different concentrations on the root (a,c) and shoot (b,d) length of Adana 99 and Nevzatbey varieties.

Aliabadi et al. [31] used both nano TiO_2 and nano aluminum in four different concentrations (0, 100, 1000, and 2000 mg/L). Their study shows that high doses of nano aluminum and TiO_2 have a negative and toxic effect on wheat. Generally, it was determined that high NP concentration decreased shoot and root length and showed a small positive effect at 100 mg/L concentration. Nano titanium dioxide application at a concentration of 100 mg/L positively affected growth parameters. It was reported that it could alleviate the negative effects of nano aluminum in combined effects. This study found no negative and toxic effects of TiO_2 on wheat. It has been observed that TiO_2 NPs up to a certain dose positively affect plant root and shoot length.

According to the 7th-day variance analysis results of the Adana 99 variety, there was no significant difference between shoot lengths but a considerable difference between root lengths. However, significant differences were found between the shoot and root lengths according to the analysis of variance on the 10th, 15th, and 21st days (Table S1). Considering the 7th-day variance analysis results of the Nevzatbey variety, there was no significant difference between shoot lengths but a significant difference in root lengths. Additionally, significant differences were found between the shoot and root lengths according to the analysis of variance on the 10th, 15th, and 21st days (Table S2). When the analysis results of the Adana 99 and Nevzatbey varieties are evaluated, it can be stated that TiO_2 concentrations up to 2000 mg/L increase wheat root and shoot length, while a concentration of 3000 mg/L negatively affects shoot and root length. The highest shoot and root lengths were observed in the 1000 and 2000 mg/L TiO_2 treatments.

Considering the experimental findings by applying different TiO_2 concentrations to wheat grown in pots at greenhouse conditions with equal treatments, at the end of the 15th day, the shoot length of the plant increased up to 60 mg/kg compared to the control, while it decreased at 80 and 100 mg/kg TiO_2 at Adana 99 variety grown in the soil (Figure 2a). As a result of the measurements made at the end of the 30th day in the Adana 99 variety, it was seen that TiO_2 application up to 60 mg/kg provided elongation in shoot length. However, it was determined that TiO_2 caused a decrease in shoot length at the rates of 80 and 100 mg/kg. Also, for the Nevzatbey variety, the lowest shoot length (26.2 cm) was observed at the concentration of 100 mg/kg on the 15th day, followed by the lowest shoot length of 26.3 cm in the control group ((Figure 2b). The shoot length was highest at 60 mg/kg and 40 mg/kg TiO_2 concentrations, measuring 28.6 cm and 28.4 cm, respectively. At the end of the 30th day, the lowest shoot length was observed at 34.2 cm and 34.3 cm at 100 mg/kg and control group, while the highest shoot length was observed at 36.8 cm and 36.4 cm at concentrations of 60 mg/kg and 40 mg/kg.

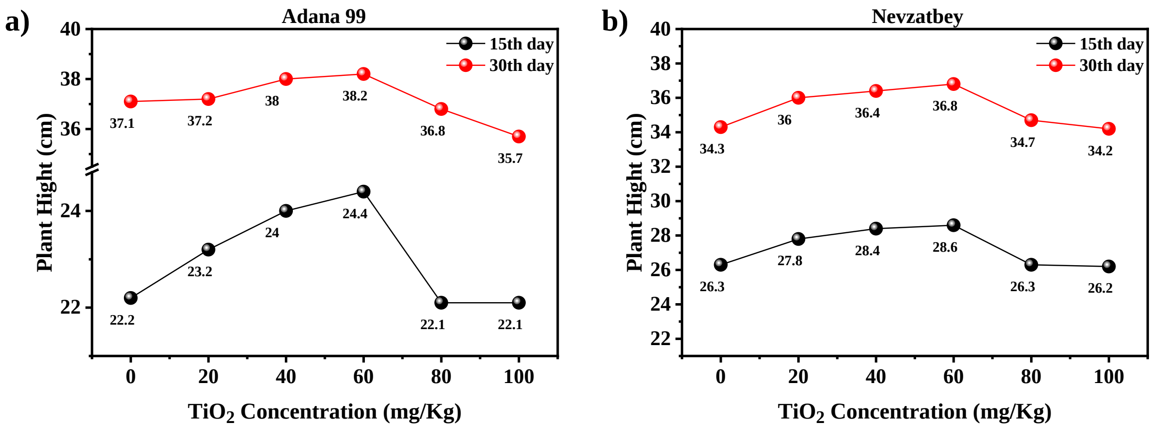

Figure 2. Plant height (cm) graph of a) Adana 99 and b) Nevzatbey variety planted in soil on the 15th and 30th days.

