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Effect of Titanium Dioxide Nanoparticles on Growth and Biomass Accumulation in Lettuce (*Lactuca sativa*)

Channa B. Rajashekar*, Brenden Armstrong

Department of Horticulture and Natural Resources, Kansas State University, Manhattan, KS, USA
Email: *crajashe@ksu.edu

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Abstract

The use of titanium dioxide nanoparticles ($nTiO_2$) is gaining interest in agriculture because of their impact on many aspects of plant growth. The present study examines the effects of $nTiO_2$ (5 nm and 10 nm) applied to seeds and the seedlings as a foliar application on various aspects of growth characteristics and biomass accumulation in lettuce (*Lactuca sativa*, cv. Grand Rapids). Application of 10 nm $nTiO_2$ to seeds through imbibition resulted in a significant reduction in shoot biomass accumulation while 5 nm $nTiO_2$ did not affect the biomass accumulation in lettuce. The application of 10 nm $nTiO_2$ reduced the fresh shoot biomass accumulation by about 18% compared to the control plants. Other growth characteristics such as shoot dry biomass, root fresh and dry biomass, plant height, and leaf area were not affected by the application of both 5 nm and 10 nm $nTiO_2$. In addition, foliar application of these nanoparticles to the lettuce seedlings did not have a significant effect on most of the growth parameters examined, and the increasing concentration ranging from 5 nm/L to 400 mg/L did not produce a consistent response in lettuce. Thus, $nTiO_2$ application to lettuce seeds had a notable negative impact on shoot growth while foliar application did not have a significant effect on many plant growth characteristics. However, foliar applications produced some symptoms of toxicity to the foliage in the form of necrotic or chlorotic patches on the leaves, which were more pronounced with increasing concentrations of both 5 nm and 10 nm $nTiO_2$. However, these symptoms were apparent at a concentration as low as 50 mg/L of $nTiO_2$. Thus, foliar application of $nTiO_2$ may not have a significant impact on many of the growth characteristics in lettuce, but it can result in foliar toxicity.

Keywords

Growth Characteristics, Lettuce, Nanoparticles, Titanium Dioxide, Toxicity

1. Introduction

There is an increasing interest in using titanium dioxide nanoparticles ($nTiO_2$) in crop production to improve the overall growth of plants and enhance the yield potential of many cultivated crops [1]. Many studies show a positive impact of $nTiO_2$ on various aspects of plant growth characteristics such as improved seed germination, photosynthetic efficiency, root growth, biomass accumulation, nutrient assimilation, plant resilience under stress, and the overall growth and performance of many crop plants [1] [2] [3] [4] [5].

Titanium dioxide is a naturally occurring mineral and is considered safe and hence extensively used in many industrial and household products such as paints, construction materials, cosmetics, medicine, and many food products [1] [6]. TiO_2 nanoparticles show photocatalytic properties and produce reactive oxygen species in the presence of UV and even under visible light with some modification of pristine TiO_2 [7]. The photocatalytic property of $nTiO_2$ and the resulting production of oxidative species has been harnessed extensively in the reclamation of soil, water, and air contaminated with pollutants, and as an antimicrobial agent to improve food safety and in the self-sterilization of surfaces [6] [8] [9] [10] [11].

Despite the increasing interest in the application of $nTiO_2$ in agriculture to improve plant growth and performance, its effects on many aspects of plant growth are not clearly understood because plant responses to $nTiO_2$ are complex and widely variable. Several plant factors can impact the effects of $nTiO_2$ depending on plant species, type of plant tissues, growing conditions, and plant-environment interactions [4] [5] [12] [13]. Several studies have shown a positive impact of $nTiO_2$ on many aspects of plant growth [1] [14] [15] [16] [17]. However, some studies show either negative effects [18] [19] [20] or no effects [14] [21] [22] [23] of $nTiO_2$ in many crop species. Thus, there is no clear understanding of the factors that may contribute to the differential responses to $nTiO_2$ in crop plants including lettuce.

