

Interaction effect of size and type of zinc oxide nanoparticles with iron nanoparticles on increasing the growth of wheat plants under drought stress

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Article information	Abstract
<p>Available online: Mar. 2024 Copyright © 2024 Kerman Graduate University of Advanced Technology. All rights reserved.</p>	<p>Drought, as a multidimensional stress, has various destructive and adverse effects on plants and affects many morphological characteristics and physiological, biochemical and nutritional processes. Wheat response to water deficit stress has many mechanisms. Therefore, the general purpose of this research was to investigate the interaction effect of iron oxide nanoparticles (25 nm) with two types of zinc nanoparticles (25 and 50 nm) at a concentration of 50 mg/liter on greater resistance of wheat (Arg cultivar) and improving its growth under drought stress conditions. After applying drought treatments and the nanoparticles to the plants, some growth and pigments parameters were measured. The occurrence of drought stress compared to the control led to a decrease in shoot length and other growth parameters. In this way, drought stress in the form of 7 days of water deprivation caused a decrease in root and stem length as well as other growth parameters such as fresh weight, dry weight, leaf water content, etc. compared to the control plants without drought stress. According to results, in conditions without drought stress, the application of both types of zinc oxide nanoparticles without or combined with iron oxide nanoparticles at the level of 5% had a significant effect on these parameters compared to the control. The results showed that in the conditions of applying drought stress, the interaction of 25 nm zinc oxide nanoparticles and iron oxide led to a significant increase in chl_a, b and total more than the control. However, the results of our research showed that the resistance mechanism for drought resistance such as ion leakage parameter and leaf surface was improved to improve growth in wheat. In this research, it was proved that the interaction between nanoparticles and wheat plants was observed in dry conditions and the results were significant.</p>

Keywords:
Drought stress
wheat, growth
iron oxide
zinc oxide
nanoparticle

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1. Introduction

Drought, as a multidimensional stress, has many destructive and adverse effects on sensitive and semi-sensitive plants (Anjum et al., 2011; Ndou et al., 2023). This important abiotic stress affects many morphological characteristics and physiological processes of plants related to growth and development (Afshari–Behbahanizadeh et al., 2016). This stress causes the production of reactive oxygen species, damage to cell membranes, inhibition of electron transfer, reduction of photosynthetic pigments and protein (Ben Ahmed et al., 2009). The reaction of different plants to drought stress is a complex process that includes molecular changes and its spread to all metabolic activities and its effect on the growth, morphology and phenology of plants under this stress. Osmotic active ions, including soluble sugars and amino acids, can also be tolerated or adapted to stress by carefully controlling the entry and exit of ions and the activation of the inhibitory system and removal of reactive oxygen species (Gong et al., 2005).

When the plant is affected by stress such as drought, the synthesized free radicals cause toxicity in the plant and cell death (Ahmad et al., 2021). Foliar spraying of zinc in conditions of stress such as drought can improve photosynthetic processes and accumulation of carbohydrates and reduce the negative effects of stress (Hasheminasab et al., 2012).

Nanotechnology has advanced in all fields of human life, such as biological sciences, especially medical devices and biotechnology, and plays an important role in improving agricultural and crop management techniques (Kumar et al., 2019). It provides a way to study the defense systems and improve the growth of organisms such as higher plants (Cheng et al., 2016). Nanotechnology has a prominent place in the transformation and production of agricultural food. Few studies have been done on the effects and mechanisms of nanomaterials in the resistance of plants to abiotic stresses (Lowry et

al., 2019). Therefore, in this article, the application of zinc and iron nanoparticles in order to improve the resistance of wheat plants to drought stress was investigated (Ndou et al., 2023).

The development of metal nanoparticles such as zinc and iron can provide new and better effects in plant and agricultural biotechnology (Rico et al., 2015). These nanoparticles can even be used to replace pesticides and chemical fertilizers and reduce damage in plants under stress and adverse conditions. Nanoparticles can be used to transfer fungicides, insecticides or plant hormones and elicitors (Saranya et al., 2019). The element zinc together with iron as a cofactor of antioxidant enzymes has caused the increase of these enzymes and has played an essential role in creating the cell's defense system against reactive oxygen species, modulating free radicals and their destructive effects in membrane systems, and as a result, plants tolerance increases to drought stress (Semida et al., 2021). In the condition of deficiency of this element, the occurrence of oxidative damage caused by the attack of free radicals such as active oxygen by disrupting the function of cell membranes and producing hydroxyl and superoxide radicals causes damage to the cell, and plants are resistant to stress (Dimkpa et al., 2019). Physiological results from the inefficiency of numerous enzyme systems and other metabolic actions related to zinc will not be safe. The occurrence of drought 5 to 15 days before the appearance of the spike reduces the number of grains in wheat by 40% (Dimkpa et al., 2019).

The interaction of plant cells with nanoparticles leads to changes in plant gene expression and related cell pathways, which ultimately affects plant growth and development. It has been proven that silver nanoparticles produced by biological methods are more stable compared to chemical methods (Sharma et al., 2019). Silver nanoparticles have many applications in the field of antimicrobial diagnosis, treatment, analysis

and microelectronics (Gubbins et al., 2011). Some recent advances in plant and agricultural sciences show the use of nanoparticles in the effective and safe use of chemicals for plants, plants as a source of synthesis of nanoparticles (Alabdallah et al., 2021). They show different effects on growth and metabolic function and genetic modification using nanoparticles. Recently, it has been observed that silver nanoparticles have harmful environmental effects and also cause heavy metal stress in plants. The use of more compatible and useful nanoparticles such as zinc and iron nanoparticles can help to solve this problem (Taran et al., 2017).

However, the interaction of plant cells with nanoparticles leads to the change of plant gene expression and related biological pathways, which ultimately affects the optimal growth and development of the plant (Ndou et al., 2023; Ali et al., 2021). Recently, from different plants as a tool for synthesis and the production of green and environmentally friendly nanoparticles is used. These obtained nanoparticles are used to improve the growth and metabolic performance of plants under stress and even to investigate genetic changes related to stress. Exogenous application of nanoparticles with optimal conditions and suitable type such as zinc and iron with specific size and concentration can cause better and greater resistance to drought stress in wheat, which was investigated in this research (Behboudi et al., 2019).

According to the recent researches and published reports on the unique properties of zinc and iron nanoparticles, as well as the high sensitivity of wheat plants to drought stress conditions, it is likely that the combined use of iron oxide and zinc oxide nanoparticles will increase the resistance of this plant in drought conditions (Behboudi et al., 2019).

