



## RESEARCH ARTICLE

# The Effect of Pre-Applied Titanium Dioxide Nanoparticles on Germination in *Carthamus tinctorius* L. Varieties

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## ABSTRACT

In the present study, to promote sustainable nano-farming, the apparent effects of different concentrations (0, 100, 200, 300, 400, 500 ppm) of titanium dioxide nanoparticle (TiO<sub>2</sub>NPs) solutions on the germination percentage, index and duration of seeds belonging to Balcı, Dincer, Hasankendi, Koc, Olas, and Zirkon safflower varieties were investigated. Moreover, scanning electron microscopy (SEM) was employed to analyze TiO<sub>2</sub>NPs in germinated safflower varieties. Germination performance was TiO<sub>2</sub>NPs concentration and variety depended. It was determined that the seed samples displayed different responses to TiO<sub>2</sub>NPs concentrations; germination percentages were between 20.0±1.15 and 82.9±0.44%, germination durations were between 2.01±0.021 to 3.82±0.017 days, and germination indices were between 9.97±0.606 and 38.97±0.959. While the highest germination percentage (82.9±0.44%) was obtained from Dincer variety with 100 ppm TiO<sub>2</sub>NP pre-application, the lowest germination percentage (20.0±1.15% and 20.0±1.92%) was obtained from Balcı and Hasan Kendi varieties with 100 and 300 ppm TiO<sub>2</sub>NP pre-application. According to this result, although the highest germination percentage based on variety was obtained from the Dincer variety, the Balcı variety with the lowest germination percentage provided the most significant increase in the 200 ppm TiO<sub>2</sub>NPs application dose compared to the control. According to the germination percentage, it can be said that the most effective TiO<sub>2</sub>NPs application dose in Safflower varieties is 200 ppm. Further research on nanoparticles is needed to determine both the economical doses of TiO<sub>2</sub>NP pre-application and its uptake by the plant.

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## 1. Introduction

Fats and lipids are sources of energy storage and important in human and animal nutrition (Gürsoy, 2019). Vegetable oils are the sources of the most widely used lipids in human nutrition as they are rich in polyunsaturated omega fatty acids and do not contain cholesterol (Roccisano et al., 2016; Zhou et al., 2020). Safflower (*Carthamus tinctorius* L.) is an important

oil seed plant for oil production due to its deep root system, which helps its tolerance to drought where dry farming is carried out (Omidi et al., 2012). Safflower seeds contain 30-50% oil and provide quality edible oil owing to its high (63-75%) linoleic acid (omega-6) content (Toma et al., 2014; Conte et al., 2016). The growth and yield of crop plants depend on rapid seed germination and emergence and good seedling formation. Seed germination is the first growth stage of a plant

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to limit its development under stress (Jabeen & Ahmad, 2013). Therefore, it is important to investigate effective seed treatment methods such as TiO<sub>2</sub>NP to improve the germination percentage and seedling growth is of great importance.

