INVESTIGATING THE EFFECT OF USING NANOPARTICLES OF TITANIUM, SILICON, AND SUPERABSORBENT POLYMER ON SOME BIOLOGICAL TRAITS AND OXIDANT ENZYMES OF CUMIN PLANT UNDER DROUGHT STRESS CONDITIONS

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Abstract

The present study investigates the effect of using nanoparticles of titanium, silicon, and superabsorbent polymer on some biological traits and oxidant enzymes of cumin plants under drought-stress conditions. In this regard, a split factorial experiment was conducted in the form of a randomized complete block design with 3 replications. The availability of water at three levels of 50 mm (control), 100 mm, and 150 mm (dry stress) of evaporation from the class A evaporation pan were considered the primary factor, and the foliar application levels of nanoparticles of titanium, silicon, and the superabsorbent polymer was considered secondary factors. The primary factor was drought stress and the secondary factor was different levels of titanium nano oxide, silicon, and superabsorbent polymer each in 2 levels. The results revealed that the maximum level of carbohydrates (14.29 µmol/g) was observed in drought stress treatment of 150 mm of evaporation from the pan, and the maximum level of leaf proline (6.81 µmol/g) was obtained in the irrigation treatment of 150 mm of evaporation from the pan. Also, the maximum relative water content (77.18%) was observed in the irrigation treatment of 50 mm of evaporation from the pan and the minimum level (45 58.0%) was obtained in the treatment of 150 mm of evaporation from the pan. The results of the analysis of the variance of the data revealed that the simple effect of drought stress on the content of chlorophyll a of cumin leaves was significant and the content of chlorophyll b of cumin leaves was significantly affected by drought stress. The results of the analysis of the variance of data also showed that the simple and interaction effects of the experimental treatments on the carotenoid content of cumin leaves were significant. The primary effect of drought stress and fertilizer experimental treatments on catalase antioxidant activity was significant at probability levels of 1% and 5%, respectively.

Keywords: Cumin plant, Drought stress, Titanium nanoparticles, Silicon, and Superabsorbent polymer

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Introduction

Cumin is scientifically named Cuminum cyminum. It is a plant belonging to the Apiaceae family. It is an annual, aromatic, hairless (except fruit) plant. It has herbaceous stems and two and sometimes three branches. The stem of the plant is grooved and has peripheral collenchyma tissue. It has long, narrow, and white roots. Its stem is straight, and its leaves are narrow, thread-shaped, and green. It has beneficial and positive impacts on bloating caused by indigestion, swallowing air, eliminating intestinal gas, eliminating vaginal secretions (feminine secretions), and stopping menstruation in young women. Applying its poultice to the breast has beneficial impacts on the collection of milk in the breast (Omid Beighi, 2017). It increases the secretion of bile enzymes and improves the digestion of fats (Platel, 2002).

The results of studies conducted on cumin irrigation regimes are conflicting (Kondratenko, et al., 2021). Rahimian Mashhadi (1991) concluded that the maximum yield of cumin was obtained in a full irrigation regime (without applying drought stress) in the Mashhad region. However, in a three-year research on the climatic conditions of Mashhad, Sadeghi (1991) observed that the effect of irrigation in increasing the yield of cumin was not significant and it caused a reduction in the yield in normal years of rainfall (250 mm per year). The local experience of the farmers of Torbat-e Jam, Sabzevar, and Torbat-e Heydariyeh regions is also consistent with the results of Sadeghi's study (1991). Irrigation is useful when the total rainfall of the 120 days of plant growth is less than 150 mm (dry spring). The superabsorbent polymer can be used in piles (inside the pit), strips, and complete mixing with the soil (Khafar, et al., 2022). The significant issue in using these materials is that they should be well mixed with the soil and not used on the soil surface. It is due to the effect of the sun and ultraviolet rays on the superabsorbent, which causes it to break quickly. These materials become a swollen gel and retain water and dissolved food after contact with water.

The application of superabsorbent reduces water consumption by 40-50% depending on the type of plant, soil texture, and climatic conditions (Rahmani et al., 2009). Salar et al. examined the hydrophilic polymer effect on the irrigation cycle in melon cultivation. They examined the freshness characteristics of biomass and fruit weight. The results revealed that the use of hydrogel positively affected the tested characteristics. Najafi Alishah et al. (2012) also reported an increase in the number of fruits in cucumber by using superabsorbent polymer. In a study conducted on the effect of superabsorbent materials on the growth of Thomson seedlings, the results revealed that adding superabsorbent materials greatly increases the storage capacity of soils. Thus, the use of superabsorbent polymer increases the absorption and retention of gravity water in the soil and prolongs the irrigation cycle for the plant (Shirdel Shahmiri et al., 2009).

The effect of titanium nanoparticles on the germination of some plants and the growth of the willow tree was investigated. Oxide nanoparticles did not affect the growth of willow, although it was absorbed faster by the roots (Seeger et al., 2008). After the foliar application of titanium dioxide nanoparticles on wheat, Moaveni et al. (2011) showed that the seed and biological yield of this plant increased under the effect of nanoparticles compared to the control. The results revealed that water stress significantly reduces plant growth, yield, and yield components. Moreover, among the different titanium treatments, 0.02% titanium dioxide nanoparticles increased almost all agricultural traits including gluten and starch (Miere, et al., 2021). Thus, it is recommended to use titanium dioxide nanoparticles in the water stress condition (Jaberzadeh et al., 2013). In an experiment on corn, Morteza et al. (2013) confirmed that the content of chlorophyll a and the ratio of chlorophyll a to chlorophyll b (a/b), and other evaluated parameters were at the maximum range after applying 0.03% of titanium dioxide. The results revealed that nano TiO_2 has an increasing effect on the photosynthetic rate, so the treatment with rutile nano TiO_2 at a concentration of 2.5% showed the maximum photosynthetic rate (3.13 times more than the control).