Considering that studies have been done to identify zinc oxide and iron oxide nanoparticles, but some characteristics of these two important nanoparticles in terms of relieving drought stress

and more resistance of plants to stresses such as drought remain to be studied further. Therefore, the mutual examination of these two types' nanoparticles are important from the aspects of size and type of nanoparticles in the conditions of drought stress in important plants such as wheat. Therefore, the general purpose of this research was to investigate the interaction effect of iron oxide nanoparticles (25 nm) with two types of zinc nanoparticles (25 and 50 nm) at a concentration of 50 mg/liter on greater resistance of wheat plant and improving its growth under drought stress conditions. Therefore, in this research, the possibility of increasing the resistance of Arg variety of wheat plants under drought stress was investigated using zinc oxide nanoparticles in two sizes of 25 and 50 nm with or without 25 nm iron oxide nanoparticles. And then the growth, morphological and physiological parameters were measured under drought stress conditions.

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 4 replications in the greenhouse of Graduate University of Advanced Technology (Kerman, Iran) in 1400-1401. The target plant for this research was the Arg variety of wheat, whose seeds were obtained from Agricultural and Natural Resources Research Center of Kerman Province, Seed and Seedling Research Unit. The treatment groups of this study included iron oxide and zinc oxide nanoparticles. It should be noted that the nanoparticles used were obtained from Pishgaman Nano Iranian Company-Mashhad. Also, Wheat plants were treated with drought stress including two control levels and 7 days of water deficit. The specifications of prepared nanoparticles are listed in Table 1.

The selection of the type of experimental design and concentrations of nanoparticle treatments and

the level of dehydration has been made after study and laboratory optimization. For example, the initial drought and drought levels were 5, 7, 10, and 15 days, and finally the 7-day drought level was selected. Regarding the concentration of nanoparticles, it should be stated that among the

concentrations of 25, 50 and 75 mg/liter, the application of the concentration of 50 mg/liter nanoparticles was chosen because it does not have a stress-causing effect. Of course, other researches were also considered to some extent.

Table 1: Characteristics of nanoparticles (Pishgaman Nano Iranian Company) used in the experiment

<i>Characteristics of nanoparticles</i>	Zinc oxide (ZnO)-50nm	Zinc oxide (ZnO)-25nm	Iron oxide (Fe₂O₃) 25nm
<i>nanoparticle diameter</i>	50-60	10-30	40-20
<i>purity</i>	99.92%	99%	98%
<i>Color</i>	Milk white	white	red brown
<i>Shape</i>	Almost spherical	Almost spherical	spherical
<i>pH</i>	-	-	7.5
<i>apparent density</i>	5.56 g/cm ³	-	1.20 g/cm ³
<i>Real density</i>	-	5.606	5.24 /cm ³

2.2. Preparation of pot culture medium and Cultivation of seeds:

At this stage, wheat seeds (Arg variety) were planted in plastic pots with an opening of 15 cm, 3 seeds per pot, and then the pots were transferred to the greenhouse with the same 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 8/16 hours of darkness/light, the humidity was 45%, and the light intensity of the greenhouse was 12 kilolux (KLx) (Fig. 1). 64 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, which was obtained from Kerman agricultural companies. 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) was used. A stock solution of high-use elements (macro element) with a concentration ratio of 200 times the requirement (200X) and low-use elements (microelement) with a concentration ratio of 1000 times the requirement (1000X) was made. Then, to make 5 liters of Hoagland's original solution with one-tenth of Hoagland's dilution, 2.5 cc of macro stock solution was mixed with 0.5 cc of micro stock solution and the volume was 5 liters with distilled water and the acidity (pH) was adjusted to 6.25-6.5 (Hoagland et al., 1950).

The treatment of the plants started after the plants reached the 3-leaf stage and iron and zinc nanoparticles were given to the plants twice a day at 10 am and 4 pm for 10 days. And out of 60 pots, 32 pots were given drought stress (Dehydration period of 7 days) and nanoparticles, which were our treated plants and 32 pots, were not given drought stress, only nanoparticles were given,

which were our control plants. Solutions containing test treatments including expressed nanoparticles were given to wheat plants through the root growth medium. (Figure 1; Tables 1, 2). After the 10-day treatment period, the plants were collected, the morphological and growth indicators were measured, and then the samples were freeze in liquid nitrogen and kept at -80 freezer to measure other desired parameters. 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 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 sonic bath and then added to the plants in an amount of 15 cc.

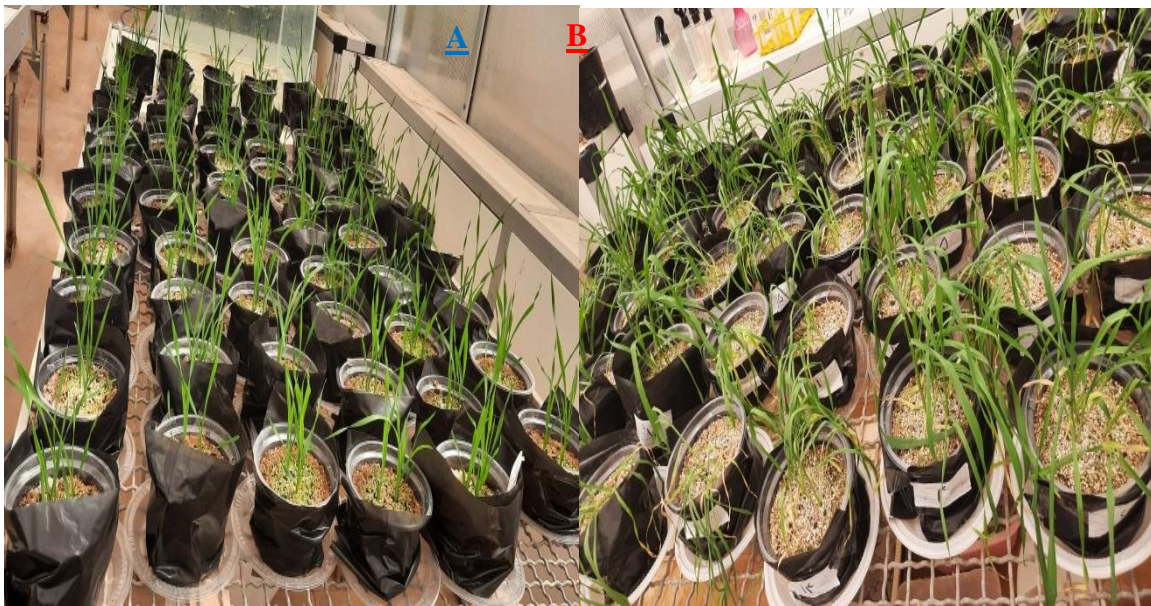


Fig. 1: The image of the respective cultivated pots of wheat plants in the 3-leaf stage before the treatments (A) and the pots with the treatment code after receiving drought stress (7 days of dehydration period) (B). Black nylons were used to prevent the growth of algae or fungi in the plant growing medium inside the pot and to prevent light from reaching the root growth environment.

