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EFFECTS OF TiO_2 NANO-PRIMING ON TOMATO SEED GERMINATION AND PLANT DEVELOPMENT

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ABSTRACT

The effect of nano-TiO_2 on seed germination and plant development as a priming agent has not been thoroughly studied. This study aimed to evaluate the effect of TiO_2 nano-priming on seed germination, plant growth and yield of tomato plants relying on experiments that have been conducted both under laboratory and greenhouse conditions. During the laboratory experiments, the seed germination, seed vigor index and water uptake of seeds were determined after 5, 10, 50 and 100 mg L^{-1} of TiO_2 nano-priming while hydropriming was used as control. The biomass of tomato seedling was increased the most for the 10 mg L^{-1} TiO_2 nano-priming condition. With follow-up experiments, the effect of 10 mg L^{-1} TiO_2 nano-priming was investigated further under greenhouse conditions, where hydropriming was also used as control. The physiological traits of tomato plants, like chlorophyll content, stomatal conductance and transpiration rate were increased by TiO_2 nano-priming treatment. Although the photosynthesis rate was boosted by nano-priming, the yield was not affected. The application of 10 mg L^{-1} TiO_2 as nano-priming agent increased plant development and chlorophyll content under both laboratory and greenhouse conditions without translocation in the plant, which is one of the most important concerts of using nanoparticles in plant production.

Key words: *Nano-priming, priming, TiO_2, tomato, seed*

INTRODUCTION

Seed germination has a vital role in crop production. Even if the seed germination is high, the homogeneous seed germination is irrefutable in plant cultivation. Moreover, the synchronization of plant development enables further treatments in agricultural production (Azimi *et al*., 2014; Khalaki *et al*., 2021). For these purposes, priming was developed as a useful technique and currently, there are various types of priming (Finch-Savage *et al*., 2004; Moammeri *et al*., 2018; Bekhrad *et al*., 2016). One of these techniques, nano-priming, is described simply as the soaking of seeds in nanomaterial suspension (Khalaki *et al*., 2021). Considering that the introduction of nanoparticles (NP) to agriculture does not go back a long way, the ethical and environmental issues about their use in agriculture is still under discussion. This especially relates to the uncertainties regarding NPs, such as their environmental effect as well as durability in nature. Studies have established the toxic effects of NPs, such as prohibition of seed germination, stem and root growth, decrease in the seedling numbers, and reduction of the yield (El-Temsah and Joner, 2010; Li *et al*., 2015). The toxicity of a NP depends on elemental composition, porosity, average size, surface area, surface charge, aggregation propensity, and hydrodynamic diameter (Peralta-Videa *et al*., 2011). However, the positive effects of the nano-priming in the plant production are undeniable. The treatment of NPs can increase the seed germination rate, seedling development, growth and yield in different plant types (Dutta, 2018; Feizi *et al*., 2013).

The combination of these two factors makes using nanoparticles as a priming agent attractive. Considering that a major concern is the uncontrolled release of NPs into nature, the advantages of using NPs as priming agent allows for preventing the spread of NPs to the environment. Furthermore, NPs both increase the solubility and decrease the premature degradation of active ingredients and can be designed of target focusing particles. For this purpose, two strategies, which are both not yet entirely understood, might be followed: developing the effect of active ingredients or introducing new ingredients (Kah *et al*., 2013).

NPs have unique properties in comparison to their bulk structure. Plant growth, resistance to abiotic stresses, photosynthetic rate and seed germination was increased after subjected to nano-size metals and their oxides such as TiO_2, SiO_2, carbon nanotubes (CNT), ZnO (Almutairi, 2016; Almutairi and Amjad, 2015; Dhoke *et* *al*., 2013; Gao *et al*., 2006). Many different studies have shown that TiO_2 increases the photosynthesis ratio and chlorophyll content of plants (Gao *et al*., 2006, 2008; Hong *et al*., 2005; Mahmoodzadeh, 2013). Hong *et al* (2005) pointed out that, TiO_2 raised the photosynthesis ratio of spinach by promoting photochemical reactions of chloroplast (Hong *et al*., 2005). Formerly, the studies performed by Gao *et al*. in 2006 and 2008 illuminated that TiO_2 caused a conformational change in rubisco activase enzyme, which increased the activity of the enzyme (Gao *et al*., 2006, 2008). TiO_2 nanoparticles were also used under N-deficient conditions and managed nitrogen deficiency in spinach by increasing the nitrate reductase activity (Yang *et al*., 2006, 2007).

In the presented study TiO_2 was used as the priming agent for tomato (*Solanum lycopersicum* L.) seeds with the aim to assess any change in plant development and growth. Therefore, tomato seeds were primed with TiO_2 nanoparticles at different concentrations and the effect of TiO_2 nano-priming on tomato seeds and plantlets' development was investigated. Later on, the optimal concentration was selected and the experiments were continued under the greenhouse condition. The physiological responses and changes in the yield of tomato plants were observed.

MATERIALS AND METHODS

Characterization of TiO_2 nanoparticles: The phase analysis of the TiO_2 nano powder was conducted with X-ray diffractometer (XRD) between 2θ: 20-80° with 2°/min speed and Cu-Kα radiation. The structure of the TiO_2 NPs was observed using a field emission scanning electron microscope (SEM) at the accelerating voltage of 5.0 kV.

