

Research Paper

Effect of iron oxide and zinc oxide nanoparticles on growth improvement and tolerance to salinity stress in tomato plantsHossein Mozafari^{1*}, Masoumeh Hejabi², Hakimeh Oloumi¹ and Salari Hassan¹

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Article information	Abstract
<p>Available online: Sep. 2023 Copyright © 2023 Kerman Graduate University of Advanced Technology. All rights reserved.</p> <p>Keywords: salt stress tomato iron oxide nanoparticle zinc oxide nanoparticle</p>	<p>More than 22% of the world's agricultural land is saline, and this trend continues to increase with climate changes. Salinity stress causes leaf color change, osmotic stress, ionic toxicity, prevents growth, photosynthesis and plant performance. Due to their size less than micron, metal nanoparticles have a great absorption and transmission power in plants. Salinity stress is a major problem in hot and dry areas under tomato cultivation. For this purpose, investigating the mutual effects of the size and type of zinc oxide and iron oxide nanoparticles on the improvement and change of growth and increasing the resistance to salt stress in tomato plants of the early urbana variety were carried out in the form of a completely randomized and factorial design with 4 replications, at a significant level of 5%. In this research, zinc oxide nanoparticles in 25 and 50 nm sizes, iron oxide in 25 nm sizes and sodium chloride in 0 and 75 mM levels were used. Nanoparticles and salinity treatments were both applied to the plants. The results showed that salt stress led to a decrease in plant growth parameters such as shoot and root length, leaf area, RWC, ion leakage. Also, NaCl led to an increase in the accumulation of prolin and other aldehydes, sodium, iron and zinc. The application of nanoparticles had a slight effect in stress-free conditions, but in stressed conditions, these two nanoparticles alone and especially in combination neutralized the effect of salinity and reduced the damage caused by salinity stress.</p>

1. Introduction

Nowadays, due to facing the problem of water shortage and also due to the excessive increase of saline water, the production of agricultural products in saline land has received much attention. By increasing the amount of salt, especially sodium chloride in the soil, salt accumulates around the roots of plants. These salts around the roots have increased to such an extent that they are beyond the plant's tolerance

and have disrupted the plant's vital activities, photosynthesis, absorption and transfer of nutrients, as well as physiological and biochemical processes and the production of primary and secondary metabolites. (Faizan et al., 2021; Amini, 1999) Salinity stress is one of the most important factors that limit the cultivation of agricultural products, and more than 50% of the world's land is saline (Faizan et al., 2021).

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Nanoparticles include atoms and molecules with a diameter between 10 and 100 nm (Ashraf et al., 2007). The change of nanotechnology in the field of agriculture is one of the factors of creating modern agriculture (Behovdian et al., 1384). Nanotechnology is taking place as a revolution that has affected the economic status of countries (Amini, 1999). Nanoparticles are superior to larger particles due to their very small particle size (Faizan et al., 2021; Carmen, 2003). Today, the production and consumption of nanoparticles is increasing in various fields. In agriculture, the application of nanoparticles in production technology is to repel plant pests and diseases (Carmen, 2003). The use of many nanoparticles leads to an increase in plant resistance against pests and diseases (De Lacerda et al., 2003). Nanoparticles in low concentrations play an important role in increasing stem length, root length, collar diameter and increasing main roots during waterlogging stress (El-Sherif, 1990).

Iron element is one of the essential micronutrients for the survival of plants, so that if iron is not available to the plant in sufficient quantity, the plant will suffer from iron deficiency, disease or leaf yellowing, in which the photosynthesis cycle is disrupted and with a decrease serious crop growth and productivity will be faced. Iron is one of the scarce food elements, which is necessary for the growth of agricultural and garden plants, and plays a valuable role in the formation of plant roots.

This element exists in various mineral forms in the soil. Iron element is one of the most important nutritional elements required by plants, which has been proven necessary in the nutrition of plants during the last few decades (Faizan et al., 2021; Fernandez-Garcia, 2004). It is the main component of the structure of various enzymes and some pigments and is essential in the biochemical processes of plants such as photosynthesis, cellular respiration, carbohydrate metabolism and enzyme formation. Although iron is not used in the composition of chlorophyll

(the green pigment in leaves), it is essential for its formation. Therefore, iron-deficient plants show chlorosis in new leaves (Faizan et al., 2021).

Due to the antimicrobial and anticancer activity of zinc oxide nanoparticles, they are used as a promising platform in biomedical research and drug carriers in the body (Fatma, 2016; Faizan et al., 2021). ZnO nanoparticles are transparent in the visible range of the light spectrum and act as a UV-resistant physical filter (Fernandez-Garcia, 2004).

Tomato is one of the most important agricultural products in the Middle East, which belongs to the potato family (Gao, 2006). Tomato is an important source of minerals, vitamins and antioxidants. This plant is rich in vitamins C and D and free from cholesterol (Gao, 2006; Gardea-Torresdey, 2002).

Research has shown that increasing the concentration of salt leads to increasing the concentration of phenolic compounds in the plant (Gao, 2006; Gül, 1992). Destruction of chlorophyll is one of the oxidative damages caused by salt stress (Haghighi et al., 2013). Siuritep and his colleagues (2017) stated that salinity stress leads to, reduction of plant chlorophylls. The reason is that during stress the production of free amino acids such as proline increases in the plant. Nitrogen plays a role in the synthesis of proline and as a result chlorophyll decreases. Also, proline prevents cell membrane destruction by removing free radicals in plants (Haghighi et al., 2013; Gao, 2006; Gül, 1992).

Nanoparticles lead to different effects on morphological, physiological and biochemical properties of plant species. According to previous studies, nanoparticles regulate salt tolerance in different plants by changing hormones concentration, activity of antioxidant enzymes, ion homeostasis, gene expression, and defense systems (Hasegawa, 2000; Heath et al., 1968). In one study, mycorrhiza can reduce the absorption of silver nanoparticles into plant roots. Therefore,

metal nanoparticles can improve the symbiosis of plants and fungi (Jian-Kang *et al.*, 2002).

Rossi *et al.* (2016) investigated the physiological and biochemical responses of *Brassica napus* under the influence of cerium nanoparticles and salt stress. The findings showed that cerium nanoparticles induced higher photosynthetic efficiency and biomass in treated plants than in untreated plants. Zinc is an essential plant micronutrient, while zinc oxide nanoparticles containing this element increased the chlorophyll content in peanuts (Kaveh, 2011). It has also been reported that Zn nanoparticles can affect photosynthetic systems under salinity stress with ribulose-1, 5-bisphosphate carboxylase activity more than oxidase (Kazemzadeh, 2019). In the study of Faizan *et al.* 2016, the application of foliar spraying of zinc nanoparticles on tomato plants under salt stress significantly increased the amount of chlorophyll and the amount of photosynthesis (Faizan *et al.*, 2021).