In addition to plant factors, many nanoparticle characteristics can notably influence the impact of $nTiO_2$ on plant growth. For example, particle size can significantly influence the absorption, translocation, and accumulation of $nTiO_2$ in various plant parts, which can affect the plant responses to $nTiO_2$. Smaller molecules are typically taken up by the plants and translocated more readily than the larger ones [24]. Studies on wheat by Larue *et al.* [24] showed that plants may have a limiting threshold of nanoparticle size for absorption and the subsequent translocation of $nTiO_2$ to other parts of the plants. Similarly, the concentration of $nTiO_2$ also has a significant impact on plants' responses. Typically, lower concentrations of $nTiO_2$ are known to produce a positive impact on many plant functions [1] [14] while on the other hand, higher concentrations have been shown to produce a negative impact on plant growth [20] [25]. Furthermore, high concentrations of $nTiO_2$ can also lead to the production of oxidative stress and toxicity in plants due to their photocatalytic activity [26] [27] [28].

The negative impact of the photocatalytic activity of $nTiO_2$ and the resulting oxidative stress has been demonstrated in many plant species and soil microbiota [13] [29] [30] [31] [32] [33]. However, there is very little known about the impact of $nTiO_2$ nanoparticle size and its concentration on the growth of lettuce plants.

Therefore, the focus of the present study was to examine the effect of various concentrations of two different sizes of $nTiO_2$, applied to seeds and to seedlings as foliar sprays, on various growth characteristics and biomass accumulation in lettuce plants.

2. Materials and Methods

The study consisted of two greenhouse trials to determine the impact of $nTiO_2$ nanoparticles on the growth of lettuce plants (*Lactuca sativa*, cv. Grand Rapids) in Manhattan, KS during 2023. Lettuce seeds were obtained from Ferry-Morse Seed Co. (Fulton, KY, USA), and the TiO_2 nanoparticles (anatase; 5 nm and 10 - 30 nm, referred to herein as 10 nm) were obtained from SkySpring Nanomaterials, Inc. (Houston, TX, USA) and were stored in the dark in desiccators until use. Treatments of $nTiO_2$ consisting of two nanoparticle sizes were applied either through seed imbibition or as foliar sprays to the seedlings.

2.1. Seed Imbibition

Aqueous suspensions of $nTiO_2$ (5 nm and 10 nm) were used for all the treatments. Lettuce seeds were imbibed with water (control) or $nTiO_2$ suspensions (250 mg/L) for up to 12 hours and placed on moist paper towels in Petri dishes for germination. The $nTiO_2$ suspensions were freshly prepared and thoroughly agitated before all the treatment applications. Following the germination (after 10 days of sowing), the seedlings were transplanted into a peat-based mix growing medium, BM6 (Berger, Quebec, Canada) contained in 15 cm plastic pots (1 seedling/pot) (HC Companies, Twinsburg, OH, USA). The plants were grown in a greenhouse maintained approximately at 20˚C with solar radiation as the light source. Plants were watered every other day and fertigated approximately once a week with 100 mL of solution/plant containing 250 ppm N (Jack's Peat-Lite, NPK: 20-10-20, JR Peters, Inc., Allentown, PA, USA). Plants were harvested after 4-5 weeks of transplanting. The experiment was conducted on a completely randomized design with 6 replications for each nanoparticle size.

2.2. Foliar Spray Treatments

In the first greenhouse study, the lettuce seeds were imbibed in water for up to 12 hours and directly sown in the moist peat-based growing medium (BM6) on July 17, 2023. After germination, the seedlings were thinned to retain one plant/pot. Seedlings were watered every other day and fertigated once a week as described above. The 20-day-old lettuce seedlings were sprayed with freshly prepared $nTiO_2$ suspensions (5 nm and 10 nm) at 5, 10, 50, 100, 150, mg/L. Each

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plant was evenly sprayed with water (control) or $nTiO_2$ suspension ensuring its uniform coverage of the canopy and avoiding $nTiO_2$ contamination of the growing medium. The plants were irrigated before the foliar applications of $nTiO_2$ suspensions. The 5-week-old plants were harvested to measure their growth characteristics. The experiment was conducted on a completely randomized design with 6 replications.

A second greenhouse study was conducted to assess the impact of higher concentrations of $nTiO_2$ on the growth of lettuce plants. Lettuce seeds were imbibed in water for 12 hours and were germinated and then, transplanted into the peat-based growing medium as described above. After thirteen days of transplanting, seedlings were treated with a foliar spray of freshly prepared $nTiO_2$ (5 nm and 10 nm) suspensions at 500 mg/L, 2500 mg/L, and 4000 mg/L as described above. With the high concentration of TiO_2, agglomeration can be a problem, so, care was taken to apply well-dispersed $nTiO_2$ evenly over the foliage. The control plants were sprayed with water. Plants were irrigated every other day and fertigated once a week as described above. Plants were irrigated before the foliar applications of $nTiO_2$ suspensions. The experiment was conducted on a completely randomized design with 6 replications for each nanoparticle size.

