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Research Article

The Physiological Role of Titanium Dioxide Nanoparticles in Alleviating the Effect of Salinity Stress on Faba Bean Plants

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Abstract:

This research studied the effect of titanium dioxide nanoparticles (TiO_2NPs) on faba bean plants grown under salinity stress. Plants exposed to two different salinity levels (40 and 80 mM NaCl) were treated with two different concentrations of TiO_2NPs (5, 10 ppm) during the growth period. Data showed that the high salinity levels decreased the plant height, fresh and dry weights of the shoots by (12.4 %, 37.2 %, and 34.8 %) respectively, as well as decreased Chl a, Chl b, Total Chl, and carotenoids content by 43.7 %, 45.7 %, 44.2 %, and 12.1 % respectively compared with the control. The application of TiO_2 NPs at (10 ppm) significantly improved faba plant height, fresh and dry weights of the shoots by 10.9 %, 67.3 %, and 33.4 % respectively, as well as significantly enhanced Chl a, Chl b, Total Chl, and Carotenoids by 58.8 %, 19.7 %, 47.6 %, and 37.7 % respectively compared with the untreated plants. It could be concluded that spraying plants with TiO_2NPs at the two used concentrations mitigated the harmful effects of salinity on faba bean plants.

1. Introduction

Many agricultural fields worldwide have suffered from salt stress, one of the most damaging biotic stress on plants. By raising the concentration of sodium and chlorine ions in plant cells (Etesami et al., 2021). The major causes of the negative consequences caused by salinity include ion toxicity, formation of reactive oxygen species, hormonal disruption, and decreased water absorption (Elsheery et al., 2020). Salinity affects over 1500 million hectares, or one-fifth of all farmed land globally (Petretto et al., 2019). Osmotic potential and soil composition affect the amount of water plants can access. As the osmotic potential, the ability of the plant to change its osmotic pressure, and the specific toxicity of salt all contribute to determining the severity of the salinity effect (Sheldon et al., 2017). The salt level, the solute component in the water, and the crop of choice determine whether low-quality water is suitable for irrigation or not. Salt stress is a significant potential barrier for faba beans (Bimurzayev et al., 2021). One of the main obstacles to faba bean output in many nations, including Egypt, is high salinity (Desouky et al., 2023). The phases of seedling development and seed germination are those most susceptible to salt stress. When seeds germinate, salt stress leads to unfavorable physiological and biochemical changes; through osmotic stress, ion-specific effects, and oxidative stress, which can impact seed germination and stand establishment(Ibrahim, 2016).

Faba beans (*Vicia faba L.)* are the third most significant legume, after soybeans (*Glycine max L.*) and peas (*Pisum Sativum* L.). Like other legumes, it is abundant in carbs, vital minerals, and protein rich in lysine, one of the necessary amino acids. It is sometimes referred to as the least expensive source of protein (Rahate et al., 2021). The use of faba bean seeds in the development of novel products has attracted greater attention from the food industry, academics, food scientists, and consumers due to their advantageous nutritional qualities, affordability, ease of accessibility, environmental advantages, and positive health effects (Badjona et al., 2023). Whole faba beans have the following nutritional values: 20–35% protein, 1-2% fat, 55–65% carbohydrates, 10-15% fiber, and several vitamins and minerals such as calcium, magnesium, zinc, iron, and potassium (Samaei et al., 2020). The major agricultural producers of faba beans are the United Kingdom, Germany, Spain, China, Egypt, Ethiopia, and Australia (Dhull et al., 2022).

One of the newest technologies in agriculture is nanotechnology, which is applied to enhance plant growth and performance in salt stress (Elsheery, Sunoj, et al., 2020a). To enhance plant growth and productivity while exposed to salt treatments, many tactics have been used recently including tissues Culture (Chaleff, 1983). as well as genetic modification techniques (Peleg & Blumwald, 2014). Nowadays, nanotechnology has the ability to tackle most problems that face modern society. It is highly recommended to utilize Nano fertilizers as foliar sprays since they have a great deal of potential to increase crop yield. Nano fertilizers are game-changers in agriculture, helping to maximize crop productivity while reducing environmental pollution issues (Abdel-Aziz et al., 2019). NPs are incredibly small particles, with a minimum size of one nanometer in one dimension and a maximum size of 100 nanometers. Differentiating them from their bulk counterparts through their unique physical, chemical, and electrical properties opens up new research avenues and applications, especially in agriculture (Khan and Upadhyaya, 2019). Which used to improve plant performance and development under salt stress (Elsheery et al., 2020b).