Effect of TiO_2 on tomato seed germination and seedling growth: For nano-priming treatments, 0 (hydropriming as control), 5, 10, 50 and 100 mg L^{-1} of TiO_2 nanoparticle suspensions were prepared using distilled water and sterilized in the autoclave at 121°C for 20 minutes. After sterilization the suspensions were incubated in the sonicator for 30 minutes for homogenization.

Experiments were laid out using a Completely Randomized Design with five replications, and each replication was conducted with 30 seeds. Tomato seeds (*S. lycopersicum* L. cv. Altar F1) were surface sterilized with 0.7% NaClO for 4 minutes and rinsed with sterile distilled water. The seeds were then soaked in TiO_2 suspensions for 24h at 120 rpm for priming. The seeds were germinated on quarter-strength Murashige and Skoog (MS) media supplied with 1.5% sucrose (Duchefa) and 8% phytoagar (Duchefa). The germination experiment was conducted in germination cabinets at 20°C under 16/8 h light/dark conditions.

On the 21st day of germination, the germination ratio, length and biomass of the shoot and root were measured. Seed vigor index (SVI) was calculated according to the following formulas:

Seed Vigor Index I= Germination % x Seedling Length (mm)

Seed Vigor Index I= Germination % x Seedling Weight (g)

Water uptake (WU) of seeds was measured with five replications. The seeds were weighed and priming was conducted. After priming, seeds were blotted dry and weighed again and WU was calculated according to the following formula:

WU%= ((SW after priming-SW before priming)/SW before priming) * 100

SW: Seed weight

Effect of TiO_2 on tomato plant development and yield: According to the laboratory experiment, 10 mg L^{-1} of TiO_2 was selected for further experiments due to having the highest SVI and hydropriming (0 mg L^{-1}) was used as a control. Experiments were laid out in Completely Randomized Design with 5 replications. Each experimental unit comprised three pots (12 L, 26 cm in diameter) with one plant in each pot. Before sowing the seeds, the seed nano-priming was conducted with the same method. The pots were filled with peat and perlite mixture in 2:1 ratio (v/v). A composed fertilizer of NPK (18+18+18+ microelements) was incorporated into the peat-perlite mixture before sowing. Three seeds were sown at 3 cm in each pot and thinned to one plant in each pot after emergence. The stem length of the tomato plants was measured at the flowering stage.

Gas exchange, photosynthesis rate and chlorophyll content measurements: Photosynthetic rate (Pn), transpiration rate (E) and stomatal conductance (Gs) were measured on the fully expanded youngest leaves using a portable photosynthesis system (LI-6400 XT, LICOR, USA). The measurements were performed under the following conditions: 400 μmol mol^{-1} of CO_2 concentration and photosynthetically active radiation (PAR) at 1500 $\mu molm^{-2}s^{-1}$. Three measurements were taken from one plant of each replication for both treatments on 28th, 56th, 96th and 123rd day after planting.

Chlorophyll index was measured on the fully-expanded youngest leaves with SPAD-502 Plus chlorophyll meter (Konica Minolta). An average of three measurements were taken for each replication. Thereafter, the same leaves, which were used for chlorophyll index measurement, were harvested for chlorophyll content determination. The fresh weight of the leaf samples was measured (around 0.2 g), and the leaves were ground very well using a tissue laser. Then, 1 ml of 80% acetone was added to the samples and shaken and incubated overnight at +4°C. After overnight incubation, the samples were centrifuged for 5 min. at

13.000 g and the supernatant was collected. The supernatant, which had the chlorophyll, was measured at 470, 646.8 and 663.2 nm. The chlorophyll content was calculated according to the following formulas:

Chlorophyll a content = (7,15 x A663.2)/1000/Fresh weight (A)

Chlorophyll b content = (18,71 x A646.8)/1000/Fresh weight (B)

Total chlorophyll content = A + B

Tomato fruits were harvested three times; on 110th, 128th and 154th days after planting. The yield was calculated as the number and the weight of the fruits per plant.

The root, stem, leaf and fruit samples were collected after the final harvest and TiO_2 content of each sample was measured by X-Ray Fluorescence (XRF).

Statistical analysis: The data obtained from experiments were examined by ANOVA test by using MINITAB software. The differences between groups were determined by Fisher's post-hoc analysis. A 2-paired t-test was performed by using MINITAB.

RESULTS

Characterization of TiO_2 nanoparticles: The purchased TiO_2 nanoparticles were analyzed with XRD and SEM. According to the XRD pattern, the nanoparticles showed strong peaks at 25° and 48° specific to the TiO_2 anatase phase, which confirmed the purification of the nanoparticles (Figure 1) (Theivasanthi and Alagar, 2013).

Besides, the image of TiO_2 nanoparticles was obtained using SEM and the sizes of TiO_2 nanoparticles were measured (Figure 2). According to the image, the sizes of particles were less than 100 nm and confirmed the suitable size for the treatments.