According to the recent researches and published reports on the unique properties of zinc and iron nanoparticles, as well as the high sensitivity of tomato plants to salt stress conditions, it is likely that the combined use of iron oxide and zinc oxide nanoparticles will increase the resistance of this plant in salt stress conditions. Therefore, in this research, the possibility of increasing the resistance of Early Urbana variety of tomato plants under 75 mM salt stress was investigated using zinc oxide nanoparticles in two sizes of 25 and 50 nm with or without 25 nm iron oxide nanoparticles. Then the morphological and physiological parameters, the accumulation of sodium, potassium, zinc and iron elements as well as the activity level of some antioxidant enzymes were measured.

2. Material and methods

2.1. Research design and treatment groups

This research was carried out in the form of a completely random factorial design and four

replications in the greenhouse of Graduate University of Advanced Technology-Kerman-Iran in 2022-2023. The target plant for this research was the early Urbana variety tomato, whose seeds were obtained from Iran Falat Ghaareh Company-Tehran. The treatment groups of this study included iron oxide and zinc oxide nanoparticles. It should be noted that the nanoparticles were obtained from Pishgaman Nano Iranian Company. Also, salt stress was used in two concentrations (0 and 75 mM). The specifications of prepared nanoparticles are listed in Table 1.

2.2. Preparation of seeds

Tomato seeds (Early Urbana variety) were planted in plastic pots with an opening of 15 cm, 3 seeds per pot that were kept in were kept in the greenhouse with the standard conditions of temperature, light and humidity. The temperature of the greenhouse was 23 degrees Celsius in the dark and 25 degrees in the light, the photoperiod was 16/8 hours of darkness/light, the humidity was 45%, and the light intensity of the greenhouse was 12 kilolux.

2.3. Cultivation of seeds

60 pots were selected, and 3 seeds were placed in each pot at a depth of 1 cm in the culture bed (Fig. 1). It should be noted that the growing medium of each pot was a mixture of perlite and peat moss with a ratio of 4 to 1. Then they were washed and moistened with distilled water until they were ready for seed cultivation.

During the period when the seeds were planted, the pots were watered with distilled water twice a day, in the morning and in the evening, and after the emergence of the sprouts, Hoagland solution was also given to the plants. It should be noted that Hoagland's solution with one-tenth dilution of the original solution contains macro elements (potassium nitrate, calcium nitrate, potassium dihydrogen phosphate, magnesium sulfate and iron chelate) and micro elements (boric acid,

manganese chloride, copper sulfate, zinc sulfate, molybdate sodium). Macros with a concentration of 200X and micros with a concentration of 1000X were made. To make 1000cc Hoagland solution, 2.5 cc of concentrated solutions of macros and 0.5 cc of concentrated solutions of micros was taken and brought to the desired volume with distilled water (Hoagland et al., 1950).

Iron and zinc nanoparticles were given twice a day at 10 am and 4 pm for 10 days when plants reached the 3-leaf stage. And out of 60 pots, 30 pots were given salt stress and nanoparticles, which were our treated plants and 30 pots, were not given salt stress, only nanoparticles were given, which were our control plants and plant treated by root irrigation (Tables 1).

After the 10-day treatment, the plants were collected, and the morphological indicators were measured. Then the samples were placed in liquid nitrogen to measure other desired parameters and transferred to -80 freezer. It should be mentioned that in order to prepare the treatment, an amount of 50 mg/liter equivalent to 0.05 grams of each of the desired nanoparticles was weighed and a solution was prepared according to the treatment table (Table 3-2) for 16 treatment codes and then Hoagland solution was added to the weighed nanoparticles. And to homogenize the nanoparticles, they were placed in a sonication bath and then added to the plants in an amount of 15 cc.

Table 1: Table of treatment groups used in the research, which were created by factorial statistical test and applied to tomato plants.

Treatment code	ZnO25 nm (mg/L)	ZnO50 nm (mg/L)	Fe ₂ O ₃ 25 (mg/L)	Salt stress NaCl mM
1 (control)	0	0	0	0
2	0	0	50	0
3	50	0	0	0
4	50	0	50	0
5	0	50	0	0
6	0	50	50	0
7	50	50	0	0
8	50	50	50	0
9	0	0	0	75
10	0	0	50	75
11	50	0	0	75
12	50	0	50	75
13	0	50	0	75
14	0	50	50	75
15	50	50	0	75
16	50	50	50	75

2.4. Measurement of growth parameters

The fresh weight of roots and shoots of tomato samples were measured using a scale with an accuracy of 0.01 grams and the results were recorded in grams. In order to measure the length of the root and shoot, a 1 mm ruler was used, after cutting the collar area of the plant and separating the root from the shoot, and the basis for

measuring the root length is the main axis of the root.

2.5. Determining the surface of the leaf

In order to measure the leaf surface, checkered millimeter paper was used. The average surface area of the third leaf of the plant was measured and reported (Khan, 1998). One leaf was selected

from each pot and its surface was drawn on checkered paper and after counting and calculating the occupied leaf surface, a note was taken (Munns, 2008).

2.6. The relative content of leaf water

In order to measure the relative water content, the last developed leaves of the plants were selected and separated. As soon as it was separated, it was placed in ice. In the laboratory, the weight of the leaves was measured with a scale. Then the samples were placed in distilled water at laboratory temperature for 24 hours. After that, the weight of the turgescence state of the leaves was measured. Then the same leaves were placed in aluminum foil and placed in an oven at 72°C for 24 hours. After 24 hours, the dried samples were taken out from inside the foil and their weight was measured by an accurate scale. The relative content of leaf water was measured according to the method of Ritchie *et al.*, 1990 (Mahmoodzadeh, 2013).

(Relation 1) $\times 100$ (dry weight - turgor weight / dry weight - fresh weight) = (%) RWC

Fw: fresh weight of the leaf immediately after sampling, DW: dry weight of the leaf after placing the sample in the oven, Sw: saturated weight of the leaf after placing the sample in distilled water.