Rafique et al. [32] evaluated the effects of different concentrations of TiO_2 (0, 20, 40, 60, 80, 100 mg/kg) on morphological parameters such as root, shoot length, and biomass of wheat plants (*Triticum aestivum*). In general, TiO_2 NPs positively affected shoot length up to 60 mg/kg, while it negatively impacted 80 and 100 mg/kg TiO_2 treatments. These results concluded that while TiO_2 NPs positively affected plant growth up to a particular concentration, they had a negative effect at higher concentrations [32]. Our results are consistent with the study of Rafique et al. (2014). It was observed that root, shoot length, and biomass were significantly affected by TiO_2 NP treatment. It was determined that the root and shoot lengths of the plant increased up to 60 mg/kg TiO_2 NPs. However, it has been reported that high TiO_2 NP concentrations increase the root and shoot lengths of the plant while decreasing its biomass.

The effect of TiO_2 applications at different concentrations of both wheat varieties grown in the soil on the shoot length of the plant was analyzed by the SPSS program and given in Table 3. In Adana 99 wheat varieties, the highest shoot length was measured at 38.13 cm on the 30th day, while the lowest was 35.72 cm at a 100 mg/kg concentration. In the Nevzatbey variety, the highest shoot length was observed at 60 mg/kg TiO_2 treatment on the 30th day, while the lowest shoot length was determined at 100 mg/kg TiO_2 concentration on the 30th day.

Table 3. The effect of TiO_2 treatment at different concentrations and days on shoot length of Adana 99 and Nevzatbey cultivars

TiO_2 (mg/kg)	Wheat cultivar	Day	Shoot Length (cm)
Control	Adana 99	15	22,15 a
		30	37,08 a
20	Adana 99	15	23,18 a
		30	37,12 a
40	Adana 99	15	23,96 a
		30	38,03 a
60	Adana 99	15	24,16 a
		30	38,13 a
80	Adana 99	15	22,10 a
		30	36,78 a
100	Adana 99	15	22,05 a
		30	35,72 a
Control	Nevzatbey	15	26,33 a
		30	34,25 ab
20	Nevzatbey	15	27,81 a
		30	35,98 ab
40	Nevzatbey	15	28,41 a
		30	36,41 ab
60	Nevzatbey	15	28,55 a
		30	36,81 b
80	Nevzatbey	15	26,30 a
		30	34,66 ab
100	Nevzatbey	15	26,21 a
		30	34,15 a

It was determined that TiO_2 increased the shoot length of the plant up to a concentration of 80 mg/kg, caused a decrease in shoot length at 100 mg/kg, and adversely affected plant growth. Based on these results, it was stated that high concentrations of TiO_2 NPs might be inhibitory, and that further investigation is needed to determine the possible consequences and effects of applying NPs to crops (Table 3). The results we obtained in this study supported the study of Rafique et al. (2014).

4. CONCLUSION

In this study, we evaluated the effects of TiO_2 NPs on the growth and germination of wheat. For this point, cultivars of Adana 99 and Nevzatbey were treated with varying concentrations of TiO_2 (0, 500, 1000, 2000, and 3000 mg/L) in Petri dishes, and observations regarding germination and growth were recorded at 7, 10, 15, and 21 days. As a result of TiO_2 treatments, the same results were obtained in both wheat varieties. The highest root and shoot length was determined at 2000 mg/L and 1000 mg/L TiO_2 concentrations. Compared to the control group, it was observed that TiO_2 NPs up to 2000 mg/L had a positive effect on wheat root and shoot length, while it had a negative effect at 3000 mg/L TiO_2 concentration. At the same time, TiO_2 positively affected wheat germination rate up to 2000 mg/L, while a decrease was found at 3000 mg/L. Consequently, it was observed that TiO_2 NPs had a positive effect on the root growth of wheat up to a specific concentration. In both cultivars grown in soil, it was determined that the shoot lengths increased up to 60 mg/kg compared to the control and decreased at 80 and 100 mg/kg TiO_2 concentrations. This study suggested that the most significant stage in demonstrating the favorable impacts of TiO_2 NPs on plant germination rate and root and shoot growth in a dose-dependent way is to determine the optimal concentration. On the other hand, we may conclude that further in-depth research under various environmental conditions, dosages, and durations should be conducted in the future to completely understand the mechanism of action of TiO_2 NPs on germination and growth of wheat.

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Declaration of Ethical Standards

The authors declare that the study complies with all applicable laws and regulations and meets ethical standards.

Credit Authorship Contribution Statement

Ozlem ATES SONMEZOGLU: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing. Alaa KAMO: Visualization, Writing-original draft. Busra BOZKAYA: Investigation, Methodology. Savas SONMEZOGLU: Conceptualization, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Research data will be made available on request.

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