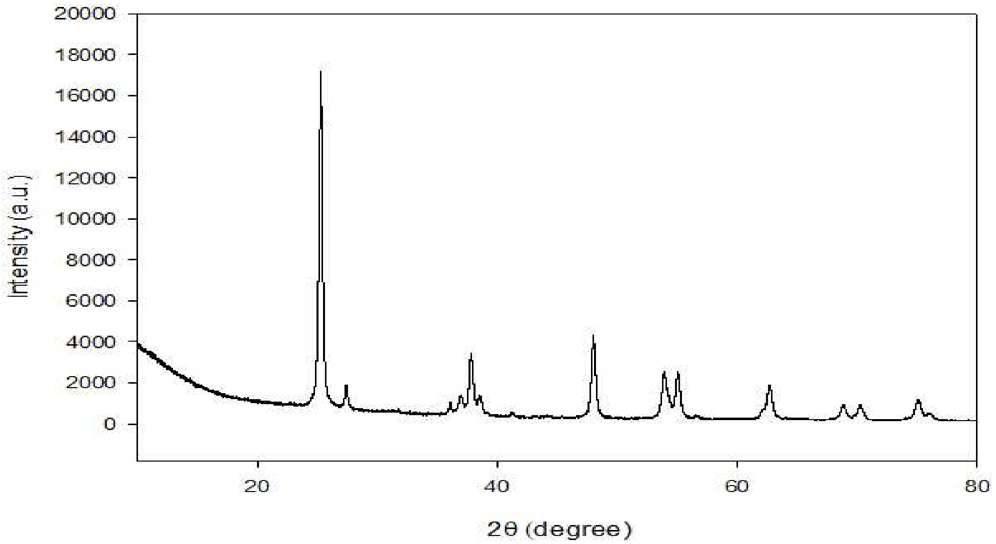

Figure 1. XRD pattern of TiO_2 nanoparticles.

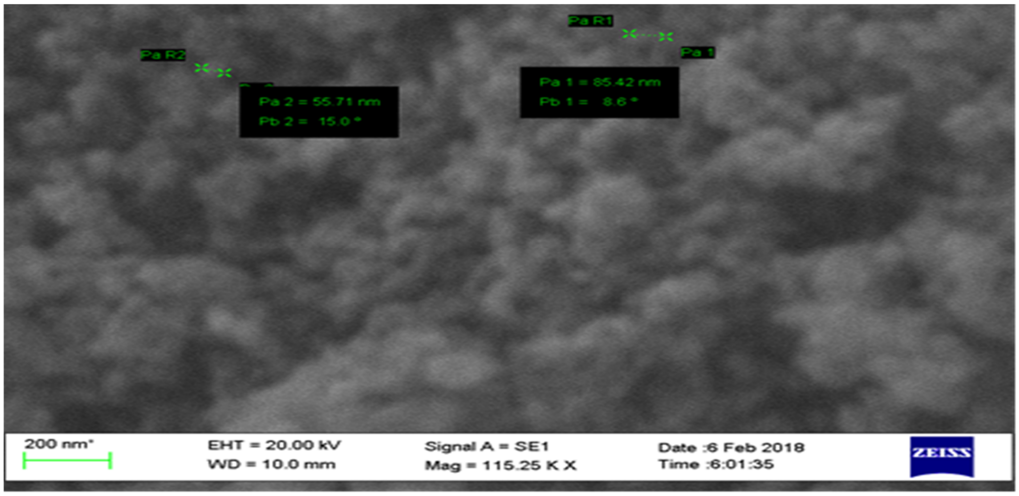

Figure 2. SEM image of TiO_2 nanoparticles.

Effect of TiO_2 on tomato seed germination and seedling growth: The effect of nano-priming on seed germination rate and seed vigor index according to the length and the weight of plantlets can be seen in Table 1. The nano-priming did not affect the seed germination rate. The seeds had more than 97% of germination rate after all treatments. On the other hand, the seed vigor index was affected for hydro and nano-priming treatments. When the 10 mg L^{-1} of TiO_2 nano-priming treatment increased seed vigor index by length, 50 mg L^{-1} of TiO_2 nano-priming increased the seed vigor index by weight the most. On the other hand, 100 mg L^{-1} of TiO_2 nano-priming decreased the seed vigor index by length. The water uptake ability of the seeds was increased for the TiO_2 nano-priming treatment. 10 mg L^{-1} of TiO_2 treatment increased the water uptake ability of the tomato seeds the most.

Table 1. Effect of hydro and TiO_2 nano-priming on germination rate, seed vigor index.

Treatment	GR (%)	SVI-I	SVI-II	WU (%)
T_0	97.3	18852b	51.5d	44.0c
T_5	97.3	19217b	59.5b	45.8bc
T_{10}	98.0	20538a	62.4b	53.0a
T_{50}	97.5	18792b	67.8a	49.1abc
T_{100}	98.0	17219c	55.0c	49.8ab

Means, in each column, followed by a similar letter are not significantly different at the 5% probability level using Fisher's Multiple Range Test.

GR: Germination rate, SVI-I: Seed vigor index by length, SVI-II: Seed vigor index by weight, WU: Water uptake, T_0: Hydropriming, T_5: 5 mg L^{-1} TiO_2 nano-priming, T_{10}: 10 mg L^{-1} TiO_2 nano-priming, T_{50}: 50 mg L^{-1} TiO_2 nano-priming, T_{100}: 100 mg L^{-1} TiO_2 nano-priming.