2.7. Measurement of leaf ion leakage percentage

To measure cell membrane damage, ion leakage was measured by the method (Ben Hamed *et al.*, 2007). For this purpose, after washing with deionized water, 0.2 grams of fresh plant tissue was placed in a test tube with a screw cap, and 10 cc of distilled water was added to it, and it was kept in the laboratory environment after 2 hours. The initial electrical conductivity of the solution (Ec1) was measured with the help of EC meter. Then the tubes are transferred to the autoclave for 20 minutes to heat and release the rest of the ions. After cooling in the laboratory environment, the

secondary electrical conductivity (EC2) of the solution is measured again. Finally, the amount of ionic leakage of the desired tissue is calculated through the opposite formula and in terms of percentage (Mahmoodzadeh, 2013).

2.8. Measurement of photosynthetic pigments

Photosynthetic pigments were measured according to Lichtenthaler's 1987 method. At first, 0.2 grams of fresh plant tissue was weighed using a digital scale in the laboratory, and each leaf was ground in a Chinese mortar with 15 cc of 80% acetone, and centrifuged for 15 minutes at 9000 rpm at 4 degrees. The supernatant solution was poured into the cell, then their absorbance was read at wavelengths of 646.8, 663.20, and 470 nm with a spectrophotometer. The concentration of pigments was calculated using the following relations and in terms of milligrams per gram of fresh weight of the plant (Lichtenthaler, 1987).

2.9. Measurement of total protein

To measure total protein, the method (Bardford, 1976) was used. To prepare the extract, 1 gram of fresh plant tissue was grinded in 3 ml of 50 mM potassium phosphate buffer (pH=7) containing 1% polyvinyl pyrrolidone (PVP) and 1 mM EDTA. All extraction steps were done on ice. The extracts were centrifuged for 15 minutes and 9000 revolutions at 4 degrees. The clear supernatant solution was used to measure the amount of total protein. To measure protein concentration, test tubes containing 50 microliters of protein extract were added to 2.5 milliliters of bioreagent and vortexed immediately. After 5 minutes, its absorption was read using a spectrophotometer at a wavelength of 595 nm. Total protein concentration was calculated using albumin standard curve (Bardford, 1976).

2.10. Measurement of proline

The method (Bates et al. 1973) was used to measure proline. First, 0.2 grams of fresh plant tissue, including shoots and roots, were weighed separately and ground in a mortar with 10 ml of 3% sulfosalicylic acid, a uniform mixture was obtained. The resulting mixture was centrifuged for 15 minutes and 9000 revolutions at a temperature of 4 degrees, and then the supernatant mixture was used to measure proline. The absorbance of the samples was read at 518 nm wavelength and the standard curve was used to calculate the proline concentration and the results were calculated in mg/g fresh weight.

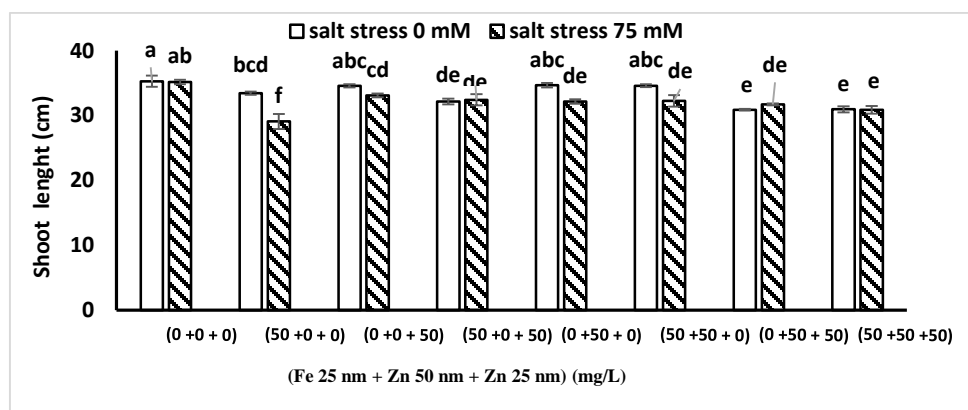
2.11. Statistical Analysis

The data obtained from the experiment were statistically analyzed in the form of a completely random design in the form of a factorial experiment in 4 repetitions using SPSS version 24 statistical software. The averages of growth, biochemical, enzymatic and chemical parameters

(elements) were compared using Duncan's multi-range test at the 5% level. The graphs of the mentioned parameters were drawn using Excel 2019 software.

3. Results

Compared to the control, the application of salt stress plus Fe NP led to a decrease in the length of aerial parts. In this way, 75 mM salinity stress plus Fe NP led to a decrease in root length and a 14.31% decrease in shoot length compared to the control. According to Figure 1, in the conditions without stress, the application of 25 and 50 nm zinc oxide nanoparticles and 25 nm iron oxide did have a significant decreasing effect at the level of 5% on root length compared to the control, but in the conditions of applying stress, the interaction of zinc oxide nanoparticles 25 and 50 nm and 25 nm iron oxide led to a significant increase of 12.47 times the length of shoot compared to saline control (Fig. 1).



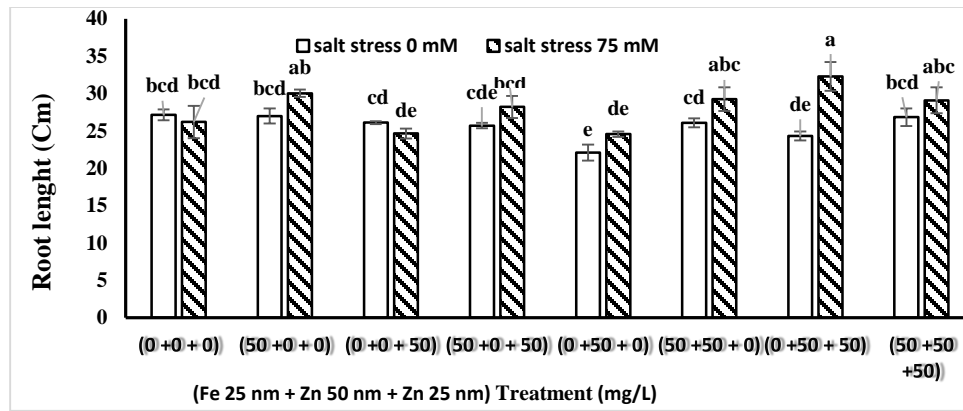
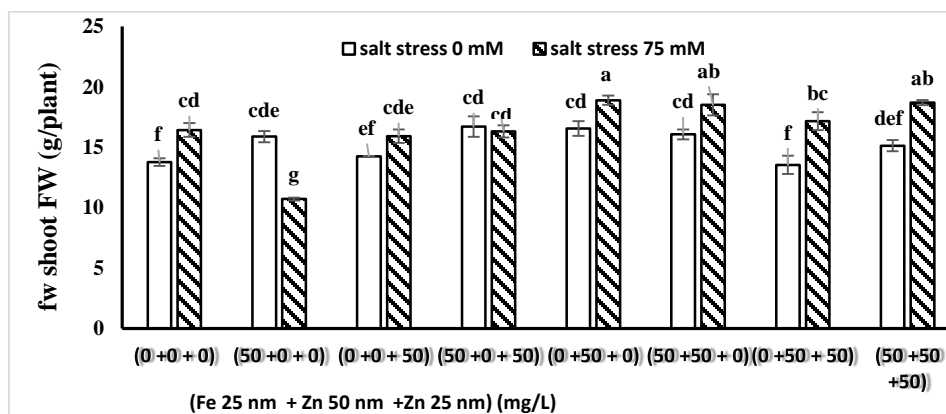


Fig. 1: The effect of zinc oxide and iron oxide nanoparticles under salinity stress (NaCl) conditions on the shoot and root length in Early Urbana tomato plants.