2.3. Plant Growth Measurements

All plant growth measurements were made on the plants grown from seeds imbibed with $nTiO_2$ and on those treated with $nTiO_2$ through foliar sprays before harvest.

As the lettuce variety has a loose-leaf canopy, plant height was measured from the base of the stem to the tallest leaf. The other growth characteristics measured were the number of fully opened leaves/plant and the leaf area of all the fully opened leaves using a leaf area meter (LI-3100, LI-COR, Inc., Lincoln, NE). To measure the plant biomass, plant shoots were separated from roots. The roots were washed free of the growing medium and were blotted dry with paper towels to remove the surface moisture. Shoot and root fresh weights from each plant were measured. They were then transferred to a drying oven at 70˚C and were dried for 4 days or until a constant weight was achieved before measuring the dry weights.

2.4. Statistical Analyses

All the experiments were conducted on a completely randomized design with 6 replications with $nTiO_2$ nanoparticle sizes and their various concentrations as treatment variables. The Tukey HSD test was conducted at alpha 0.05 and *p-values* were obtained to perform multiple comparisons of the treatment means.

3. Results and Discussion

3.1. Seed Imbibition and Plant Growth

The response of lettuce plants grown from seeds treated with 5 nm and 10 nm

$nTiO_2$ in relation to various plant growth characteristics including shoot fresh and dry biomass, root fresh and dry biomass, plant height, leaf area, and leaf number was studied at the time of harvest.

Lettuce plants grown from the seeds imbibed with $nTiO_2$ at 250 mg/L produced variable responses. The fresh shoot biomass of lettuce plants was significantly reduced by the seed treatment with 10 nm $nTiO_2$ by 18.1% compared to the control while, on the contrary, there was no significant impact on the fresh shoot biomass in plants receiving seed treatment with 5 nm nanoparticles (**Figure 1(a)**). This was partly due to a significant reduction in the number of leaves/ plant with 10 nm $nTiO_2$ seed treatment (**Figure 1(b)**). This shows that larger nanoparticle size had a significant negative impact on shoot biomass in lettuce compared to the smaller particle size. However, it should be noted that the size of nanoparticles can impact not only their absorption but more importantly their translocation leading to their differential accumulation in various plant parts [24].

Many growth characteristics including plant height, dry shoot biomass, leaf area, and both fresh and dry root biomass were not affected by the seed treatment of either 5 nm or 10 nm nanoparticles (**Table 1** and **Figure 1(b)**). Also, there was no significant impact on the seed germination in response to the different nanoparticle sizes (data not shown). Similar results were observed in kidney beans where $nTiO_2$ did not affect the germination but had a significant impact on early root growth [14].

Although most of the growth characteristics were unaffected by $nTiO_2$ treatment, it is noteworthy that the negative impact of $nTiO_2$ (10 nm) on fresh shoot biomass has practical implications for the growers as it can notably affect the marketable yield. The reduction of shoot growth (biomass accumulation) when