Table 2: Table of treatment groups and codes used in the research, which were created by factorial statistical test and applied to wheat plants Arg cultivar.

Treatment code	ZnO25 nm (mg/L)	ZnO50 nm (mg/L)	Fe ₂ O ₃ 25 (mg/L)	Drought stress (days of water deficit)
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	7
10	0	0	50	7
11	50	0	0	7
12	50	0	50	7
13	0	50	0	7
14	0	50	50	7
15	50	50	0	7
16	50	50	50	7

2.3. Measurement of growth parameters:

The fresh weight of roots and shoots in fresh wheat samples was 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 (Crusciol et al., 2009).

2.4. 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. 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 (Hayatu, et al., 2014).

2.5. 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 24 hours, 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.

2.6. 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 (Ben Hamed et al., 2007).

2.7. Measurement of photosynthetic pigments:

Photosynthetic pigments were measured according to Lichtenthaler's 1987 method (Lichtenthaler, 1987). 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).

2.8. Statistical Analysis

The data obtained from the experiment were statistically analyzed in the form of a completely random design and in the form of a factorial experiment and 4 repetitions using SPSS version 24 statistical software. The averages of growth parameters were compared using Duncan's multi-range test at the 5% level. The graphs of the mentioned parameters were drawn using Excel 2016 software. However, in this study, 4 independent factors were used in the form of 16 treatment codes with 4 repetitions to affect wheat plants of Arg variety.

The raw data were entered into the statistical software after practical measurements. Data normalization and variance homogenization were performed. The coefficient of variation (CV) was checked according to the used experimental design.

3. Results

After normalizing the data and controlling the coefficient of variation, the overall average of the test error rate and the ANOVA table were obtained for all measured parameters; which is shown in Table 3. The analysis of variance table showed that 12 codes and treatment groups had a significant effect on the change of all measured parameters. It can also be said that the nanoparticles in the treatments had a significant effect on the improvement of the parameters in plants under stress (Table 3).

The occurrence of drought stress compared to the control led to a decrease in shoot length. In this way, drought stress in the form of 5 days of water shortage caused a 27% decrease in root length and a 28% decrease in stem length compared to the control plants without drought stress. According to Figure 1, in conditions without drought stress, the application of both types of zinc oxide nanoparticles without or combined with iron oxide nanoparticles at a level of 5% had a significant effect on root length compared to the control. The results showed that in the conditions of applying drought stress, the interaction of 25 nm zinc oxide nanoparticles and 25 nm iron oxide led to a significant increase in stem and root length more than the control (Figure 1).

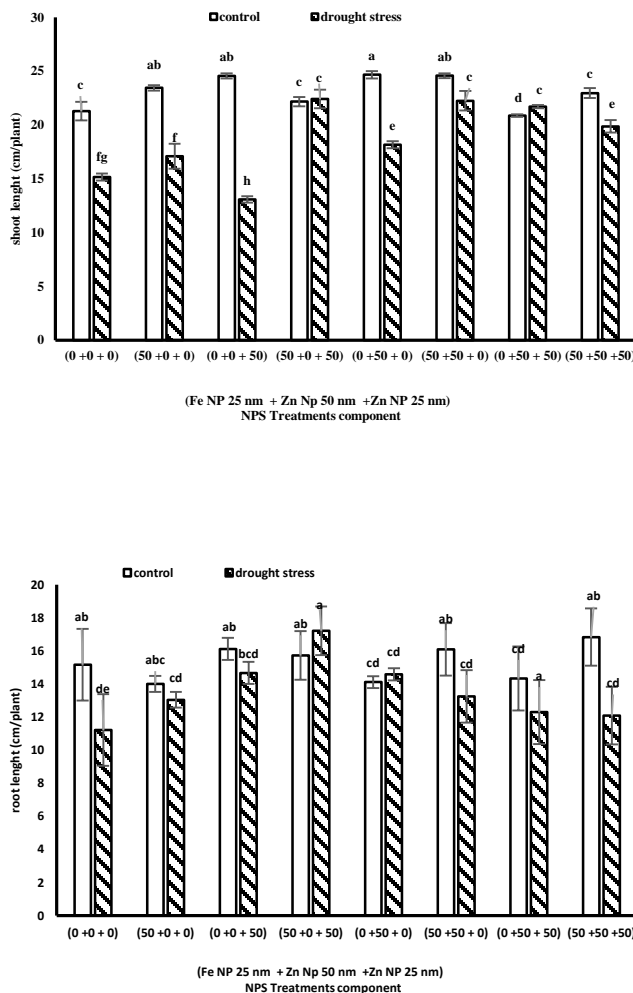


Fig. 2: The effect of zinc oxide (25 or 50 nm) and iron oxide nanoparticles (Size?) under control or drought stress conditions on the shoot and root length mean in Arg cultivar of wheat plants. Different letters indicate significant difference based on Duncan post hoc test ($P < 0.05$). Error bars show standard error (SE). The numbers 0 and 50 under the horizontal axis of the graph represent the concentrations of 0 and 50 mg/L of each type of nanoparticle used.

Also, in the conditions of drought stress, the application of 25 nm zinc oxide nanoparticles along with iron improved the longitudinal growth of the stem by 46% and the longitudinal growth of the root by 54% compare to what. Therefore, it can be said that the longitudinal growth of root and stem under stress conditions has given a better response to the combined treatment of 25 nm nanoparticles of iron and zinc. However, all the treatments containing nanoparticles had

positive and improving effects in the conditions of drought stress and dehydration.

The separate application of zinc oxide 50 nm or nanoparticles under stress conditions had a less significant effect on shoot length. For example, the use of iron nanoparticles without the presence of zinc led to a 13% improvement in shoot length growth, and the use of only 50 nm zinc oxide nanoparticles led to a 20% improvement in the same parameter compared to stress conditions. The separate application of 25 oxide

nanoparticles together had a negative effect of 14% on the longitudinal shoot growth of wheat plants under stress, while the interaction of this nanoparticle with iron nanoparticles was very effective.