Also, in saline conditions, the application of 25 nm zinc oxide nanoparticles by 6.2% and 25 nm iron oxide by 17.55% reduced the length of shoot compared to the saline control. The application of iron oxide nanoparticles led to a 10.6% increase in root length compared to the saline control.

The interaction of zinc oxide and iron oxide nanoparticles, as well as the separate application of zinc oxide in stress-free conditions, had no significant effect on root length. 75 mM salt stress resulted in a 2-fold decrease in root length compared to the control. The interaction of zinc oxide and iron oxide nanoparticles in salinity conditions led to a 37.8 times increase in root

length compared to the salinity control. The use of zinc oxide and iron oxide separately under stress conditions led to a significant increase in root length by 2 and 8.51 times compared to the saline control. The use of nanoparticles in aerial organs also reduced their length compared to the saline control. But in the roots, with the application of nanoparticles, the root length increased compared to the salinity control in most cases, except for the application of both zinc nanoparticles separately, in these two cases, the application of the nanoparticles reduced the root length compared to the saline control (Fig. 2).



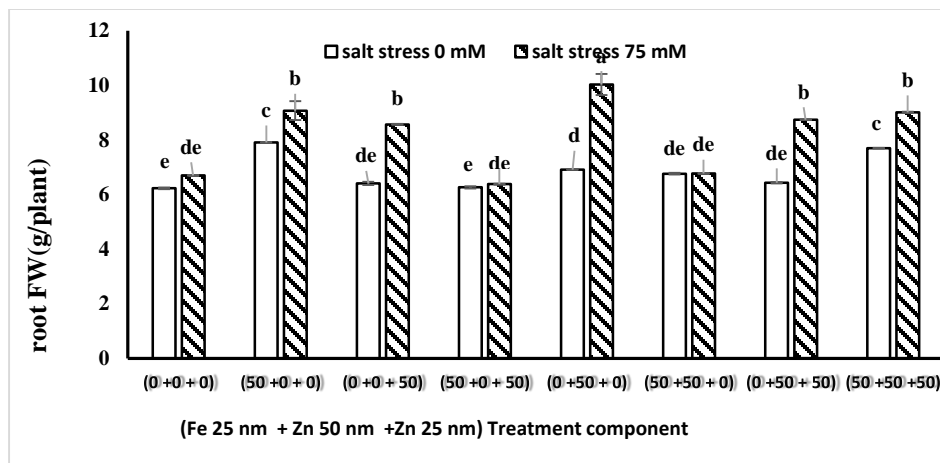


Fig. 2: The effect of zinc oxide and iron oxide nanoparticles under salinity stress (NaCl) conditions on the shoot and root fresh weight (FW) in Early Urbana tomato plants.

Salinity stress alone without the application of nanoparticles caused an increase in the weight of aerial parts compared to the control. The use of nanoparticles in most cases caused an increase in the fresh weight. Only when iron nanoparticles were used alone and iron and zinc nanoparticles of 25 nm were used together, the fresh weight of aerial parts decreased compared to the control. The combined use of both zinc and iron nanoparticles led to a significant decrease in the fresh weight of aerial parts by 35.8% compared to the saline control.

In general, the separate or combined application of iron and zinc nanoparticles has improved the leaf surface to the extent of the control or even more. For example, the combined application of

50 mg/liter of each of iron nanoparticles and both zinc nanoparticles caused the amount of leaf surface to be even higher than the control. This issue is also true for the combined application of 50 nm iron and zinc nanoparticles. It can be said that the data of the leaf area show that in stress conditions, the use of three nanoparticles has improved the leaf area better and more favorably than in non-stressed conditions, so that in non-stressed conditions, the application of 50 mg/liter of zinc nanoparticle 50 The nanometer significantly reduced the leaf area by 35% compared to the control. Now, under stress conditions, the amount of leaf surface was more and it has improved (Fig. 3).

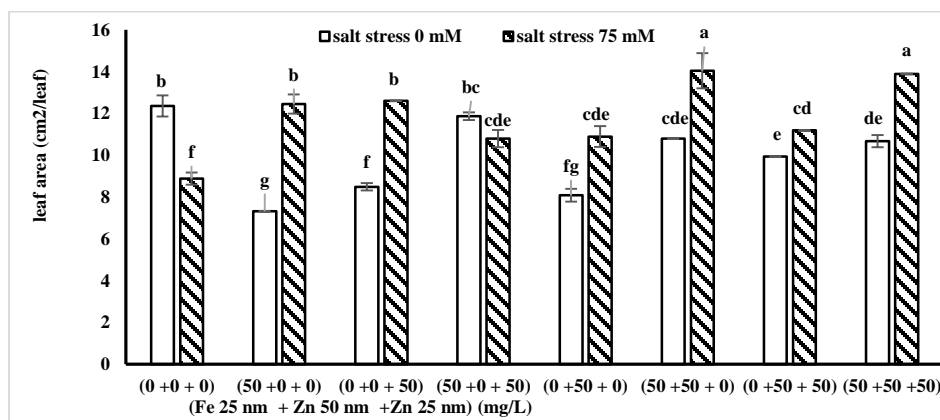


Fig. 3: The effect of zinc oxide and iron oxide nanoparticles under salinity stress (NaCl) conditions on the leaf area in Early Urbana tomato plants.

The use of nanoparticles in all cases has significantly increased the relative water content compared to the control. The application of iron nanoparticle alone increased the relative water content by 2 times, the application of 25 nm zinc nanoparticle alone increased the relative water content by 2 times. The application of 50 nm zinc and iron nanoparticles significantly increased the

relative water content compared to the control. The highest increase in relative water content is related to the combined use of all 3 nanoparticles, so that the combined use of all three nanoparticles significantly increased the relative water content by 25.88% compared to the salinity control (Fig. 4).