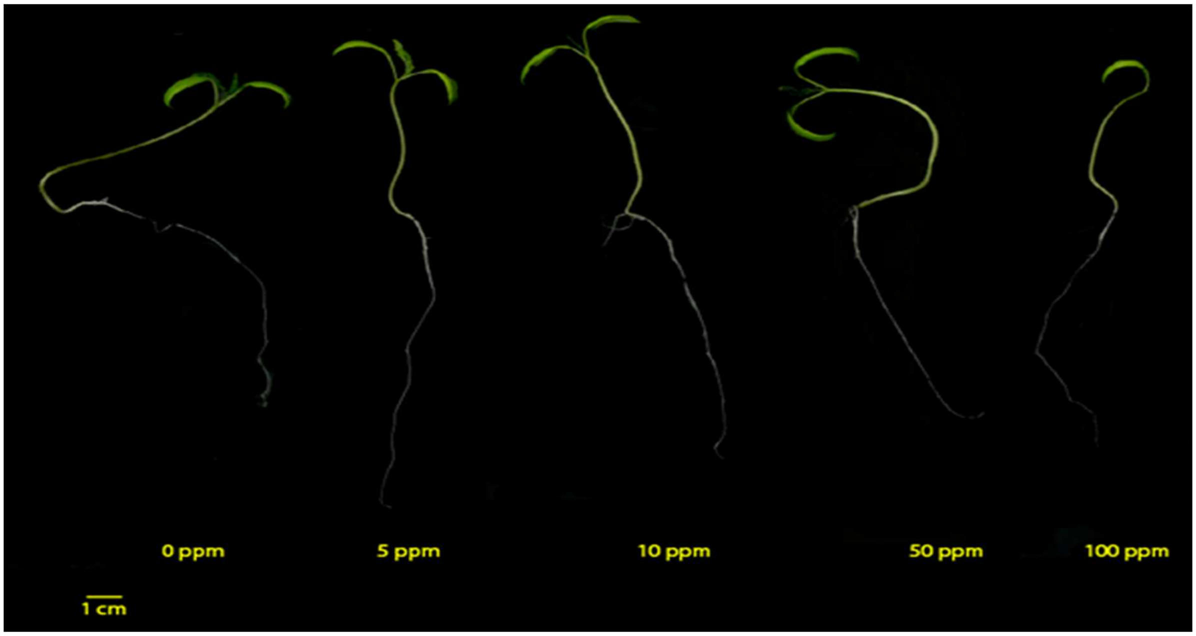

Figure 3. Tomato plantlets developed after TiO_2 nano-priming.

The effect of TiO_2 nano-priming on plantlets' development was also observed (Figure 3). The shoot length was not affected by TiO_2 nano-priming. However, root length was increased with 10 mg L^{-1} of TiO_2 nano-priming treatment. Besides, 100 mg L^{-1} of TiO_2 nano-priming decreased the root length of the tomato plantlets. The shoot fresh weight of tomato plantlets was increased with TiO_2 nano-priming treatments at all concentrations, and 50 mg L^{-1} of TiO_2 treatment holds the first rank. The heaviest shoot dry weight was measured after 10 mg L^{-1} TiO_2 nano-priming and root dry weight after 5 and 100 mg L^{-1} TiO_2 treatment (Table 2).

Effect of TiO_2 on plant growth and yield: Primed seeds with 10 mg L^{-1} of TiO_2 nanoparticles were planted under greenhouse conditions. The stem length of the plants was measured at the flowering period, and it was observed that the nano-priming treatment did not affect the stem length of the plants (Figure 4).

Table 2. Effect of hydro and TiO_2 nano-priming on shoot and root length, shoot and root fresh and dry weight.

Treatment	SL	RL	SDW	RDW
T_0	73.5	120.3b	0.0124c	0.0021b
T_5	76.1	121.3ab	0.0137abc	0.0027a
T_{10}	78.6	131.0a	0.0155a	0.0025ab
T_{50}	76.6	116.1b	0.0141bc	0.0026ab
T_{100}	73.1	102.6c	0.015ab	0.0027a

Means, in each column, followed by a similar letter are not significantly different at the 5% probability level using Fisher's Multiple Range Test.

SL: Shoot length, RL: Root length, SDW: Shoot dry weight, RDW: Root dry weight, T_0: Hydropriming, T_5: 5 mg L^{-1} TiO_2 nano-priming, T_{10}: 10 mg L^{-1} TiO_2 nano-priming, T_{50}: 50 mg L^{-1} TiO_2 nano-priming, T_{100}: 100 mg L^{-1} TiO_2 nano-priming.

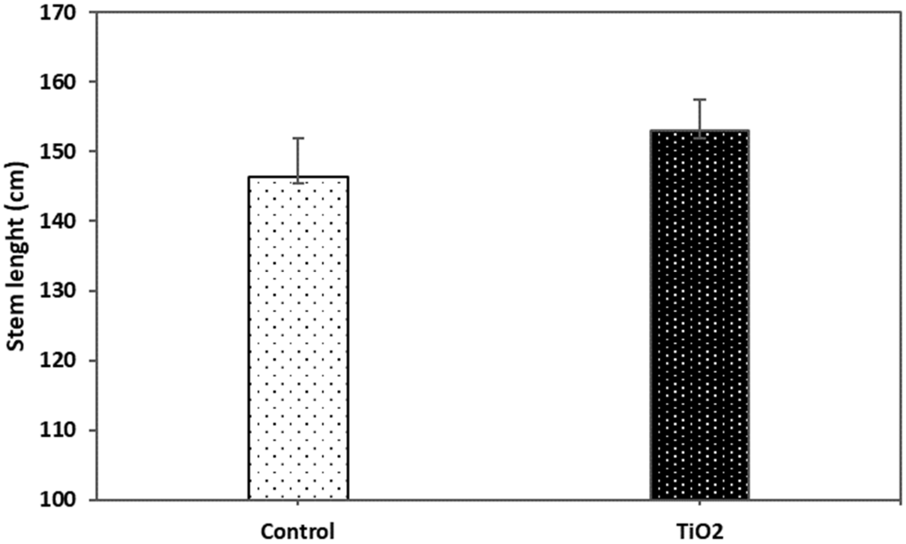

Figure 4. The effect of TiO_2 nano-priming on stem length of tomato plants. Data are shown as the mean ± SD of five independent biological replicates. The significant differences among the treatments were estimated by 2- pair t test with the least significant difference at a probability threshold level of $p < 0.05$.