TiO_2NPs improve growth and are used as an ameliorative measure to address soil salinity. The results were linked to the ability of TiO_2 NPs to increase photosynthesis and antioxidant defense, both of which help maximize plant growth potential (Abdel Latef et al., 2018). Among the most widely used natural elements, titanium dioxide (TiO_2) has a beneficial effect on plants and is widespread. It has a high ability to oxidize. It may accelerate energy transfer and stimulate electrons on material surfaces (Abdel Latef et al., 2018). It claimed that plants might better withstand a variety of environmental challenges when exposed to TiO_2NPs, since it modulates a number of physiological and biochemical processes (Khan et al., 2020).In relation to the above, the purpose of this work is to cheek the effect of TiO_2NPs nanoparticles on the growth of *V. faba* under salinity stress and their impact on growth and photosynthetic pigments.

2. Materials and Methods

2.1. Experiment location

Two pot experiments were conducted under greenhouse conditions at the experimental station of Agric. Botany Dept. Faculty of Agriculture, Tanta University, Egypt ((30° 47' 00.2") N latitude, (30° 59' 53.9") E longitude and the altitude is 11 m above sea level) during the growing season of 2021.

2.2. Plant material

Faba bean seeds (*Vicia faba L.)* cultivar of Giza 716 obtained from Sakha Agricultural Experiments and Research Station, Agricultural Research Center (ARC), Kafr Al-Shekh, Egypt. Seeds were soaked in wet tissue for 48 h. They were sown in plastic pots (diameter 25 cm and height 35 cm) 7 seeds /pot rate. Sowing was taken place on the 15th of October in the growing season. Pots were filled with a mixture of Patmos/vermiculite (1:1).

2.3. Experimental conditions and treatments

The experiment was divided into nine groups and three replicates for each group. One was left as a negative control, while other groups were divided into two concentrations of salinity [(40 mM and 80 mM) of NaCl] and two concentrations of TiO_2NPs [5 ppm and 10ppm of TiO_2NPs]. All treatments began after ten days of planting. Thus, salt and nanoparticles proceeded simultaneously with irrigation every four days, starting from the ten days of culture. Plant samples were collected 15 and 30 days after processing for new measurements or stored at −80 ◦C for further determination.

2.4. Nano titanium Dioxide (TiO_2NPs)

TiO_2NPs imported from nanostructured and amorphous material, sourced from Rhawn Company, China ,with an average size of 5–10 nm, were used in the current study.

2.5.Growth Parameters

At each sampling date plant height (cm), fresh and dry weights (g) were recorded. Shoot, height was measured from the soil surface to the top.

The fresh weight of the root and the shoot were measured using the sensitive scale. The samples dried at 105 °C for 15 minutes in a hot air oven, then maintained at 70 °C for two days (48 hours) until reaching a constant weight, and weighted to determine the dry weight (g/plant).

2.6. Photosynthetic pigments

Chlorophyll a, b and carotenoid concentrations were analyzed from 0.5 grams of the 3rd leaf fresh weight. The plant leaf extract was incubated in dark conditions overnight and then the dyes were measured accordingly (Lichtenthaler and Wellburn, 1983). Acetone (80%) was used for extraction. Homogenized mixture is separated by centrifugation at 3000 rpm, for 10 minutes. The analytical determination was performed with a spectrophotometer (UV1901PC) at the following wavelengths: 646, 663, and 470 nm, for chlorophyll a, b and carotenoids respectively. They were calculated according to the following equations:

Chlorophyll *a* (mg g^{-1} FW) = [(12.21x A663 – 2.81 x A646) x (V/W)]/ 1000

Chlorophyll *b* (mg g^{-1} FW) = [(20.13 x A646 – 5.03 x A663) x (V/W)]/ 1000

Carotenoids (mg g^{-1} FW) = [(1000 x A470) – (3.27 x Chl a – 104 x Chl b))/229 x (V/W)]/ 1000

Where (V) is the volume of acetone extract (ml) and W is the weight of plant leaf sample (g).