Titanium dioxide nanoparticles (TiO<sub>2</sub>NPs) are widely used catalysts in areas such as sewage treatment, food preservation, and environmental improvement, thanks to their low toxicity and high chemical stability (Arezoo et al., 2020; Li et al., 2021; Wu et al., 2018). Titanium dioxide nanoparticles are the most used nanomaterials in agricultural production thanks to their wide range of benefits in plants, such as promoting nutrient absorption in plants, increasing stress resistance, stimulating antioxidant enzyme activity, and increasing product yield and quality (Torres et al., 2020; Kamal & Mogazy, 2021; Shoja et al., 2022). Because nanoparticles (NPs) are very small, they affect very wide areas in chemical and biological applications (Khalil et al., 2017; Rizvi & Saleh, 2018). Nanotechnology is the production of structures with completely new physical, chemical and biological properties, developed by working at the level of atoms with sizes of 1-100 nm. Since nanomaterials can be produced from many different materials, such as metals (Ni, Pt, Au), semiconductors (Si, InP, GaN) and insulators (SiO<sub>2</sub>, TiO<sub>2</sub>), they can be used in various industries, including the mechanical, medical, pharmaceutical, manufacturing and environmental sectors (Ateş et al., 2015; Castillo et al., 2020). In recent years, many studies have indicated that they are involved in the main metabolic events that control plant growth and development (Arora et al., 2012; Regier et al., 2015). Seed applications are an important field for the potential usage of NPs (Mahakham et al., 2016), where NPs can affect seed germination, seedling establishment, root and shoot growth, and photosynthesis (Paparella et al., 2015; Guha et al., 2018). They stated that titanium dioxide nanoparticle (TiO<sub>2</sub>NP) applied to spinach leaves improved the growth of spinach (*Spinacia oleracea* L.) by promoting the reduction of nitrogenous fertilizer in the form of NH<sub>3</sub> to ammonium (NH<sub>4</sub>) and nitrate (NO<sub>3</sub>) for nitrogen (N) adsorption (Yang et al., 2008). They stated that TiO<sub>2</sub>NPs significantly reduce salt stress and H<sub>2</sub>O<sub>2</sub> concentration, increase antioxidant enzyme activity, and reduce disease index and biological stress in plants (Gohari et al., 2020; Satti et al., 2021). It was also reported that titanium application from seed reduced cadmium (Cd) stress in safflower plants (Sardar et al., 2022). Pre-treatment methods (such as a nanoparticle, gibberellic acid and salicylic acid) have been developed for seeds to minimize environmental stresses by increasing the germination quality of seeds (Liu & Lal, 2015). The pre-application method applied to seeds is determined by factors such as the osmotic state of plants, water potential, temperature, light conditions, oxygen availability, soil moisture, and seed quality (Hussain et al., 2016). Nanoparticle applications in the seed are promising techniques in the agricultural sector under normal conditions (Rastogi et al.,

2019), and it was tested under environmental stress factors (Ahmad & Akhtar, 2019).

To increase the yield in safflower production, one of the most important considerations is that the seeds show good germination and emergence. Therefore, it is necessary to develop different methods against unfavorable environmental factors affecting the germination and exit process. It was added also above. In the study, it was investigated how titanium dioxide (TiO<sub>2</sub>) nanoparticle pretreatment in different doses will have an effect between varieties to break the dormancy and increase productivity that occurs depending on genetic characteristics and promote germination in the germination performance of safflower seeds.