The stomatal conductance and transpiration rate showed the same trend under the same treatments (Figures 5a and 5b). The stomatal conductance increased after TiO_2 nano-priming treatment (Figure 5a). Likewise, the transpiration rate was positively affected by TiO_2 nano-priming treatment and the control group had a lower transpiration rate (Figure 5b).

TiO_2 nano-priming affected the chlorophyll content of tomato plants (Figures 5c-f). When the chlorophyll content of tomato plants was analyzed in detail, the chlorophyll a content increased with the TiO_2 treatment (Figure 5c). Chlorophyll b content also increased with the TiO_2 nano-priming treatment (Figure 5d). As a result, total chlorophyll content also increased when the chlorophyll a and chlorophyll b content were increased (Figure 5e). In contrast to chlorophyll content, relative chlorophyll content was not affected by TiO_2 nano-priming treatment (Figure 5f).

The Pn of plants developed from nanoprimed seeds were higher than the control group (Figure 6). On 56th day after planting, the photosynthesis performance of the plants was 18% higher than the control (16,20 vs. 19,05). Besides, when the plants started to die, the Pn increased 55% (from 6,96 to 10,85) following the TiO_2 treatment.

The yield of tomato plants was not changed by treatment of TiO_2 nano-priming (Figures 7a and 7b). When the yield was analyzed by fruit number / plant, there was no effect of TiO_2 nano-priming on the yield of tomato plants (Figure 7a). In the same trend, yield of tomato plants as weight was not affected by TiO_2 nano-priming treatment (Figure 7b).

Translocation of TiO_2 nanoparticles in the tomato plant: Translocation of TiO_2 nanoparticles to different parts of the tomato plants was investigated. For this purpose, root, stem, leaf and fruit samples were collected and TiO_2 accumulation in these parts were analyzed. As result, accumulation of TiO_2 nanoparticles was observed in the root (Figure 8).