2.7.Statistical Analysis

Statistical analyses were performed using EXCEL® VBA macro add-in (DSAASAT) version 1.101 to estimate the variability by analysis of variance (ANOVA). Differences between the mean values were compared by Duncan's multiple range test (DMRT) at $P \leq 0.05$.

3. Results

3.1. Mechanism of TiO_2NPs to enhance salt tolerance

3.1.1. Growth parameters

The effects of salinity, TiO_2 NPs and their interactions on the growth parameters (Plant height, fresh and dry weights) of faba bean plants grown in the growing season throughout the growth period are illustrated in Figure (1).

Data indicated that salinity decreased the plant growth parameters, whereas foliar application of TiO_2 NPs increased faba bean growth parameters. In addition, it was found that TiO_2 NPs treatments reduced the harmful effect of salinity throughout the growth period during the growing season.

15 days after the first treatment, the obtained results showed that the high salinity levels decreased significantly plant height, fresh and dry weights of the shoots by 26.9 %, 46.7 %, 36.0 % respectively as compared with the control. Foliar application of TiO_2 NPs at 10 ppm significantly improved the plant height, fresh and dry weights of the faba shoots by 19.2 %,42.7 %, 50.9 % respectively, compared with the control.

Similarly, in the second sampling date ,30 days from the first treatment, the obtained results showed that the high salinity levels decreased the plant height, as well as fresh and dry weights of the shoots by 12.4 %, 37.2 %, and 34.8 % respectively compared with the control (Table 1). The application of TiO_2NPs at 10 ppm significantly improved faba plant height, as well as fresh and dry weights of the shoots by 10.9 %, 67.3 %, and 33.4 % respectively compared with the control.

3.2. Photosynthetic pigments

The effects of salinity, TiO_2 NPs and their interactions on the recorded photosynthetic pigments (Chlorophyll a, Chlorophyll b, Total Chlorophyll, Chlorophyll a /b and carotenoids) of the faba bean plant leaves grown throughout the growth period during the growing season are presented in Table (2).

Data showed that photosynthetic pigments in the leaves were decreased significantly under salt stress, whereas the foliar application of TiO_2 NPs increased in this respect. The interaction treatments showed that TiO_2 NPs decreased the harmful effect of salinity throughout the experimental period.

At the 1st sampling date (15 days from the NPs application) the obtained results showed that the process of photosynthesis is very sensitive to saline conditions. The highest level of salinity decreased chlorophyll a, Chl b, total Chl and Chl a/b by 28.4 %, 15.4 %, 25.7 %, 14.9 % respectively, carotenoids content was increased by 1.3% as compared to the control. TiO_2 NPs (10 ppm) treatments improved significantly the Chl a, Chl b, Total Chl, Chl a/b by 41.4 %, 28.5 %, 38.5 %, 8.1 % respectively and carotenoids by 27.6%, compared with the control. As for the interaction effects, the same Table showed that TiO_2 NPs application counteracted the inhibiting effects of salinity on chlorophylls. In contrast, it showed additive effects on carotenoids.

At the 2nd sampling date (30 days from the first NPs application) the obtained results showed that the high level of salinity decreased Chl a, Chl b, Total Chl and carotenoids content by 43.7 %, 45.7 %, 44.2 %, 12.1 % respectively. However, Chla/b was increased by 9.6 % compared to the non-saline condition. The foliar application of TiO2 NPs (10 ppm) improved significantly Chl a, Chl b, Total Chl and Chl a/b by 58.8 %, 19.7 %, 47.6 %, 35.8 % respectively and Carotenoids by 37.7 % compared with the untreated plants. Moreover, TiO_2 NPs application significantly increased plant photosynthesis under saline conditions.