Silicon is the second mineral element found in the soil after oxygen and occupies about 31% of the earth's crust (Epstein, 1999). The silicon solution in the soil is in the silicic acid $Si(OH)_4$ form, which is present in soil with a concentration of 0.1 to 0.6 mM. Pereira et al (2013) reported an increase in the synthesis of proline in a study conducted to examine the effect of using silicon under water stress conditions in pepper plants. Li et al (2007) examined the effect of drought stress and silicon on corn plants under greenhouse conditions. The results of their experiment revealed that silicone treatment increased growth and yield under drought stress conditions, and drought stress increased the content of proline in coriander plants (Alibadi Farahani et al., 2016). There are several reports on reducing the effects of different stresses, including heavy metal toxicity, dryness, and salinity with proper silicon nutrition.

Liang et al (1996) showed that silicon caused potassium absorption in two varieties of barley and decreased sodium absorption. As a result, silicon reduced salt toxicity in two varieties of barley. They also reported that silicon increased salt tolerance in two varieties of barley. Gunes et al (2007) also examined the effects of silicon on the enzymatic and non-enzymatic activity of antioxidants in 10 cultivars of *Spinacia oleracea* L. under drought stress. They observed that the activity of the superoxide dismutase (SOD) enzyme decreased in some cultivars and increased in others. However, in their study, the activity of this enzyme increased in all cultivars under silicon treatment. They also found that under drought stress, the activity of the catalase enzyme decreases significantly, while the treatment with silicon led to an increase in the activity of this enzyme in some *Spinacia oleracea* L. cultivars.

Genetics, environmental factors, and their interaction affect the quality and quantity of medicinal plants (Abdalla and El-Khoshiban, 2007). The water stress in different stages of development, especially the reproductive stage, is due to the reduction of the length of the photosynthetic period and the transfer of materials resulting from current photosynthesis to the seed, caused by the premature aging of the leaves and the reduction of the leaf area, and a reduction in the share of re-transfer of the materials stored in the stem to the seed. It causes a decrease in yield due to a decrease in the weight of the seeds. Given the significance of the cumin plant and its low water requirement in the flowering and fruit formation stages, the present study investigated the effect of using nanoparticles of titanium, silicon, and superabsorbent polymer on some biological traits and oxidant enzymes of the cumin plant in dry stress conditions.

Materials and Methods

The present study was performed in the crop year of 2018-2019 in two research farms in Pariz and Balvard regions in Sirjan city. Pariz region is located at 49°40' east longitude and 29°35' north latitude at an altitude of 1937 meters above sea level. Balvard region is also located at 56°11'east longitude and 29°18'north latitude at an altitude of 2050 meters above sea level. The experiment was conducted as a split plot as a randomized complete block design with 3 replications. Sampling was done from three points at a depth of 0-30 cm to evaluate the physical and chemical characteristics of the soil of the test site before planting (Table 1).

Table 1: Physical and chemical characteristics of the soil of the test area (Pariz) at a depth of 0-30 cm

Electrical conductivity	pH	Organic matter	N	P	K	Fe	Mn	silt	clay	sand	Soil texture
$ds.m^{-1}$		%			ppm				%		
1.8	7.6	0.61	0.07	13	137	2.2	3.1	27	31	42	Clay loam

Table 2: Physical and chemical characteristics of the soil of the test area (Balvard) at a depth of 0-30 cm

Electrical conductivity	pH	Organic matter	N	P	K	Fe	Mn	silt	clay	sand	Soil texture
$ds.m^{-1}$		%			ppm				%		

1.84	7.8	0.58	0.06	14	142	2	2.95	25	30	45	Clay loam

Studied traits

1. Soluble carbohydrates

Soluble carbohydrate was measured using the Keles and Oncel method (2004). In this regard, 0.2 g of dry leaf tissue with 10 ccs of 95% ethanol (or 5 ccs of 96% ethanol) was first heated in closed test tubes for one hour in a Bain-Marie bath at a temperature of 80 °C. After cooling, 1 cc of the samples was removed and 1 cc of 0.5% phenol and 5 cc of 98% sulfuric acid were added to it. Using a spectrophotometer, the amount of light absorbed at 483 nm was read. The amount of extracted carbon hydrate was obtained in µg/g of weight from the standard table.

2. Proline

Bates et al (1973) method was used to measure proline content using dry plant material. First, the aerial parts of the plant were harvested. Then, the samples were dried in an oven at 70°C for 48 hours.

3. Relative water content

Three fully-grown leaves were selected from each plot to measure the relative water content (RWC) of the leaves in the full flowering stage. The leaves were separated from the plant, placed in a nylon freezer, and placed in an ice flask (Moradi et al., 2008), and were transferred to the laboratory. In the laboratory, three leaf discs with a diameter of 1 cm were prepared from each leaf and after weighing (FW: fresh weight of the plant), they were placed in distilled water and kept at room temperature for 18 hours. Then, the discs were removed from the distilled water, and the surface moisture of the samples was measured using a dry towel, and their saturated weight (TW) and the samples were kept at a temperature of 70 °C for 24 hours and were placed in the oven, and the dry weight (DW: dry weight) was measured using the following formula.

(1) $WRC = [(FW - DW) /(TW - DW)]/100$

4. Chlorophyll a, Chlorophyll b, and carotenoids

Samples were prepared from the youngest fully-developed leaves and transferred to the laboratory in cold sealed containers to measure the concentration of chlorophyll a, Chlorophyll b, and carotenoids in the flowering stage. Then, discs with a diameter of 5 mm were prepared from the middle part of each leaf. After accurate weighing of them with a scale (accuracy of 0.001 g) and extraction with 80% acetone, the concentration of chlorophyll a, chlorophyll b, and carotenoids was determined using a spectrophotometer. It was determined at 663 nm for chlorophyll a, 645 nm for chlorophyll b, and 470 nm for carotenoids (carotene and xanthophyll). To calculate the concentration of chlorophyll a, chlorophyll b, and carotenoids (in mg/g of fresh leaves), the following equations were used (A: reading at the desired wavelength):

Chlorophyll a =12.25 A663 – 2.79 A645

Chlorophyll b = 21.5 A645 – 5.1 A663

Carotenoides = (1000A470 – 1.82 chl a- 85.25 chl b)/198

5- Catalase enzyme

The activity of the catalase enzyme was measured as follows. The total reaction solution is 1.5 ml (1500 µl). Then, 50 µl of enzyme extract, 600 µl of sodium phosphate buffer (pH=7), 0.15 µl of EDTA, and 549.85 µl of water were poured into the tube. Then, 300 µl of hydrogen peroxide was added to it and its absorption rate was immediately measured in a spectrophotometer at a wavelength of 240. Its absorption was read again after one minute. The absorption changes obtained in one minute were divided by the molar quenching coefficient of this reaction, which is equal to $36M^{-1}cm^{-1}$, and the enzyme activity was expressed in terms of units per gram of fresh weight.