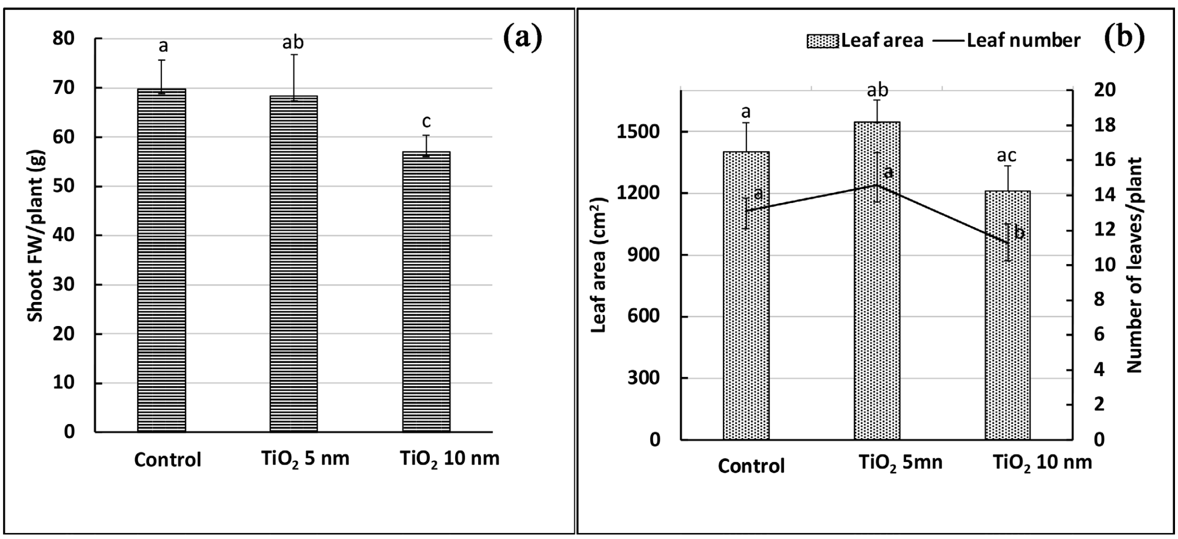

Figure 1. Growth characteristics of lettuce plants grown from seeds treated with $nTiO_2$ nanoparticles of different sizes. Lettuce seeds were imbibed with $nTiO_2$ (5 nm and 10 nm; 250 mg/L) or water (control) for up to 12 hours, and the shoot fresh weight (FW) (a), leaf area and number of leaves/plant (b) were measured at the time of harvest. The mean values with their standard deviations (SD) are presented. Significant differences at $p \leq 0.05$ are indicated by letters.

$nTiO_2$ was applied to seeds may indicate its negative impact on the early growth of lettuce [14]. Similar observations on the negative effects of seed treatment of $nTiO_2$ have been made in other species including corn, cucumber, oats, onion, and tomato [15] [34].

3.2. Foliar Spray and Plant Growth

In the first greenhouse study, lettuce seedlings were treated with both 5 nm and 10 nm $nTiO_2$ nanoparticles at concentrations ranging from 5 mg/L to 150 mg/L as a foliar spray.

Foliar spray of 5 nm $nTiO_2$ did not have any significant impact on many of the growth characteristics of lettuce except the number of leaves per plant (**Table 2**). Plants treated with 10 mg/L and 150 mg/L of $nTiO_2$ (5 nm) produced more leaves/plant compared to the control plants, the highest leaf number/plant being at 150 mg/L of $nTiO_2$. The responses to other $nTiO_2$ concentrations were not

Table 1. Growth characteristics of lettuce plants grown from seeds treated with $nTiO_2$ nanoparticles of different sizes. Lettuce seeds were imbibed with $nTiO_2$ (5 nm and 10 nm; 250 mg/L) or water (control) for up to 12 hours and the growth measurements of plants were made at the time of harvest. The mean values with their SD and *p-values* are presented.

	Plant height (cm)	Shoot DW* (g)	Root FW* (g)	Root DW* (g)
Control	20.6 ± 2.1	7.2 ± 1.4	29.8 ± 5.5	3.3 ± 1.0
$nTiO_2$, 5 nm	21.3 ± 2.7	6.4 ± 0.8	34.3 ± 8.5	3.5 ± 0.7
$nTiO_2$, 10 nm	19.3 ± 3.9	5.8 ± 0.9	35.6 ± 3.4	3.5 ± 0.7
p-value	0.953	0.150	0.530	0.925

*FW and DW represent the fresh weight and dry weight, respectively.

Table 2. Effect of foliar spray of lettuce plants with $nTiO_2$ (5 nm) on growth characteristics of lettuce plants. Lettuce plants were sprayed with $nTiO_2$ (5 nm) or water (control). The growth measurements were made at the time of harvest. The mean values with their SD and *p-values* are presented.

$nTiO_2$ 5 nm	Shoot FW* (g)	Shoot DW* (g)	Plant height (cm)	Leaf number/ plant	Leaf area (cm^2)
Control	43.6 ± 12.0	2.9 ± 0.8	20.5 ± 1.7	8.6 ± 1.1	636 ± 158.8
5 mg/L	42.3 ± 2.0	3.8 ± 0.5	21.7 ± 2.8	10.2 ± 0.4	674 ± 191.1
10 mg/L	47.8 ± 12.5	4.5 ± 1.6	22.2 ± 3.7	12.8 ± 2.3*	812 ± 131.7
50 mg/L	45.2 ± 9.9	3.6 ± 0.9	26.2 ± 4.7	11.4 ± 2.0	606 ± 89.5
100 mg/L	38.0 ± 5.9	3.9 ± 0.6	22.2 ± 3.7	11.2 ± 1.7	601 ± 97.9
150 mg/L	54.8 ± 9.6	4.9 ± 1.1	25.0 ± 4.8	13.8 ± 2.4*	796 ± 109.0
p-value	0.278	0.067	0.174	0.003	0.063