The use of 50 nm zinc oxide and iron oxide separately under drought stress conditions led to a significant increase in root length by 27 and 18% compared to plants under stress. The separate use of 25 nm nanoparticles in the aerial organs also improved the root length by 27% compared to the stress conditions. However, our

results showed that the separate application of the three types of nanoparticles used in this research was more significant on the longitudinal growth of roots compared to aerial organs, but the mutual effect of these three types of nanoparticles was quite significant according to the interaction table, and especially the combined effect of nanoparticle 25 Nanometer zinc and iron had the greatest effect on improving the longitudinal growth of wheat plants under the influence of drought stress (Figure 2).

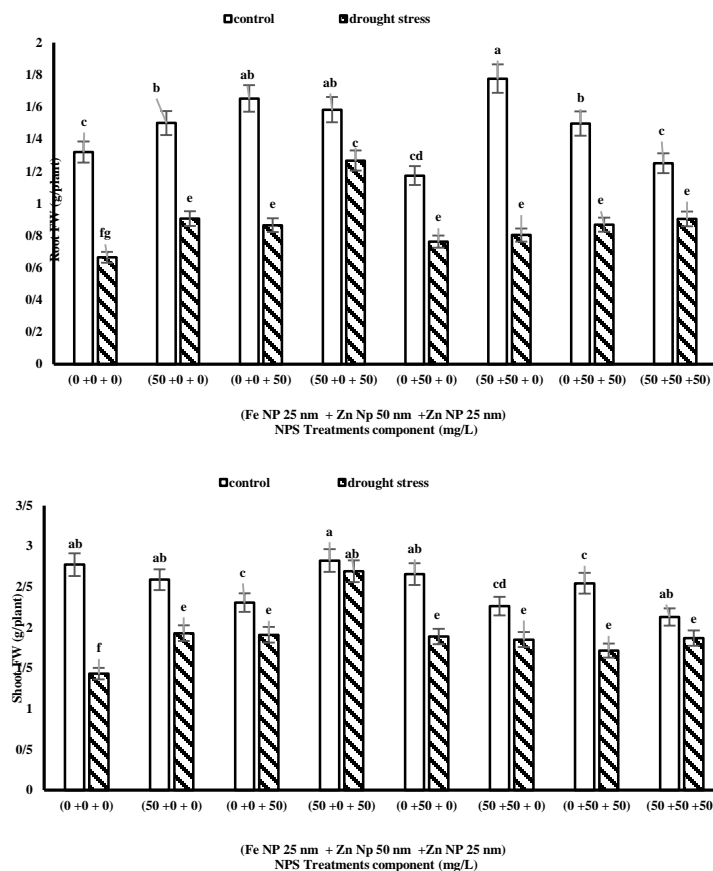


Fig. 3: The effect of zinc oxide (25 or 50 nm) and iron oxide nanoparticles under control or drought stress conditions on the shoot and root Fresh weight (FW) mean in Arg cultivar of wheat plants. Different letters indicate significant difference based on Duncan post hoc test ($P < 0.05$). Error bars show standard error (SE). The numbers 0 and 50 under the horizontal axis of the graph represent the concentrations of 0 and 50 mg/L of each type of nanoparticle used.

Drought stress alone without the use of nanoparticles caused a 50% decrease in shoot weight compared to the control. The use of nanoparticles in most cases caused an increase in weight. Only when both types of zinc nanoparticles were used together, the fresh weight of aerial parts did not increase significantly compared to the control and only a 20% significant increase was observed. The combined use of both 25 nm zinc and iron nanoparticles led to a significant improvement in shoot weight by 88% compared to plants under drought stress (Fig 3). However, the separate or combined application of iron and zinc nanoparticles improved the root wet weight in dry conditions, but the effect was less than the shoot weight parameter, and the best effect was related to the 50% increase in root wet weight, which was obtained from the 25 nm zinc nanoparticle. It was used together with iron nanoparticles under drought stress conditions (Fig 3). In general, the separate or combined application of iron and zinc nanoparticles improved the leaf

area up to 92% of the control value or even more, and the increase in leaf area was related to the combined application of 25 nm nanoparticle with iron nanoparticle. For example, the combined application of 50 mg/L of each of Fe nanoparticles and both Zn nanoparticles caused the leaf area to be close to control plants under drought stress conditions. This issue was also observed for the combined application of 50 nm iron and zinc nanoparticles (Fig. 4).

It can be said that the leaf surface data shows that in stress conditions, the use of three nanoparticles improved the leaf surface better and more favorably than in stress-free conditions, so that in stress-free conditions, the application of 50 mg/liter 50 nm zinc nanoparticles significantly reduced the leaf area by 27% compared to the control. In the stress conditions, the amount of leaf surface increased and improved under the effect of treatments containing iron and zinc nanoparticles, which was previously stated (Fig. 4).

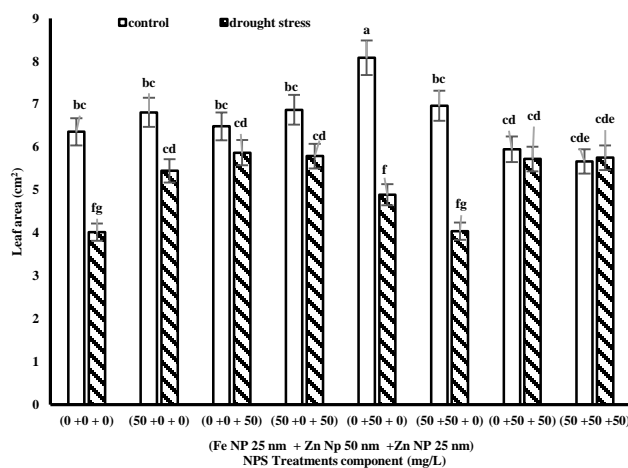


Fig.4: The effect of zinc oxide (25 or 50 nm) and iron oxide nanoparticles under control or drought stress conditions on the leaf area mean in Arg cultivar of wheat plants. Different letters indicate significant difference based on Duncan post hoc test ($P < 0.05$). Error bars show standard error (SE). The numbers 0 and 50 under the horizontal axis of the graph represent the concentrations of 0 and 50 mg/L of each type of nanoparticle used.

In the conditions of drought stress in wheat plants, the application of zinc nanoparticles with sizes of 25 and 50 nm increased the relative water content by 1.23 times compared to stressed plants. The greatest increase in relative water content is related to the combined use of both zinc

nanoparticles. But the combined use of all three nanoparticles significantly increased the relative water content by 78% compared to the second treatment code, which is the separate application of drought stress (Fig. 5).