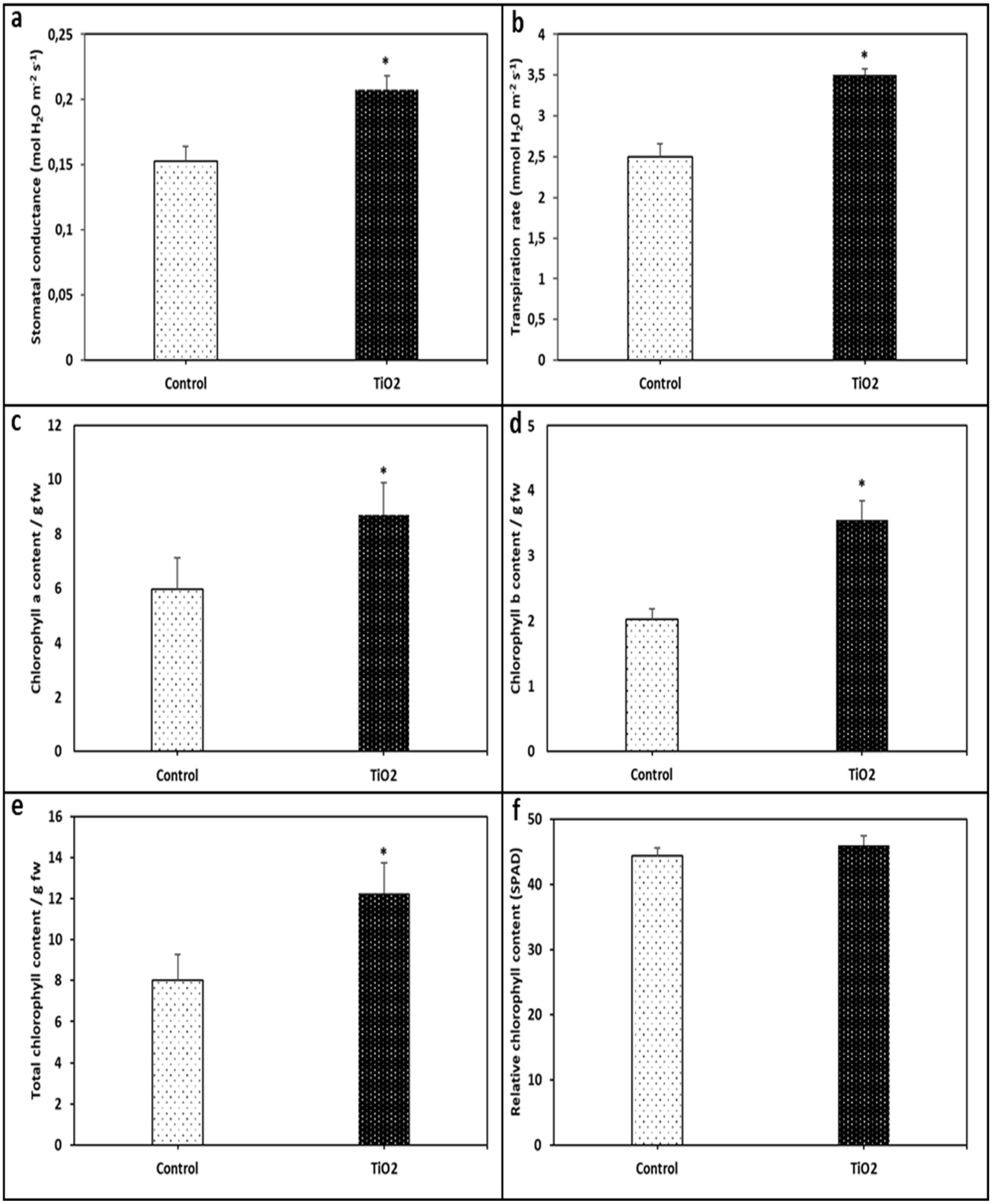

Figure 5. Effect of TiO_2 nano-priming on physiological traits of tomato plants. Stomatal conductance (a), transpiration rate (b), chlorophyll a content (c), chlorophyll b content (d), total chlorophyll content (e), relative chlorophyll content (f) measured as mentioned in the text. Data are shown as mean ± SD of five independent biological replicates. The significant differences among the treatments were estimated by 2-pair t test with the least significant difference at a probability threshold level of $p < 0.05$.

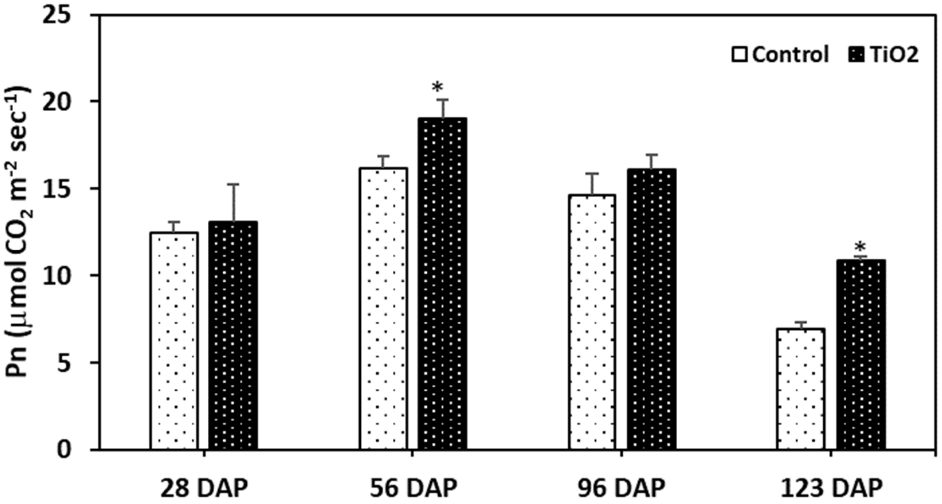

Figure 6. Effect of TiO_2 nano-priming on photosynthesis rate of tomato (DAP: Day after planting). Data are shown as mean ± SD of five independent biological replicates. The significant differences among the treatments were estimated by 2-pair t test with the least significant difference at a probability threshold level of $p < 0.05$.