## 2. Materials and Methods

### 2.1. Material Procurement

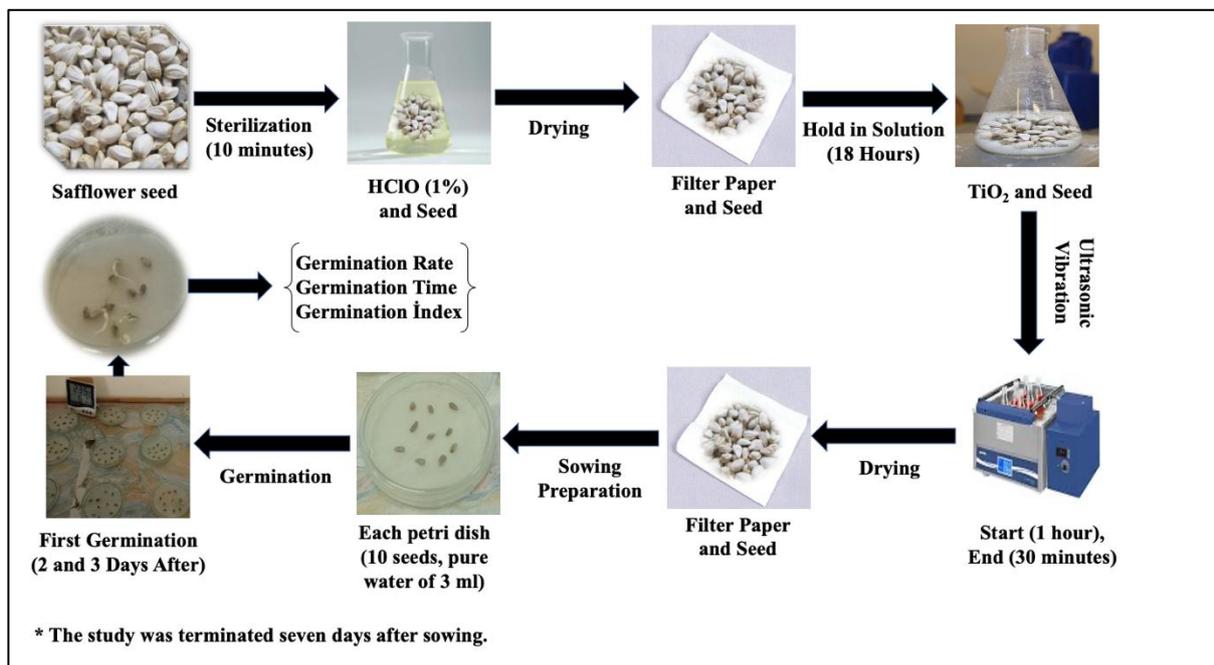
Safflower seeds of six varieties; Balcı (Average yield: 1500-2500 kg ha<sup>-1</sup>; 1000 grain weight: 40-48 g; oil content is 38-40%), Dincer (Average yield: 1000-2500 kg ha<sup>-1</sup>; 1000 grain weight: 45-49 g; Oil content: 25-28%), Hasankendi (Average yield: 1600-2800 kg ha<sup>-1</sup>; 1000 grain weight: 36-48 g; oil rate: 36-38%), Koc (Average yield: 2000-4000 kg ha<sup>-1</sup>; 1000 grain weight: 40-50 g; oil content: 37-39%), Olas (Average yield: 1500-2800 kg ha<sup>-1</sup>; 1000 grain weight: 45-50 g; oil content: 39-40%), and Zirkon (Average yield: 2500-3500 kg ha<sup>-1</sup>; 1000 grain weight: 34-53 g; oil content: 28-35%) were obtained from institutes affiliated with the Turkish Republic Ministry of Agriculture and Forestry. Titanium dioxide nanoparticles (20 nm in size, 99.9% purity) were purchased from a company (Nanografi Nanotechnology, Ankara/Türkiye).

### 2.2. Experimental Setup

The seeds were kept in a 1% sodium hypochlorite (HClO) solution for 10 min. Then washed five times with distilled water for surface sterilization. After washing, the seeds were placed on filter papers and dried at room temperature. Titanium oxide concentrations at different doses applied to safflower seeds were determined by a preliminary experiment (0-500 ppm no germination) and the most appropriate doses were selected according to this range. Sterilized seeds were incubated in 0, 100, 200, 300, 400, and 500 ppm NP solutions for 18 h. Control treatment was incubated in distilled water without TiO<sub>2</sub> and NP solutions were prepared using distilled water. To prevent heat increases due to ultrasonic treatment, the seeds were subjected to “ultrasonic vibration (Frequency: 40 kHz and power: 180 W)” twice, 1 h at the beginning of the incubation and 30 min before at the end of the incubation. After 18 h, the seeds were washed three times with distilled water and left to dry on filter papers. Filter papers were placed in the Petri dishes and 3 ml of distilled water was added. The experiment was set up with three replications and each replication contained 50 seeds in petri dishes. The seeds were placed in a dark environment with a

temperature of  $25\pm 2$  °C and a relative humidity of  $50\pm 5\%$  for germination. Initial germination data were obtained two days after planting (germination durations differed among varieties).

The experiment was terminated seven days after sowing when germination data were obtained (Figure 1).