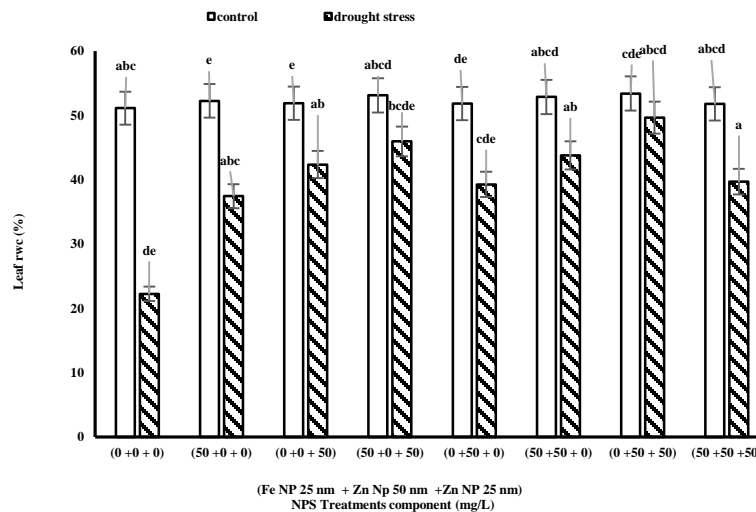


Fig. 5: The effect of zinc oxide (25 or 50 nm) and iron oxide nanoparticles under control or drought stress conditions on the leaf relative water content (RWC) mean in Arg cultivar of wheat plants. Different letters indicate significant difference based on Duncan post hoc test ($P < 0.05$). Error bars show standard error (SE).

In the conditions of drought stress without the application of nanoparticles, the percentage of ion leakage increased by 2.78 times than the control plants. The use of 50 nm zinc nanoparticles without the other two types of nanoparticles significantly reduced the amount of ion leakage in leaves under drought stress. However, the results of this research on wheat cultivar Arg showed that the use of triple nanoparticles decreased more than the separate use of ion

leakage. In the treatment code 16 according to the treatment table, this contained all three nanoparticles (Fe NPs 25 nm + Zn Nps 50 nm + Zn NPs 25 nm), the amount of ion leakage decreased by 4.58 times compared to the drought stress conditions in wheat plants (Fig. 6). The numbers 0 and 50 under the horizontal axis of the graph represent the concentrations of 0 and 50 mg/L of each type of nanoparticle used.

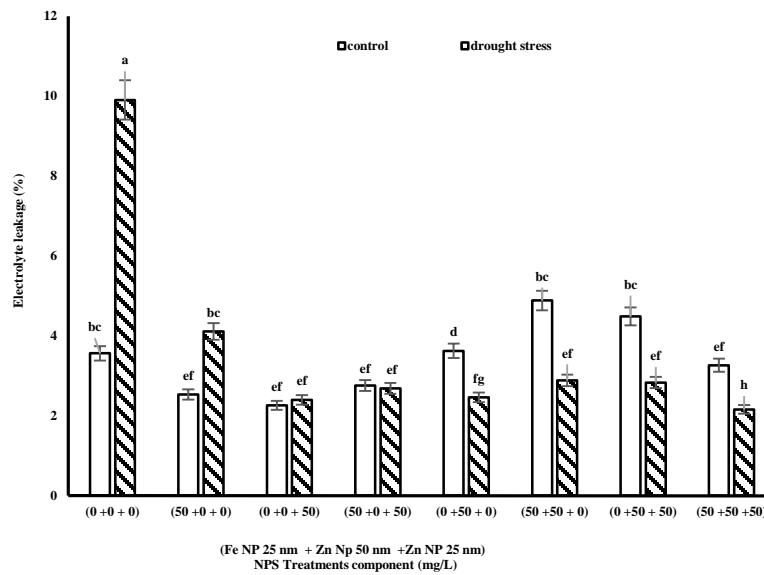


Fig. 6: The effect of zinc oxide (25 or 50 nm) and iron oxide nanoparticles under control or drought stress conditions on the percentage mean of leaf Electrolyte leakage in Arg cultivar of wheat plants. Different letters indicate significant difference based on Duncan post hoc test ($P < 0.05$). Error bars show standard error (SE). The numbers 0 and 50 under the horizontal axis of the graph represent the concentrations of 0 and 50 mg/L of each type of nanoparticle used.

The effect of the treatments had a significant and statistical effect on the change of dry weight (DW) of the plant. In this way, the stress of low water caused a 51% reduction in the dry weight of the stem of the plants under stress than the control plants, and the application of 25 nm zinc nanoparticles along with iron improved the dry weight of the lost stem and brought it to the level of the control plants (Figure 7).

The analysis of variance table shows that the experimental treatments generally had a significant effect on the amount of chlorophyll a, and drought stress alone reduced chlorophyll a in

the leaves of wheat plants by 38%. The slope of the bars shows that the application of 50 nm zinc nanoparticles without the other two types of nanoparticles improved and increased the amount of chlorophyll a in this treatment. Although the mutual effect of nanoparticles on the improvement of chlorophyll a under drought was significant, but the increase of this main leaf pigment in separate treatments of nanoparticles in conditions containing a period of dehydration in wheat plants was significant and impressive (Fig. 8).