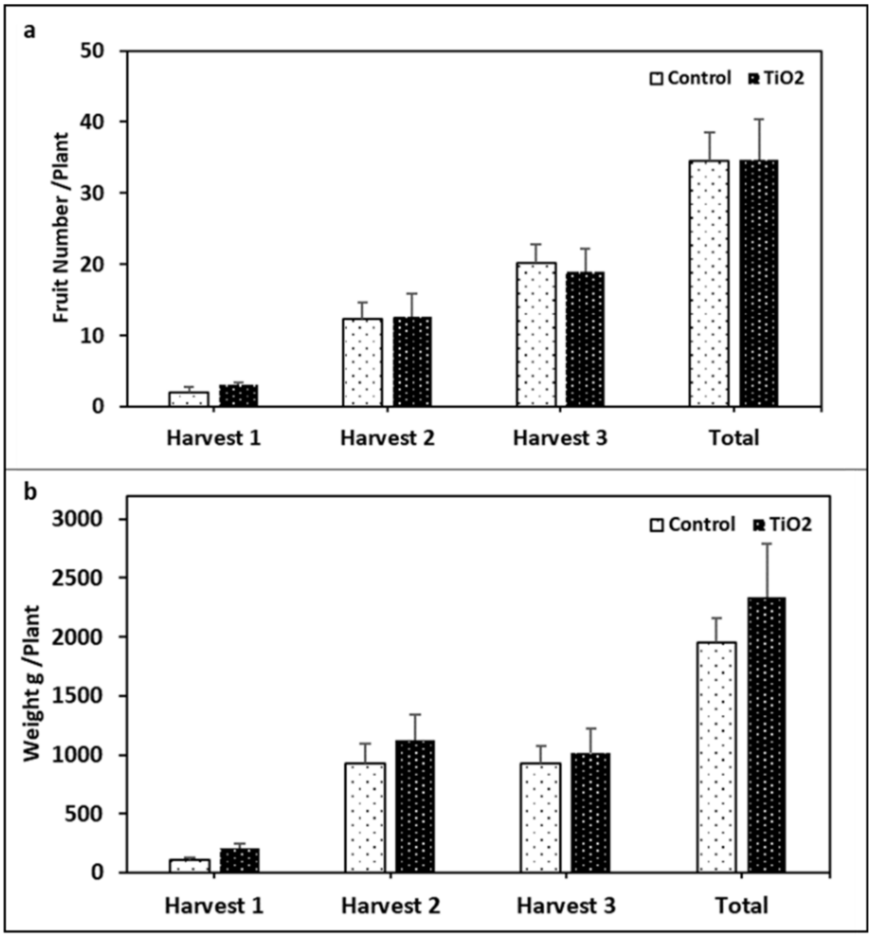

Figure 7. The effect of TiO_2 on yield of tomato. Fruit number/plant (a), Weight/plant (b) measured as mentioned in the text. Data are shown as mean ± SD of five independent biological replicates. The significant differences among the treatments were estimated by 2-pair t test with the least significant difference at a probability threshold level of $p < 0.05$.

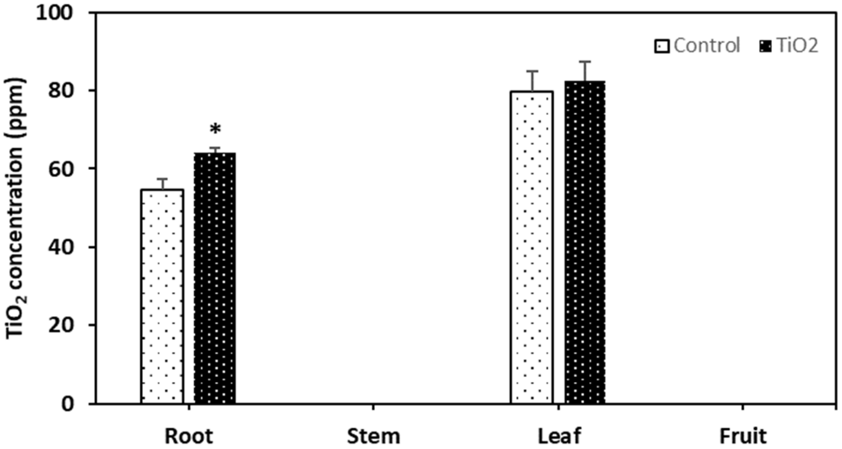

Figure 8. TiO_2 content of different parts of tomato plants. Data are shown as mean ± SD of five independent biological replicates. The significant differences among the treatments were estimated by 2-pair t test with the least significant difference at a probability threshold level of $p < 0.05$.

DISCUSSION

In this study, our focus has been on using TiO_2 NP as priming agent, in order to determine optimal concentration for increasing in tomato seed germination and plant development by minimizing the toxicity of NPs. TiO_2 nano-priming treatment did not change the germination rate of the tomato seeds because the seeds had already high germination rate. Yet, both weight- and length-dependent seed vigor index increased with the TiO_2 nano-priming treatment (Table 1). Nanoparticles have a higher binding ability to seed coat compared to other priming agents (Anand *et al*., 2019; Mahakham *et al*., 2017). Nano-priming treatment was shown to affect the hormonal balance in the seed (Yang *et al*., 2017). Seeds recognize the NP as an external agent and activate gibberellic acid (GA) and abscisic acid (ABA) dependent response mechanisms (Anand *et al*., 2019; Mahakham *et al*., 2017). When NPs enter the seed coat, ROS accumulate and seed dormancy is broken via the activation of GA synthesis (Dietz *et al*., 2016). An increase in ROS after nano-priming is reported in some studies (Chandrasekaran *et al*., 2020; Oracz and Karpiński, 2016). Thus, the accumulation of ROS probably promotes seed germination (Chandrasekaran *et al*., 2020).

On the other hand, nano-priming increases α-amylase activity and starts rapid starch degradation (Mahakham *et al*., 2017; Man *et al*., 2013). The activity of α-amylase depends on GA and this relationship increases with the integration of nanoparticles in the seed (Mahakham *et al*., 2017). The effect of nano-priming on phytohormones enhances sugar signaling factors, which promote seed germination (Laby *et al*., 2000). Even though the TiO_2 nano-priming treatment did not increase the seed germination rate, it speeded up the process by mechanisms that are mentioned above and increased the length and biomass of plantlets, thus resulting in an increased seed vigor index. Even though the root length and biomass of the tomato plants increased after nano-priming treatment, the shoot length of tomato plantlets was not affected (Table 2). The translocation of TiO_2 nanoparticle studies showed that TiO_2 nanoparticles were accumulated in the root (Figure 8). While the stem length was not affected by the TiO_2 nano-priming treatment, the probable reason for the increase in the root length is the TiO_2 accumulation. Translocation of TiO_2 nanoparticles was investigated in basil and similarly, when the TiO_2 accumulation was observed in roots, there was no accumulation in the shoots (Tan *et al*., 2018). Treatment of TiO_2 nanoparticles by sludge was shown not to affect the accumulation of TiO_2 in the stem, leaf, or fruit parts of the tomato plant (Bakshi *et al*., 2019). The size of the TiO_2 affects the accumulation of the NPs in different parts of the plant. If the NPs are bigger than 140 nm, they cannot accumulate in the root. The threshold value for accumulation in the stem is 36 nm for wheat (Larue *et al*., 2012). The size of the nanoparticles was approximately 70 nm in the present study (Figure 2), which may have prevented the translocation of NPs from the root through the stem. The absence of TiO_2 nanoparticles especially in the fruit of tomato plants is an advantage for using this material as a priming agent, considering that this technique counteracts the risks and concerns about TiO_2 contamination in the edible part of the tomato.