**Figure 1.** Scheme of preparation of the experiment.

### 2.3. Germination Percentage (%)

The germinated seeds were counted at the same time every day, and the seeds were considered as germinated when the radicle reached 2 mm and germination percentage was calculated according to Sanoubar et al. (2018).

$$\text{Germination percentage (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100 \quad (1)$$

### 2.4. Mean Germination Time (day)

Mean germination time was determined following the formula of Ellis and Roberts (1981).

$$\text{Mean germination duration} = \frac{n_1 \times d_1 + n_2 \times d_2 + n_3 \times d_3 + \dots + n_n \times d_n}{\text{Total number of days}} \quad (2)$$

Where, n = the number of germinated seeds, d = day.

### 2.5. Germination Index

The germination index was calculated according to Czabator (1962).

$$\text{Germination index} = \frac{n_1}{d_1} + \frac{n_2}{d_2} + \frac{n_3}{d_3} + \dots + \frac{n_n}{d_n} \quad (3)$$

Where, n = number of germinated seeds, d = day.

### 2.6. Scanning Electron Microscopy (SEM) And Energy-Dispersive X-Ray Spectroscopy (EDX) Analysis

For SEM imaging, the Examples of germinating safflower were left to dry in the air, fixed on carbon tapes, coated with Au-Pd on the Cressington Sputter Coater for 60 s and then

placed on the FEI Quanta FEG 250 SEM (operating at 10 kV and 20 kV) immediately. The elemental analyses of the samples were done by area and point analysis with an EDX detector (Javed & Mashwani, 2020; Javed et al., 2020).

### 2.7. Statistical Analysis

The two-year data obtained from the research were subjected to analysis of variance with the help of JMP 5.0.1 (SAS institute 2002) package according to the Randomized Complete Blocks Design. Significant differences among treatments were compared and grouped according to the Duncan Multiple Range Test at the  $p < 0.05$  probability level.

## 3. Results and Discussion

Significant differences were detected at the statistically  $p < 0.01$  level in terms of germination percentage, germination duration and germination index variety x  $\text{TiO}_2\text{NPs}$  pre-application. While significant differences were obtained in terms of  $\text{TiO}_2\text{NPs}$  pre-application at the germination index statistically level of  $p < 0.05$ , significant differences were obtained at the germination percentage and germination duration statistically level of  $p < 0.01$  (Table 1).

**Table 1.** Variance analysis material of the development parameters of safflower varieties germinated in different titanium dioxide nanoparticles pre-applications.

Source Variation	d.f	Germination Percentage	Germination Duration	Germination Index
Variety	5	30.529**	54.316**	11.062**
Pre-Application	5	2.325*	2.361*	6.040**
Variety x Pre-Application	25	116.296**	13.626**	14.506**

\*\*, \* Statistically significant at  $P < 0.01$  and  $P < 0.05$ , d.f: degrees of freedom, the difference between the averages denoted by different letters is significant.

### 3.1. Germination Percentage

The mean germination percentages of safflower varieties pre-treated with different concentrations of  $TiO_2$ NPs varied between  $20.0 \pm 1.15$  and  $82.9 \pm 0.44$  %. While the highest germination percentage ( $82.9 \pm 0.44\%$ ) was obtained from Dincer variety with 100 ppm  $TiO_2$ NP pre-application, the lowest germination percentage ( $20.0 \pm 1.15\%$  and  $20.0 \pm 1.92\%$ ) was obtained from Balcı and Hasan Kendi varieties with 100 and 300 ppm  $TiO_2$ NP pre-application.  $TiO_2$ NP pre-application doses had a positive effect on the varieties at different rates compared to the control (Table 2). The mean germination percentages of safflower varieties pre-treated with different concentrations of  $TiO_2$ NPs varied between  $20.0 \pm 1.15$  and  $82.9 \pm 0.44\%$ . This significant difference between varieties may be due to genetic, climate, and environmental factors. It can also be said that the storage life of safflower seeds varies depending on their storage life and conditions (Kumari, 2009). The highest germination percentage on a variety basis was obtained in the Dincer variety at a pre-application dose of 100 ppm  $TiO_2$ NP. It was observed that  $TiO_2$ NP pre-application