6- Guaiacol enzyme

The concentrations used for the enzyme are 100 mM sodium phosphate buffer (pH=7), 0.1 µM EDTA, 5 mM guaiacol, and 15 mM H2O2.

7- Ascorbate enzyme

The reaction complex (one ml) containing 250 µl of 100 mM phosphate buffer solution, pH=7, 250 µl of 1 mM ascorbate, 250 µl of 0.4 mM EDTA, 190 1 µliter of double-distilled water, 10 µl of 10 mM hydrogen peroxide, and 50 µl of enzyme solution were extracted to measure the activity of ascorbate peroxidase (APX) enzyme by the Sairaam et al (2002) method. The absorption of the reactive complex was recorded at a wavelength of 290 nm and the enzyme activity was calculated using the extinction coefficient of 2.8 $mmol^{-1}$ cm^{-1}.

8. Percentage of essential oil

The essential oil was extracted from dry seeds by an essential oil extraction device and water distillation method in the research laboratory of the Faculty of Agriculture of the University. After calculating the weight percentage of the essential oil in total, its performance per surface unit was determined.

Results and Discussion

1-Soluble carbohydrates

The maximum content of carbohydrates (14.29 µmol/g) was observed in the drought stress treatment of 150 mm evaporation from the pan (Figure 1). The increase in carbohydrates due to drought stress is that the plant increases its internal osmotic pressure to absorb nutrients and water from the soil. The distribution of hydrocarbons is directly affected by stresses such as water shortage and indirectly by plant hormones. The accumulation of organic compounds such as carbohydrates and amino acids in the cytoplasm plays a significant role in regulating the osmotic pressure of plants. Soluble sugars are compatible osmolytes that accumulate in dry conditions and may act as osmotic agents. They are directly affected by stresses such as water shortage and indirectly by plant hormones. The accumulation of organic compounds such as carbohydrates and amino acids in the cytoplasm plays a key role in regulating the osmotic pressure of plants.

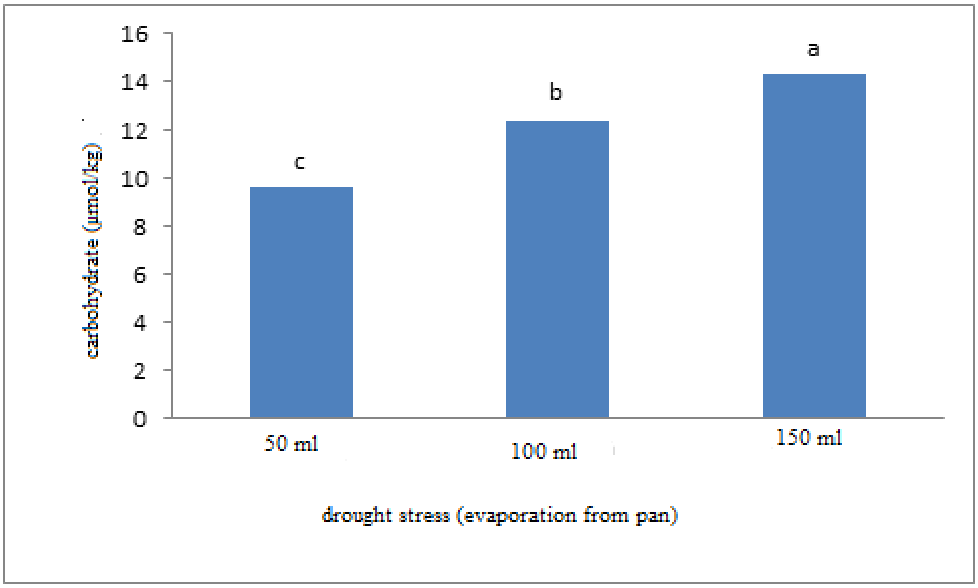

Figure 1: Simple effect of drought stress on cumin leaf soluble carbohydrates

2. Proline

The maximum content of leaf proline (6.81 µmol/g) was observed in the irrigation treatment with 150 mm of evaporation from the pan and the minimum (5.11 µmol/g) was observed in the control treatment (50 mm of evaporation from the pan) (Figure 2). A comparison of mean data showed that the maximum (6.67 µg/g) and the minimum (5.37 µg/g) amount of leaf proline was related to the foliar application of titanium and silicon nanoparticles, respectively (Figure 3). Thanks to its osmotic role, proline has beneficial effects on plants under stress. One of these strategies of plants in drought stress conditions is to increase the accumulation of organic and mineral substances in plant cells to absorb more water through the osmosis phenomenon. Proline increase in plants during stress is a defense mechanism (Kuznetsov and Shevyankova, 1997). The increase of proline as a result of a reduction in soil moisture may be due to drought stress on plant growth, which proline prevents further damage to the plant. Bits et al (1973) stated that the concentration of proline amino acid increases and seed yield decreases under drought stress conditions. Moreover, when the plant faces water shortage, the concentration of proline may increase up to 100 times under normal conditions. In some plants, in the early stages of drought stress, several amino acids increase, and with the continuation of drought stress, only proline amino acid accumulates and stores more.