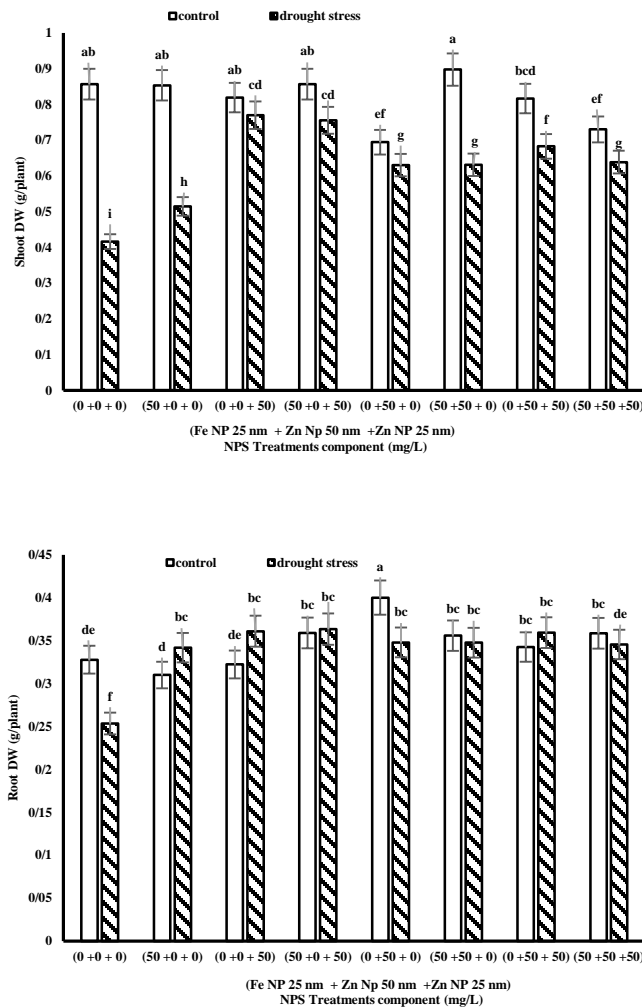


Fig. 7: The effect of zinc oxide (25 or 50 nm) and iron oxide nanoparticles under control or drought stress conditions on the shoot and root Dry weight (DW) mean in Arg cultivar of wheat plants. Different letters indicate significant difference based on Duncan post hoc test ($P < 0.05$). Error bars show standard error (SE). The numbers 0 and 50 under the horizontal axis of the graph represent the concentrations of 0 and 50 mg/L of each type of nanoparticle used.

Drought stress led to a significant decrease in the dry weight of aerial parts in aerial parts of wheat plants by 51% compared to control plants (Fig. 7). While these decreases in root dry weight was about 24%. The combined or separate use of zinc and iron metal oxide nanoparticles also led to an improvement in stem fresh weight, especially in aerial parts, compared to environmental stress conditions of dehydration in this research.

The application of 50 nm zinc and iron nanoparticles or both zinc nanoparticles together, this nanoparticle led to an increase in the amount of dry weight in aerial organs compared to stress. The use of both zincs nanoparticles separately and together with iron in the form of all three nanoparticles in both root and stem organs of stressed plants was significant at the level of 5% (Fig. 7).

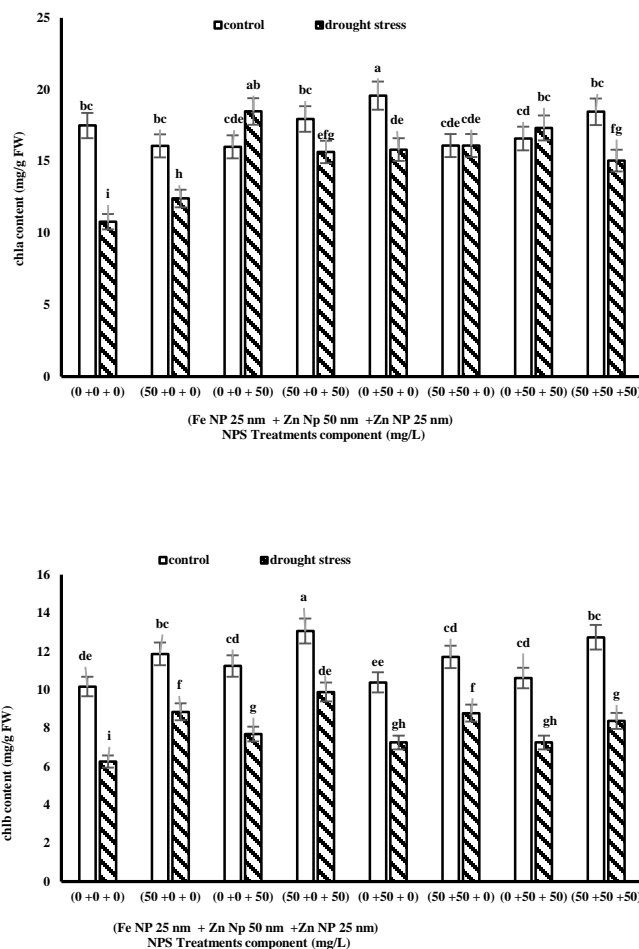


Fig. 8: The effect of zinc oxide (25 or 50 nm) and iron oxide nanoparticles under control or drought stress conditions on the leaf chl a and chl b content mean in Arg cultivar of wheat plants. Different letters indicate significant difference based on Duncan post hoc test ($P < 0.05$). Error bars show standard error (SE). The numbers 0 and 50 under the horizontal axis of the graph represent the concentrations of 0 and 50 mg/L of each type of nanoparticle used.

The 10-day irrigation period in this research reduced the amount of chlorophyll b in the leaves of wheat plants by 38% compared to the control. The application of iron nanoparticles combined with 25 nm zinc nanoparticles significantly improved the content of chlorophyll b to 63% of the control value. The combined use of all three nanoparticles significantly increased the amount of this type of chlorophyll compared to stress conditions (Fig. 8).

The highest amount of total chlorophyll in the leaves of the plants under 16 treatments used in this treatment was related to the 50 nm zinc

nanoparticle alone, which significantly increased the amount of total chlorophyll compared to the control without stress. However, the application of both 25 nm zinc and iron nanoparticles had the greatest effect in improving the chlorophyll content of the whole leaf compared to plants under drought stress. Because drought stress and water deficit caused a significant decrease in the amount of total chlorophyll by 34% compared to control wheat plants without drought stress (Fig. 9).