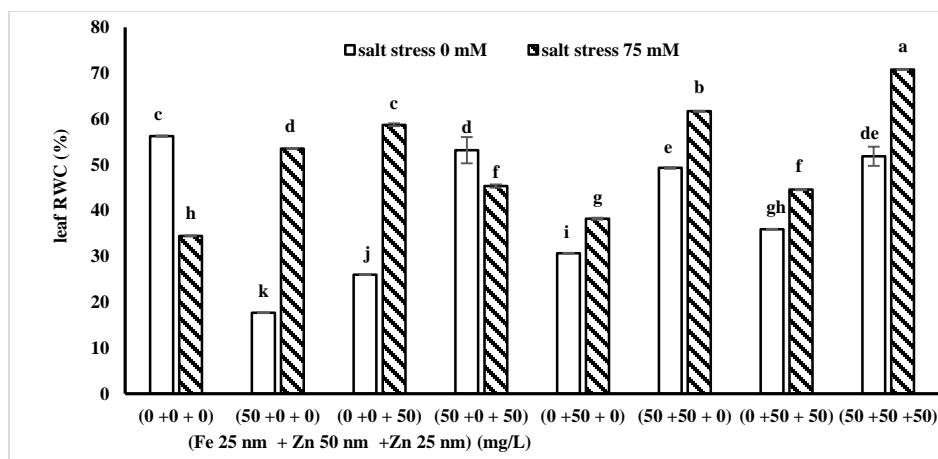


Fig. 4: The effect of zinc oxide and iron oxide nanoparticles under salinity stress (NaCl) conditions on the RWC leaf Early Urbana tomato plants.

In the conditions of salt stress without nanoparticles, the ion leakage increased, the use of nanoparticles in all cases reduced the ion leakage only in the application of all three

nanoparticles, and the 25 nm zinc nanoparticle increased the ion leakage compared to the control (Fig. 5).

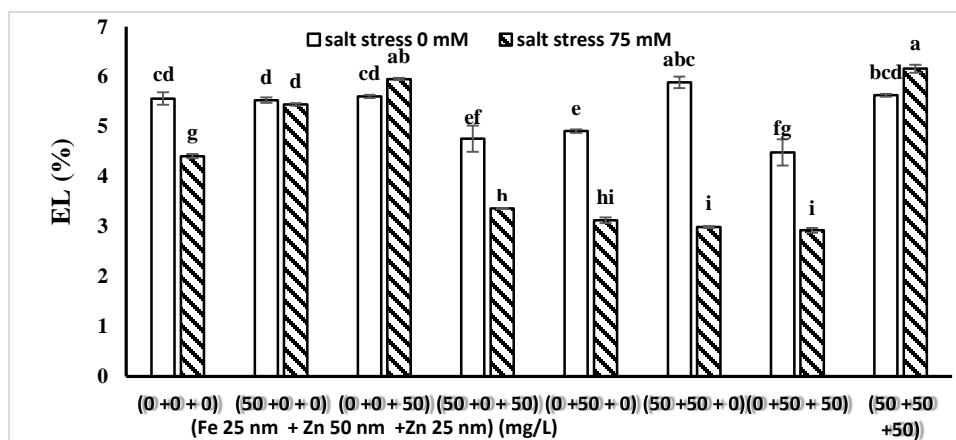


Fig. 5: The effect of zinc oxide and iron oxide nanoparticles under salinity stress (NaCl) conditions on the leaf EL in Early Urbana tomato plants.

The analysis of variance table in the appendices shows that the test treatments generally had a significant effect on the amount of chlorophyll a, even salt stress alone did not reduce chlorophyll a.

The slope of the bars shows that the application of nanoparticles over time showed an increase in the amount of chlorophyll in most of the treatments, except for the iron treatment alone. Salt stress led to a significant decrease of proline in aerial parts by 26.07% (Fig .6).

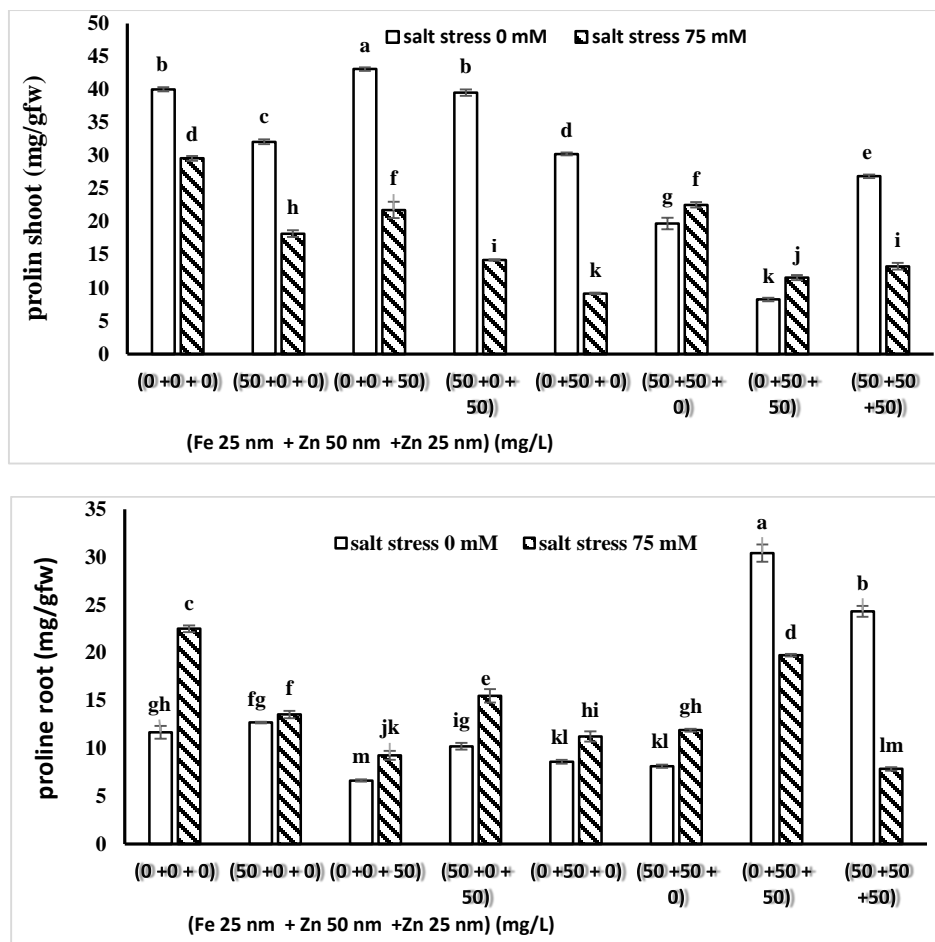


Fig. 6: The effect of zinc oxide and iron oxide nanoparticles under salinity stress (NaCl) conditions on the shoot and root proline content in Early Urbana tomato plants.