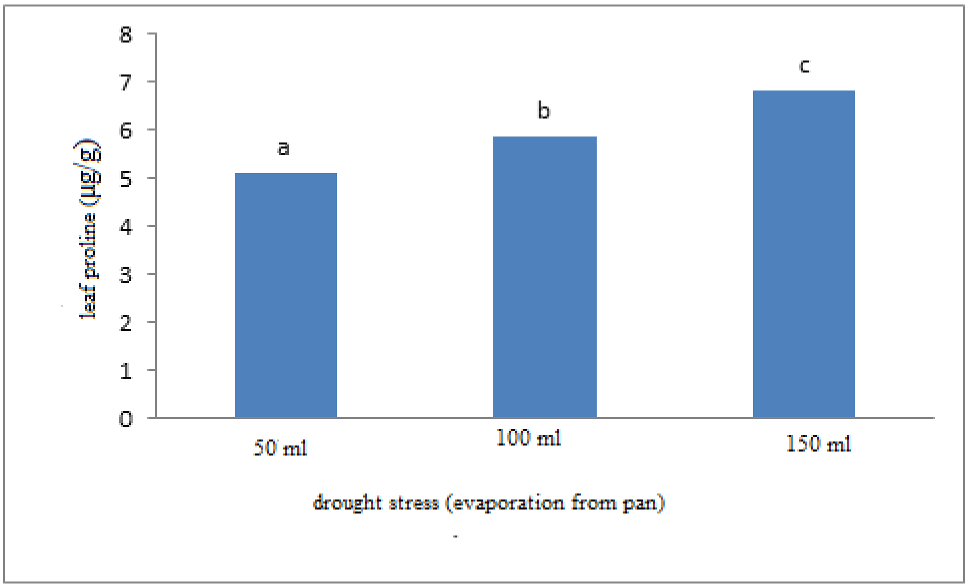

Figure 2: Simple effect of drought stress on cumin leaf proline

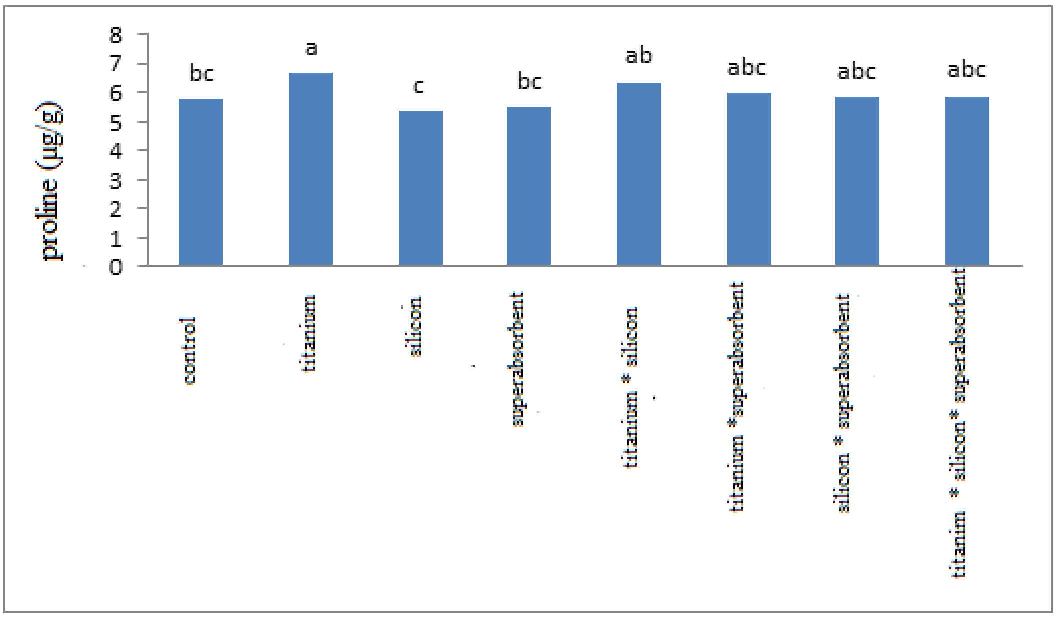

Figure 3: Simple effect of fertilizer treatments on cumin leaf proline

3. Relative water content

The maximum leaf relative water content (77.18%) was obtained in the irrigation treatment with 50 mm of evaporation from the pan and the minimum content (58.45%) was obtained in the treatment of 150 mm of evaporation from the pan (Figure 4). Drought stress leads to a reduction in water relative content (RWC), total water potential, and a reduction in plant growth (Bajji et al., 2001). However, the existence of the osmotic regulation mechanism in drought-tolerant plants helps to maintain and keep the RWC high in the plant. A reduction in the relative water content of the leaves due to drought stress has a high positive correlation with the soil moisture content. A reduction in the relative water content has been reported in the leaves of corn, soybeans, and beans during drought stress in different growth stages.

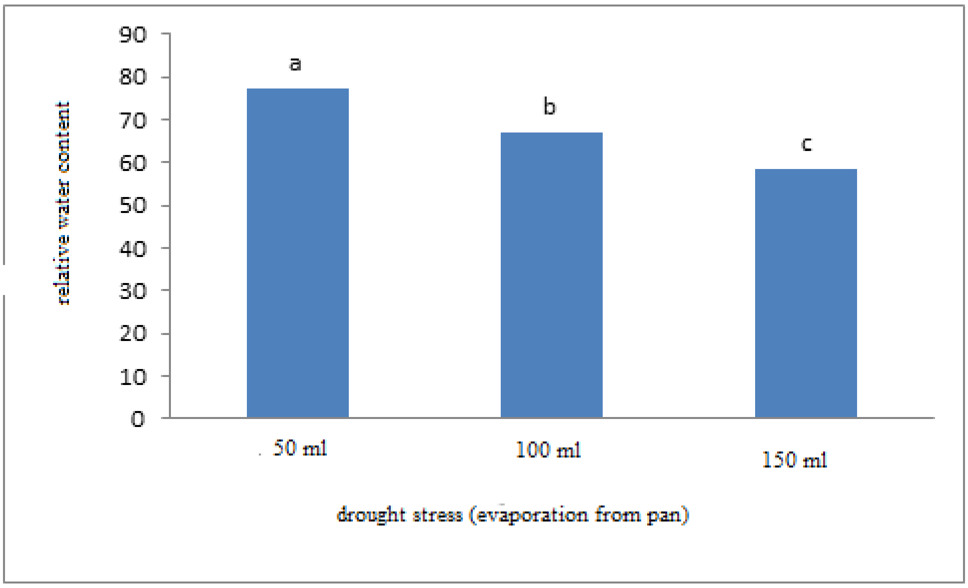

Figure 4: The simple effect of drought stress on the relative water content of cumin leaves

Table 3: Analysis of the variance of some biochemical traits of cumin under experimental treatments

		Mean of squares		
Source of variations	df	carbohydrate	proline	Relative water content
place	1	ns 0.03	ns 0.02	ns 24.6
place* replication	4	0.71	0.41	1.72
Irrigation level	2	**131.02	**17.32	**2114.4
place *Irrigation level	2	ns 0.31	ns 0.031	ns 11.81
Primary error	8	2.67	0.33	6.23
fertilizer	7	0.52 ns	*1.6	ns 1512
place *fertilizer	7	ns 0.4	ns 0.151	ns 1.21
Irrigation level * fertilizer	14	1.46ns	0.45 ns	ns 16.08
place* Irrigation level * fertilizer	14	ns 0.03	ns 0.161	ns 19.55
Secondary error	84	1.68	0.64	25.89
Total	143			
CV%	-	10.69	13.54	7.54

*, **, and ns, respectively, represent significant differences at the probability levels of 5%, 1%, and lack of significant difference.