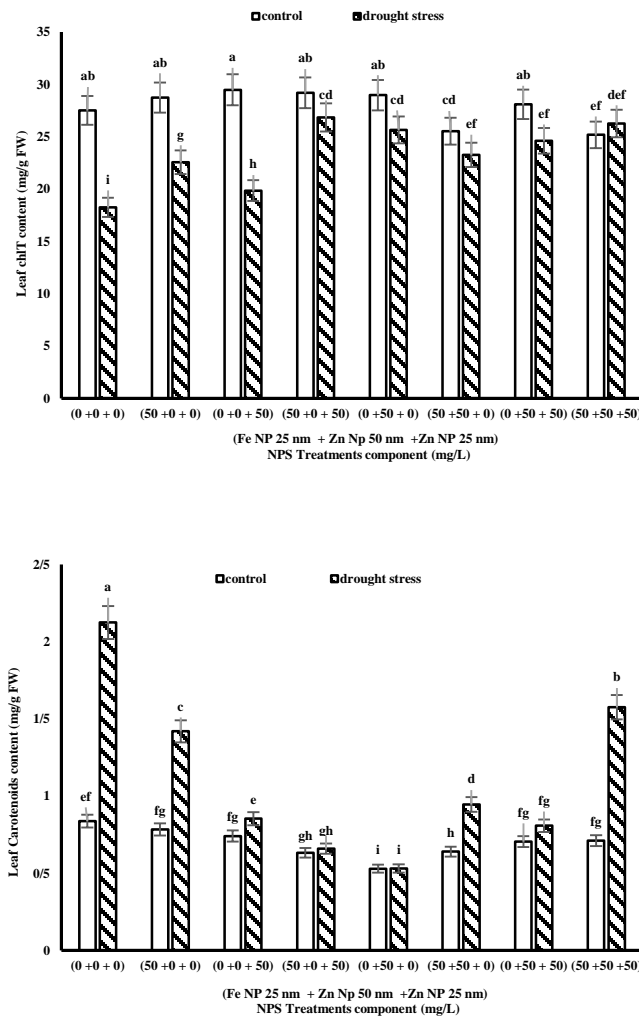


Fig. 9: The effect of zinc oxide (25 or 50 nm) and iron oxide nanoparticles under control or drought stress conditions on the leaf chlT and Carotenoids content mean in Arg cultivar of wheat plants. Different letters indicate significant difference based on Duncan post hoc test ($P < 0.05$). Error bars show standard error (SE). The numbers 0 and 50 under the horizontal axis of the graph represent the concentrations of 0 and 50 mg/L of each type of nanoparticle used.

Considering that the amount of leaf carotenoids of wheat plants increased by 60% compared to the control under drought stress conditions. But the use of nanoparticles in this leaf parameter was quite clear and obvious. The separate or combined effect of iron and zinc nanoparticles was decreasing and the amount of carotenoids returned to the control level. The greatest effect of reducing nanoparticles was related to the separate treatment of 50 nm zinc nanoparticles with a concentration of 50 mg/liter in the form of a 75% reduction in the amount of this auxiliary leaf pigment (Figure 9).

Table 3: Analyzing the variance analysis of the data obtained from the research with spss software that the effect of 16 treatment groups in three replications on Arg variety wheat plants at a significant level of 5%. The asterisk (*) means a significant effect of the treatment on the desired parameter with 95% accuracy.

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
Shoot length	Between Groups	179.237	15	11.949	1.066	.0413*
	Within Groups	482.000	63	11.209		
	Total	661.237	78			
Root length	Between Groups	262.182	15	17.479	1.107	.0179*
	Within Groups	678.750	63	15.785		
	Total	940.932	78			
Leaf area	Between Groups	177.009	15	11.801	32.680	.020*
	Within Groups	11.555	63	.361		
	Total	188.564	78			
Chla	Between Groups	682.323	15	45.488	49.788	.030*
	Within Groups	29.237	63	.914		
	Total	711.559	78			
Chlb	Between Groups	292.041	15	19.469	166.231	.041*
	Within Groups	3.748	63	.117		
	Total	295.789	78			
chlT	Between Groups	959.284	15	63.952	36.192	.034*
	Within Groups	56.545	63	1.767		
	Total	1015.829	78			
Cart	Between Groups	88.955	15	5.930	184.974	.024*
	Within Groups	1.026	63	.032		
	Total	89.981	78			
EL	Between Groups	57.433	15	3.829	107.376	.008*
	Within Groups	1.141	63	.036		
	Total	58.574	78			
Leaf RWC	Between Groups	.225	15	.015	124.762	.000*
	Within Groups	.004	63	.000		
	Total	.229	78			
shoot FW	Between Groups	5707.016	15	380.468	505.314	.038*
	Within Groups	24.094	63	.753		
	Total	5731.110	78			
Root FW	Between Groups	480.546	15	32.036	29.073	.042*
	Within Groups	35.262	63	1.102		
	Total	515.808	78			
Shoot DW	Between Groups	430.509	15	28.701	13.529	.049*
	Within Groups	67.885	63	2.121		
	Total	498.394	78			
Root DW	Between Groups	320.638	15	21.376	8.352	.012*
	Within Groups	81.898	63	2.559		
	Total	402.536	78			

4. Discussion

Drought is one of the important abiotic stresses that reduce the yield of agricultural products (Abedi and Pakniyat, 2010). One of these solutions to reduce the destructive effects of drought stress is the use of iron oxide and zinc oxide nanoparticles with new features, which was the main goal of this research. Nanoparticles (NPs) are considered as an effective and promising tool to increase crop yield in order to resist current and future limitations in terms of the occurrence of stresses in sustainable agricultural production by improving plant tolerance mechanisms in abiotic stress conditions, including drought (Chen, 2018).

In this research, the use of nanoparticles such as zinc oxide and iron oxide in optimal and controlled conditions in the hydroponic culture environment was proposed and evaluated as a suitable solution to relieve drought stress, increase resistance and improve growth in wheat plants of the new Arg variety.

Micronutrient elements are important for the growth, natural and physiological activities of plants. Zinc is one of the essential elements of low consumption for plants and plays important roles including the synthesis of proteins and carbohydrates, the metabolism of photosynthetic pigments, especially chlorophyll, increasing the photosynthetic power and metabolic actions of the cell, protecting the cell membrane in conditions of oxidative stress and the presence of free radicals. Iron also has its specific roles like zinc in plants. In this research, the role of zinc and iron nanoparticles in resistance to drought stress in Arg cultivar wheat plants was given importance, and the results obtained from both nutritional and stress resistance aspects are discussed (Scott et al., 2012).

The stated results showed that in this study, the amount of chlorophyll a and b types and total chlorophyll decreased significantly under

drought stress compared to the control (Figs 8, 9). Other researches also proved that reducing the accumulation of leaf pigments and their destruction in relatively sensitive plants to this stress is one of the symptoms of stress damage in plants (Sheikhbaglou, 2018). In an experiment, the levels of silicon, potassium and phosphorus nanoparticles in *Pisum sativum* were investigated and the positive effect of nanoparticles in improving the photosynthetic system of the plant has been proven. In our study, the application of zinc and iron nanoparticles also increased the level of chlorophyll pigments in the leaves of Arg wheat plants (Shi, 2002).

However, nanotechnology has been praised as a useful and practical field in the agricultural, food and medical industries (Alabdallah and Hasan, 2021). Recently, various nanoparticles (NPs) including titanium dioxide (TiO₂), iron oxide (Fe₃O₄), zinc oxide (ZnO), silicon oxide (SiO₂), copper (Cu-NPs) and selenium (Se-NPs) have been used for They are more threatening in the agricultural sector (Chen and Yada, 2011).

Various researches have proven that nanoparticles have positive effects on the growth and development of plants. However, these effects of nanoparticles can vary based on the origin, size, concentration, and time of application of the products (Auffan et al., 2009). Recently, nanoparticles have improved plant resistance against biotic and abiotic stresses such as drought. By enhancing antioxidant activity, nanoparticles protect plants against secondary and induced oxidative stress (Ahmad et al., 2021). Nanoparticles can reduce the toxic effects of periods of dehydration and drought stress by reducing the accumulation of hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) and maintaining the efficiency of the photosynthetic apparatus (Adrees et al., 2020; Ahmed et al., 2009).