The use of nanoparticles also led to a decrease in proline in aerial parts in all but two cases compared to the control.

In the application of 50 nm zinc nanoparticle and iron, and both zinc nanoparticles together, the nanoparticle led to an increase in the amount of

proline in aerial parts compared to the control by a significant decrease of 69.77%. The use of both zinc nanoparticles separately and together with iron and all three nanoparticles was significant at the 5% level (Fig. 7).

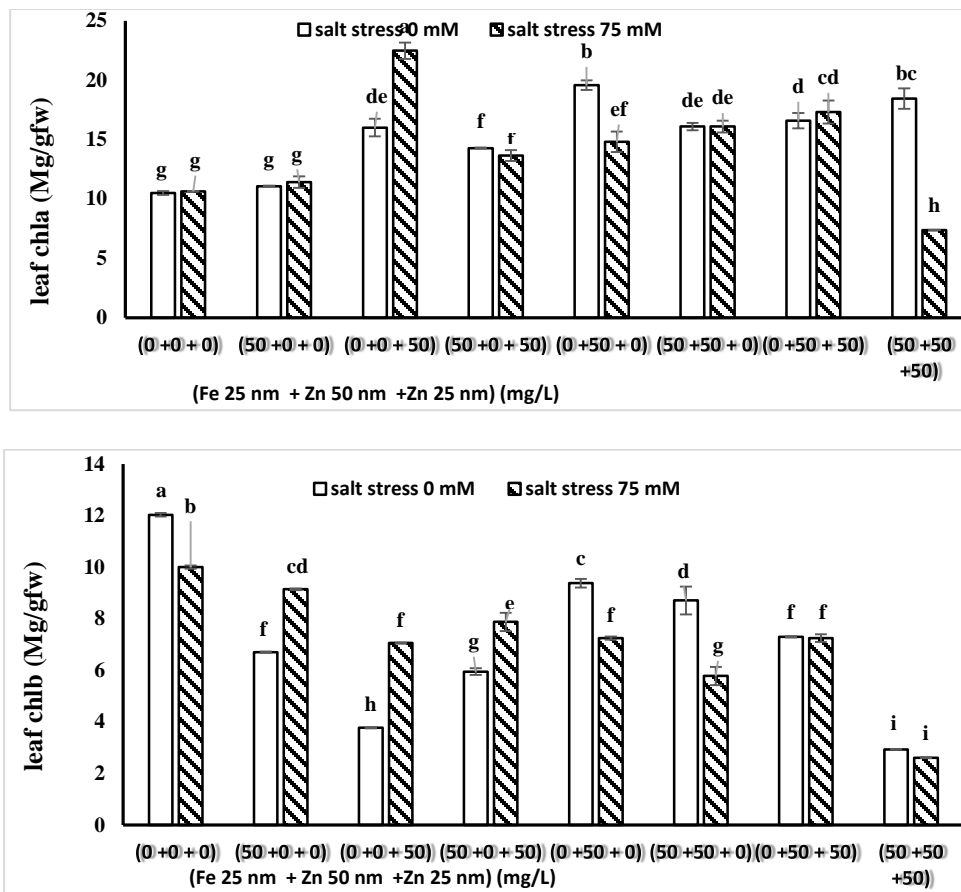


Fig. 7: The effect of zinc oxide and iron oxide nanoparticles under salinity stress (NaCl) conditions on leaf chl a and chl b in Early Urbana tomato plants.

Sodium chloride reduces the protein content of the Root by 60.58% compared to the salt control. The application of iron nanoparticle significantly increased the protein content of the whole stem by 149.48% compared to the saline control. The combined use of all three nanoparticles and two zinc nanoparticles significantly reduced the amount of total stem protein compared to the control. The highest amount of total protein in the

shoot was related to the 50 nm zinc nanoparticle alone, which increased the total protein content 4 times and significantly compared to the saline control. The application of both zinc nanoparticles showed the lowest amount of total stem protein compared to the control, the amount of total stem protein decreased significantly by 55.21% compared to the control (Fig. 8).