4. Chlorophyll a

The results of the analysis of the variance revealed that the simple effect of drought stress on the chlorophyll content of cumin leaves is significant (Table 4). The maximum amount of leaf chlorophyll a (25.03 mg/g) was observed in the drought stress treatment of 50 mm evaporation from the pan (Figure 5). Water stress causes chloroplasts to break and a reduction in the amount of chlorophyll. New plastids are formed, chlorophylls a and b decrease and the ratio of chlorophyll a to b also changes under the water stress. Rezvani et al. (2013) stated that the reduction of chlorophyll biosynthesis in two sainfoin species under drought stress was caused by the competition of glutamate kinase enzyme (proline catalyzing enzyme) and glutamate ligase enzyme (the first enzyme of chlorophyll biosynthesis pathway), which caused glutamate precursor will consume more proline and thus, chlorophyll biosynthesis will be limited. Also, one of the reasons for this reduction is the increase in the activity of chlorophyllase enzyme, which is induced under drought stress conditions (Ranjan et al: 2001). The reduction of chlorophyll under water stress conditions observed in this study was also reported in a study by Remroudi et al. One of the significant physiological processes of plants is photosynthesis, the intensity of which is reduced in a water shortage. Reduction and degradation of photosynthetic pigments under drought stress conditions have been reported in other studies such as Karimi (2009) in basil and Karmaka (2007) in sunflower.

The results revealed that foliar application of titanium dioxide and silicon nanoparticles affected the amount of chlorophyll and increased it, which is in line with the results of the study by Morteza et al. in corn. These researchers stated that the reason for the increase of these pigments compared to the control treatment is the stabilization of the chloroplast membrane and the protection of the chloroplast from aging during the flowering of the corn plant by titanium dioxide nanoparticles. In other words, these nanoparticles can improve the structure of chlorophyll and receive sunlight through these pigments.

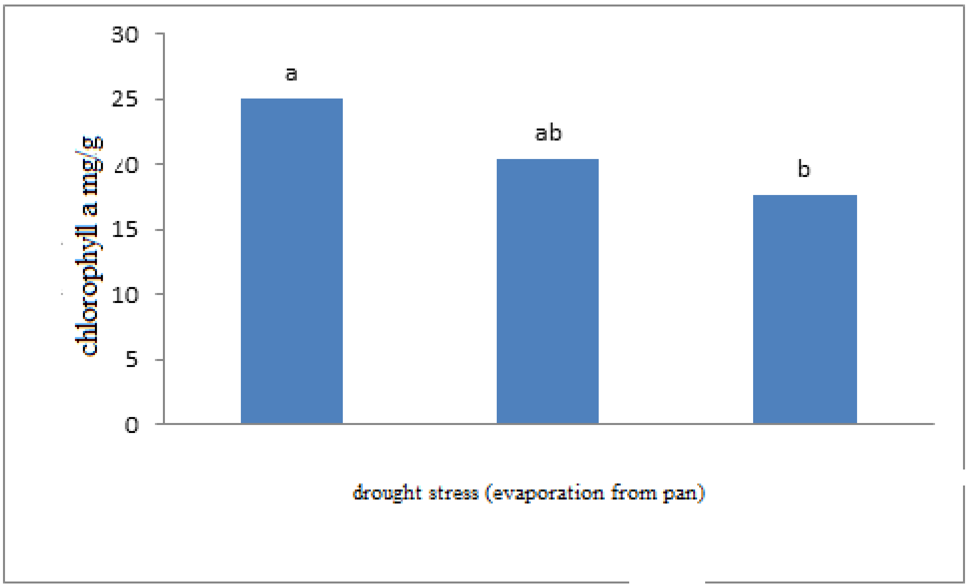

Figure 5: Simple effect of drought stress on chlorophyll a content of cumin leaves

5. Chlorophyll b

The analysis of the variance of the data showed that the chlorophyll b content of cumin leaves was significantly affected by drought stress (Table 4). The results of comparing the mean data showed that the maximum content of chlorophyll b in cumin leaves (9.98 mg/g) was obtained in the irrigation treatment of 50 mm of evaporation from the pan, which did not show a significant difference with the irrigation treatments of 100 mm of evaporation from the pan. The minimum content of leaf chlorophyll b (7.72 mg/g) was also obtained in the treatment of 150 mm of evaporation from the pan. Moreover, the results of the analysis of the variance of the data showed that the content of leaf chlorophyll b was affected by fertilizer treatments at a probability level of 1%.

Comparing the mean data showed that the maximum content of chlorophyll b leaves (9.67 mg/g) was obtained in the treatment of the foliar application of titanium nanoparticles along with silicon nanoparticles, and the minimum content of chlorophyll b leaves was observed in the control treatment. Water stress is one of the significant sources of abiotic stress, so it causes a reduction in growth, development, and yield during the vegetative, reproductive, and ripening stages of the crop (Santos: 2004). Drought stress can cause oxidative stress (Chaoz v Aolioyra: 2004), which can lead to a reduction in the content of chlorophyll a and b pigments (Etorbormaks:

1998), resulting in a reduction in the photosynthesis capability.

6. Carotenoids

The results of the analysis of the variance of the data revealed that the simple and interaction effect of the experimental treatments on the carotenoid content of cumin leaves was significant (Table 4). A comparison of the mean data showed that the maximum amount of leaf carotenoid (7.23 mg/g) was obtained in the treatment of drought stress of 50 mm evaporation from the pan along with the foliar application of silicon and titanium nanoparticles, and the minimum content of leaf carotenoid (4.85 mg/g) was obtained in the drought stress treatment of 150 mm evaporation from the pan and the control treatment.