Through the method of osmotic regulation by using the accumulation of a limited number of

osmotic active factors such as soluble sugars and amino acids, as well as by accurately regulating the entry and exit of ions and activating the system for removing different types of active oxygen, the plant can withstand drought stress or adapt it (Gong et al., 2005).

The yield and growth of the wheat plant are affected by stresses such as heat and drought, depending on the type of variety and the phenological stage of the plant. Other research has shown that foliar spraying of zinc oxide nanoparticles has increased the yield and resistance of plants compared to the application of conventional agricultural fertilizers. Drought stress reduces the relative water content, and foliar application of nano oxides of zinc and iron can moderate a part of the relative decrease of water under stress conditions and increase grain yield (Hasheminasab et al., 2012). The results of this research also showed this issue and the application of 25 nm nanoparticles of zinc and iron were able to improve and increase the length growth rate, dry and wet weight in root and stem as well as the relative water content in the leaves of Arg wheat plants (Fig. 1).

Nanoparticles can affect the organelle structure of plant leaf chloroplast and improve the activity of photosystem center 2 (PS-II) and the reactions of electron transfer and light absorption in chloroplasts under DS, and as a result, photosynthetic efficiency, organic matter production and plant growth (Shivakrishna et al., 2018). In this research, the improvement of the accumulation and preservation of chlorophyll pigments was observed (Figs 8, 9).

The accumulation, transfer and absorption of nanoparticles largely depends on factors such as plant species, size and type of nanoparticles, stability of nanoparticles and interaction of nanoparticles with roots, soil and soil microorganisms (Ali et al., 2021). For example, it has been confirmed that the accumulation of metal oxide nanoparticles by roots is significantly influenced by the chemical properties of

nanoparticles and the environmental conditions of plant growth (Ali et al., 2021). In another study, it was stated that the application of silver nanoparticles significantly increased the concentration of these nanoparticles in the roots and stems of lettuce plants (Silveira et al., 2019). However, according to previous research, water deficiency reduces chlorophyll synthesis, PS-II efficiency, and electron flow rate, and thus adversely affects the overall efficiency of plant photosynthesis (Semida et al., 2021). The use of NPs (ZnO) improved the synthesis and accumulation of chlorophyll, chlorophyll fluorescence and the activity of chlorophyll synthesis enzymes (chlorophyllase), which in turn improved the photosynthetic efficiency under drought stress (Liu et al., 2018). Exogenous application of nanoparticles also preserves the ultrastructure of chloroplasts and mitochondria, which increases photosynthesis under stress (Djanaguiraman et al., 2018).

Due to the lack of zinc in the soil, the use of this element as a fertilizer is very important in increasing the yield and quality of crops, but excessive use of this element can cause toxicity in plant growth and processes (Raliya et al., 2017). The negative effects of indiscriminate application of zinc fertilizer cause a decrease in the growth of roots and shoots, impaired absorption of other nutrients, especially phosphorus, and an increase in excessive absorption of iron. Drought is one of the important abiotic stresses that reduce the yield of agricultural products. One of these solutions to reduce the destructive effects of drought stress is the use of iron oxide and zinc oxide nanoparticles with new features, which is the main goal of this research (Taran et al., 2019).

Nanoparticles such as zinc and iron show mitigating effects against drought stress by resetting physiological and biochemical processes, modulating gene expression involved in response and tolerance to drought stress. Also, nanoparticles compensate carbon absorption

caused by drought by increasing photosynthetic activity (Semida et al., 2021).

Improving root length growth and its wet and dry weight, regulating the activity and structure of membrane aquaporin channels, improving intracellular water retention, accumulation of compatible ions and ionic homeostasis are the main effects of nanoparticles to reduce osmotic stress caused by water deficiency in plants (Afshari–Behbahanizadeh et al., 2016). NPs prevent the loss of water from the leaves by closing the stomata due to the accumulation of abscisic acid (ABA) and reduce oxidative stress damage by reducing reactive oxygen species and activating the antioxidant defense system (Mahmoud et al., 2020).

The results of our research and the measurements carried out in this article also showed that a resistance mechanism has been improved to withstand drought and improve growth in Arg wheat plants (Table 3). In this research, it was proved that there are interactions between NPs and wheat plants under drought and the results were significant. The mutual effect of iron and zinc nanoparticles on increasing and improving plant growth to withstand drought stress was observed. In general, this research highlights the importance of NPs in dealing with the problem of water deficit period in the plant growth environment which is also faced in the field.

5. Conclusion

In general, in this research, the mutual effect of two sized 25 and 50nm zinc oxide nanoparticles with 25 nm iron nanoparticles under drought stress conditions (a 7-day water deficit period) on the Arg variety of wheat plant was investigated. The results obtained from the investigation of the morphological characteristics of shoot and root growth, leaf pigments characteristics, as well as the investigation of some indicators of water content and ion leakage showed that the drought stress led to a decrease in the mentioned growth characteristics and an increase in ion leakage and

a decrease in the amount of chlorophyll a and b and the increase of carotenoids was significantly compared to stressed plants. The use of 25 nm zinc nanoparticles and iron oxide at a concentration of 50 mg/liter individually or especially together reduced the damage caused by drought stress on the growth of wheat plants. The effect of nanoparticles on roots and shoots under stress was not the same in most parameters and had significant differences. In other words, the root and stem response to the application of these two types of nanoparticles were different. In this way, the growth rate of the treated plants with the application of nanoparticles in the root and stem was different.

This means that the reactivity of roots and aerial organs to treatments containing nanoparticles is completely different under drought stress conditions. However, it should be stated that the 25 nm zinc oxide nanoparticle had a better effect than the 50 nm type in this research, that this nanoparticle together with the iron oxide nanoparticle improved the growth of Arg wheat plants under drought stress and made the plant more resistant to this stress made it more resistant. In line with the research, the following suggestions are presented: The effect of zinc oxide and iron oxide nanoparticles in the reproductive phase of the wheat plant should be investigated. The interaction effect of zinc oxide and iron oxide nanoparticles on wheat plant in field conditions should be studied similarly.

However, due to some laboratory limitations and the standard way of growing wheat plants in the greenhouse, this research should also be conducted in the field environment and the effect of environmental and climatic factors on the effect of this type of nanoparticle treatments in water shortage conditions should be investigated. It is also necessary to investigate this research in terms of antioxidants and the expression of important genes related to low and controlled error.

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