doses had a positive effect on other varieties compared to the control. The administration dose of  $TiO_2$ NPs may have increased the germination rate by fully penetrating the seed coat and endosperm and acting on the embryo (Song et al., 2013). Dogaroglu and Köleli (2016), stated that the increase in  $TiO_2$  and  $TiO_2$ Ag nanoparticle dose increases the germination percentage in lettuce seeds, so the same kind of nanoparticles may have different effects on other plants. (Ravindran et al., 2012), due to AgNP applications to tomato and corn plants, stated that tomato seeds are more sensitive than corn seeds and cause inhibition of 20-40% in germination percentages depending on the increase in concentration. The seed coat protects the embryo against adverse environmental conditions and may not always allow the inhibition of nanoparticles into the seed with its semi-permeable structure (Boonyanitipong et al., 2011; Song et al., 2013).  $TiO_2$ NPs, which do not ionize in water, can see the seed coat and endosperm as effective barriers, especially when they become clumped. The germination percentage results we found show similarities with the results of some researchers.

**Table 2.** The average germination percentage, germination duration and germination index of safflower varieties germinated in different titanium dioxide nanoparticles pre-applications.

Varieties	Germination Percentage (%)					
	Control	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm
Balcı	24.0±1.15qr	20.0±1.15r	47.0±0.58gi	43.0±2.89ij	46.3±0.33gi	37.0±0.58km
Dinçer	78.4±0.96b	82.9±0.44a	63.6±1.94d	70.7±0.67c	81.6±0.96ab	48.6±0.98gh
Hasankendi	47.6±1.24gh	34.8±1.13mo	46.4±0.22gi	20.0±1.92r	28.2±0.97pq	33.1±0.59mo
Koç	50.4±0.44fg	40.01±0.00jl	31.4±0.99op	54.8±0.98e	48.2±0.98gh	36.8±1.93kn
Olas	48.0±1.15gh	36.01±1.55ln	40.3±3.48jk	46.0±2.31hi	54.0±0.00ef	32.7±0.67no
Zirkon	61.4±0.98d	46.7±1.26gi	65.0±2.89d	40.0±3.85jl	73.3±0.87c	50.0±1.92fh
Varieties	Germination Duration (day)					
	Control	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm
Balcı	3.04±0.151de	3.19±0.097bd	3.36±0.125bd	3.38±0.095bc	3.51±0.017ab	3.82±0.017a
Dinçer	2.39±0.205gi	2.31±0.124gj	2.41±0.011gi	2.33±0.049gj	2.62±0.130fg	3.04±0.119ce
Hasankendi	2.01±0.021j	2.11±0.032hj	2.23±0.071hj	2.30±0.087gj	2.34±0.023gj	2.35±0.115gj
Koç	2.16±0.023hj	2.24±0.017hj	2.20±0.115hj	2.31±0.055gj	2.36±0.031gi	2.38±0.035gi
Olas	2.09±0.060ij	2.16±0.081hj	2.24±0.062hj	2.36±0.086gi	2.34±0.172gj	2.40±0.226gi
Zirkon	2.32±0.072gj	2.40±0.160gi	2.39±0.144gi	2.45±0.072gh	2.45±0.358gh	2.83±0.192ef
Varieties	Germination Index					
	Control	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm
Balcı	12.94±0.727qr	9.97±0.606r	24.10±0.02in	22.16±1.385ko	24.87±0.069im	18.09±0.382oq
Dinçer	37.48±0.149ab	35.73±1.299ac	27.09±0.823fk	30.59±0.247ch	32.60±1.566be	15.57±0.658pq
Hasankendi	28.42±1.727ei	28.34±1.213ei	22.19±0.206ko	31.41±0.535cf	27.19±7.459fk	18.99±2.994np
Koç	23.94±0.050in	18.96±0.147np	14.71±0.618pr	26.23±0.461gl	21.76±1.404lo	14.33±0.919pr
Olas	33.63±1.307bd	27.37±0.637fj	22.34±5.502jo	31.77±0.564cf	38.97±0.959a	25.50±0.316hm
Zirkon	26.79±0.267fl	18.65±0.352op	28.72±1.668di	22.91±1.411jo	30.74±0.399cg	20.84±0.584mo

The difference between the averages denoted by different letters is significant.