Table 4: Analysis of the variance of some cumin traits under experimental treatments

		Mean of squares		
Source of variations	df	chlorophyll a	chlorophyll b	carotenoid
place	1	ns 30.13	ns 4.82	ns 0.6
place* replication	4	4.22	5.34	1.95
Irrigation level	2	**338.2	**31.06	**12.19
place *Irrigation level	2	ns 2.31	ns 2.01	ns 0.81
Primary error	8	33.72	4.34	0.31
fertilizer	7	5.98 ns	**3.21	*0.58
place *fertilizer	7	ns 2.4	ns 0.151	ns 0.21
Irrigation level * fertilizer	14	6.81ns	0.61 ns	*0.4
place* Irrigation level * fertilizer	14	ns 4.03	ns 0.161	ns 0.55
Secondary error	84	5.48	0.7	0.21
Total	143			
CV%	-	11.13	9.36	7.75

*, **, and ns, respectively, represent significant differences at the probability levels of 5%, 1%, and lack of significant difference.

7. Catalase enzyme

The results of the analysis of the variance of the data showed that the primary effect of the experimental treatments of drought stress and fertilizer on the level of catalase antioxidant activity was significant at the probability level of 1 and 5%, respectively (Table 5). The maximum amount of catalase antioxidant activity (0.192 µmol/mg) was observed in the drought stress treatment of 150 mm evaporation from the pan. Also, among the fertilizer treatments, the maximum (0.193 µmol/mg) was obtained from the treatment of the foliar application of titanium nanoparticles (Figures 6 and 7).

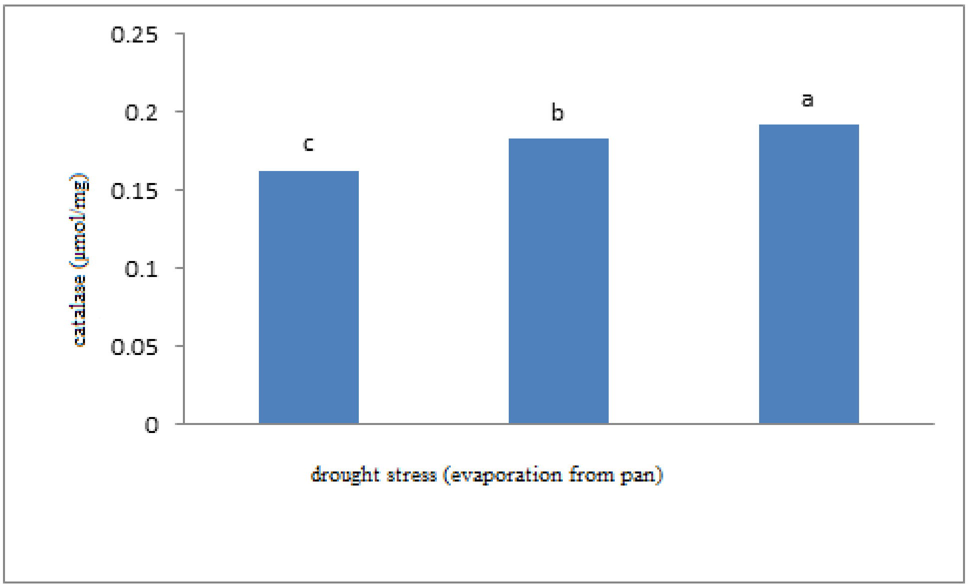

Figure 6: Simple effect of drought stress on catalase enzyme activity of cumin leaves

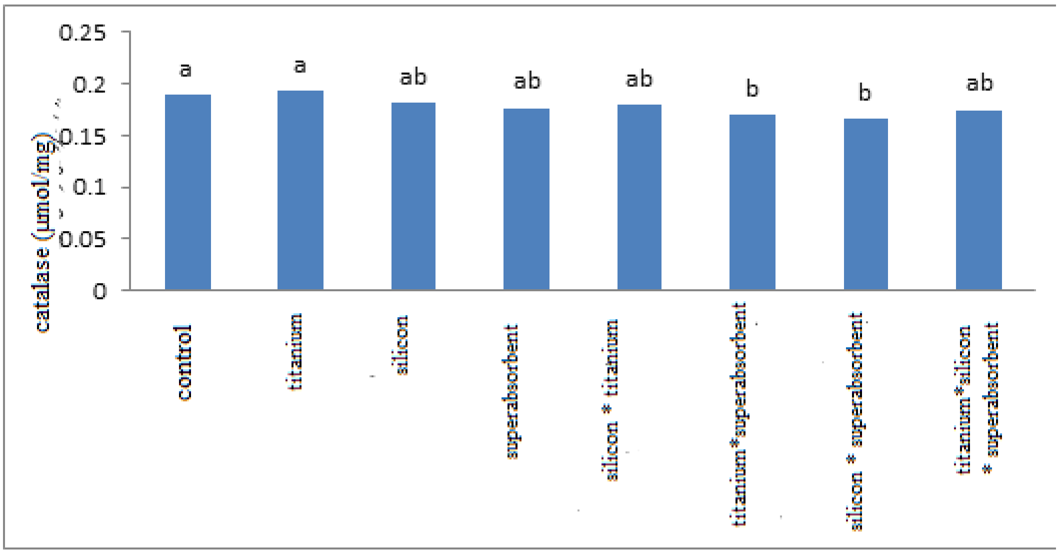

Figure 7: Simple effect of fertilizer treatments on the activity of leaf catalase enzyme in cumin

8-Guaiacol enzyme

Drought stress significantly affected the antioxidant activity of guaiacol at the 1% level (Table 5), so the maximum level of activity of this enzyme was obtained in severe drought stress (150 mm of evaporation from the pan) with a mean of 0.169 µmol/mg and the minimum level was obtained in control plant in mild stress (50 mm of evaporation from the pan) with a mean of 0.129 (µmol/mg) (Figure 8). The effect of fertilizer treatment and the interaction effect of the experimental treatments on the antioxidant catalase did not have a significant effect