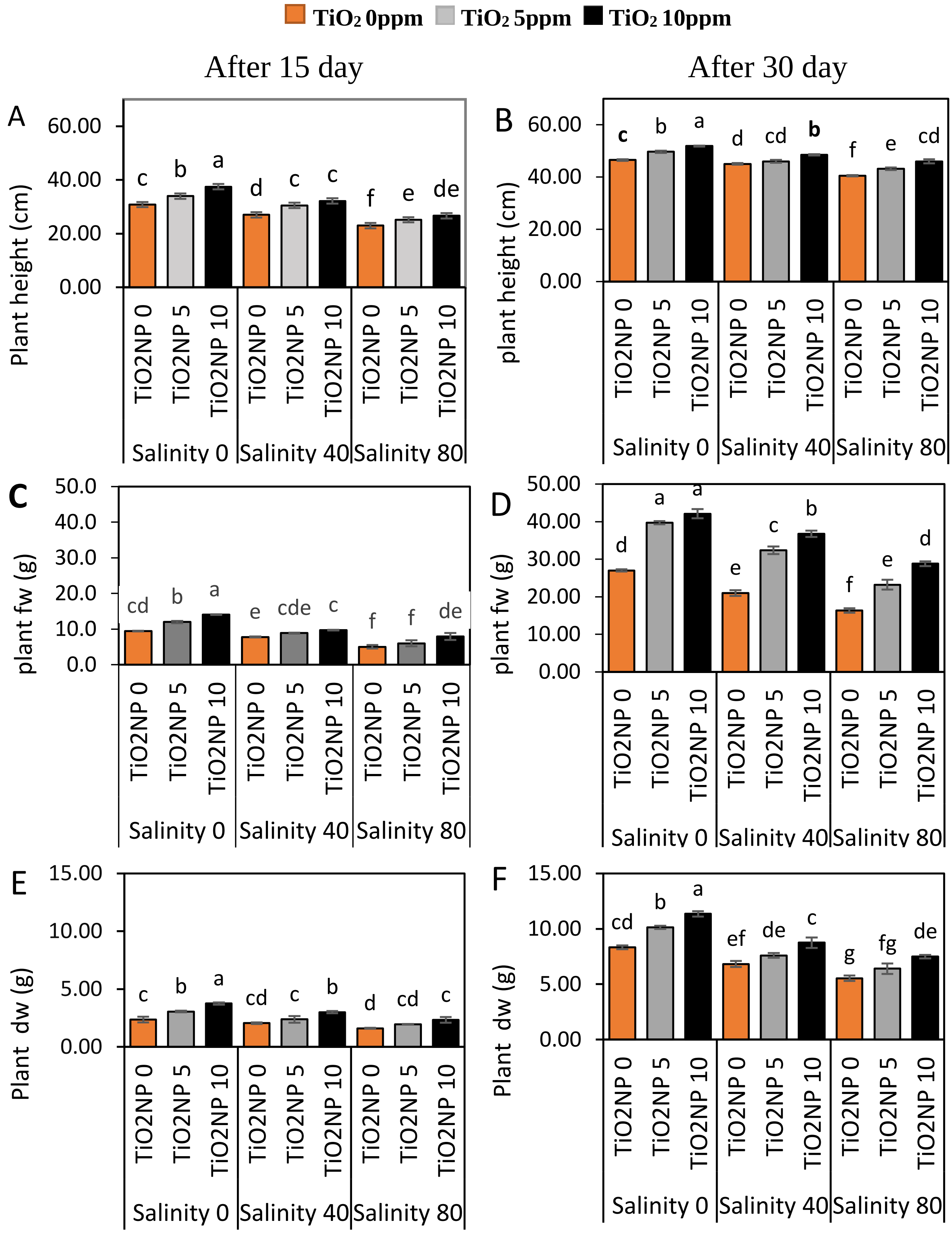

Fig (1): Effects of NaCl salinity, TiO_2 NPs foliar application and their interaction on certain growth parameters of faba bean plant throughout the growth period (Plant height (A,B), fresh weight (C,D) and dry weight (E,F) of shoot) of the faba bean plant grown under salinity levels(40 and 80 mMol) (after 15 days and 30 days).

Table 2: Effects of NaCl salinity, TiO_2 NPs foliar application and their interaction on (Chl *a*, Chl *b*, Total Chl and CAR) of faba bean plant throughout the growth period (after 15 and30 days) during the growing season.