*FW and DW represent the fresh weight and dry weight, respectively.

different from those in the control plants. Unlike the seed treatment of $nTiO_2$ (10 nm), the foliar sprays did not result in the reduction of shoot fresh biomass compared to the control plants (**Figure 2(a)**). Although there was an increase in shoot dry biomass in response to $nTiO_2$ (10 nm) at 10 mg/L, both shoot fresh and dry biomass accumulations at higher concentrations of $nTiO_2$ were similar to the those in the control.

Similarly, $nTiO_2$ concentrations did not have an impact on the leaf area compared to the control (**Figure 2(b)**). Overall, these observations suggest that the foliar application of $nTiO_2$ up to 150 mg/L did not significantly impact most of the growth characteristics in lettuce plants. Therefore in a separate study, we used higher concentrations of $nTiO_2$ as a foliar spray, to determine their impact on the growth of lettuce plants. Foliar sprays of both 5 nm and 10 nm $nTiO_2$ at concentrations ranging from 500 mg/L to 4000 mg/L were applied to the lettuce seedlings. Again, these treatments produced variable results on the shoot and root biomass with regard to the nanoparticle concentrations. The notable response of lettuce at higher concentrations of $nTiO_2$ was the reduction in fresh shoot biomass with 5 nm $nTiO_2$ and in dry shoot biomass with 10 nm $nTiO_2$ (**Figure 3(a)** and **Figure 3(b)**).

Fresh shoot biomass was reduced by approximately 20% compared to that in the control plants with the application of 5 nm $nTiO_2$ at 500 mg/L. However, 10 nm $nTiO_2$ at 500 mg/L reduced the shoot dry biomass by about 29% compared to that in the control plants (**Figure 3(a)** and **Figure 3(b)**).

However, these responses were variable at higher concentrations. Foliar applications of 5 nm or 10 nm $nTiO_2$ did not have any impact on other growth characteristics such as plant height, leaf area, root fresh and dry biomass (**Table 3** and **Table 4**). Our results support the previous studies that show no impact of $nTiO_2$ on many aspects of plant growth [3] [14]. Furthermore, previous studies have also shown that $nTiO_2$ can have a negative impact on plant growth and even be toxic, especially at higher concentrations [17]. Hu *et al.* [25] showed that