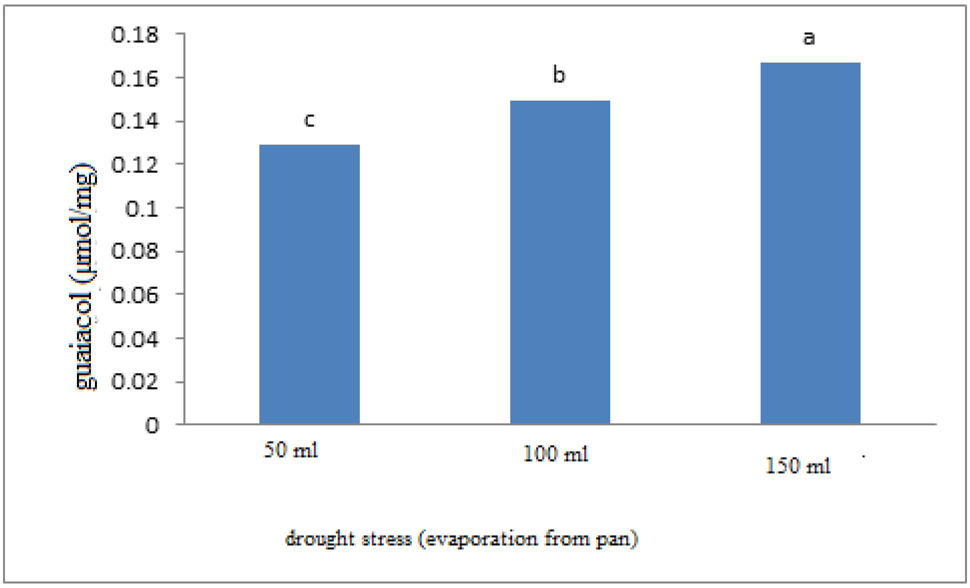

Figure 8: simple effect of drought stress on guaiacol enzyme activity of cumin leaves

9- Ascorbate enzyme

As shown in Table 1, the interaction of the experimental treatments significantly affected the activity of the antioxidant enzyme ascorbate peroxidase (Table 5). The maximum level of its activity (0.173 µmol/mg) was related to the treatment of drought stress of 100 mm of evaporation from the pan and the control treatment and the minimum level (0.107 µmol/mg) was related to the treatment of drought stress of 50 mm of evaporation from the pan and by foliar application of silicon nanoparticles (Figure 9). A large number of reactive oxygen species such as superoxide anion, hydroxyl radicals, and hydrogen peroxide are produced when the plant is exposed to stress. In many plants, the enzyme system is activated to destroy these radicals (Jangir et al., 1996). The activities of antioxidant enzymes are often increased when exposed to environmental stress, and accordingly, plants can reduce the damage caused by free radicals. Under water stress conditions, the stomata are closed in the plant, and subsequently, the 2CO concentration in the mesophyll tissue decreases. It results in a disruption in dark reactions and the products of the light reactions, including NADPH and ATP, are not consumed.

Under such conditions, NADP+ decreases to receive electrons due to the non-oxidization of the consumed NADPH molecule. Thus, the oxygen molecule acts as an electron substitute acceptor in the path of the electron transfer chain and leads to the formation of superoxide radicals ($O2^-$), hydrogen peroxide radicals (H2O2), and hydroxyl (OH^-). ROS potentially can react with many cellular compounds and cause damage to the membrane and other essential macromolecules such as photosynthetic pigments, proteins, nucleic acids, and lipids (Barker et al., 2003). The foliar application of titanium on pepper leaves increased ascorbic acid and capsanthin (responsible for causing red pigment) in pepper fruit. The titanium also affects the biochemical activities of the plant and increases the activity of catalase, nitrate reductase, and peroxidase enzymes.

These researchers attributed the increase in the activity of these enzymes under the effect of titanium to the increase in iron absorption. The growth of the pepper plant was due to the increase in the length of the plants, and titanium led to an increase in photosynthesis and the length of the pepper plants by increasing the absorption of nitrogen. The increase in the pepper and its sugar content due to the increase in chlorophyll caused by the application of titanium treatment has also been reported (Martínez-Sánchez et al., 1990). Treatment with Tio2 increases the activity of antioxidants: superoxide dismutase (which converts O2- to H2O2 and O2), catalase, and peroxidase (which converts H2O2 to H2O and O2) significantly compared to control and TiO2 (Zheng et al., 2008).

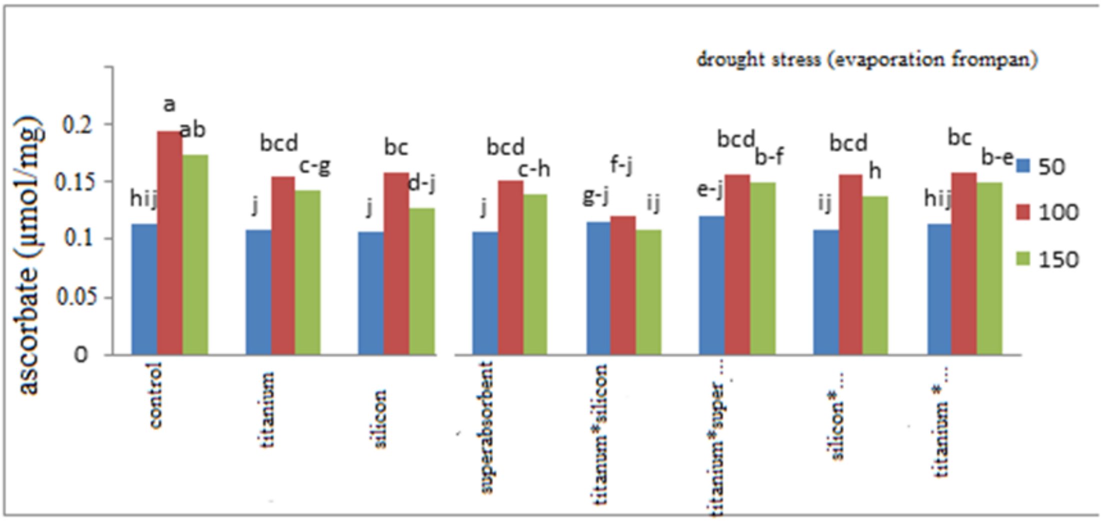

Figure 9: The interaction effect of drought stress and fertilizer treatments on the level of leaf ascorbate enzyme activity in cumin