After *in vitro* conditions, the effect of TiO_2 nano-priming on tomato plants was observed under the greenhouse conditions. For this purpose, 10 mg L^{-1} of TiO_2 was selected for greenhouse experiments according to the seed vigor index results. The stem length of the tomato plants was not affected by TiO_2 nano-priming treatment, which was expected, similar to the shoot length of the tomato plantlets.

Some physiological traits of the tomato plants were increased by TiO_2 nano-priming treatment (Figure 5). The stomatal conductance and transpiration rate of the tomato plants presented a similar trend after TiO_2 nano-priming (Figures 5a and 5b). Stomatal conductance and transpiration rates increased due to the opening of stomata (Qi *et al.*, 2013). This may be caused by the increases in the root length and water uptake, which decreased the water need of the plant and increased the stomatal conductance and transpiration rate. However, the mechanisms behind the increases in stomatal conductance and transpiration rate after TiO_2 nano-priming treatment are not yet fully understood.

As the core reactive molecule in the photosystem I and the photosystem II, Chlorophyll a content was increased following the TiO_2 nano-priming (Figure 5c) (Blankenship, 2013). Similarly, chlorophyll b content increased with the TiO_2 treatment (Figure 5d), which increased the wavelength range and amount of light absorbed by plants during photosynthesis (Conway *et al.*, 2015). The increases in both chlorophyll a and b molecules lead inevitably to an increase in total chlorophyll content (Figure 5e). However, relative chlorophyll content was not affected by TiO_2 nano-priming treatment. Similar results were observed in a study where, nano and bulk-TiO_2 treated spinach plants had higher chlorophyll content 37.48% and 13.34%, respectively (Yang *et al.*, 2007). Furthermore, an increase in chlorophyll content week by week after continuous TiO_2 treatment in wheat was reported (Refique *et al.*, 2018). However, it was highlighted that TiO_2 treatment in tomatoes did not affect the chlorophyll a and b content (Bakshi *et al.*, 2019). The method of application is also a factor that needs to be taken into account for the chlorophyll content of tomatoes since the foliar application leads to a higher increase in the total chlorophyll content in comparison to priming (Perveen and Siddique, 2022).

The photosynthesis rate of the tomato plants increased after 56 DAP and 123 DAP with the TiO_2 nano-priming treatment (Figure 6). The photosynthesis rate is correlated with chlorophyll content. As mentioned above, the chlorophyll content of the tomato plant was increased by TiO_2 nano-priming treatment in the current study. By increasing the chlorophyll content a and b increased the photosynthesis ratio individually. Besides, TiO_2 promoted photochemical reactions and increased the photosynthesis activity in spinach (Hong *et al.*, 2005). TiO_2 treatment also caused conformational changes in the rubisco activase enzyme and increased photosynthesis rate (Gao *et al.*, 2006, 2008). TiO_2 nanoparticles have a role as photocatalysts and promote the redox reaction (Higashimoto, 2019). Consequently, the activation of Rubisco by TiO_2 treatment increases, thereby leading to an increase in the photosynthesis ratio (Yang *et al.*, 2008). In the current study, the photosynthesis rate increased when the plant was in the flowering stage and before dying, which were critical periods for the plant's development.

Although TiO_2 nano-priming increased plant development and growth, the yield of tomato plants was not affected by TiO_2 nano-priming treatment (Figures 7a and 7b). There are various studies that have shown increases in the yield of tomato plants after TiO_2 treatment. For instance, the yield of tomato plants was reported to increase after 100 mg L^{-1} by foliar spray application (Choi, 2021). In another study, the positive effect of TiO_2 treatment on the yield of tomato plant by foliar application was reported (Parveen and Siddique, 2022). The reason why there was no effect on yield in tomato plants in the study is thought to be due to the use of the priming method.

Conclusion: Use of traditional chemicals during plant production has hazardous effects on the environment and human health. Thus, different technologies have been developed to decrease this toxic effect and one of them is nanoparticles. However, using nanoparticles can cause phytotoxicity; therefore, the determination of the correct concentration and application technique is very important. With the *in vitro* experiments, it was observed that using a very small amount of TiO_2 (10 mg L^{-1}) as a priming agent induced plant development. Under greenhouse conditions, the TiO_2 nano-priming increased the chlorophyll content and photosynthesis rate of the tomato plants. During this process, the nanoparticles did not translocate in the plant, which was a fundamental issue regarding the use of nanoparticles in crop production. TiO_2 nano-priming application had a positive effect on tomato plant development and increased chlorophyll content and photosynthesis rate, which could be a possible alternative to traditional fertilizers. However, the impact of TiO_2 nanoparticles on plant development should be investigated in more detail.

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Author contributions: AKY and MEC designed the study. AKY performed the experiments and collected data. AKY and MEC analyzed data. AKY wrote the manuscript and MEC reviewed and edited the manuscript.

Conflict of Interest: The authors declare that they have no conflict of interest.

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