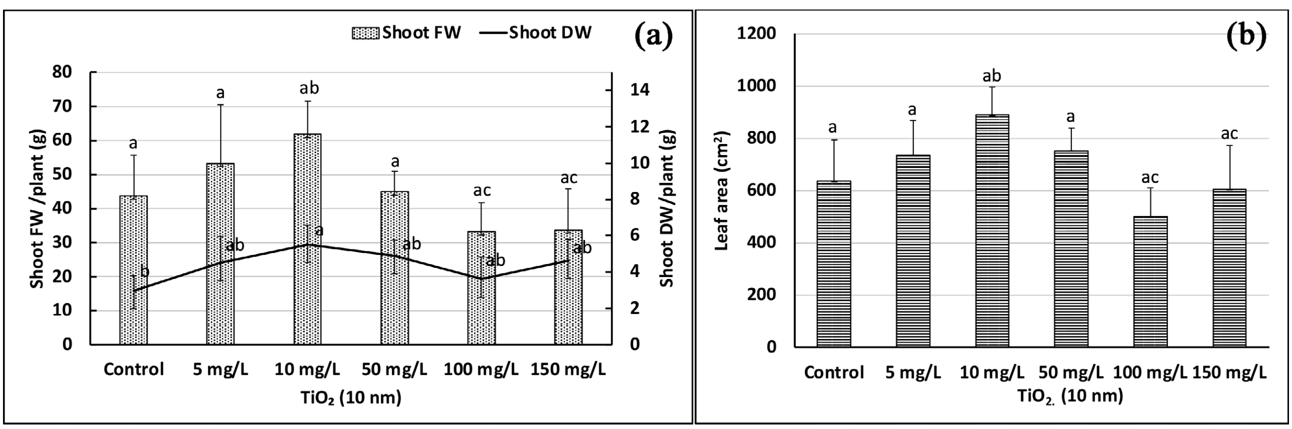

Figure 2. Effect of foliar spray with 10 nm $nTiO_2$ on growth characteristics of lettuce plants. Lettuce seedlings were sprayed with $nTiO_2$ (10 nm), and the growth measurements of plants were made at the time of harvest. Control plants were treated with water. The mean values for the shoot fresh weight (FW) and dry weights (DW) (a) and leaf area (b) with their SD are presented. Significant differences at $p \le 0.05$ are indicated by letters.

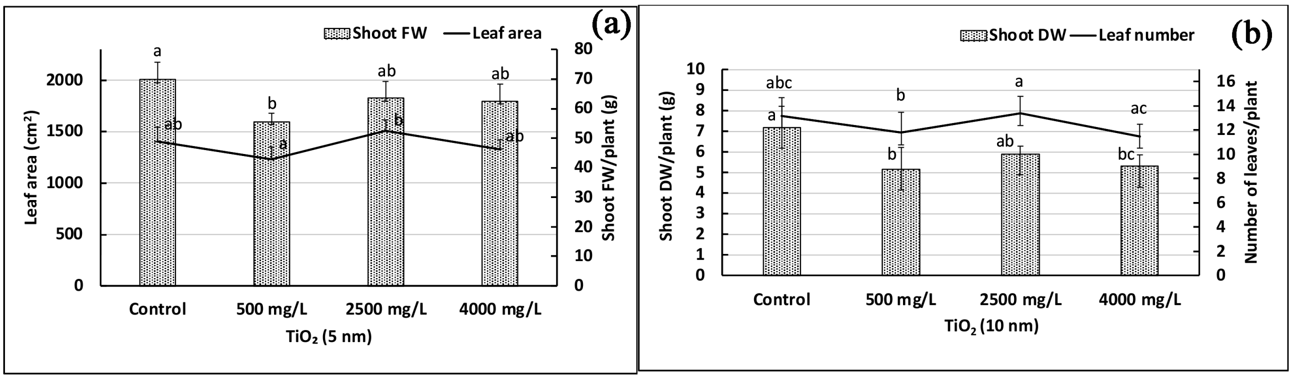

Figure 3. Effect of foliar spray with $nTiO_2$ (5 nm and 10 nm) on growth characteristics of lettuce plants. Lettuce seedlings were sprayed with $nTiO_2$ (5 nm and 10 nm) or water (control), and the growth measurements of plants were made at the time of harvest. The mean values for the leaf area and shoot fresh weight (FW) for 5 nm $nTiO_2$ (a), shoot dry weight (DW) and leaf number for 10 nm $nTiO_2$ (b) with their SD are presented. Significant differences at $p \leq 0.05$ are indicated by letters.

Table 3. Effect of foliar spray of lettuce plants with 5 nm $nTiO_2$ on growth characteristics of lettuce plants. Lettuce seedlings were sprayed with $nTiO_2$ (5 nm) or water (control). The growth measurements were made at the time of harvest. The mean values with their SD and *p-values* are presented.

$nTiO_2$ 5 nm	Shoot DW* (g)	Plant height (cm)	Leaf number/ plant	Root FW* (g)	Root DW* (g)
Control	7.2 ± 1.4	20.6 ± 2.1	13.2 ± 0.7	29.8 ± 5.5	3.3 ± 1.0
500 mg/L	5.3 ± 0.9	21.3 ± 2.9	12.3 ± 0.8	34.8 ± 12.9	3.6 ± 1.6
2500 mg/L	6.2 ± 0.6	21.7 ± 2.1	14.5 ± 2.8	25.4 ± 2.4	3.6 ± 1.6
4000 mg/L	6.1 ± 1.0	18.8 ± 2.3	12.3 ± 1.4	31.7 ± 4.1	3.2 ± 0.5
P-value	0.048	0.683	0.096	0.511	0.560

*FW and DW represent the fresh weight and dry weight, respectively.

Table 4. Effect of foliar spray of lettuce plants with 10 nm $nTiO_2$ on growth characteristics of lettuce plants. Lettuce seedlings were sprayed with $nTiO_2$ (10 nm) or water (control). The growth measurements were made at the time of harvest. The mean values with their SD and *p-values* are presented.