Table 5: Analysis of the variance of antioxidant enzyme activity of cumin under experimental treatments

		Mean of squares		
Source of variations	df	catalase	Guaiacol	Ascorbate
place	1	0.0005 ns	0.003ns	ns 0.0005
place* replication	4	0.00003	0.0002	0.001
Irrigation level	2	**0.005	**0.0086	**0.012
place *Irrigation level	2	0.0015ns	0.0023ns	ns 0.0025
Primary error	8	0.00004	0.002	0.0006
fertilizer	7	*0.0007	0.003ns	0.001**
place *fertilizer	7	0.0011ns	0.0043ns	ns 0.0015
Irrigation level * fertilizer	14	0.0005ns	0.002ns	*0.004
place* Irrigation level * fertilizer	14	0.005 ns	0.0003ns	ns 0.0005
Secondary error	84	0.0003	0.0003	0.0002
Total	143			
CV%	-	9.69	12.44	10.22

*, **, and ns, respectively, represent significant differences at the probability levels of 5%, 1%, and lack of significant difference.

10. Percentage of essential oil

The results revealed that the interaction effect of water availability at different levels of titanium on the percentage of essential oil was significant at the 5% probability level (Table 6). The maximum percentage of essential oil in the irrigation treatment was obtained after 50 mm of evaporation from the pan and foliar application of titanium. When exposed to water shortage (no limitation of light energy), a severe decrease was observed in the activity of their photosystems, resulting in closing the stomata to minimize the waste of water due to transpiration. This process disrupts the absorption of CO2 by the leaves, and thus, lower amounts of CO2 are stabilized by the

Calvin cycle. Also, the consumption of reduction coenzymes (NADPH + H +) decreases significantly and a large amount of these reduction equivalents are accumulated. Therefore, only a small amount of oxidized reduced coenzymes (NADP +) will be available as an electron acceptor. Thus, large amounts of NADPH+ H+ accumulate in the cell. Given the decreasing process of coenzymes (H+ NADPH), reactions should also work toward their consumption, which will increase as a result of the synthesis of secondary compounds such as phenols, terpenes, alkaloids, cyanogenic glycosides, and glucosinolates (Khodakovskaya, 2009).

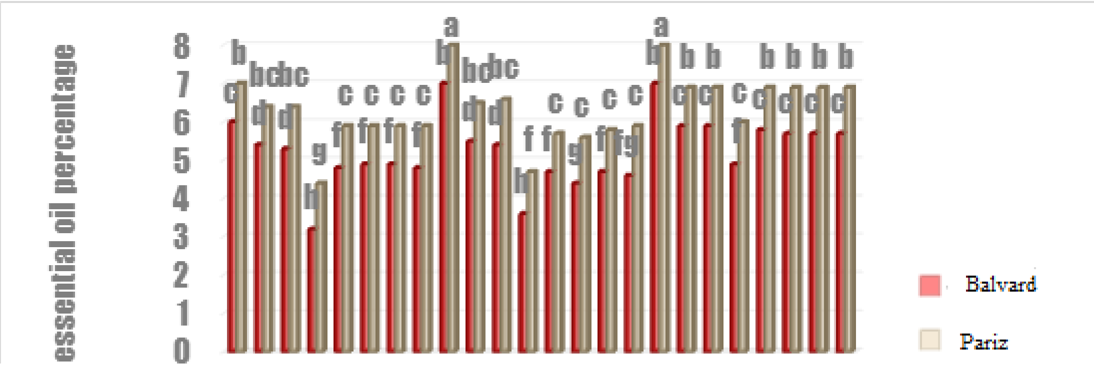

Figure 10- The interaction effect of drought stress, fertilizer treatments, and place on the percentage of cumin essential oil

Based on Table 6, the interaction of the experimental treatments significantly affects the percentage of essential oil (Table 6). The maximum amount of essential oil 8% is related to the treatment of the Pariz area, the stress of 150 mm evaporation from pan A, and titanium * silicon * superabsorbent, and the minimum of 3.4% was related to the treatment of the Balvard area, the stress of 50 mm of evaporation from pan A without fertilizer (control).

Table 6: Interaction effects of experimental treatments

		Mean of squares
Source of variations	df	Percentage of essential oil
place	1	0.412**
place* replication	4	0.014
Irrigation level	2	0.119**
place *Irrigation level	2	214.9**
Primary error	8	0.002
fertilizer	7	0.173**
place *fertilizer	7	0.251^{ns}
Irrigation level * fertilizer	14	0.0903*
place* Irrigation level * fertilizer	14	0.0403*
Secondary error	84	0.005
Total	143	
CV%	-	4.71

Conclusion

The results revealed that the maximum amount of carbohydrates (14.29 µmol/g) was observed in the treatment of drought stress with 150 mm of evaporation from the pan. The maximum amount of leaf proline (6.81 µmol/g) belonged to the irrigation treatment of 150 mm of evaporation from the pan and the minimum (5.11 µmol/g) belonged to the control treatment (50 mm evaporation from the pan). The maximum relative water content of the leaf (77.18%) was observed in the irrigation treatment with 50 mm evaporation from the pan, and the minimum amount (58.45 percent) was obtained in the treatment of 150 mm of evaporation from the pan. The results of the analysis of the variance of the data revealed that drought stress significantly affected the chlorophyll content of cumin leaves.

The analysis of the variance of the data indicated that the chlorophyll b content of cumin leaves was significantly affected by drought stress. The results of the analysis of the variance of the data revealed that the simple and interaction effects of the experimental treatments on the carotenoid content of cumin leaves were significant. The results revealed that the primary effect of the experimental treatments of drought stress and fertilizer on the level of catalase antioxidant activity was significant at the probability levels of 1 and 5%, respectively. Drought stress significantly affected the level of 1% on the level of guaiacol antioxidant activity. The interaction effect of the experimental treatments also significantly affected the activity of the ascorbate peroxidase antioxidant enzyme. The interaction effect of water availability at different levels of titanium on the percentage of essential oil was significant at the 5% probability level.

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