Salinity (mM)	TiO2-NPs					
	Control (0 ppm)		5 ppm		10 ppm	
	After 15 day	After 30 day	After 15 day	After 30 day	After 15 day	After 30 day
Chl *a* (mg.g^{-1} FW)						
0 mM	1.44±0.05cd	1.67±0.02c	1.74±0.01b	2.11±0.04b	1.94±0.03a	2.43±0.13a
40 mM	1.23±0.04e	1.14±0.02e	1.40±0.02d	1.64±0.01c	1.67±0.05b	2.07±0.01b
80 mM	0.94±0.01f	0.91±0.01f	1.22±0.01e	1.18±0.06e	1.50±0.04c	1.41±0.02d
Mean	1.20±0.03C	1.24±0.02C	1.46±0.01B	1.64±0.04B	1.7±0.4A	1.97±0.05A
Chl *b* (mg.g^{-1} FW)						
0 mM	0.39±0.00ab	0.62±0.02a	0.45±0.02ab	0.65±0.03a	0.49±0.00a	0.70±0.04a
40 mM	0.35±0.06b	0.56±0.00a	0.37±0.08ab	0.57±0.01a	0.44±0.02ab	0.69±0.01a
80 mM	0.32±0.01b	0.31±0.01b	0.37±0.03ab	0.36±0.07b	0.43±0.03ab	0.40±0.09b
Mean	0.35±0.03B	0.50±0.01B	0.40±0.04AB	0.53±0.04AB	0.45±0.2A	0.60±0.05A
Total Chl (mg.g^{-1} FW)						
0 mM	1.83±0.05cd	2.30±0.01c	2.19±0.03b	2.76±0.02b	2.43±0.03a	3.13±0.09a
40 mM	1.58±0.03e	1.70±0.02d	1.77±0.07d	2.22±0.00c	2.12±0.03b	2.76±0.00b
80 mM	1.26±0.02f	1.22±0.01f	1.60±0.02e	1.53±0.05e	1.92±0.02c	1.81±0.10d
Mean	1.56±0.03C	1.74±0.01C	1.85±0.04B	2.17±0.02B	2.16±0.03A	2.56±0.06A
Total CAR (mg.g^{-1} FW)						
0 mM	0.34±0.01e	0.77±0.01f	0.43±0.02bcd	0.98±0.02c	0.50±0.00a	1.28±0.03a
40 mM	0.38±0.03de	0.84±0.01ef	0.41±0.04cde	0.97±0.00c	0.48±0.01ab	1.17±0.01b
80 mM	0.41±0.00cde	0..85±0.02e	0.41±0.01cde	0.90±0.02de	0.47±0.02abc	0.92±0.04cd
Mean	0.38±0.01C	0.82±0.01C	0.42±0.02B	0.95±0.02B	0.48±0.01A	1.12±0.03A

4. Discussion

Many complex reactions affecting plant growth and metabolism can be induced by salt. It has been documented that the physiological characteristics of faba bean and its growth performance was negatively affected by salt stress (Abdelhamid et al., 2010;Assimakopoulou et al., 2015;Eldardiry et al., 2017). Plant growth may be retarded by inhibition of certain growth metabolic processes, or by loss of cell turgor, which impairs cell division and expansion (Farooq et al., 2012; Ludwiczak et al., 2021).

Plant membranes are strongly affected by salinity, so the water content in the plant is reduced due to the osmotic deficiency resulting from salinity. Under conditions of salt stress, ABA is produced, which causes the stomata to close, which reduces the amount of water absorbed by the roots and affects the transpiration process, reducing the water content in the cell(Blatt, 1992;Meneguzzo et al., 1999;Jaleel et al., 2007). These findings are also agree with Özdemir *et al*. (2004) they found that specific physiological reactions, such as stomatal closure, increased rates of leaf senescence, and reduced plant growth, occur when plants are exposed to salt stress. In general, the opposite effect on enzymatic activities resulting from specific bonds between salts and some organic elements in the cell may also be responsible for the inhibitory effect of salinity on pea growth (Yin et al., 2018). Results in this study also agree with those obtained by Abd-Allah et al. (2015).