### 3.2. Germination Duration

According to the results of the variance analysis, the effect of variety and TiO<sub>2</sub>NP applications on germination duration was significant, while the effect of variety x TiO<sub>2</sub>NP interaction was insignificant (Table 1). The shortest mean germination duration among TiO<sub>2</sub>NP doses was obtained from the control group (2.01±0.021 days) and the shortest germination duration was obtained from the 500 ppm TiO<sub>2</sub>NP dose (3.82±0.017 days). Although the effect of different TiO<sub>2</sub>NP doses on the germination duration was significant, the germination duration values recorded at the application doses were close (Table 2). The highest germination duration (3.82±0.017 days) was obtained from the 500 ppm TiO<sub>2</sub>NP pre-application group in the Balçı variety. In comparison, the shortest germination duration (2.01±0.021 days) was obtained from the control group in the Hasankendi variety. In some cases, adverse effects were observed on some growth parameters, which may indicate a toxic effect of TiO<sub>2</sub>NPs doses. The increase in different TiO<sub>2</sub>NPs doses have restricted the germination duration compared to the control (Table 2). In this case, the seed pods and endosperm may have acted as filters that absorb metals but pass through the water. Indeed, Song et al. (2013) determined that 2.2 ± 0.6 mg kg<sup>-1</sup> TiO<sub>2</sub> passed through the TiO<sub>2</sub>-treated corn seeds (*Zea mays* L.) and that the nanoparticles did not penetrate the seed coats. (Younes et al., 2020), stated that the mean germination time of seeds belonging to the *Solanaceae* family was reduced when coated with 100 mgL<sup>-1</sup> TiO<sub>2</sub>NPs. According to the results we obtained, like some researchers, the increase in the pre-application dose of TiO<sub>2</sub>NP<sub>s</sub> extended the germination duration.

### 3.3. Germination Index

In the present study, 300 and 400 ppm concentrations of TiO<sub>2</sub>NPs applied to safflower seeds showed a positive effect by increasing the germination index compared to normal conditions by 8.6%, respectively. However, 100, 200, and 500 ppm concentrations negatively affected the germination index by 14.76, 14.72, and 30.03%, respectively. The germination indexes of safflower seeds varied, and the highest mean index was obtained in Dincer (37.48±0.149) and Olas (38.97±0.959) varieties, whereas the lowest mean germination index was obtained in Balçı (9.97±0.606) varieties. Although the germination indexes of the Dincer and Olas varieties were the highest, as the pre-treatment concentrations of TiO<sub>2</sub>NPs increased, the germination indexes decreased to a certain extent. Furthermore, although there were increases in 300 and 400 ppm concentrations, these were not extremely high compared to the standard conditions. In the Balçı variety, which had the lowest germination index, a 23.1% decrease occurred in the 100 ppm treatment compared to the control (12.94±0.727) group. In contrast, increases of 84.6, 69.2, 93.3 %, and 38.5% occurred in the pre-treatments of 200, 300, 400, and 500 ppm TiO<sub>2</sub>NP, respectively. Differences were