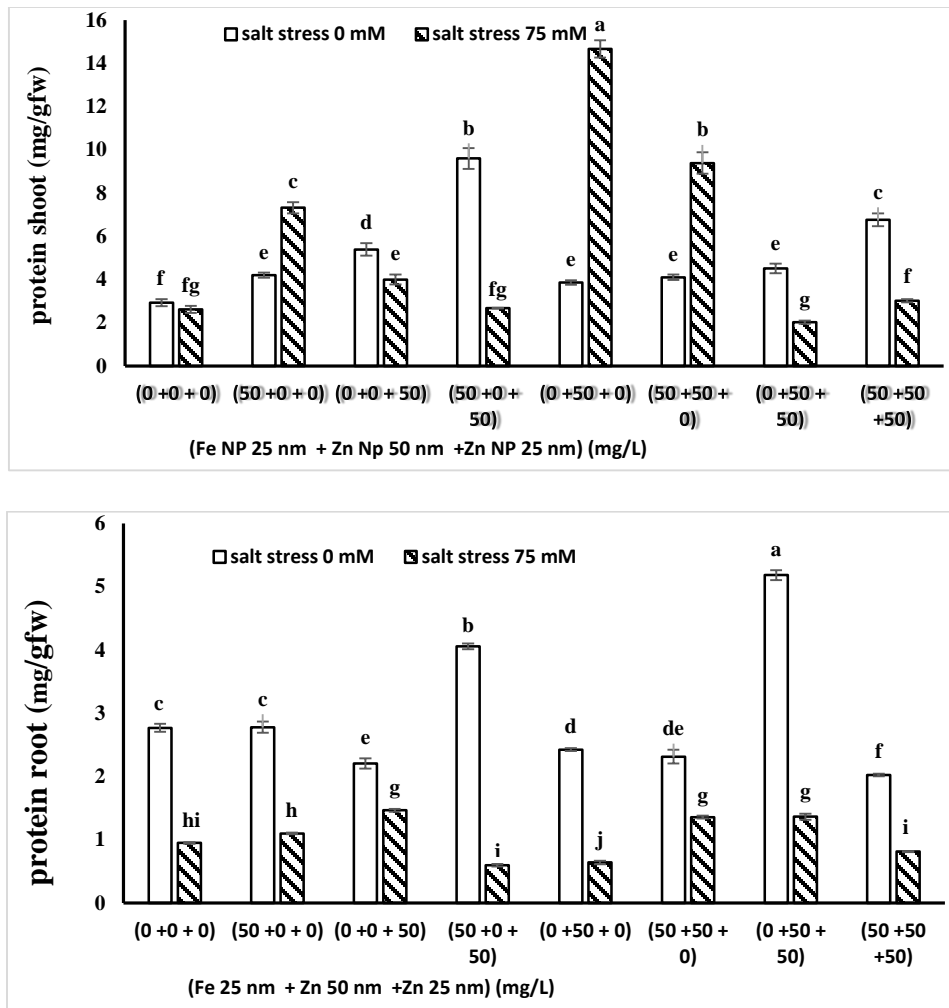


Fig. 8: The effect of zinc oxide and iron oxide nanoparticles under salinity stress (NaCl) conditions on the shoot and root total protein content in Early Urbana tomato plants.

4. Discussion

Salinity stress is one of the most important factors limiting the growth and production of crops in dry regions of the country. In line with the obtained results, previous research showed that salt stress prevents the growth of tomato seedlings (Nair, 2010). According to Kazemzadeh's studies, salt stress reduces seed germination and seedling growth in fodder sorghum (Kazemzadeh, 2019). The results of this research are also compatible with other researches.

In line with the conducted research, the relative content of water, the ratio of transpiration to absorption, carbon dioxide have decreased, which

leads to a decrease in the absorption and transfer of nutrients, which in turn limits plant growth (Prasad et al., 2012). In this study, salinity stress reduced the relative water content (Fig. 4).

A plant that is placed in saline conditions, the concentration of salts in the soil or water around its roots increases, which increases the osmotic pressure, which causes the energy required to absorb water and nutrients consumed by the plant. Increase, and as a result of this action, the respiration process increases and the length and basic functions of the plant decrease (Ritchie, 1990; Romero, 2006). In high concentrations of salt, the concentration of proline increases in

tomato plants (Romero, 2006). In line with the present research, salinity stress increases proline content in melon (Sariam, 2002).

The reason for the accumulation of proline in plants under stress is to maintain acidity, turgorecence pressure and cell volume, and the greater adaptability of the plant (Scott *et al.*, 2013; Sheykhbaglou, 2010). When the plant is under salinity stress, the level of sodium ions in the plant increases, and the plant must remove these sodium ions by spending energy, and because it consumes energy to remove sodium ions, the growth of the plant decreases (Shi, 2002). In the studies, salt stress increased the amount of sodium ion in shoots and roots, and as a result, the longitudinal growth of shoots and roots decreased (Siddiqui, 2013).

In another study, the effect of copper and zinc nanoparticles on the drought stress of wheat was done, and these two nanoparticles increase the stability of photosynthetic pigments and relative water content and enzyme activity and reduce the destructive effects of stress (Sonald, 1999; Siddiqui, 2013; Shi, 2002).

Zinc is one of the elements that act as a structural or regulatory cofactor; Therefore, it is used in the synthesis of proteins and photosynthesis, etc. (Tripathi *et al.*, 2017). Studies show that zinc plays an important role in reducing the harmful effects of stress and improving growth (Yin, 2013). In Sorghum bicolor plant, the use of silicon nanoparticles increased the wet weight of shoots and roots compared to salt stress (Sonald, 1999; Siddiqui, 2013 Yin, 2013).

Salinity stress, by affecting the stomatal factors, reduces the entry of carbon dioxide into the leaf, and as a result, reduces phytochemical activity and free radical production, and ultimately reduces chlorophyll (Sonald, 1999; Siddiqui, 2013). Proline causes osmotic adaptation and its accumulation has been reported in response to osmotic stress (Fig. 6) (Shi, 2002; Kazemzadeh, 2019). In some studies, it is mentioned that the accumulation of proline in stress conditions

indicates the increase of plant tolerance to sensitive types (Scott *et al.*, 2013; Sheykhbaglou, 2010). In this study, the amount of chlorophyll b and total chlorophyll also decreased. In another research, it has been said that the accumulation of proline is a sign of stress damage in sensitive plants compared to the tolerant type (Scott *et al.*, 2013; Sheykhbaglou, 2010). In an experiment, silicon nanoparticle levels of potassium and phosphorus in Pisum sativum plant. In our study, the nanoparticles also increased potassium levels in tomatoes (Shi, 2002) (Fig 5).

5. Conclusion

In this research, the interaction effect of 25 and 50 nm zinc oxide nanoparticles and 25 nm iron nanoparticles under salinity stress conditions was investigated on growth of tomato plants. The results obtained from the investigation of morphological traits and physiological traits as well as the investigation of some of growth parameters showed that the use of 75 mM sodium chloride led to a decrease in the morphological traits of plants such as the length of shoot and root, leaf surface with or without Fe and Zn NPs. Also generally, relative percentage of water, chlorophyll b and total chlorophyll pigments significantly reduced the plant growth compared to the control.

Also, salt stress decreased the content of total protein and shoots hydrogen peroxide, shoot proline. Salinity stress reduced weight of shoot and root, chlorophyll a, proline of root. The use of zinc and iron oxide nanoparticles individually and especially in combination reduced the damage caused by salt stress. The effect of nanoparticles on roots and shoots under stress was not the same in most of the parameters, and it was even the opposite, in that the parameter that increased with the application of nanoparticles in the roots decreased in the shoots, and even the improvement effect of nanoparticles in the roots and shoots was different. This means that the reactivity of roots and shoots to treatments

containing nanoparticles in salt stress conditions is completely different.

In line with the research, the following suggestions are presented; the effect of zinc oxide and iron oxide nanoparticles in the reproductive phase of tomato plants should be investigated. The mutual effect of zinc oxide and iron oxide nanoparticles on tomato plants in field conditions should also be studied.