$nTiO_2$ 10 nm	Shoot FW* (g)	Plant height (cm)	Leaf area (cm^2)	Root FW* (g)	Root DW* (g)
Control	69.8 ± 5.9	20.6 ± 2.1	1403.8 ± 139.1	29.8 ± 5.5	3.3 ± 1.0
500 mg/L	58.0 ± 5.2	21.2 ± 4.1	1310.7 ± 149.8	41.6 ± 11.2	4.3 ± 1.5
2500 mg/L	61.9 ± 8.7	19.3 ± 2.4	1290.3 ± 254.9	31.6 ± 1.5	3.1 ± 0.5
4000 mg/L	58.6 ± 8.8	20.7 ± 3.4	1374.5 ± 258.7	32. 3 ± 1.7	3.1 ± 0.3
p-value	0.109	0.809	0.531	0.240	0.419

*FW and DW represent the fresh weight and dry weight, respectively.

the effects of $nTiO_2$ were dose-dependent in lettuce plants in that its higher concentrations suppressed chlorophyll content, biomass, and the nutritional quality of lettuce.

Considering the observation that the negative impact of plant growth (biomass accumulation) by $nTiO_2$ when it was applied to the seeds while foliar sprays produced little or no impact on overall growth may suggest that early stages of seedling growth including the germination process are more sensitive to $nTiO_2$ [14]. This is further supported by previous studies that find early growth in many plants including corn, oats, cabbage, and oilseed rape is sensitive to $nTiO_2$ [14] [15].

Furthermore, foliar applications of $nTiO_2$ to lettuce seedlings, regardless of their size, showed some signs of toxicity. The foliar applications resulted in many brown necrotic spots or etiolated blotches on the leaves, which were more pronounced at higher concentrations of $nTiO_2$ (**Figure 4**). However, these symptoms were apparent with the foliar application of $nTiO_2$ even at concentrations as low as 50 mg/L. It is noteworthy that although the growth responses were variable with foliar application of $nTiO_2$, the higher concentrations of $nTiO_2$ produced signs of toxicity in lettuce. These observations support many previous studies involving the applications of high concentrations of $nTiO_2$ that result in injury caused by oxidative stress in several plant species [25] [28] [31] [35]. As $nTiO_2$ is a photocatalytic substance, it can generate reactive oxygen species in the presence of light, especially UV, which can be highly damaging to cells [23]. The primary effect of oxidative species induced by $nTiO_2$ is the damage to the chloroplast membranes resulting not only in reduced photosynthetic function but also in promoting autophagy, a process through which impaired cell organelles could be eliminated [31] [36].

Figure 4. Effect of $nTiO_2$ foliar spray on lettuce plants. Foliar damage to leaves (highlighted in red rectangle) by $nTiO_2$ (5 nm) at 50 mg/L (left) and 100 mg/L (right).

Similar findings of the negative impact of $nTiO_2$ on photosynthetic function and the overall growth in many plant species including lettuce have been reported [20] [25] [31] [32]. Our results on lettuce show that the application of both 5 nm and 10 nm $nTiO_2$ as a foliar spray can cause toxicity to the leaves in lettuce even at moderate concentrations of $nTiO_2$ (50 mg/L or higher).

4. Conclusion

The impact of two nanoparticle sizes of $nTiO_2$ applied through seed imbibition and as a foliar spray at various concentrations (5 mg/L to 4000 mg/L) on the growth characteristics of lettuce was studied in a greenhouse trial. Seed imbibition of $nTiO_2$ (10 nm) significantly reduced shoot biomass accumulation in lettuce plants. However, the other growth characteristics including dry shoot biomass and both fresh and dry root biomass were not affected by $nTiO_2$ treatments. The foliar applications of $nTiO_2$ showed variable responses with regard to many of the growth parameters studied. Considering the observation that there was a significant negative impact on shoot growth with seed treatment but with no significant effects on many growth characteristics with foliar applications, early growth in lettuce appears to be more sensitive to $nTiO_2$ than the later stages of plant growth. However, foliar spray, especially at higher concentrations (≥50 mg/L), showed signs of toxicity as evidenced by localized leaf necrotic and etiolated patches. Thus, the results show that lettuce plants are more sensitive to $nTiO_2$ when applied to seeds, suppressing shoot growth, while the foliar applications had no significant effect on many of the growth characteristics but can produce foliar toxicity.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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