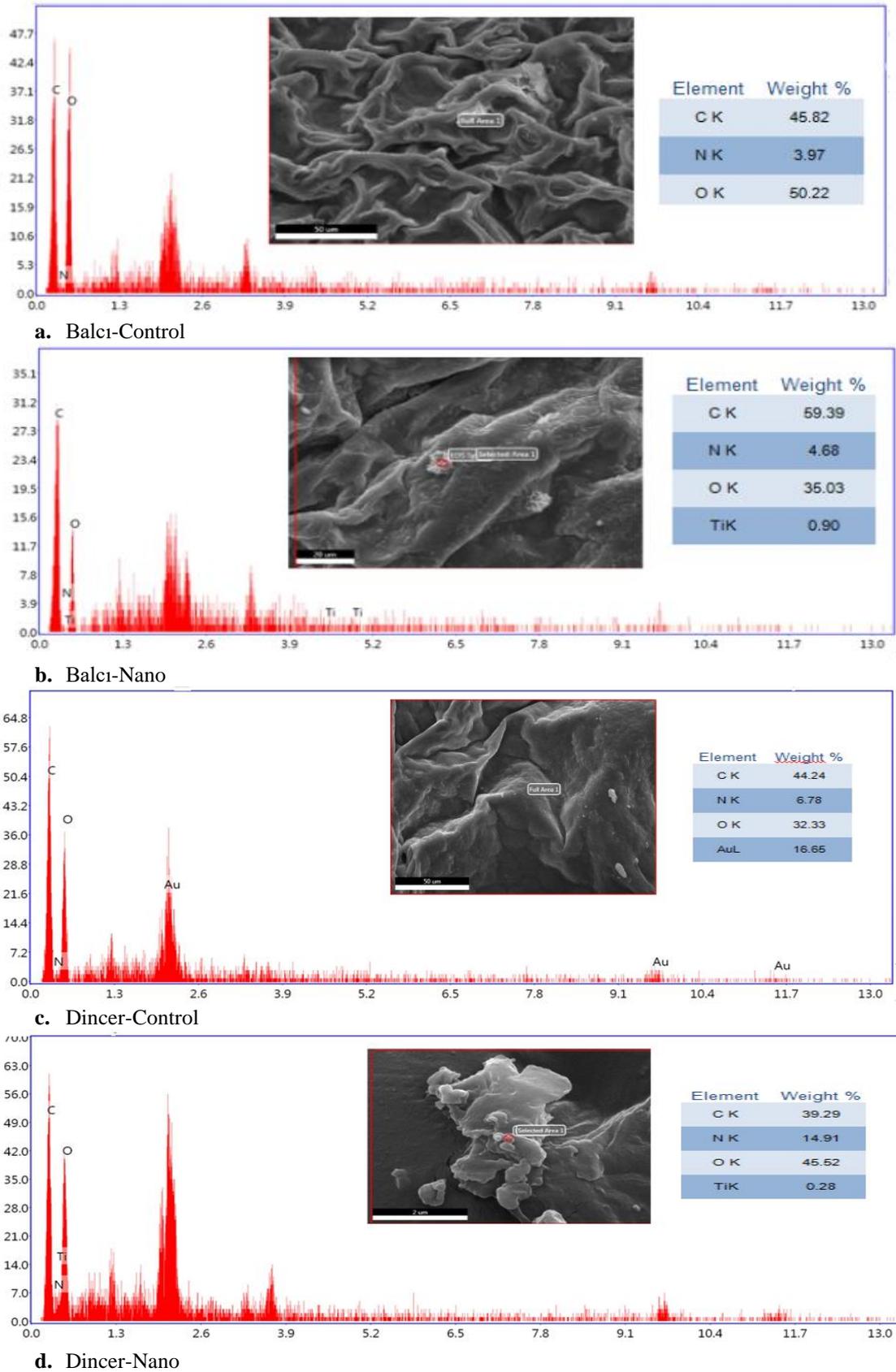
determined between varieties regarding their germination indices depending on the titanium dioxide nanoparticle concentration increase. Different responses to the pre-treatment concentrations of TiO<sub>2</sub>NPs caused variety x pre-treatment interaction to be significant in terms of the germination index. Although the germination index of Dincer and Olas cultivars was the highest, germination indexes decreased at certain rates as TiO<sub>2</sub>NPs pre-application doses increased. Although there are very few studies on the effect of TiO<sub>2</sub>NPs on seed germination, the results of different studies show that each plant is affected differently by TiO<sub>2</sub>NPs. Differences were determined in terms of the germination index of varieties depending on the increase in TiO<sub>2</sub>NPs dose. (Mehrian et al., 2016), reported that the germination index of some tomato seeds increased with AgNP application, while there was no change in some. (Hatami et al., 2014), reported that applying different doses of TiO<sub>2</sub>NPs to five different medicinal species seeds stimulated seed germination, but this response was dependent on the application of TiO<sub>2</sub>NP<sub>s</sub> and plant species (Faraji et al., 2018).