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7. References

1. Amini M. Evaluation of soil salinity and alkalinity using geostatistical in the selected soil of rodas. MSc Thesis; department of agriculture, Isfahan University of technology, 1999; 119 pages (in Farsi).
2. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress and resistance. *Environ. Experiment. Bot.* 2007; 59: 206-216.
3. Behovdian B, Elhoti M, Nizami A. Investigate the effects of salinity on the germination of chickpea cultivars. *Sci. J Agri*: 1384; (2)2:127-137.
4. Bradford M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 1976; 72(1-2): 248-254.
5. Carmen M.I. A New Frontier in Food Science Understanding the special properties of materials of nanometer size will allow food scientists to design new, healthier, tastier, and safer foods. *Nanotech.* 2003; 57(12): 12-16.
6. De Lacerda CF, Cambraia J, Oliva MA, Ruiz HA, Prisco JT. Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. *Environ. Experiment Bot.* 2003; 49: 107-120.
7. El-Sherif AF. Response of tomato seedlings to zinc application under different salinity levels. *Egypt. J. Hort.* 1990; 1(7): 131-142.
8. Faizan M, Bhat JA, Chen C, Alyemeni MN, Wijaya L, Ahmad P, Yu F. Zinc oxide nanoparticles (ZnO-NPs) induce salt tolerance by improving the antioxidant system and photosynthetic machinery in tomato. *Plant Physiol. Biochem.* 2021; 161, 122–130.
9. Fatma M. Interplay between nitric oxide and sulfur assimilation in salt tolerance in plants. *Crop J.* 2016; 4(3): 153-161.
10. Fernandez-Garcia N. Effect of salinity on growth, mineral composition, and water relations of grafted tomato plants. *J plants nut. Soil Sci.* 2004; 16(7): 616-622.
11. Gao, F., Hong, F., Liu, C., Zheng, L., Su, M., Wu, X., Yang, F., Wu, C., Yang, P., 2006. Mechanism of nano-anatase TiO₂ on promoting photosynthetic carbon reaction of spinach. *Biol. Trace Elem. Res.* 111, 239–253.
12. Gardea-Torresdey J. Formation and growth of Au nanoparticles inside live alfalfa plants. *Nanoletters.* 2002; 2: 397.
13. Gül A. Effect of growing media on glasshouse tomato yield and quality. *Acta horticulturae.* 1992;3030: 145-150.
14. Haghighi M, Afifipour Z, Mozafarian, M. The effect of N-Si on tomato seed germination under salinity levels. *J biodiver. Environ Sci.* 2012; 6(16): 87-90.
15. Haghighi, M. and Pessarakli, M. (2013) Influence of silicon and nano-silicon on salinity tolerance of cherry tomatoes (*Solanum lycopersicum* L.) at early growth stage. *Scientia Horticulturae* 161: 111- 117.
16. Hasegawa P. Plant cellular and molecular responses to high salinity. *Annu. rev. plant physiol. plant mol. biol.* 2000; 5(1): 463-499.
17. Heath RL, Packer L. Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophys.* 1968; 12(5): 189-198.

18. Hoagland DR, Arnon DI. 1950. The water-culture method for growing plants without soil. California Agricultural Experiment Station, Circular-347.
19. Hwang N. Rapid hippurate hydrolysis method for presumptive identification of group B streptococci". J. Clin Microb. 1975; 1(1): 114-115.
20. Jian-Kang Zhu. University of California, Riverside, California, 2002. USA. doi:10.1002.97870470015902. a0001300. pub2.
21. Kaveh H. How salinity affect germination and emergence of Tomato Lines". J. biodivers. environment sci. 2011; 5(15): 159-163.
22. Kazemzadeh A. 2019 evaluation of salinity tolerance based on germination in 9 fodder sorghum cultivars. Plant sci res. 2011; 5(15): 159-163
23. Kerista BT. Salinization processes in irrigated lands of Turkmenistan. J. prob. des develop. 1993; 1: 12-15.
24. Khan A. Effect of sodium chloride on growth and mineral composition of sorghum. Acta Physio Planta. 1998. 10(3): 257-264.
25. Lichtenthaler HK. Methods in enzymology. Elsevier. 1987; 350-382.
26. Mahmoodzadeh H. Physiological effects of TiO₂ nanoparticles on wheat (*Triticum aestivum*). Tech J. engin. appl sci. 2013; 3(14): 1365–1370.
27. Munns R. Mechanisms of salinity tolerance. Ann Rev. Plant Biol. 2008; 59: 651-681.
28. Nair R. Nanoparticulate material delivery to plants. Plant Sci. 2010; 17(9): 154-163.
29. Nayyar H, Walia DP. Water stress induced proline accumulation in contrasting wheat genotypes as affected by calcium and abscisic acid. Biolo Planta. 2003; 46: 275-279.
30. Prasad T, Sudhakar P, Sreenivasulu Y, Latha P, Munaswamy V, Reddy KR, Sreeprasad TS, Sajanlal PR, Pradeep T.. Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. J. Plant Nutr. 2012; 35: 905–927.
31. Ritchie S. Leaf water content and gas-exchange parameters of two wheat genotypes differing in drought resistance. Crop Sci. 1990; 3(0): 105-111.
32. Romero-Aranda M. Silicon alleviates the deleterious salt effect on Tomato plant growth by improving plant water status. J. Plant Physiol. 2006; 16(3): 847-855.
33. Sariam R. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Sci. 2002; 16(3): 1037-1046.
34. Scott N, Chen H. nanoscale science and engineering for agriculture and systems. Indust biotech. 2013; 9(1): 17–18.
35. Sheykhbaglou R. Effect of nano iron particles on agronomic traits of soybean. Not Scient Biolo. 2010; 2(2): 112–113.
36. Shi Z. Effect of different EC management on yield, quality and nutraceutical properties of Tomato grown under supplemental lighting. Acta Hort. 2002; 58(0): 241-247.
37. Siddiqui MH, Al-Whaibi MH. Effects of hematite and ferrihydrite nanoparticles on germination and growth of maize seedlings. Saud J. Biolo Sci. 2013; 2(1): 13-17.
38. Siuritep NS, Schmöckel SM and Tester M. Evaluating physiological responses of plants to salinity stress. Ann Bot. 2017; 119(1): 1–11.
39. Sonald S. Phenolics and cold tolerance of Brassica napus. Plant Agricul. 1999; 1: 1-5.
40. Sreenivasulu N. Different response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedling of foxtail millet. Physiol Planta. 2000; 10(9): 435-442.
41. Tripathi DK, Shweta Singh S, Singh S, Pandey R, Singh VP, Sharma, Chauhan DK. An overview on manufactured nanoparticles in plants: Uptake, translocation, accumulation and phytotoxicity. Plant Physiol. Biochem. 2017; 110: 2–12.
42. Yin L, Wang S, Li J, Tanaka K, Oka M. Application of silicon improves salt tolerance through ameliorating osmotic and ionic stresses in the seedling of Sorghum bicolor. Acta Physiol Plant. 2013; 35: 3099-3107.