Using NPs is a viable way to overcome the challenges posed by oxidative stress on plant growth and productivity (Omar et al., 2023). It is necessary to evaluate their effects on plants and consider the changes in treated plants resulting from them. The most notable changes associated with salt stress in this study were the observed reduction in *V. faba* plant growth parameters. The two salinity levels showed a decrease in fw and dw of the shoot. The use of nanoparticles significantly increased mitotic indices that had decreased due to salt stress (Omar et al., 2023). Plant metabolism and cell division are enhanced by TiO_2NPs, which have been shown to have stimulatory effects on root and shoot growth (Frazier et al., 2014). TiO_2NPs, application in this study-increased photosynthesis, which raises Chl levels similar result, were reported by Zheng et al., (2005) in spinch.

The primary indicator of oxidative stress in salt-stressed plants is a decrease in chlorophyll levels because of an excessive buildup of sodium ions in the leaf tissues, which reduces biosynthesis and photosynthetic efficiency (Najar et al., 2019). Additionally, one important factor in reducing chlorophyll concentration under salt stress may be due to the activity of the enzyme chlorophyllase, which degrades chlorophyll (Santos, 2004). There is also a significant decrease in stomatal conductance and a subsequent reduction in carbon dioxide concentration, followed by a decrease in photosynthesis (Chaves et al., 2009; James et al., 2002). Increased ROS-induced damage to pigments in cells, abnormalities in stomatal motility function, and instability of the pigment protein complex in response to salinity stress (Gohari et al., 2020). Oxygen radicals are produced mainly in chloroplasts and mitochondria (Qureshi et al., 2013). This causes oxidative damage to lipids, proteins and nucleic acids, which affects the basic functions of respiration and photosynthesis and ultimately hinders plant growth (Qureshi et al., 2013). Therefore, one of the main causes of tissue damage in plants under stress from the environment is the generation of oxygen radicals (Küçük et al., 2007)

Carotenoids is essential for plant protection. They also act as antioxidants and scavengers of single oxygen molecules, preventing lipid peroxidation and maintaining membrane integrity. In situations of abiotic stress, carotenoids are essential in preventing autoxidation of the photosynthetic reaction center (Rainha et al., 2011; Anand Gururani et al., 2015). They also act as collectors of light energy for photosynthesis, stimulates the hormone abscisic acid, which promotes the closure of stomata when exposed to stress (Elsheery, Helaly, et al., 2020).

NPs with specific traits have been shown to have a significant impact on the growth and physiology of salt-stressed agricultural plants. One of the most important factors that affect a plant's ability to grow and develop is the level of chlorophyll in it. It has been shown that at high concentrations of salt, the amount of chlorophyll decreases significantly, as shown in Table (2). Under salt stress,application of the majority of NPs (Si, Ag, ZnO, TiO_2, CeO_2, Fe, and Se) increased the amount of chlorophyll(Sarkar & Kalita, 2023).

It wase found that applying TiO_2NPs spray delayed the chloroplast senescence process and increase the amount of time needed for photosynthesis to take place(Yang et al., 2006). TiO_2NPs stimulates Rubisph carboxylase activity, increasing the amount of chlorophyll and the rate of photosynthetic activity (Akbari et al., 2014; Yamori et al., 2012). TiO_2 NPs treatments regulate the activity of nitrogen metabolism-related enzymes and improve the process of converting inorganic nitrogen to organic nitrogen, as well as the production of proteins and chlorophyll (Mishra et al., 2014; Yang et al., 2006).

5. Conclusions

TiO_2NPs has the potential to mitigate the negative effects of salinity stress on faba bean plants. It may act as a free radical scavenger, reducing oxidative stress caused by salinity. The results of this experiment showed that the application of TiO_2NPs resulted in increased growth parameters by increasing photosynthesis parameters under sub-salinity stress conditions. Finally, application of TiO_2NPs (5 and 10 mg $L^{-1)}$ increased the measured traits of faba bean. Therefore, the use of TiO_2NPs (10 mg L^{-1}) can be considered as a useful strategy to increase growth and photosynthetic pigments in faba bean plants under salt stress. This can help maintain optimal levels of photosynthesis, which is crucial for plant growth and productivity. Further research is needed to fully understand the effects of TiO_2NPs on faba bean plants under salinity stress. Additionally, the potential risks associated with the use of TiO_2NPs taken into consideration, and thorough assessments of its environmental impact and human health concerns conducted before widespread application in agriculture.

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