### 3.4. Scanning Electron Microscopy (SEM-ED) Analysis

Surface morphology and topography of the TiO<sub>2</sub>NPs were observed by using SEM. When the SEM images in Figure 2 were examined, it was observed that the particles appeared in cubic shapes and were located in the radicle and plumule of the seeds that were treated with TiO<sub>2</sub>NPs. While nanoparticles were spread on the surface in both the radicle and plumula in the Balçı variety, it was determined that there was an agglomeration in the Dincer variety.

As seen in Figure 2, it shows that the morphology of TiO<sub>2</sub>NP residues in the germinating safflower samples is close to spherical in shape and is in an aggregated state. Elemental detection of TiO<sub>2</sub>NPs was observed with an energy-dispersive X-ray. Energy dispersive X-ray detector confirms the presence and purity of TiO<sub>2</sub>NPs. While TiO<sub>2</sub>NPs were detected at a rate of 0.90% in the Balçı variety and 0.28% in the Dincer variety in the applied groups, TiO<sub>2</sub>NPs could not be seen on the surface of control plants. TiO<sub>2</sub>NPs are less detected in Dincer than Balçı variety, which can be interpreted as the better development of this variety.

EDS spectra recorded from the TiO<sub>2</sub> nanoparticles are shown in Figure 2, respectively. SEM image showed that the TiO<sub>2</sub>NP<sub>s</sub> were spherical in shape or cubic (Figure 2). The qualitative and quantitative elemental profile involved in materialized synthesis was ascertained using EDS microanalysis of numerous distinct particles. EDS profile shows a strong Ti signal along with oxygen and carbon peak, which may originate from the biomolecules bound to the TiO<sub>2</sub> nanoparticles' surface. Observation of some peaks in the vicinity of the Ti and O other than C and O were Au peaks used in the coating of plant material.



**Figure 2.** SEM and EDX-based analysis of safflower varieties of Balcı and Dincer (a. Balcı-Control, b. Balcı-TiO<sub>2</sub>NPs, c. Dincer-Control, d. Dincer-TiO<sub>2</sub>NPs). The difference between the averages denoted by different letters is significant.

#### 4. Conclusion

Based on the results obtained, the germination performance of safflower seeds has shown changes depending on the doses of TiO<sub>2</sub>NPs and the variety. According to TiO<sub>2</sub>NPs concentrations and the variety of safflower seed samples, germination percentages varied between 20.0±1.15-82.9±0.44%, germination duration varied between 2.01±0.021-3.82±0.017 days and germination indexes varied between 9.97±0.606-38.97±0.959. It can be said that the significant differences between safflower varieties in terms of germination rate vary depending on the genetic, climate, and environmental factors of the varieties, as well as the storage life and conditions of the seeds. The highest germination percentage (82.9±0.44%) was obtained from Dincer variety with 100 ppm TiO<sub>2</sub>NP pre-application, while the lowest germination percentage (20.0±1.15% and 20.0±1.92%) was obtained from Balcı and Hasankendi varieties with 100 and 300 ppm TiO<sub>2</sub>NP pre-application. Different TiO<sub>2</sub>NP pre-application doses had a positive effect on the germination percentage of safflower varieties compared to the control. According to this result, although the highest germination percentage based on variety was obtained from the Dincer variety, the Balcı variety with the lowest germination percentage provided the most significant increase in the 200 ppm TiO<sub>2</sub>NPs application dose compared to the control. According to the germination percentage, it can be said that the most effective TiO<sub>2</sub>NPs application dose in Safflower varieties is 200 ppm. Further research on nanoparticles is needed to determine both the economical doses of TiO<sub>2</sub>NP pre-application and its uptake by the plant.

#### Conflict of Interest

The authors declare that they have no conflict of interest.

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